



Ecology of fouling assemblages associated with mangrove's roots: An artificial substrate for manipulative experiments



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ABSTRACT

Several of the most influential studies in marine ecology are based on results obtained from experiments using units of artificial habitats. However, there are few studies that have measured the effectiveness of those experimental substrates as surrogates of natural ones, in terms of reflecting natural patterns of colonization and development of the natural assemblages. Such considerations are necessary to extrapolate the described patterns and processes in artificial habitats to natural communities. This situation is particularly noticeable in ecological studies dealing with the identification of process that sustain the high levels of species diversity reported for epibiont communities that grow on the roots of *Rizophora mangle* in the Caribbean Sea. That is, no study has demonstrated the validity of artificial substrate as surrogate of mangrove roots. Consequently, in this study we tested whether wooden sticks can be used as surrogates for natural prop roots. Furthermore, and in order to propose possible mechanisms explaining the observed patterns, we also tested whether differences in epibiotic assemblages on natural mangrove prop roots were due primarily to intrinsic (biological) differences among roots. The experiment was conducted in four randomly chosen localities of La Restinga National Park, Venezuela, to test for the generality of the observed outcomes. We used two types of substrates: broomsticks as artificial mangrove roots (AMR) and natural mangrove roots (NMR) denuded from epibionts. We recorded the presence and estimated the abundance of sessile species growing on the experimental treatments after 30, 130 and 200 days. No significant differences in species richness between the two substrates at any locality and at any time were detected. However, the effect of the substrate on the composition and abundance of species varied among localities and increased over time. Multivariate dispersion was not different between substrates, indicating that intrinsic biological characteristics of prop roots are not responsible for the observed differences among neighboring roots. Despite spatial differences at the scale of localities, most of the species recorded in this study colonized both types of substrates, although some species were more abundant over AMR (e.g. cirripeds, bryozoans), while others were more abundant on NMR (e.g. oyster, algae). Typical mangrove sponges and tunicates colonized similarly both types of substrates. Results obtained in this work suggest that broomsticks are appropriate surrogates of mangrove roots to experimentally evaluate processes associated with species diversity, but should not be used to evaluate processes that affect the abundance and structure of epibiont assemblages.

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1. Introduction

Assemblages associated with prop roots of the Caribbean red mangrove, *Rhizophora mangle* L., are very diverse and they can be considered as a particular assemblage of epibionts as they grow over a substrate that is alive (Wahl, 1989). More than 550 species, from almost every single existing phylum and from a very diverse range of algae classes, have been found living as epibionts on roots of *R. mangle* in the Caribbean

Sea (Díaz and Rützler, 2009; Lacerda et al., 2002; Nagelkerken et al., 2008; Rützler, 1969). Consequently, the diversity and ecology of these assemblages have been the topic of various regional revisions and analyses in the Caribbean (Collin, 2005; Diaz, 2012; Rützler et al., 2004). A common finding in studies dealing with these assemblages is the great variability in composition of species at different spatial scales, but particularly, among neighboring roots (Farnsworth and Ellison, 1996; Guerra-Castro et al., 2011a, 2011b; Hunting et al., 2008). Proposed mechanisms to explain this common pattern of spatial distribution, however, have varied considerably. In this sense, a myriad of models or explanations have been proposed, ranging from mechanisms related with larval supply and dispersal limitations (Bingham, 1992; Farnsworth and Ellison, 1996), biological interactions as predation

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(Wulff, 2005) and variability in the epibiosis relationship (Hunting et al., 2008, 2010b) to environmental mechanisms (Álvarez, 1989; Díaz and Rützler, 2009; Pawlik et al., 2007), where factors such as tidal amplitude, suspended sediment or salinity play a crucial role. In any case, in almost all these studies, ecologists have treated these assemblages as actual units of “natural” communities (e. g. Bingham, 1992; Farnsworth and Ellison, 1996; Sutherland, 1980), because natural temporal variations of the different species (recruitment and local extinctions) can be observed, and most importantly, can potentially be related to different ecological processes operating at different spatial and temporal scales. Identifying “natural units of habitats” of communities (sensu stricto of an ecological island) is of foremost importance in ecology, as the operational concept of “community” has traditionally been elusive for ecologists (Leibold et al., 2004; Ricklefs, 2008; Simberloff, 2004).

This property (i.e. each root can be considered as a “natural unit of habitat”) can be very useful to test hypotheses about ecological processes that determine and affect the development of benthic marine communities, because it allows the design and implementation of properly replicated and controlled manipulative experiments (Underwood, 1990). These assemblages, however, have a particular characteristic in comparison to other benthic marine assemblages, that is: they strive over a substrate that is alive (mangrove roots) and consequently it is very heterogeneous in space and variable in time (Gill and Tomlinson, 1969). Namely, all intrinsic characteristics of each root (e.g. length, shape, diameter, anatomy, physiological conditions) vary among regions, among patches of mangroves within a region, among different trees within a patch, among roots within a tree and even within the same root along time (Brooks and Bell, 2005; Gill and Tomlinson, 1977). Due to this “intrinsic characteristic”, it is difficult, if not impossible, to do properly controlled experiments using mangrove roots as replicate units; consequently, the use of artificial units that can perform as mangrove’s root surrogates is essential.

The need of using an artificial unit has traditionally been recognized in experimental ecology (Gill and Tomlinson, 1977; McGuinness, 1989), and especially in these systems (i.e. mangrove root epibiosis), because it allows standardized comparisons by eliminating historical effects associated with natural substrata and by reducing variability associated with physical differences such as position, orientation, size, etc. (McGuinness, 1989). Many artificial substrates have been attempted in this sense, with many different characteristics to test many different types of hypotheses. These artificial units ranged from asbestos plates used to describe succession patterns of mangrove root epibionts (Sutherland, 1980); wooden stakes to experimentally examine the potential importance of mangrove epibionts to fish habitat (MacDonald et al., 2008), or ceramic tiles to describe recruitment rates of invertebrates (Bingham, 1992), going through PVC tubes to monitor survival and condition of typical mangrove and reef sponge species in mangrove and reef environments (Hunting et al., 2013b). However, some of these authors have pointed out that species composition on artificial substrates is very different from those found on surrounding natural roots (e. g. Bingham, 1992; Sutherland, 1980). Even though it is generally accepted that artificial units are not supposed to exactly mimic assemblages found in natural environments (McGuinness, 1989) as they are designed to “manipulate” or “represent” a particular characteristic of the natural system; it is of foremost importance that artificial units represent basic features of natural substrates, as the physical properties of the surface (e.g. texture, availability of microhabitats, shape) and host typical epibionts (Bulleri and Chapman, 2009; Glasby and Connell, 2001).

Various studies have shown that the development of different coastal assemblages depends on the nature of the substratum which they associate with (Anderson and Underwood, 1994; Bulleri and Chapman, 2004; Glasby et al., 2007; McGuinness, 1989). In particular, it has been shown that there are various cues and characteristics of the substrate that could stimulate and trigger the settlement of larvae of sessile

invertebrates such as: 1) physical properties (Crisp and Ryland, 1960; Eckman, 1990; Menge et al., 1983), 2) biochemical composition (Hunting et al., 2010a, 2010b; Pawlik, 1992; Rodríguez et al., 1993), 3) facilitation due to previous settled biota (Zardus et al., 2008; Zobell, 1939) and 4) a combination or interaction of the above. These processes determining the early settlement of larvae will then have a strong influence on the subsequent development of the assemblage (Bulleri, 2005; Lewin, 1986; Underwood and Fairweather, 1989). Despite these important considerations, the effectiveness of artificial units to mimic the natural conditions of mangrove roots has not been evaluated up to date.

To evaluate this, we are proposing to employ wooden sticks (i.e. broom sticks in this case) as artificial units mimicking mangrove roots, called from now on: AMR (Artificial Mangrove Roots). These AMR, have very similar shape, texture and heterogeneity to that presented by natural mangrove roots; however, they lack the biological ability of mangrove roots to produce biochemical substances like tannins or polyphenolic compounds. This feature also allows testing for potential effects of the “biological” component of the natural mangrove roots on the recruitment and colonization processes of epibenthic assemblages associated with them. Consequently, in this study we evaluated the following two models: (A) wooden stick can be used as surrogates for mangrove prop roots, and (B) potential differences in epibionts between AMR and natural roots are explained by “biological” characteristics of natural prop roots. Deriving from these models we proposed then the following null hypotheses: Structure and composition of assemblages will not differ between substrates. If this null hypothesis is not rejected, then we can directly support Model A (i.e. wooden stick can be used as surrogates of prop mangrove roots) without further considerations. If rejected, then we can propose an additional hypothesis derived from models aimed at explaining differences between substrates. In particular the model evaluated was that “biological” characteristics of the natural roots are important for the development of assemblages. If that model is correct the following hypothesis can be proposed: Variability among AMR would be smaller than that among natural prop roots. Consequently, the two main objectives of this study were to: (1) evaluate the null hypothesis that there are no differences between early stages of development of the assemblages associated with AMR and those associated with NMR, and (2) test whether high variability in epibiotic assemblages among neighboring natural roots are due primarily to intrinsic (biological) differences among the roots.

2. Methods

2.1. Study area

This experiment was done between October 2007 and April 2008 at “Laguna de La Restinga National Park” (LRNP) located in Margarita Island, Venezuela (Fig. 1). This marine reserve protects a coastal lagoon that has an area of ~30 km² and a maximum depth of 6 m. This lagoon is completely surrounded by very well developed mangrove forest (up to 5 m high). These mangroves extend all the way from the entrance of the lagoon, where the conditions of the water are similar to the marine system (37–39); to the internal systems of small lagoons, where the conditions are hypersaline (44 ± 2.5) (Gómez Gaspar, 1991).

2.2. Artificial units of recruitment and selection of natural roots

Pine wood sticks, commercially sold as broomstick, were used as artificial units of mangrove’s roots (AMR). Only broomsticks that did not receive any chemical treatment such as anti-termite sprays were used in this experiment. These units were 118 cm long and 2.2 cm wide (diameter) and had a perimeter of 7.5 cm. In the field, AMR were vertically fixed (around 90° and 75° on inclination) to branches of natural mangrove’s roots using two inch nails, always ensuring that 60–100 cm of the AMR was submerged into the water at low tide and

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