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EARLY COLONIZATION OF *POSIDONIA OCEANICA* (L.) DELILE BY EPIPHYTES: COMPARISON BETWEEN NATURAL AND ARTIFICIAL SEAGRASS LEAVES

Abstract

Posidonia oceanica is an important marine Magnoliophyta of the Mediterranean coastal zone that can form dense meadows. The leaves of this seagrass are colonized by a lot of organisms, from bacteria to polychaetes and algae. However, the early stages of colonization are not well known.

A preliminary is proposed to examine the nine first days of colonization by epiphytes on natural leaves and on Artificial Seagrass Units (ASUs). Aims of this work were to understand which species are the first to set up, to compare colonization on both leaves and to determine the interest of ASUs in ecological studies.

It was shown that the setting up of epiphytes on the bases of both leaves is rapid (first algae on the second day) but the number of organisms increases quicker on ASUs than on natural leaves and on the smooth faces than the rough ones. Shannon-Wiener diversity and evenness were higher for the *P. oceanica* leaves and the use of Bray-Curtis similarity index showed that colonisation is not similar (between 20 and 30 % similarity) on both leaves for the same day. It can be explained by the lower colonization rate of natural leaves. This lower rate is probably due to a less developed biofilm on natural leaves than on ASUs, to a difficult access to the bases of *P. oceanica* leaves for epiphytes and microorganisms, and the production of phenol compounds by the plant. Even if ASUs used here do not seem to be similar to natural leaves in early stages of colonization, they could be used in ecological studies thanks to their rapid covering by epiphytes.

Keywords: *P. oceanica*, ASUs, epiphytes, colonization.

Introduction

In the Mediterranean coastal zone, one can find dense meadows form by the marine Magnoliophyta *Posidonia oceanica*. This seagrass plays important roles like trapping particles in suspension, stabilizing the sediment, attenuating hydrodynamism of the waves, importing and exporting an important vegetal biomass and producing a large amount of oxygen. These meadows are also involved in many food webs and represent a place of nursery, reproduction and predation for a lot of ecological and economical interesting species. Thanks to that, they are sensitive to environmental perturbations and are often used as a pollution descriptor (Boudouresque & Meneisz, 1982; Gobert *et al.*, 2005; Pergent-Martini *et al.*, 2005; Procaccini *et al.*, 2003)

There are lots of different organisms on *P. oceanica* leaves. These epiphytes are as diverse as bacteria, diatoms, macro-algae, bryozoans, hydrozoans, foraminifera or annelids and represent 20 to 40 % of the biomass of seagrass leaves (Gobert *et al.*, 1995; Lepoint *et al.*, 1999). They take also actively part in total primary production of the meadows (27,5 %; Dauby *et al.*, in prep.) and to many food webs (Bell *et al.*, 1984; Lepoint *et al.*, 2000; Mazzella *et al.*, 1995).

Early stages of colonization by those epiphytes are not well known because only very few studies were realised on this subject, despite the importance of those species, especially on the ecological point of view (De Troch *et al.*, 2005; Novak, 1984; Virnstein & Curran, 1986). Indeed, epiphytes are useful in pollution studies because they react more quickly to

environmental perturbations than the plant itself thanks to their rapid turnover (Delgado *et al.*, 1999; Pergent-Martini *et al.*, 2005; Piazzzi *et al.*, 2004; Ruiz & Romero, 2001).

To study the colonization by epiphytes, the use of ASUs seems interesting because they can be put anywhere, whatever the conditions (Barber *et al.*, 1979; Lee *et al.*, 2001) and because it is a non destructive method. It also has the advantage to make possible the control of parameters like the number of leaves, the shoot density, leaves length and width, as well as the surface available for colonization (Bologna & Heck Jr, 1999; Trautman & Borowitzka, 1999).

For all these reasons, aims of this study are (1) to understand the setting up of epiphytes during the first days of colonization on the bases of *P. oceanica* leaves and on ASUs, (2) to compare the colonization on both types of leaves and (3) to determine the interest of ASUs in ecological studies.

Material and methods

This study was realised in the port of the oceanographic research station STARESO, Revellata Bay, Calvi Gulf (42° 35' N, 8° 43' E), Corsica, France.

This site is characterized by a continuous *P. oceanica* meadow which present a shoot density from 400 to 700 shoot/m² at 10 m (Soullard *et al.*, 1994) and a relatively low hydrodynamic regime (Bay, 1984). This meadow presents low anthropic perturbations because of the sewage of the station. However, the discharge was not important during this study (from March 6th to 15th 2005) because of the low frequenting rate in March.

The study was led at 10 m depth. Each ASU was made of a PVC band of 1 cm width and 50 cm length with a float at the extremity, fixed on a ballasted PVC tube and positioned inside the meadow. At the same time, *P. oceanica* shoots were marked with Zieman's method (Zieman & Wetzel, 1980) to compare adequately data on both types of leaves.

Each day of the study, one ASU and one marked natural shoot were taken by scuba diving. For the *P. oceanica* shoot, only the younger leaf was taken. The all length of ASUs was observed with 50x magnification, separating data from rough faces and smooth ones, in order to determine the number of colonizing epiphytes by cm². The same observations were done with parts of natural leaves situated underneath the hole made by the Zieman's method.

Data coming from determinations of organisms were also used to calculate Shannon-Wiener diversity index, Pielou's evenness and Bray-Curtis similarity with PRIMER 5 (Clarke & Warwick, 2001; Jørgensen *et al.*, 2005).

Results

The basal parts of both leaves were compared according to the density of organisms. This density increases with time and reaches until 15 organisms/cm² on *P. oceanica* leaves and 249 org./cm² on smooth faces of ASUs (Fig. 1). For the same day, there are more organisms on the ASU than on the natural leaf, so, the epiphyte community seems to develop more rapidly on ASUs. Indeed, curves of the two faces of one ASU present density values from 7 (rough face) to 16 (smooth face) times higher than that of the natural leaf (Fig. 1).

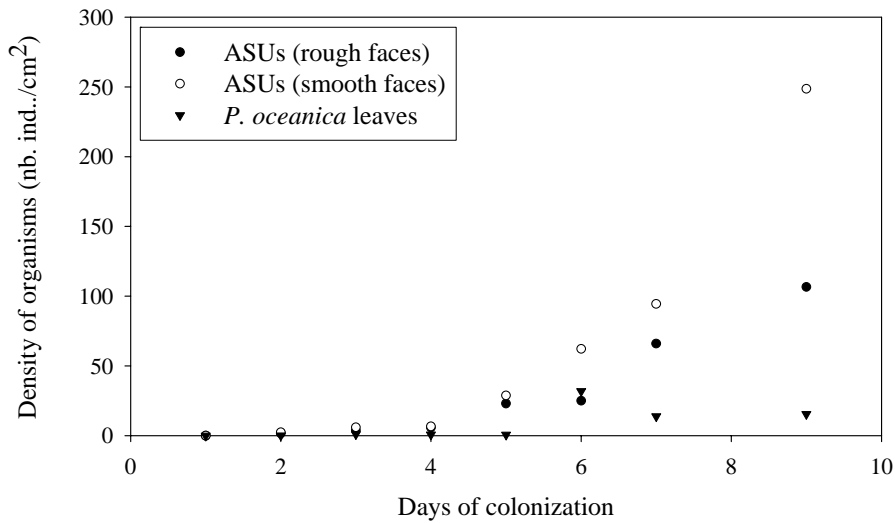


Fig. 1: Evolution of the density in number of individual by square centimetre in basal parts of ASUs rough faces (full discs), ASUs smooth faces (empty discs) and *P. oceanica* leaves (inverted triangles), with time.

Concerning the observed taxa (algae, foraminifera, bryozoans, ciliates and nematods), the most important groups found in the first days of colonization are algae and foraminifera. They represent between 82 % (natural leaf) and nearly than 100 % (ASU) of the observed organisms in the end of the study. So, they are mainly responsible of the increase of density.

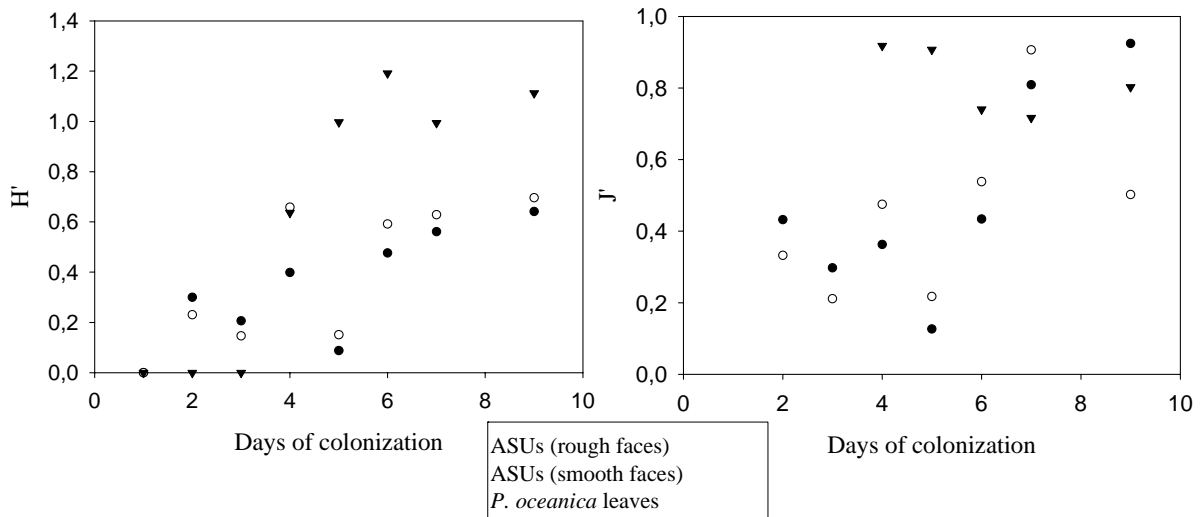


Fig. 2: Evolution of the Shannon-Wiener diversity index, H' (left) and Pielou's evenness, J' (right) according to days of colonization. The legend is the same as for Fig. 1.

The calculation of diversity index for those taxa shows that Shannon-Wiener diversity index (Fig. 2) is higher for natural leaves than ASUs since the fifth day of colonization, while the evolution of the evenness (Fig. 2) is more fluctuating. Indeed, during the second and third days, evenness can only be calculated for ASUs because there are only algae on natural leaves. From the third to the sixth day, evenness is higher for natural leaves than for ASUs, what means that the epiphytic community of the bases of *P. oceanica* leaves is not really

dominated by one taxon, unlike ASUs. From the seventh day, the difference in evenness between both leaves become low, what means that the distribution of taxa becomes more similar. During all the experiment, there was no clear difference between the two faces of the ASUs.

The analysis of the Bray-Curtis similarity index (Fig. 3) shows two distinct clusters, which can also be separated in two additional clusters. First clusters separate days 2, 3, 4, 5 (cluster A) and days 6, 7, 9 (cluster B) with less than 20 % similarity. In the cluster A, there is a cluster with ASUs (cluster C) and a cluster with natural leaves (cluster D) separated with more than 20 % similarity. Separation between ASUs (cluster E) and *P. oceanica* (cluster F) is also observed in cluster B, with 30 % similarity. So, there are differences in colonization according to time, but also according the type of leaves.

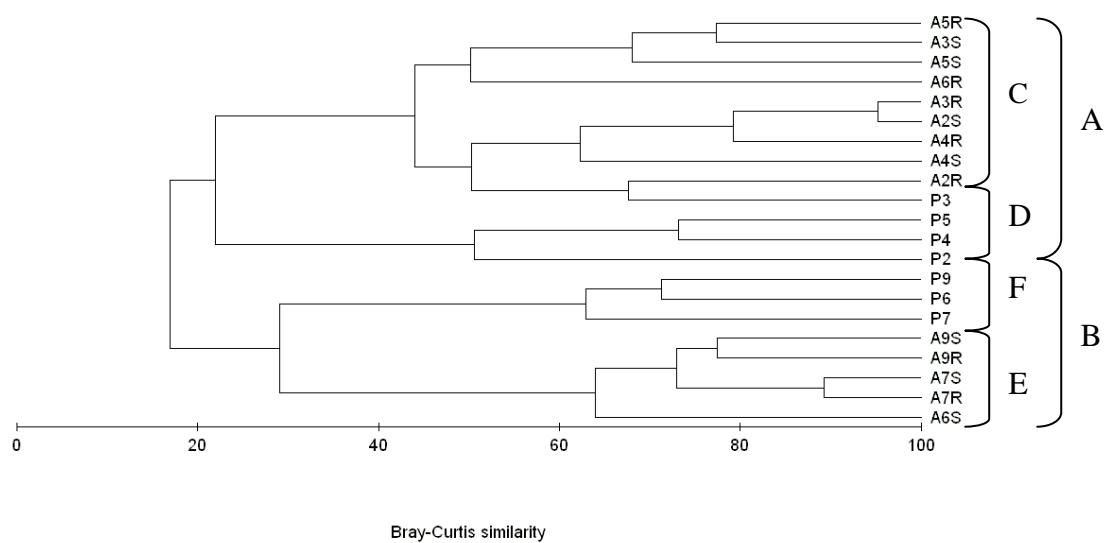


Fig. 3: Bray-Curtis similarity index analysis. In the dendrogram, A = ASU, P = *P. oceanica* leaf, number = day, S = smooth face and R = rough face.

Discussion

The colonization dynamics of *P. oceanica* leaves, concerning epiphytes biomass and according to time, is characterized by a sigmoid evolution (Cebrián *et al.*, 1999). The stabilization of the curve is reached when equilibrium between epiphytes growth and losses, due mainly to grazing pressure, exists (Alcoverro *et al.*, 1997; Cebrián *et al.*, 1999; Van Montfrans *et al.*, 1984). Curves obtained in this study (Fig. 1) don't show a sigmoid tendency because of the shortness of the envisaged period. Indeed, the study lasts only 9 days while Cebrián *et al.* (1999) had shown that epiphytes biomass reached the upper part of the sigmoid after 270 days. On artificial seaweeds, Edgar (1991) reached this part of the curve after two months, which is shorter than 270 days but still longer than our sampling period. However, the curves corresponding to ASUs show a highly increasing density of organisms towards the end of the experience, what means that colonization is in its rising phase.

The increase in epiphytes density on *P. oceanica* leaves is low in comparison with ASUs even if Shannon-Wiener diversity index and Pielou's evenness are higher. Those observations are in accordance with many authors (Bologna & Heck Jr, 1999; Cattaneo & Kalff, 1978; Edgar, 1991; Novak, 1984). This difference between both types of leaves gives the dendrogram of similarity (Fig. 3) which suggests a difference in colonization patterns.

This can be explained by a difference in bacterial communities living on the leaves or in the maturity of the biofilm, which sets up during the early stages of colonization and plays an important role in the installation of larvae and algae (Dahms *et al.*, 2004; Keough & Raimondi, 1995, 1996; Novak, 1984). This difference in the maturity of the biofilm was observed on SEM samples where a more developed biofilm on ASUs was present. That is probably one of the causes of the observed differences between both leaves.

Another cause often cited to explain the low colonization of natural leaves in comparison with artificial ones is the production of phenolic compounds by *P. oceanica* in more or less important quantity according to environmental stress (competition, sewage ...). Their concentration is higher in intermediate leaves than in adult ones and presents a seasonal variation (Agostini *et al.*, 1998; Cuny *et al.*, 1995; Dumay *et al.*, 2004; Novak, 1984). Moreover, they can act on the development of the biofilm, what would increase the impact on epiphytes colonization (Harrison & Chan, 1980). As the studied zones of *P. oceanica* are those of maximal phenolic compounds production, it is possible that these compounds have influenced the coverage by epiphytes.

A third possible reason to this low colonization rate on natural leaves is linked to experimental protocol. The observed fragments of *P. oceanica* leaves correspond to younger ones. So, they are situated in a zone where leaves are tightly put side by side, what makes the access to leaf surface difficult for epiphytes, unlike ASUs which are well separated.

The differences in diversity and evenness could also be attributed to a difference in the morphology of the leaf (Schneider & Mann, 1991) or in the density of the leaves (Boström & Bonsdorff, 2000; Lee *et al.*, 2001). However, it doesn't exist a real difference in the density of both leaves types in this study because ASUs were put inside the meadow, as close as possible as *P. oceanica* shoots. So it can't explain the difference in diversity and evenness.

The difference between textures of the faces of one ASU was also observed by Bologna & Heck Jr. (1999) for the epiphytic fauna. This difference and the rapid increase in epiphytes density on ASUs is maybe due to a snowball effect. Indeed, it was proved that epiphytes colonization modifies the leaf and favours the setting up of new colonizers (Bologna & Heck Jr, 1999; Irving *et al.*, 2007; Novak, 1984).

Conclusion

Colonization by epiphytes in the early stage begins quickly and, on both types of leaves, algae and foraminifera are dominant groups. The increase in epiphytes density is higher on ASUs than on natural leaves, even if diversity and evenness are higher for *P. oceanica*. However, even if the colonization seems to be different for both leaves types, one can't exclude that, in a longer experiment, epiphytes communities would be more similar. Moreover, ASUs could be useful tools in ecology, thanks to their rapid colonization by epiphytes. That would permit a use in rapid assessment studies.

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