Journal of Applied Phycology (2006) 18: 583–589
DOI: 10.1007/s10811-006-9067-1

Group recruitment and early survival of *Mazzaella laminarioides*

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Received 21 June 2003; accepted 6 January 2005

Key words: coalescence, recruitment, *Mazzaella*, Ca^{++}, spore abundance

Abstract

Several phycocolloid-producing Rhodophyta of significant economic importance are coalescing species, able to fuse with conspecifics during recruitment, reach larger sizes and increase their survival. In these species spores are needed to start cultivation (e.g. *Gigartina*, *Mazzaella*) or to increase the seed stocks, to renew senescent clones or to enlarge the base of genetic variation of vegetatively propagated species (e.g. *Chondrus, Gracilaria, Eucheuma*). This study uses *Mazzaella laminarioides* to evaluate some key features that influence recruitment success. Field measurements indicate that in any recruitment event a variable amount of the spores reaching a given place may form groups of 2 to over 100 coalescing spores, while field experiments support the idea that early recruitment success is a function of the number of coalescing spores forming the individual, as multisporic, coalescing recruits have higher survival rates than sporelings formed by one or a few spores. Therefore, group recruitment (spores settling and recruiting in close spatial proximity) appears as a prerequisite for sporeling coalescence and early recruitment success. In turn, laboratory experiments suggest that the frequency of group recruitment and coalescence increases with increasing spore abundance and with slight Ca^{++} additions to the culture medium. These last two factors could be handled by farmers to improve the success of spore inoculations of coalescing species.

Introduction

Several economically important red seaweeds are coalescing species, with the capacity to fuse and form genetically composite entities (e.g. *Chondracanthus, Chondrus, Eucheuma, Gigartina, Gracilaria, Mazzaella, Sarcothalia*). Fusion can occur among crustose basal portions of grown thalli (Santelices et al., 2003a), but occurs more frequently among spores, spore groups and sporelings (see Santelices, 2004, for a review). Laboratory results indicate that coalescence leads to larger sizes, reduced susceptibility to herbivory, enhanced competitive performance for space and higher survival (Maggs & Cheney, 1990; Santelices et al., 1996, 1999, 2003b).

Many of the above coalescing species are farmed for commercial purposes, with intensive use of spore inoculations. In species without commercial vegetative propagation (e.g. *Gigartina, Mazzaella, Sarcothalia*), spores are needed to propagate the crop and to initiate the farm (Buschmann et al., 1999). In species propagated by vegetative fragments (e.g. *Eucheuma, Gracilaria, Chondrus),* spores are needed to enlarge the base of genetic variation of the farmed stock (Santelices, 1992), to increase the seed stock (Azanza-Corrales et al., 1996) and to renew senescent clones (Alveal et al., 1995).

Among the above species, group recruitment (spores settling and recruiting in close spatial proximity) is a prerequisite for spore and sporeling coalescence, and therefore, a condition for higher survival during early recruitment. In spite of its biological and economic importance, however, no study seems to have addressed the question of how to increase group recruitment among these species. Available information (see Santelices, 1990, for a review) suggests that mode and periodicity of spore release, total spore abundance and dissolution rates of the mucilage layer maintaining

the released spores in close proximity are important factors affecting group recruitment. Group recruitment is more likely to occur among species exhibiting massive spore shedding during comparatively short periods (see data in Boney, 1978; Ngan & Price, 1983) than among species releasing spores one by one over comparatively longer periods. After massive spore shedding, groups of spores may remain together, settling and recruiting as a group, although a few spores may break away from the main spore mass, drifting away and eventually settling in isolation from other spores. It would be expected, therefore, that increasing spore abundance will increase the frequency of group recruitment over solitary recruitment.

On the other hand, the chemical charges of the sulphated polysaccharides and glycoproteins of the mucilage around the spores (Pueschel, 1979; Chamberlain & Evans, 1981; Diannelidis & Kristen, 1988; Apple & Harling, 1995) probably influence the dissolution rate of mucilage while it is free-floating. As in other phycocolloids, adding positive ions to the culture medium (e.g. Ca^{++}) would probably promote gelification of the mucilage layer (Craigie, 1990), thereby delaying its dissolution rate and promoting group recruitment.

In this study we first compare the relative representation of solitary and group recruitment in a natural recruitment event of a coalescing species *(Mazzaella laminarioides).* Then we evaluate survivorship in the field during early recruitment of sporelings formed by a large number of spores in comparison to sporelings formed by one or a few spores. Finally, we measure the relationships between the relative frequency of group recruitment and increasing spore abundance, and Ca^{++} additions to the culture medium.

Materials and methods

Relative frequency of solitary and group recruitment at different levels of spore abundance

Relative frequency of solitary and group recruitment in a recruitment event were measured using 10 recruitment plates exposed in the middle level of two *Mazzaella laminarioides* belts on rocky outcrops in the Parque Pedro del Río Zañartu, Hualpén Peninsula, Concepción (36°48′S; 73°10′W) on September 7th, 2002. Plates were made of epoxy resin (Sea-Goin Poxy Putty), 5 cm. diam., 1 cm thick and with a coarse surface (see Brawley & Johnson 1991 for fabrication details). Average depth of the rugosity (measured as in Norton & Fetter 1981) was 783.35 ± 64.9 μm. They were attached to the substratum with stainless steel screws. After 72 h in the field, they were removed, transported to the laboratory in Santiago in glass containers containing 0.2 μm-filtered seawater and maintained at 8 ± 3°C. In the laboratory, the plates were placed in Petri dishes inside growth chambers and cultured under constant conditions of temperature (14°C $\pm$ 1 °C), photon flux density (40 μmol photons m^{-2} s^{-1}) and photoperiod (12:12) for 20 h. Previous experimental studies with this species (e.g. Hannach & Santelices, 1985; Santelices et al., 1999, 2003b) have shown that the above conditions are most suitable to transport the fertile blades from the field and to incubate spores in laboratory cultures.

A total of 24, 1.3 mm^2 circular microscopic fields were randomly marked on each of the 10 plates. Using a camera Cool Snap-Pro (Media Cybernetics) on a compound stereo-microscope Nikon Sm2-Udia, the image of each field was captured and stored in a computer. Images were then analysed using an Image Proplus 4.5 Program (Media Cybernetics). All images on all plates were captured within 20 h. The number of spores recruiting individually or in groups was quantified in each microscopic field and the data used to evaluate the relative frequency of solitary and group recruitment. Two or more spores settling at distances of 10 μm or less (cell wall to cell wall distance) were considered aggregated settlement.

The above spore recruitment data were also used to compare the frequency distribution of spore groups in plates with different values of spore abundance. Analysis of covariance (ANCOVA) was used (Snedecor & Cochran, 1967) to compare the percent contribution of either type of recruitment (solitary or in group) as a function of spore density.

Field survival of solitary and coalescing recruits

Experimental evaluation of differential survival in the field between sporelings built with different numbers of spores was done using thin rectangular (5 cm × 4 cm × 4 mm) ceramic plates that were previously detoxified by maintaining them for 48 h in running seawater. Spore groups formed by 1, 10, 50 ± 5 or 100 ± 10 spores were seeded on each plate in the laboratory, incubated for 5 days under the above controlled conditions and then transferred to the field. Germination rate of spores in each treatment was measured 2 days after seeding to assure that all the plates transferred