

# The effects of coalescence on survival and development of *Mazzaella laminarioides* (Rhodophyta, Gigartinales)

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**Abstract** The potential benefits associated with coalescence in red algae have been best documented with early stages of development. In this study, we report on the effects of the original number of spores on branching and fertility during later stages of development. Measurements for 20 months of survival rates, field growth, and fertility of a set of 96 sporelings produced in the laboratory with different number of spores, and outplanted at mid levels of the *Mazzaella laminarioides* belt at a locality in central Chile, indicate significant differences between multisporic holdfasts (50 and 100 coalescing spores) and uni- or oligosporic (ten spore) holdfasts in the number of branches and erect axes produced 10 and 15 months after germination. The difference is related to the persistence of clusters of axes and branch initials in deep portions of holdfasts of multisporic plantlets and their absence from equivalent portions of uni- or oligosporic holdfasts. This is an example in macroalgae of an event occurring during germination and expressing its effects in the next growing season (some 10 months after).

**Keywords** Coalescence · Cluster of initials · Erect axis formation · Growth long-term effects · *Mazzaella* · Survival

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## Introduction

Coalescence is widespread in natural populations of red, green, and brown seaweeds (Santelices 2004). Using the red alga *Mazzaella laminarioides* (Bory) Fredericq as a model organism, we explored some of the costs and benefits of this interaction on recruitment and growth and the consequences this may have on reproduction and biomass production.

The potential benefits associated with coalescence in red algae have been best documented in early stages of development. Germinating spores of coalescing species do not exhibit crowding effects, and their germination rates are linear functions of the initial number of spores (Santelices et al. 1999). Inter-individual fusions during early stages of recruitment lead to immediate size increments, thus increasing the survival probabilities of the fused sporelings (Santelices and Alvarado 2008), while at later growth stages, coalescence accelerates erect axis formation and growth, also increasing the survival probabilities of the sporeling (Santelices et al. 2010).

It is, as yet, unknown if coalescence may have any effect on later stages of juvenile development. Experimental studies have shown that the differences in holdfast area gained by fusion of a larger number of spores disappear within 30–60 days after germination (Santelices et al. 2010). Therefore, long-term effects of coalescence, as consequence of holdfast size due to differences in the number of original spores, are unlikely. However, the number of coalescing spores contributing to the sporeling is a significant variable in determining both the time of primordia formation and the number of axes formed. In fact, in the two species studied (*M. laminarioides* and *Gracilaria chilensis*), the number of erect axes per sporeling formed in the first 30 days of life increased asymptotically with increasing number of initial spores (Santelices et

al. 2010). If the cell clusters differentiating primordia remain within the holdfast and maintain their capacity to differentiate or regrow new uprights during growing seasons, the original number of coalescing spores in a given sporeling may have some effects on later stages of development, affecting the number of branches produced. In this study, we test the hypothesis that multisporic plants will have more branches with higher potential fertility as a result of more axes.

## Materials and methods

### Measurements of survival, growth, and reproduction

Fertile thalli of the middle intertidal belt of *M. laminarioides* were collected in January 2007 in Maitencillo (32° 30'S; 71°29'W) in central Chile. The intertidal habitats and the collection and transportation methods have been extensively described elsewhere (e.g., Santelices and Alvarado 2008; Santelices et al. 2010).

Carpospores released by mature cystocarps were mixed and deposited inside 4-mm-wide holes excavated in each corner of a 4×4×0.6-cm square ceramic plate. Each hole received 1, 10, 50, or 100 carpospores, each treatment being randomly assigned within a plate. A total of 24 ceramic plates were used as replicates. Each plate was then placed within an individual deep Petri dish (100×80 mm; 400 mL) filled with SFC culture medium (Correa and McLachlan 1991) and cultivated for 90 days under controlled temperature (14±2°C), photon flux density (20 ±10 μmol photons m<sup>-2</sup> s<sup>-1</sup>), and photoperiod (12 h of daily light). The salinity of the culture medium was 35‰, and this was exchanged every 3 days.

After 90 days, the ceramic plates were transferred to the field, attached to the substratum using removable screws, placed haphazardly at the middle level of a *M. laminarioides* belt at Maitencillo, and maintained for field experiments between April 2007 to December 2008. Every 1–3 months (depending on weather and accessibility to the sea), plates were removed from the field and transported to the laboratory within temperature-insulated containers (8–10°C). There they were maintained under the above controlled conditions while they were cleaned of epiphytes, photographed, measured, and returned to the field within 4–7 days.

The diameter of the holdfasts and the number and length of erect axes were measured with a caliper (larger plants) or using the program Image Pro-Plus v 4.5 (Media Cybernetics, Silver Spring, MD, USA). Regular monitoring of the plates allowed us to measure the rates of disappearance of plates as well as the disappearance and survival rates of the sporelings in the different treatments. Disappearance and

survival data were analyzed by regression models of survival times (Proc Lifereg, SAS Institute Inc., 1999) where the independent variable (days of permanence) was transformed logarithmically, and the errors were modeled with a Weibull distribution. Quantification of erect axes and blades only considered axes longer than 1 mm. Data on growth of the disc area, number of erect axes, and average length for each date were analyzed as a mixed model design (Proc mixed, SAS Institute Inc., 1999) with plates as blocks (random variable) and treatment as a fixed variable. Comparisons between 1 and 10 and between 50 and 100 spores indicated lack of significant differences (Proc Mixed, with LSMEANS procedure, SAS Institute Inc., 1999); therefore, contrast comparisons were made between treatments formed by 1 and 10 spores and those formed by 50 and 100 spores.

### Histological analysis

Sporelings were fixed for histological analysis using a 10% solution of formaldehyde in seawater. Fixed holdfasts were sectioned using a Leitz freezing microtome. Sections were stained with 1% aniline blue and mounted in 50% Karo brand corn syrup.

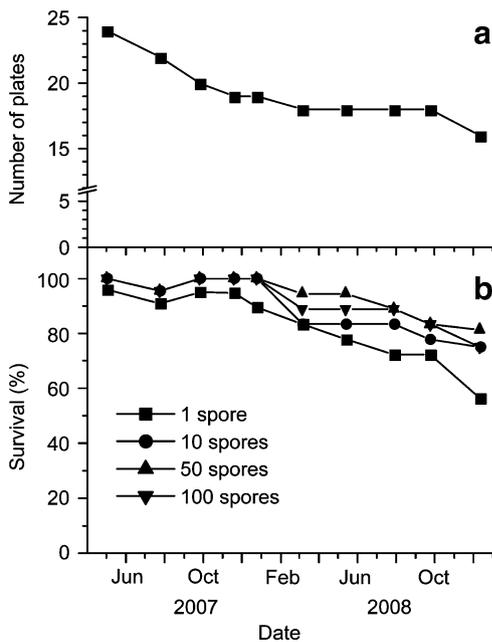
## Results

### Survival, growth, and reproduction

**Survival** There was a gradual decrease in the number of experimental plates over the experimental period (Fig. 1a), decreasing from 24 in April 2007 to 19 by December 2007 and to 18 by September 2008 (75%). Water movement was responsible for this gradual decrease in numbers due to removal of plates. The high level of permanence of the plates allowed evaluation of sporeling survival as a function of the initial number of spores.

Sporeling survival was similar for all treatments for most of the first year of the experiment (May 2007 to May 2008; Fig. 1b). It was only after 14 months of exposure (July 2008) that some sporelings exhibited slightly lower survival than any of the other three treatments. Inter-treatment differences, however, were not significant (Wald  $\chi^2=3.15$ ;  $p=0.37$ ).

**Growth of the disc area** In all treatments, the disc area showed a steady growth over the 20-month period, from initial disc areas of 2–3 mm<sup>2</sup> to areas of 30–50 mm<sup>2</sup> (Fig. 2a). Individual treatments show growth fluctuations, but there is little seasonality. Only in July 2008, a reduction in disc area occurred in most treatments, probably due to heavy waves and sand scour during the winter months. No



**Fig. 1** Persistence of experimental plates and sporelings of *Mazzaella laminarioides* over the experimental period. **a** Persistence of the number of experimental plates. **b** Sporeling survival during the experimental period. Treatments include sporeling formed by 1, 10, 50, and 100 spores

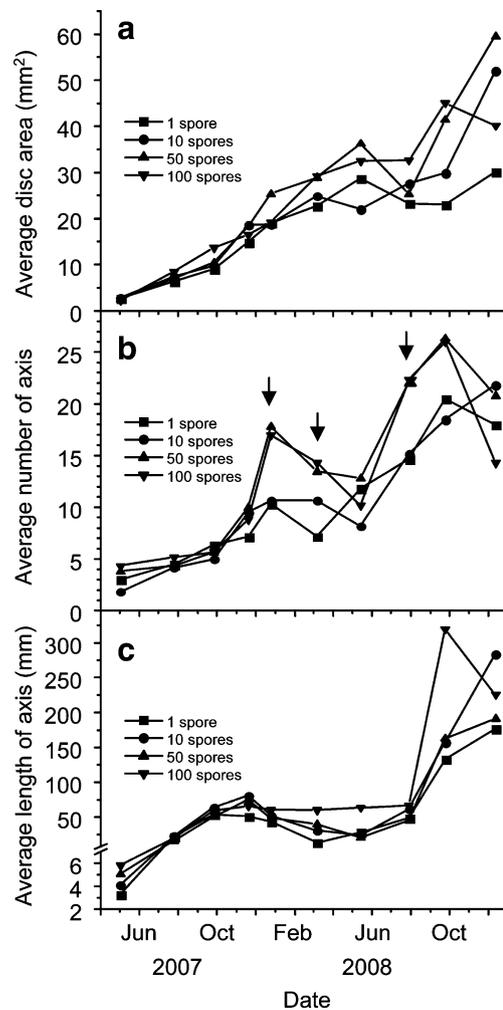
significant inter-treatment differences (1 and 10 vs 50 and 100 spores) were found during the study period (Proc Mixed, with CONTRAST procedure, SAS Institute Inc., 1999). As holdfast size increased, the treatment variability among treatment results also increased.

**Average number of erect axes** Similar to holdfast area, the average number of erect axes showed an overall increase during the study period, from 3–5 to 20–25 erect axes per plant. This morphological characteristic shows (Fig. 2b) a clear seasonality over the study period, with two peaks either in early summer (December 2007) or in spring (October 2008). After the growth period, there is a reduction in the average number of erect axes (March to May 2008; Fig. 2b) probably due to axis destruction by high insolation and high temperature during the summer. In our experimental population of plants, a new growth cycle started in late winter (August 2008) extending to October 2008, and then it started to decline (December 2008).

Statistical analysis of the inter-treatment differences in number of erect axes (Proc Mixed, with LSMEANS procedure, SAS Institute Inc., 1999) indicated a lack of significant differences between treatments starting with one and ten original spores. Similarly, there was a lack of differences between treatments again with 50 or 100 spores. However, differences were significant in the number of axes produced by the first two treatments compared with the last two treatments (Table 1). Differences become

significant during periods of active growth of erect axes, in spring to summer (January 2008) or in periods of regrowth, after winter storms (July 2008; see arrows in Fig. 2b).

**Average length of axes** This character also showed two peaks during the study period (Fig. 2c). A slight increase occurred in December 2007 in line with the increment in number of erect axes. However, during the following months, the average length of axes and branches became shorter, probably due to the emergence of new, shorter axes and by die-off of axes exposed to summer radiation. The second peak was shown in late spring and early summer (September to December 2008), although some treatments (100 spores) exhibited reductions in the average length of



**Fig. 2** Growth response of the sporelings of *Mazzaella laminarioides* during the experimental period. **a** Temporal changes in the average holdfast area. **b** Temporal changes in average number of axes and branches. Arrows indicate dates in which significant differences occur between oligosporic (1- and 10-spore sporelings) and multisporic (50- and 100-spore sporelings). **c** Temporal changes in average length of axes

**Table 1** *Mazzaella laminarioides*: planned contrast comparisons of the average number of erect axes at different dates between germlings formed with few spores (one and ten) and those built with large number of spores (50 and 100)

Date	F value	P
May 2007	8.73	0.0067
July 2007	1.39	0.2487
September 2007	0.08	0.7775
November 2007	0.28	0.6001
December 2007	9.08	0.0057
March 2008	7.18	0.0126
May 2008	0.69	0.4131
July 2008	5.86	0.0228
September 2008	2.19	0.1506
December 2008	0.59	0.4477

Results were obtained with a mixed model analysis (Proc Mixed, SAS Institute Inc.) for random block design. Degrees of freedom are 1 for treatments and 25 (May 2007) or 26 for errors

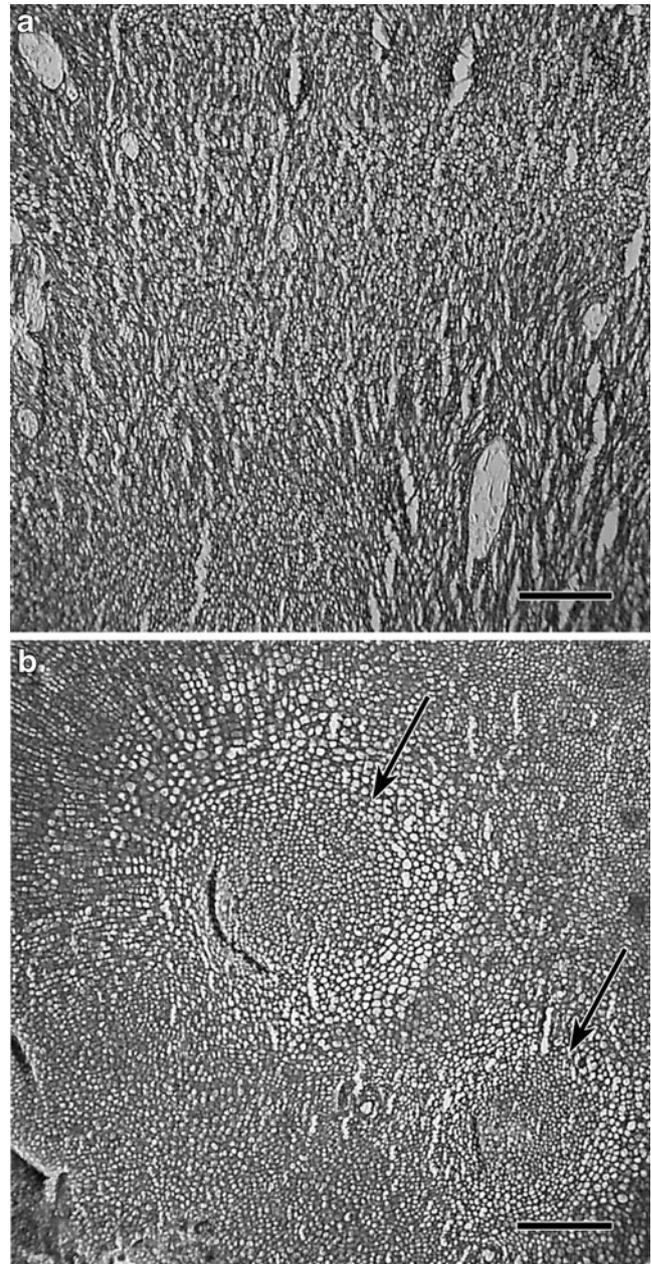
erect axes already in December. No statistically significant inter-treatment differences were found in the inter-treatment comparisons (Proc Mixed, with CONTRAST procedure, SAS Institute Inc., 1999).

**Reproduction** By December 2008, tetrasporangial sori occurred in plants of all the experimental treatments. Their frequencies, measured either as the percentage of the total number of plants surviving (7.7–22.2%) or as the percentage of fertile blades (4.95–25.0%), were low. The number of fertile blades in fertile plants was larger in multisporic (nine and four) than in uni- (2.5) or oligosporic (1.5) individuals, and the same happened with the relative abundance of fertile blades (8.8% in unisporic, 4.95% in 10-spore, 25% in 50-spore, and 15.4% in 100-spore sporelings). The number of fertile plants in all treatments was too low to allow statistical analysis of the above differences.

#### Histological analysis

Median sections through deep portions of unisporic holdfasts (Fig. 3a) exhibit homogeneous tissue with a few empty scars that were caused by encrusting organisms (cyanobacteria and barnacles remains) or substratum grains originally present in the substratum that were later surrounded and overgrown by the holdfast of *Mazzaella*. During the fixation and dehydration processes to prepare sections for the histological studies, these encrusted remains tore loose from the tissues, leaving the empty scars in the holdfast tissue. In some holdfasts, more than one layer of cells of different sizes could be observed resembling

growing rings and suggesting more than one growing period in the life of these holdfasts. Apart from that, the deep tissues of unisporic holdfasts are homogeneous consisting of filaments extending from the central parts of holdfasts to the margins. By contrast, median sections through deep portions of multisporic holdfasts show clusters of initials forming the base of erect axes (arrows



**Fig. 3** Median sections through deep parts of uni- and multisporic holdfasts of *Mazzaella laminarioides*. **a** Median section through the deep part of a unisporic holdfast. Cells are homogeneous in size, and empty spaces correspond to encrusting cyanobacteria or substratum particles. Scale bar, 100 µm. **b** Median section through the deep part of a multisporic holdfast (50 spores) showing the clusters of initials (arrows). Scale bar, 100 µm

in Fig. 3b). Similar clusters are found in surface sections of both unisporic and multisporic holdfasts.

## Discussion

The evidence gathered in this study indicates that for *M. laminarioides*, differences in the original number of coalescing spores forming the sporeling have a significant effect on the number of branches and erect axes produced at later (10 and 15 months after germination) stages of development. Multisporic holdfasts (50 and 100 coalescing spores) produced significantly more branches and axes than uni- and oligosporic (ten spores) holdfasts. By contrast, no effects associated with the original number of spores were detected on sporeling survival or holdfast growth.

The lack of effects on sporeling survival and holdfast growth is consistent with previous results on coalescence. They support the idea that survival benefits, due to coalescence of larger spore numbers, are restricted mainly to recruitment and post-recruitment stages (Santelices and Alvarado 2008). The lack of effects on holdfast size also supports previous results (Santelices et al. 2010), indicating that spore-dependent size differences disappear within 1–2 months after germination.

Since there are no significant differences in holdfast areas between the different treatments, it is unlikely that the significant differences found in average number of axes could be explained by differences in the size of the holdfasts. Histological observations suggest that the differences are related to an earlier initiation, during post-recruitment, of erect axes in multisporic sporelings, as compared to unisporic or oligosporic plantlets. Upright production is earlier and more numerous in multisporic than in unisporic sporelings (Santelices et al. 2010), and such clusters of initials persist in deeper parts of the multisporic holdfasts. Seemingly, they maintain a capacity to regenerate the uprights whenever they are destroyed by abiotic (e.g., extreme desiccation) or biotic factors (e.g., grazing). Similar clusters of initials with the capacity to generate their own axes within a larger holdfast have been described in previous morphological studies with young sporelings (Santelices et al. 2004), and such an autonomy seems to persist even after the spores and derivatives have coalesced with other spores and derivatives within the holdfast. From this perspective, the potential number of erect axes to be differentiated by holdfast, at least in this species, would depend not only on disc size but also on the number of clusters of initials and derivatives, with the capacity to form primordial axes that might persist in the holdfast.

The low number of fertile plants found in our experiments allows for just a few conclusions on fertility. A capacity to differentiate tetrasporangia was found in all

treatments, including plants formed by few and many spores. Since plants formed by a larger number of spores have a tendency to produce more blades and since all these blades, after reaching a given size, seem to have the capacity to become fertile, it is suggested that the number of original spores coalescing to form a sporeling might also affect, at a later stage of development, the reproductive potential of the plants. In the future, more extended and more highly replicated experiments seem necessary to statistically evaluate this idea.

The relationship between the number of spores originally forming the sporeling and the abundance of axes and branches differentiated in the next growing season (10 months after) involves the maintenance in the holdfast of clusters of branch initials, differentiated early in the life of the sporeling, that maintain their capacity to start or re-assume growth at every growing season. This is an example of delayed effects of early ontogenic processes that later determine morphological attributes in the grown plant. It is possible that this type of response could be common among seaweeds, but seemingly, it has been reported only infrequently.

The accumulated evidence on coalescence has previously indicated that it increases survival during recruitment (Santelices and Alvarado 2008), and survival and growth during post-recruitment (Santelices et al. 2010). The present evidence indicates that coalescence of a larger number of spores might also increase branching at later stages of development and reproductive potential. Coalescence is strongly dependent on spore density (Santelices and Aedo 2006), and its occurrence could be easily handled in culture conditions of selected species. Previous (e.g., Santelices et al. 1999; González and Santelices 2008) and ongoing research (e.g., Santelices 2010) indicate that coalescence occurs in a diversity of economically important red, green, and brown algae. It is therefore concluded that adequate handling of coalescence in culture might maximize recruitment, growth, reproduction, and biomass production in commercially cultivated species.

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