

Coalescence increases abiotic stress tolerance in sporelings of *Mazzaella laminarioides* (Gigartinales, Rhodophyta)

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Abstract Chimerism theoretically increases the genetic heterogeneity of coalescing organisms, which in turn may increase phenotypic variability in chimeras, allowing them greater tolerance to environmental changes when compared with non-coalescing individuals. In order to test this hypothesis, we compared abiotic stress tolerance between coalescing and non-coalescing organisms. The specific daily growth was compared using discs formed with 1, 5, and 20 *Mazzaella laminarioides* carpospores. These were cultivated under three different temperature (6, 12, and 25 °C) and salinity (10, 35, and 50 g L⁻¹) conditions. Growth of the disc area was measured after 30 days of cultivation under controlled conditions. Sporeling survival similarly was calculated under each of these temperatures and salinities in order to record whether stress-resistant phenotypes were present. The results showed that, under stressful conditions, non-coalescing specimens experienced a significant reduction in the specific growth rate compared with those under non-stressful conditions. The reduction in growth falls off, however, as the number of coalescing spores increases. The cultivation of spore populations also indicates the presence of sporelings with the capacity to survive alone in stressful situations. The results suggest that the coalescing discs exhibit higher tolerance to environmental stressors than non-coalescing discs, allowing them to survive and grow under these conditions. This can be explained by an increase in phenotypic plasticity, resulting from greater genetic heterogeneity due to somatic fusion of a larger number of spores.

Keywords Coalescence · Chimerism · *Mazzaella laminarioides* · Phenotypic plasticity · Tolerance

Introduction

Somatic fusion (coalescence) of two or more macroalgae individuals results in the formation of a new biological entity with a unique set of morphological, anatomical, and functional attributes (Santelices et al. 1999; Pineda-Krch and Lehtilä 2004). Coalescence has been described in members of seven orders of the Rhodophyta (Santelices et al. 1999), in the genus *Codium* among the Chlorophyta (González and Santelices 2008), and has recently been found to be widespread among kelps (Phaeophyceae; González et al. 2014). Two types of adaptations have been described for coalescing individuals; the first is a direct increase in size, which reduces the mortality rate resulting from herbivory or other types of disturbances (Santelices et al. 1999, 2010). Moreover, this implies an increase in the rate of recruitment, growth, and fertility, as well as the capacity to acquire resources (Pineda-Krch and Lehtilä 2004; Santelices et al. 2010). The second type of adaptation described is the increase in phenotypic variance to cope better with environmental factors, compared to that exhibited by non-coalescing individuals (DeWitt et al. 1998; Sultan 2000). Coalescing organisms may therefore show greater fitness than non-coalescing ones, owing to synergistic effects on their phenotypic variance (Santelices et al. 1996; Santelices 2004; Pineda-Krch and Lehtilä 2004). Some of these benefits may even suggest heterosis (Rinkevich and Weissman 1987; Hughes 2002). Experiments have yet to be conducted, however, in order to measure this second type of response.

This study assesses whether or not coalescing macroalgae, formed by medium (5) or high (20) number of spores, exhibit

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greater phenotypic variance than non-coalescing individuals, permitting them higher growth rates under abiotic stress. To achieve this, thermal and saline tolerance was evaluated for the red alga, *Mazzaella laminarioides* (Bory) Fredericq. Extensive experimental studies with this species have previously shown (Santelices and Alvarado 2008) that somatic fusions may increase the survival, attachment, and growth capacity of this species. In order to evaluate levels of individuals' stress tolerance among spore populations, in a second experiment, we measured the rate of survival of individual spores when exposed to these stressful conditions.

Materials and methods

General experimental conditions

For these experiments, a cystocarpic frond from each of 50 clumps of *M. laminarioides* was gathered by hand from mid-intertidal zones in Maitencillo, central Chile (32° 30' S; 71° 29' W) during July and August 2013. They were then transported to the laboratory in plastic bags, while a temperature of 4 °C was maintained according to the protocol described by Santelices et al. (2010). In the laboratory, sporulation was induced and the spores collected, making sure the spores used in the different density treatments came from fronds from different clumps (i.e., that all were replicated truly independently). The experiment then proceeded to the incubation stage while following the procedures described by Santelices et al. (2003b). The spores were cultivated under controlled laboratory conditions of temperature (12±2 °C), salinity (35 g L⁻¹), light intensity (25±5 μmol photons m⁻² s⁻¹), and photoperiod (12:12 light/dark).

Coalescence and environmental stress

One experiment was tested for thermal tolerance. Three levels of coalescence were considered under three different temperature conditions. The levels of coalescence were: non-coalescing (1 spore, NC), 5±1 coalescing spores (Co-5), and 20±3 coalescing spores (Co-20). The temperature conditions were: high (25±2 °C), medium (12±2 °C), and low (6±1 °C). Eight replicates were used per treatment, and the specific growth rate was compared between the nine treatments at 30 days of cultivation. Initial measurements were made at 15 days of incubation, under the conditions given above, and after complete fusion in the multisporic treatments (Co-5 and Co-20) had been confirmed. At day 16, the replicates were photographed with an inverted Nikon Eclipse microscope (TE3000, Japan) equipped with a

CoolSNAP-Proof color (Media Cybernetics) camera, and using the program Image-Pro Plus version 4.1.0.0 (Media Cybernetics). During the 30-day experiment, the culture was monitored and photographed, and the SFC medium (Correa and McLachlan 1991) replaced on a weekly basis. Differences in the specific growth rate between the different treatments were calculated by analyzing changes in the cultivated disc area using the formula $\mu = \frac{\ln(\frac{T_{30days}}{T_{1day}})}{30days} \times 100$ (Littler and Littler 1985), where T is the disc area.

The data were tested for homoscedasticity and normality using the Levene and Kolmogórov-Smirnov tests, respectively. Average daily specific growth rates were compared using ANOVA (Sokal and Rohlf 1995), followed by a Bonferroni test. All tests were conducted using the program Minitab version 16.2.4 (Minitab Inc.).

The second experiment was tested for saline stress. This experiment was conducted under the same coalescence conditions applied in the previous experiment, using three levels of salinity: high (50±3 g L⁻¹), medium (35±3 g L⁻¹), and low (10±3 g L⁻¹). The high salinity condition was obtained by adding 20 g of NaCl to 1 L of SFC culture medium. The low salinity condition was obtained by diluting the SFC culture medium with distilled water (2:3 proportion) and adding the corresponding quantity of nutrients, vitamins, and trace metals to the culture medium. Replicates of between 8 and 11 were used per treatment, and the specific growth rate was compared between the nine treatments carried out. Photography, measurement, statistics, and culture comparison protocols were similar to those used for the initial experiment to test for thermal tolerance.

The thermal and saline treatments were chosen according to the natural distribution of *M. laminarioides* (Muñoz et al. 2005, 2008) in the field and on the basis of expected temperature increases resulting from El Niño (Ulloa et al. 2001) or possible future climatic changes (Clark et al. 2013).

Tolerance to environmental stress in spore populations

Eleven replicates were incubated in Petri dishes filled with 10 mL of seawater-filtered culture medium (SFC) and 1 mL spore solution (18,100 spores mL⁻¹), under the same temperature and salinity conditions used in the previous experiments. The culture medium was replaced at days 7 and 14. Care was taken to allow spores to grow far enough away from each other to avoid coalescence. Sporeling survival was calculated at days 2, 7, 14, and 21. The survival values for the different treatments over time were compared using ANCOVA. A Tukey test was subsequently performed (Sokal and Rohlf 1995) in order to determine the source of the differences.

Results

Coalescence and environmental stress

Thermal stress

Under normal conditions (12 °C), the highest daily growth rate (9.5±0.4 %) occurred among the non-coalescing (NC) sporelings (Fig. 1), whereas the sporelings formed by five coalescing spores (Co-5) grew significantly less (7.09±1.3 %), and the sporelings formed by 20 coalescing spores (Co-20) grew the least (5.13±1.0 %).

The stressful effects of high temperature (25 °C) reduced the specific growth rates for all coalescence levels (Fig. 1). The reduction in the growth rates became progressively smaller as the number of coalescing spores increased. While the growth rate reduction between NC at 12 and NC at 25 °C was 2.97 %, the difference dropped to 1.75 % for Co-5 and was only 0.82 % for the respective C-20 treatments. Thus, the percentage reduction from 12 to 25 °C was much larger in the non-coalescing spores than in the 20-spore treatments. In fact, the Bonferroni test showed no significant differences between the C-20 treatments cultivated at 12 and 25 °C (Table 1).

One equivalent response was found with the juveniles incubated at low temperatures (6 °C, Fig. 1). While the growth rate reduction between NC at 12 and NC at 25 °C was 4.43 %, the difference dropped to 2.88 % for Co-5 and was only 1.38 % for the respective C-20 treatments. The percentage reduction in the daily growth rate from 12 to 6 °C was therefore much larger in the non-coalescing spores than in the 20-spore treatments similar to the results obtained in the high-temperature experiment.

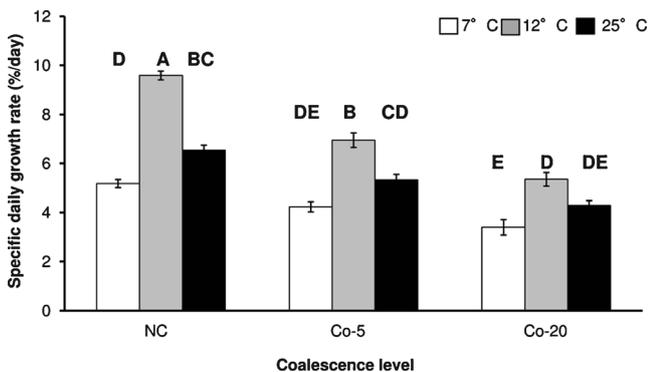


Fig. 1 Effects of increased (25 °C) and decreased (6 °C) thermal stress on the specific daily growth rate of sporelings of *Mazzaella laminarioides* formed by non-coalescing (NC) and coalescing discs with different number of spores. Co-5 sporelings formed by 5 coalescing spores. Co-20 sporelings formed by 20 coalescing spores. Different letters indicate significant differences calculated by Bonferroni test. Bars indicate standard error

Table 1 ANOVA type 1 results of the experiment to test coalescence with thermal and saline stressors. The confidence interval used was $P < 0.05$

Source	Temperature			Salinity		
	Df	F	P	Df	F	P
Coalescence	2	71.70	<0.000	2	110.20	<0.000
Stressor	2	89.37	<0.000	2	19.95	<0.000
Interaction	4	8.51	<0.000	4	0.71	0.589
Error	63			76		
Total	71			84		

Saline stress

Response patterns in the salinity experiment were similar to those already described for temperature, but there was no interaction between the coalescent treatments and salinity (Table 1). Under normal salinity levels (35 g L⁻¹), the NC individuals showed (Fig. 2) the highest specific growth rate (9.67±0.96 %), followed by the Co-5 (6.41±1.0 %) and then the Co-20 (5.39±1.3 %) juveniles. The stress provoked by high salinity (50 g L⁻¹) also resulted in a reduction of the growth rate, reaching 2.05 % for the unisporic juveniles, 1.34 % for Co-5, and 1.28 % for Co-20. As with the high-temperature experiment, the difference in growth between Co-20 incubated at 35 g L⁻¹ was not significantly different from that of the Co-20 individuals incubated at 50 g L⁻¹.

The hyposaline stress conditions showed a marked reduction in the growth rate of 1.5 % between NC cultivated at 35 and at 10 g L⁻¹ (Fig. 2). Growth variations between the Co-5 and Co-20 juveniles were lower (0.78 and 0.72 %, respectively), and no statistically significant differences were observed with their respective controls.

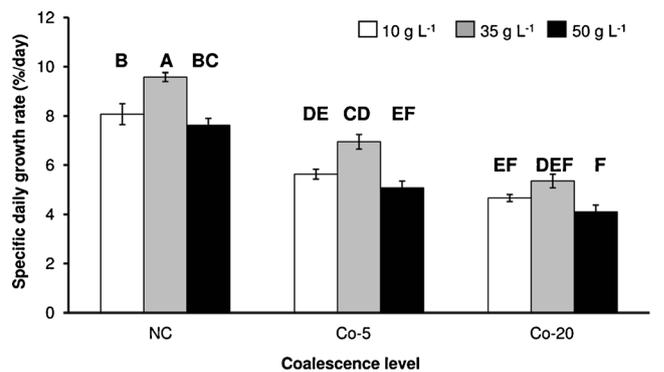


Fig. 2 Effects of increased (50 g L⁻¹) and decreased (10 g L⁻¹) saline stress on the specific daily growth rate of sporelings of *Mazzaella laminarioides* formed by non-coalescing (NC) and coalescing discs with different number of spores. Co-5 sporelings formed by 5 coalescing spores. Co-20 sporelings formed by 20 coalescing spores. Different letters indicate significant differences calculated by Bonferroni test. Bars indicate standard error

Tolerance to environmental stress in spore population

The results for the survival of spore populations exposed to different temperatures (Fig. 3a) showed that, under the three temperature conditions, some sporelings are able to survive when subject to conditions of thermal stress. Over time, the highest survival rates were found to occur at a temperature of 6 °C, with 47.76 ± 16.34 % of sporelings still alive at 2 days and 30.31 ± 11.29 % alive at the end of the experiment. The second highest survival rate occurred at a temperature of 12 °C, while the lowest survival rate occurred at 25 °C; at this temperature, only 5 ± 2 % of the sporelings had survived at 21 days. The ANCOVA and Tukey tests showed significant differences at different temperatures and times (Table 2).

Regarding the different salinities used (Fig. 3b), sporeling survival was highest at a salinity of 35 g L^{-1} and lowest at 10 g L^{-1} . The ANCOVA and Tukey tests showed significant differences between sporeling survival under different salinities and for various treatment times (Table 2). However, there was some sporeling survival under all the conditions tested.

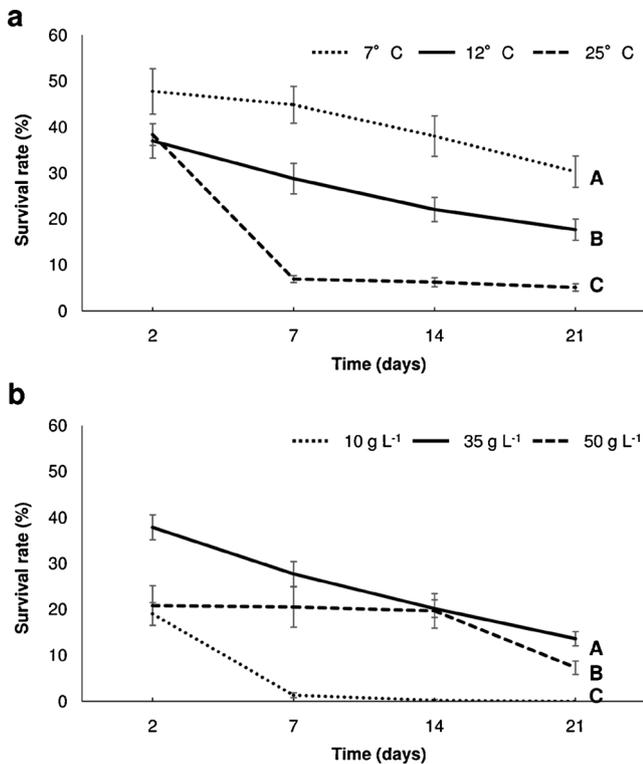


Fig. 3 Sporeling survival under different temperature and salinity conditions measured at 2, 7, 14, and 21 days after sowing. Different letters show the statistical differences calculated by Tukey test. Bars indicate standard error. **a** Shows the survival under the different temperature conditions and in **b** shows the survival under the different salinity conditions

Table 2 ANCOVA results of the experiment to test the spore population's tolerance to thermal and saline stressors. The confidence interval used was $P < 0.05$

Source	Temperature			Salinity		
	Df	F	P	Df	F	P
Time	1	127.91	<0.000	1	95.85	<0.000
Stressor	2	111.36	<0.000	2	74.18	<0.000
Error	630			624		
Total	633			627		

Discussion

The results of this study are consistent with the hypothesis that coalescing juveniles, formed by the somatic fusion of a medium (5) or high (20) number of spores, are better able to withstand the abiotic stress resulting from thermal stress and, to some extent, from saline extremes than unisporic individuals. This conclusion is supported by the observed growth patterns.

The increased tolerance to thermal stress determined by coalescence may be partially explained by a possible synergistic effect of the phenotypic plasticity exhibited by each participant spore during coalescence. This would give a greater diversity of responses to environmental stressors. Somatic fusion enables individuals to grow more efficiently under stress; this being consistent with previous findings (Santelices et al. 2003a) of potential benefits associated with coalescence in red algae, i.e., an increase in the growth and survival probabilities for each coalescing individual. This contrasts with observations made for coalescing marine invertebrates, in which there is no documented evidence of benefits derived from coalescing individuals when subject to adverse environmental stressors (Maldonado 1998; Rinkevich 2005).

As expected, these results suggest that some of the phenotypes present in coalescing discs (both Co-5 and Co-20) may tolerate better the stressful conditions, thus providing greater tolerance when subject to adverse conditions and giving the coalescing sporelings an advantage over non-coalescing individuals.

Although different numbers of spores were used throughout the experiment, none of the three temperature regimes used resulted in total mortality. Nevertheless, the plants' temperature endurance threshold is probably close to 20 °C (Eggert 2012). The survival rates at different levels of salinity are consistent with previous descriptions (Kirst 1989) on the effects of hypo- and hypersaline conditions. At all salinity levels tested, sporelings were found alive during the initial days of cultivation where survival rates for individual sporelings did not exceed 40 to 50 %. This figure appears to represent winter rates, since in other studies (for instance, Santelices et al. 2003b) undertaken in spring, sporelings

exhibited comparable survival rates of between 60 and 80 %. Nevertheless, the survival times between the salinity conditions differed, depending on the type and magnitude of stress to which they were exposed. The lower survival rates of *M. laminarioides* in hypo- and hypersaline environments are probably due to the fact that no growth was observed at salinities of 10 and 50 g L⁻¹ similar to the results for other species (De Miranda et al. 2012), which may result in complete inhibition in the early stages of growth at salinity levels of around 7 and 55 g L⁻¹. A complementary explanation (Karsten 2012) is that, owing to the energy required for the organism's osmotic regulation, survival becomes nonviable under prolonged salinity stress.

The existence of organisms with survival capabilities under different conditions of stress, and which have been studied from their very early life stages, indicates that certain individuals in a population may potentially provide survival and tolerance benefits in a coalescing disc when subject to thermal or saline stress. In the light of these results, the genetic heterogeneity provided by the coalescing algae during fusion of spores in their initial stages of development seemingly increases the tolerance levels and possibly the fitness of these chimeric organisms, thus reducing the stressful effects of adverse environmental conditions. A number of studies have shown that coalescing individuals are widespread in natural environments (Santelices et al. 1999, 2003a, 2010; Santelices and Alvarado 2008). Therefore, this benefit is likely to be having a direct or indirect bearing on the species' ecological and demographic traits as well as their ecological and geographical distribution patterns.

The benefits of coalescence described in this study seemingly result from a significant and synergistic increase in the plastic phenotypic properties of each participant spore. Notwithstanding this, it should be mentioned that it is yet unknown as to how the different genetic components of each coalescing spore interact with each other and how this interaction generates changes in the variance and phenotypic plasticity of the resulting chimeric algae. Further research is required on the alga's genetic variability and its effect on tolerance towards different environmental conditions similar to the work and findings of Clark et al. (2013).

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