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DEMOGRAPHIC CONSEQUENCES OF COALESCENCE IN SPORELING POPULATIONS OF *MAZZAELLA LAMINARIOIDES* (GIGARTINALES, RHODOPHYTA)[1]

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Coalescing macroalgae are ecologically important members of intertidal and shallow subtidal communities. However, we still lack quantitative information on the demographic consequences of coalescence. Using demographic models developed for modular invertebrates, we studied the demography of settlement and early recruitment in the coalescing macroalga *Mazzaella laminarioides* (Bory) Fredericq. Permanently marked microscopic fields on laboratory-incubated and field-incubated plates were monitored regularly (at 15, 30, 45, and 60 d) using image analysis techniques to evaluate the relative importance of settler abundance, mortality, coalescence (fusion), and fission on the changes in size and numbers of recruits. On the plates, spores settled individually or in groups. Over time, spores in close proximity may coalesce, resulting in a mixture of unisporic and multisporic crusts. When new spores arrive, they may or may not coalesce with previously settled crusts. Coalescence and mortality reduce the number of sporelings, but coalescence increases the size of the sporelings, thereby reducing further probability of sporeling mortality. Crust fissions are negligible in frequency, while the frequency of coalescence increases from ~25% after only 3 d, to ~75% after 60 d. Thus, as a result of variable settlement, mortality, and coalescence, any area colonized by *M. laminarioides* would contain a mixture of crusts of different sizes, ages, and genetic constitution. The interactions between the above three processes create a more complex survivorship curve than the ones known for unitary organisms.

Key index words: **coalescence; *Mazzaella*; red algae; settlement; survivorship curve**

Abbreviations: **SFC, seawater filtered medium C**

Coalescing macroalgae are able to fuse with conspecifics, forming composite and sometimes genetically heterogeneous entities (Santelices 2004). This kind of growth strategy occurs frequently in taxa from roughly half of the orders of the red algae (Santelices et al. 1999), many of which are dominant components of intertidal and shallow subtidal communities (Santelices et al. 2003a).

Fusion of coalescing macroalgae may occur between sporelings (Jones 1956, Tveter and Mathieson 1976, Maggs and Cheney 1990, Santelices et al. 1996), basal crusts of field-established clumps (Santelices et al. 2003a), or sporelings and grown crusts (Santelices et al. 2004). The process has not been considered in demographic studies of new sporelings or in the demography of grown thalli (see references in Santelices 1990, Norton 1992, Johnson and Brawley 1998, Wright and Steinberg 2001) probably because of the rather recent awareness of coalescence and its biological implications. In field studies, each clump has traditionally been assumed to be unisporic or to represent only one genet (Dyck and De Wreede 1995, 2006, Scrosati and De Wreede 1999, Thornber and Gaines 2003, 2004), independent of its ontogenic origin. Therefore, at present we lack quantitative information on the demographic consequences of coalescence, either among recruiting sporelings or among field-established populations.

Several factors indicate that coalescence should be considered in population studies of macroalgae. Interindividual fusions simultaneously reduce the numbers and increase the sizes of individuals in a population. If the individuals cannot be properly defined, then individual-based demographic models cannot be used (Raymundo and Maype 2004). Similarly, since coalescence also results in modifications of size, size-based demographic models are also of little use. Furthermore, since each sporeling of a coalescing species may either live, die, or coalesce, their survivorship curves would be expected to be more complex than those traditionally described for unitary macroalgae.

Complex recruitment processes occur not only in macroalgae. Some modular invertebrates, especially scleractinian corals, exhibit fusions and fissions during development (Hughes and Jackson 1980). These processes modify the size and number of individuals, the relationship between size and age (Hughes and Jackson 1980, Hughes 1984, Babcock 1991, Cochran and Ellner 1992), and predicted reproduction because, as in macroalgae (Chapman 1986), the reproductive potential of these corals is

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more related to size than to age (Hughes 1984). Quantification of colony fusion and fission during growth has allowed the application of individual-based, size-based, and age-based demographic models to corals, which have helped explain size-related growth and mortality in coral colonies (Raymundo and Maype 2004) and to correct and modify survivorship curves in clonal species (Tanner 2000, 2001).

Through the application of some of the demographic models and methods developed for corals, in this study, we made a first characterization of the demography of settlement and early recruitment of the coalescing macroalga *M. laminarioides*. For this purpose, we first measured spore settlement on experimental plates. Through the regular examination of permanently marked areas in laboratory-incubated and field-incubated plates, we evaluated the relative importance of settlement, growth, survival, mortality, coalescence (fusion), and fissions through a 60 d experimental period, and the effects of all these factors on the changes in size and number of sporelings. It is expected that, as a consequence of coalescence and growth, the total number of spores forming the sporelings and their average size would increase over time. Thus, the initial frequency distribution of sporelings formed by different numbers of spores would be different from that at the end of the monitoring period, when a larger proportion of larger sporelings is expected. Also, as a result of mortality and coalescence, the total number of sporelings is expected to decrease over time. For any cohort of individuals, the proportion of live noncoalescing, dead, and coalescing sporelings is expected to change over time, with the proportion of coalescing individuals increasing, and that of noncoalescing alive individuals decreasing over time. If mortality is a size-dependent function, it is expected that survival probabilities will increase with the size of the sporelings and coalescence frequency. Results are integrated into a recruitment model from which a different type of survivorship curve is proposed for the coalescing macroalgae.

MATERIALS AND METHODS

Study taxon. The coalescing *M. laminarioides* was used as experimental material. This species dominates midintertidal levels of rocky intertidal habitats along most of the central and southern Chilean coastline (30°S to 45°S). In the field, plants appear as clumps, 5–15 cm tall, formed by 10–60 erect blades growing from a dome-shaped or irregularly shaped holdfast. Usually, one to two blades per plant are larger than the rest, and frequently they contain reproductive sori (Santelices and Martínez 1997).

The seasonal fertility of this species in central Chile varies from one locality to another (Hannach and Santelices 1985, Santelices and Norambuena 1987). In our study sites in Caleta Maitencillo, fertile cystocarpic blades are more abundant than fertile tetrasporic blades throughout the year, but especially in autumn (late March to early June). Furthermore, the relative abundance of both phases varies spatially, with some localities dominated by cystocarpic plants and others by tetrasporic blades (Santelices and Martínez 1997).

Study sites. Field studies were carried out at two experimental sites at Caleta Maitencillo (32°39′ S, 71°29′ W), ~100 km north of Valparaíso in central Chile. The study sites consist of rocky platforms and outcroppings of large and small boulders, all exposed to the direct effects of onshore waves and surrounded by sandy beaches. Two large rocky outcroppings in this locality were used. They were judged, a priori, to experience a roughly similar wave exposure. Both presented similar slopes and contained structurally similar intertidal communities. These two large rocky outcroppings were 150 m apart and separated by a small sandy beach. In these two sites, the total tidal range is ~2.2 m, and the zone of *M. laminarioides* extends from 0.5 to 1.6 m above chart datum (lowest low water), reaching close to 100% cover in the lower belt margin and decreasing to 10%–20% in the upper zone. Single or mixed-species associations of the mussel *Perumytilus purpuratus*, acorn barnacles, and ephemeral foliose algae—such as *Ulva compressa*, *U. rigida*, and *Porphyra columbina*—intermixed with *M. laminarioides* at medium tidal levels, becoming increasingly abundant and eventually replacing *M. laminarioides* at higher levels.

The two sampling sites in Caleta Maitencillo were selected by their gametophyte-dominated populations, and experiments were performed during April 2005, a period of high carpospore production. Thus, while the propagules recruiting on the plates were expected to be mostly carpospores, it is realistic to assume that a minor quantity of tetraspores could also have recruited on the experimental plates. The average diameter of recently released carpospores is 20 ± 0.45 μm, while that for tetraspores is 27 ± 0.52 μm (Santelices and Martínez 1997). However, size cannot be used to distinguish between them since both show variable growth rates between the time of settlement and monitoring. Both kinds of propagules have been shown to effectively coalesce under laboratory conditions (Santelices et al. 2003b) and probably do so in the field as well, as Faugeron et al. (2001) found 2%–3% of 400 clumps studied in several localities of central Chile to be formed by blades of both phases arising from a single holdfast.

General experimental procedures. Early recruitment of *M. laminarioides* was assessed using circular, 6.0 cm diameter, 0.5 cm thick artificial plates with coarse surface (783 μm of average depth of rugosity). Plates were made of previously detoxified (Brawley and Johnson 1991) epoxy resin (Sea Goin Poxy Putty; Permalite Plastics, Newport Beach, CA, USA). Eleven plates were randomly bolted to the rock substrate within the *M. laminarioides* belt in both sites in Caleta Maitencillo. After 2 d of field exposure, plates were removed, placed in individual petri dishes with 0.2 μm filtered seawater, and transported to the laboratory (2–3 h) at 10°C ± 2°C in refrigerated coolers. In the laboratory, plates were maintained under controlled conditions of temperature (14°C ± 1°C), photon flux density (40 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$), and photoperiod (12 h of daily light). Previous experimental studies with this species (Hannach and Santelices 1985, Santelices et al. 1999, 2003b) have shown that the above conditions are adequate to transport and incubate spores in laboratory cultures.

On each plate, 10 randomly selected 2 mm^2 fields were permanently marked and photographed using a Cool Snap-Pro Camera (Media Cybernetics, Silver Spring, MD, USA) mounted on a stereomicroscope (Nikon SMZ-10A; Nikon, Melville, NY, USA). The image of each field was captured and stored in a computer. All images on all plates were captured within 24 h (during the third day after their initial deployment). The next day, plates were randomly separated into two groups. Ten plates (five from each field site) were incubated in the laboratory (''laboratory plates'') under the above controlled

conditions using seawater filtered medium C (SFC) culture medium (Correa and McLachlan 1991) exchanged every 6 d. The remaining 12 plates were returned to the two original sites in the field. While the patterns observed on the plates after their initial 2 d deployment are technically patterns of recruitment, we assume that they reflect the patterns of settlement (an instantaneous event) and use the terms settlers and settlement accordingly.

All plates were monitored again after 15, 30, 45, and 60 d following their initial deployment. Monitoring only lasted 60 d for the following two reasons: After 60 d, sporelings change their main growth pattern, differentiating erect axes, making measurements of their growth more complex and requiring three dimensional estimations. Furthermore, after 60 d of incubation, the surface of the plates began losing resin and some of the sporelings.

During each monitoring, the field-based plates were removed from the field, transported to the lab, photographed, and returned to the field within 48 h. Regular monitoring every 15 d allowed us to quantify the number of sporelings that disappeared from each plate (mortality), those exhibiting fissions or fusions, and those increasing their respective areas. Examples of these processes are shown in Figure 1. Images captured from all plates were analyzed using the software Image Pro plus v4.5 (Media Cybernetic, Silver Spring, MD, USA).

Incubation and regular monitoring of the plates under laboratory conditions allowed us to follow the fate of individual sporelings without the confounding effects of additional recruitment episodes to the plates. On the other hand, the field plates allowed us to follow the fate of the sporelings under natural conditions, including factors influencing mortality and new settlement and recruitment events. Comparisons of the responses of the sporelings under both conditions allowed us to evaluate the relative importance of mortality, survival, fission, and fusion as demographic determinants in both growing conditions. Due to the differences between laboratory and field conditions (e.g., regular aerial exposure in the field vs. constant submersion in the lab; absence of grazers in the lab vs. grazing activities in the field), comparisons were restricted only to the most significant patterns. On the other hand, pertinent statistical analysis for several comparisons showed absence of significant intersite differences. Therefore, data from sites 1 and 2 were pooled in all the analyses.

The fate of laboratory-incubated sporelings. Frequency distribution of sporeling numbers: The position of each sporeling within a given microscopic field on the laboratory-incubated plate was mapped in relation to substrate marks on the plate (see Fig. 1, A–D for examples). For each sporeling, its area and the number of spores forming the sporeling at day 3 were recorded. Two or more spores settling at distances of 10 μm or less (cell wall to cell wall distance) were considered group recruitment (sensu Santelices and Aedo 2006). Each sporeling was then followed through the consecutive monitoring times, and the resulting data were used to calculate fission, mortality, coalescence, and survival rates during the first 60 d of recruitment. A total of 2,158 sporelings were followed. Frequency distributions of unisporic and multisporic sporelings during the first and the last monitoring times were compared using a Kruskal–Wallis test based on Wilcoxon scores (rank sums) (Sokal and Rohlf 1969, Hollander and Wolfe 1973). The significance values were adjusted using Bonferroni's correction.

Survival, coalescence, and mortality in sporelings of different spore numbers: A more detailed analysis of the relative importance of survival, coalescence, and mortality in this cohort of laboratory-incubated sporelings was performed for all of the spores (3,288) recruiting at day 3. Sporelings (2,158) were separated into unisporic (1,623), bisporic (320), trisporic (88), and those formed by four or more spores (127). Since sporelings formed by four or more spores were infrequent, we grouped all of the possibilities into a single class. The relative importance of mortality, coalescence, and survival without additional coalescence (''in isolation'') for each sporeling group through time were compared using multinomial logistic regression (SAS Institute, Cary, NC, USA; Hosmer and Lemeshow 2000). The independent variables used were number of days of incubation and number of spores forming the sporeling. Live sporelings without additional coalescence were used as the reference category. A significant result (Wald χ^2 value) would indicate significant differences in the proportions of dead, live noncoalescing, and coalescing individuals between sporelings made up of different numbers of spores.

Frequency distribution of sporeling sizes: The frequency distributions of sporeling area recorded at the first and the last monitoring times were compared using a Kruskal–Wallis test based on Wilcoxon scores (rank sums).

The fate of field-incubated sporelings. Frequency distribution of sporeling size: Monitoring the field-incubated plates every 15 d did not allow us to quantify with certainty the number of new spores settling on the plates. For example, spores could settle, grow, and then disappear within the 15 d between monitoring times, or they could coalesce with others, leaving no sign of previous presence. Therefore, we restricted comparisons to changes in size-frequency distribution over time, also using a Kruskal–Wallis test based on Wilcoxon scores. A total of 2,897 sporelings recorded at day 3 were followed through time.

Survival, coalescence, and mortality in sporelings of different size: A more detailed analysis of the relative importance of survival, coalescence, and mortality in this population of sporelings was also performed using sporeling size as independent variable. All sporelings found in the marked fields on the plates at day 15 (n = 1,885) were classified into small, intermediate, and large. These are part of the 2,897 sporelings recorded at day 3 and still surviving at day 15, plus the new sporelings recruited between days 3 and 15. Sporelings were defined as small when their sizes ranged from 120 to 770 μm^2 (mean area = 320 ± 11 μm^2) and they lacked any evidence of coalescence in the first 15 d after settlement (e.g., abrupt size increments). At day 15, we found 503 small sporelings. The 13 sporelings defined as large (above 770 μm^2) all showed evidence of coalescence within the first 15 d after settlement and had a mean size of 1,114 ± 137 μm^2. The size range of the remaining 1,369 sporelings of intermediate size was 380 to 770 μm^2, and their mean size was 890 ± 19 μm^2. These sporelings could correspond to fast-growing unisporic sporelings, slow-growing multisporic sporelings, or unisporic sporelings that coalesced a few days just before monitoring time. These intermediate sporelings could also correspond to multisporic sporelings whose size had been reduced by some process of disturbance (e.g., grazers).

The proportions of dead, alive coalescing, and alive noncoalescing sporelings at different monitoring times for the three sporelings size classes were compared using multinomial logistic regression (Proc Logistic, SAS Institute; Hosmer and Lemeshow 2000). A significant result (significant Wald χ^2 value) would indicate differences in proportions of dead, alive, or coalescing sporelings between the three sporeling sizes. Live sporelings without additional coalescence were used as the reference category.

Survival as a function of size: This analysis evaluates the effect that sporeling size may have on sporeling survival. The size of each of the 1,385 sporelings occurring on the field-exposed plates at day 45 and day 60 was recorded. Mortality was estimated as the difference in survival between the two monitoring dates. Using multinomial logistic regression (Proc. Logistic, SAS Institute, Hosmer and Lemeshow 2000),

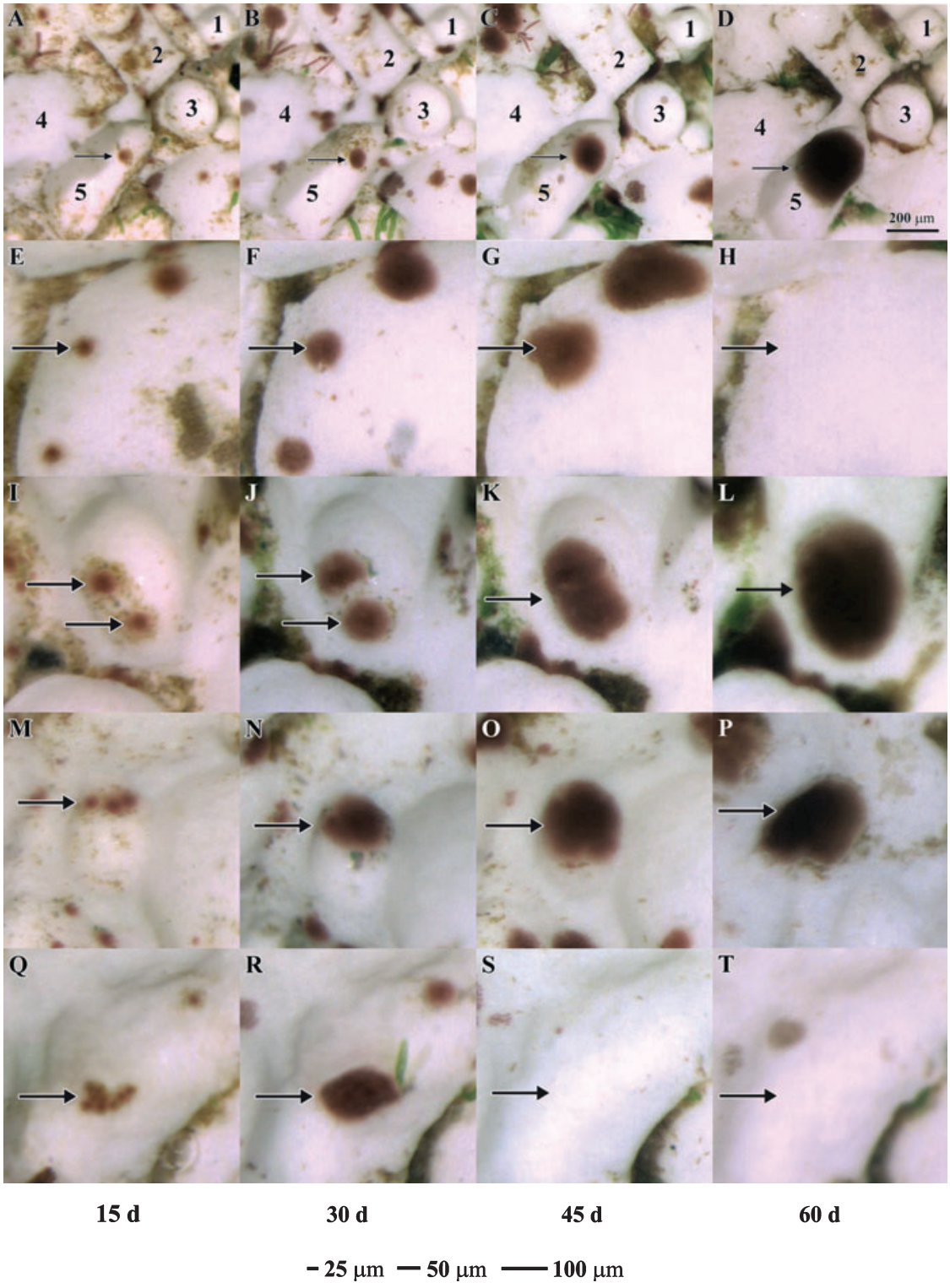

FIG. 1. Microscopic fields with sporelings of *Mazzaella laminarioides* showing the representative states in this study. (A–D) Examples of topographic landmarks (numbers) in the microscopic fields that help to locate a given sporeling (long arrow) during successive monitoring events. The sporeling in this series recruited in isolation and remained without coalescence to day 60. (E–H) Example of unisporic sporeling recruiting in isolation at day 15, growing to day 45, and then disappearing. (I–L) Two sporelings recruiting in isolation, coalescing at day 45, and remaining alive until day 60. (M–P) Three sporelings coalescing between day 15 and day 30 and growing as a multisporic sporeling to day 60 without additional coalescence. (Q–T) Group of nine recruits coalescing between day 15 and day 30 and then disappearing from the field. The scale for (A–D) is indicated in (D). The scales for all other panels are indicated at the bottom of Figure 1.

we evaluated if the size of the sporeling at day 45 was significant in determining the proportions of dead, live noncoalescing, or coalescing sporelings found at day 60. Live sporelings without additional coalescence were used as the reference category.

RESULTS

The fate of laboratory-incubated sporelings. Frequency distribution of sporeling numbers: The 10 plates later incubated under controlled laboratory conditions exhibited an average of 16.2 ± 1.7 spores·mm^{-2} (=3,288 spores · 200 mm^{-2}) at day 3 (Fig. 2A). Of these, $49.3 \pm 15.7\%$ of the spores recruited isolated, while the remaining $50.9 \pm 13.2\%$ of the spores recruited forming groups of 2–20 spores.

The total number of sporelings in the 200 mm^2 of marked fields gradually decreased throughout the incubation period from 2,158 at day 3 to 260 at day 60 (a 87.9% reduction, Fig. 2, B and C). The magnitude of reduction was different for unisporic (93.7% reduction) and multisporic (70.4% reduction) sporelings. Simultaneously, there was an increase in the average number of spores forming each sporeling. While at day 3 there were no sporelings formed by >20 spores (Fig. 2A), at day 60 (Fig. 2C), there were eight sporelings formed by >20 initial spores. In fact, the two largest sporelings found at this time in this population were both formed by 70–75 initial spores (Fig. 2C). Thus, mortality and coalescence appear to be important factors determining the number of multisporic

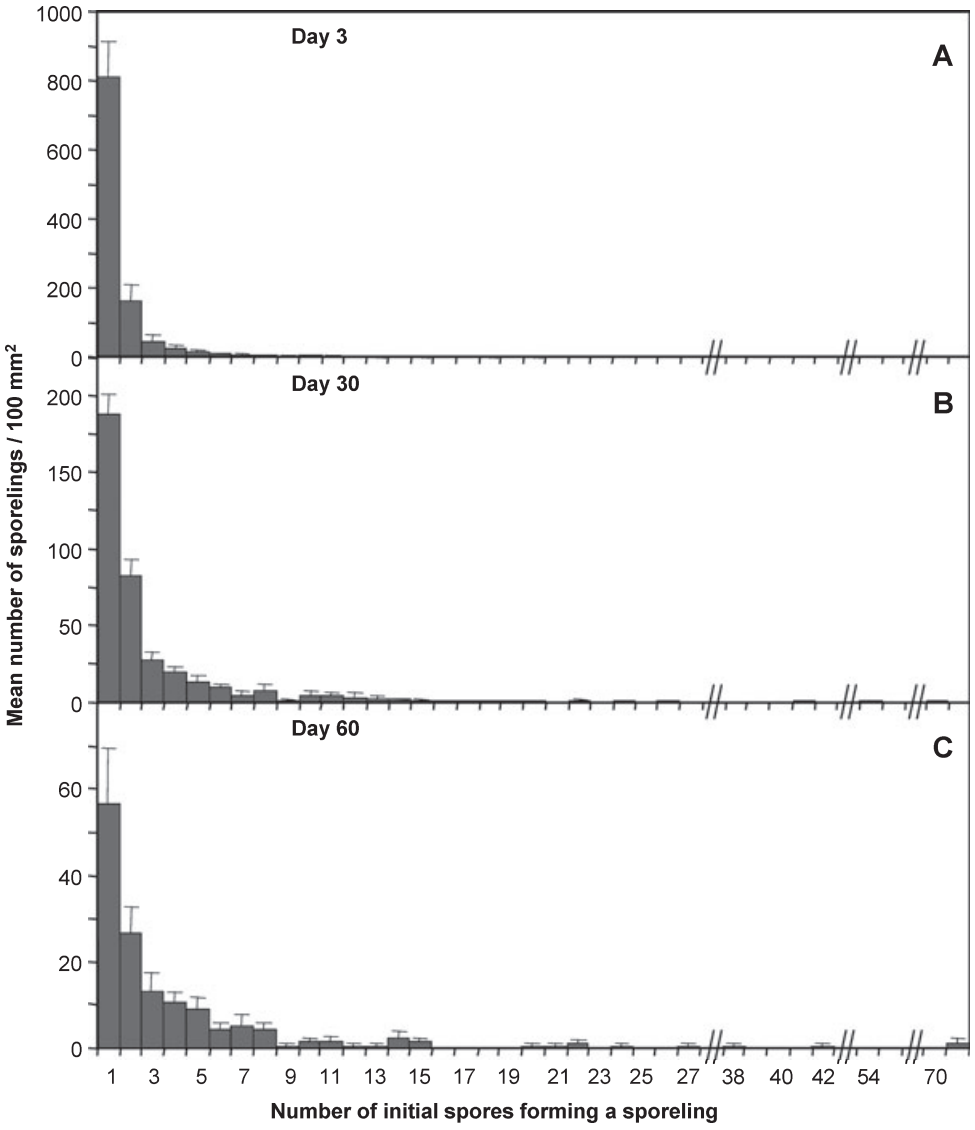

FIG. 2. Frequency distribution of sporelings formed by different numbers of initial spores (day 3), occurring on the experimental plates after 3 (A), 30 (B), and 60 (C) d of laboratory incubation. Bars are standard errors.

sporelings on these plates and their numerical differences in frequency distribution over time.

Application of the Kruskal–Wallis test to the frequency distribution of sporelings formed by different numbers of spores showed significant differences in the distribution patterns between day 3 and day 60 ($\chi^2 = 191.92$, df = 1, $P < 0.0001$).

Sporelings formed by 1, 2, 3, and 4 or more spores. Detailed monitoring of sporelings formed at day 3 by 1, 2, 3, and 4 or more spores illustrates the above dynamics in further detail (Fig. 3, A–D). Survival of 1-spore sporelings at day 60 was 6.15% (101 unisporic germlings out of the 1,641 counted at day 3). The number of sporelings that coalesced with other unisporic or multisporic sporelings was about seven times greater (707 sporelings; 42.5%), while 833 sporelings (51.3%) disappeared during the study period.

Although differing in the respective number of spores, two- and three-spore sporelings followed a pattern similar to that described above. The proportion of two- and three-spore sporelings surviving without additional coalescence up to day 60 (''isolated'' sporelings in Fig. 3, B and C) amounted to 5.0% and 2.3% of the initial number of sporelings, respectively. Dead sporelings made up 38.8% in two-spore germlings and 27.3% in three-spore germlings, while coalescing sporelings reached values of 56.3% in two-spore sporelings and 70.5% in three-spore sporelings.

The group of sporelings formed by four or more spores (Fig. 3D) is heterogeneous, as it includes 127 sporelings formed by groups of four to 20 initial spores. Yet, the pattern of change is similar to the above groups. At day 60, the population of sporelings formed by the original number of coalescing spores (''isolated'' in Fig. 3D) amounted to a minor (2.3%) proportion of the population, while 18.5% of the sporelings disappeared during the study period. Most of the multisporic sporelings (70.5%) survived to day 60 as coalescing entities.

Application of multinomial logistic regression to these results (Fig. 3, A–D) showed significant differences (Wald $\chi^2 = 90.4997$, df = 6, $P = 0.0001$) in the proportions of dead, live noncoalescing, and coalescing sporelings among the germlings formed by different number of spores (1, 2, 3, and 4 or more spores).

Frequency distribution of sporeling sizes. Similar to sporeling numbers, average germling size (measured as area) changed from the first to the last monitoring time, increasing, on average (±SE), from 815 ± 6.3 μm^2 at day 3 to 55,648 ± 2,412 μm^2 at day 60 (a 62-fold increase; Fig. 4, A–C). Changes in size and abundance affected unisporic and multisporic sporelings differently. By day 15, the number of unisporic sporelings was almost double that of multisporic sporelings (63.4% vs. 36.6%), but the average area of unisporic sporelings was almost half that of multisporic sporelings (4,857.42 ± 110.13 μm^2 vs. 12,401.64 ± 478.81 μm^2, respectively). Due to coalescence and mortality, by day 60 (Fig. 4C) coalescing sporelings were 1.5 times as abundant as unisporic sporelings, while the average (±SE) sizes had changed to 39,124.98 ± 2,722.52 μm^2 in unisporic sporelings

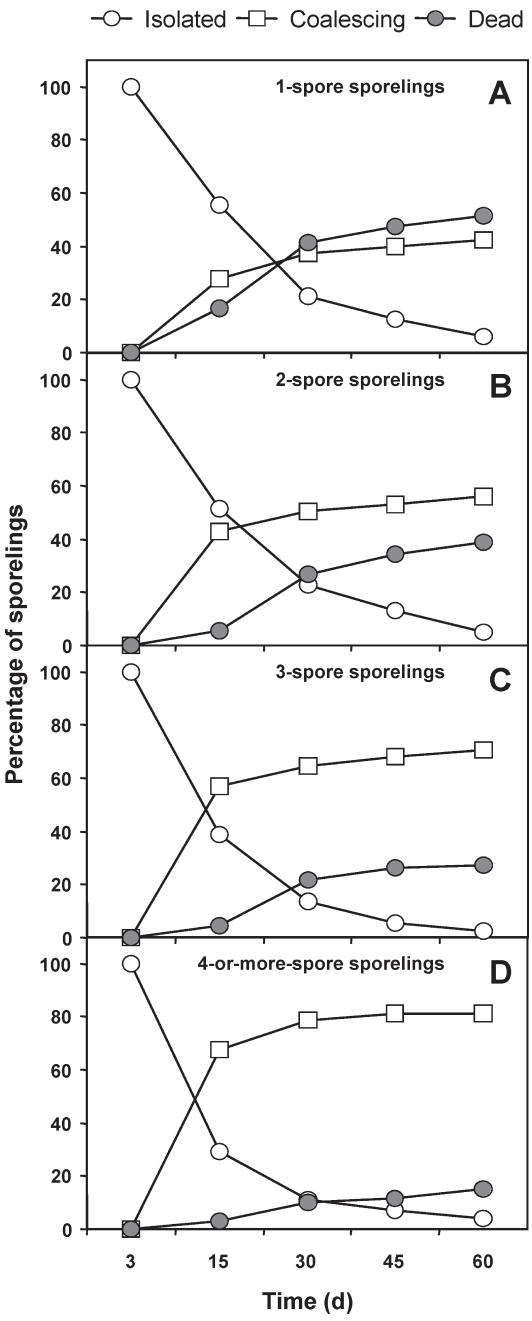

FIG. 3. Temporal changes in the percentage of sporelings dying and surviving with and without coalescence (''isolated'') in laboratory-incubated plates. Sporelings have been separated according to the number of spores forming them at day 3, including 1-spore sporelings (A), 2-spore sporelings (B), 3-spore sporelings (C), and 4-or-more-spore sporelings (D).

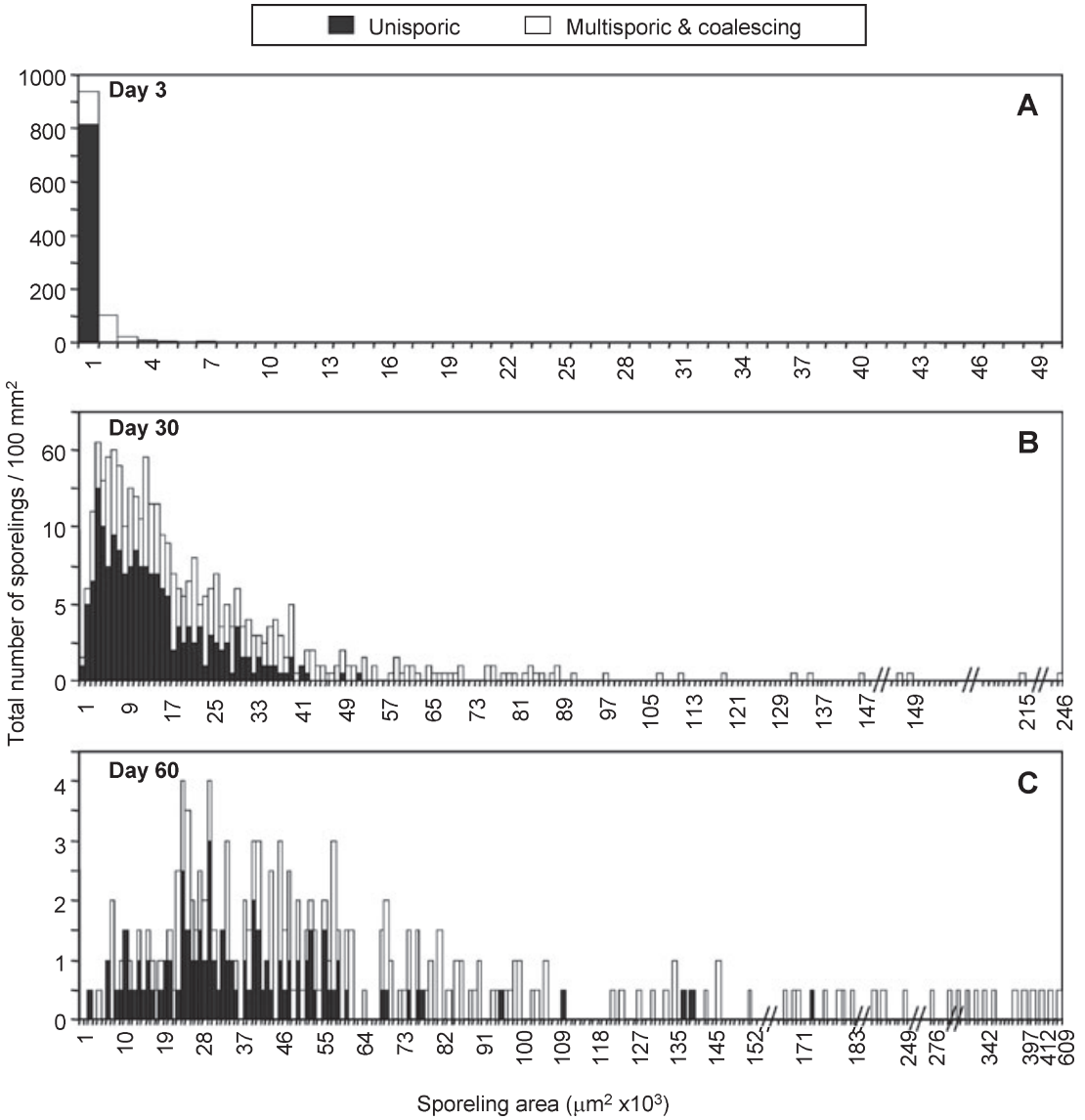

FIG. 4. Temporal changes in frequency distribution of sporelings incubated under laboratory conditions at day 3 (A), day 30 (B), and day 60 (C) of incubation. Sporelings have been arranged by size classes (based on area) and separating unisporic and multisporic sporelings.

and to 111,296.09 ± 11,898 μm^2 in multisporic sporelings. The variability in growth exhibited by both kinds of sporelings on laboratory-incubated plates overlapped in size (Fig. 4C) between the faster-growing unisporic and the slower-growing multisporic sporelings. In spite of this, by day 60, most of the sporelings >80,000 μm^2 and all of those >170,000 μm^2 were multisporic.

Comparisons of the size distributions of unisporic versus multisporic sporelings using the Kruskal–Wallis test showed significant differences between both kinds of sporelings at all monitoring times ($P < 0.0001$). However, χ^2 values are progressively smaller as time progresses (e.g., 963.9 at day 3; 6.4 at day 30; 1.3 at day 60) probably as a result of the progressive size overlap between fast-growing unisporic and slow-growing multisporic sporelings.

Crust fissions: Only two crust fissions were observed out of the 2,158 sporelings monitored during the 60 d study period. These were found on 2 different plates on day 45. Thus, based on the laboratory-incubated plates, fissions have a negligible frequency (0.45% of the 444 germlings occurring at day 45) in the early recruitment of *M. laminarioides.*

The fate of unisporic and multisporic sporelings on field-incubated plates. Frequency distribution of sporeling size: The 12 plates that were later used for the field-grown series all exhibited an overall recruitment pattern similar to the laboratory plates, but with numerical differences (compare Fig. 5A with Fig. 4A). The total number of recruiting spores was

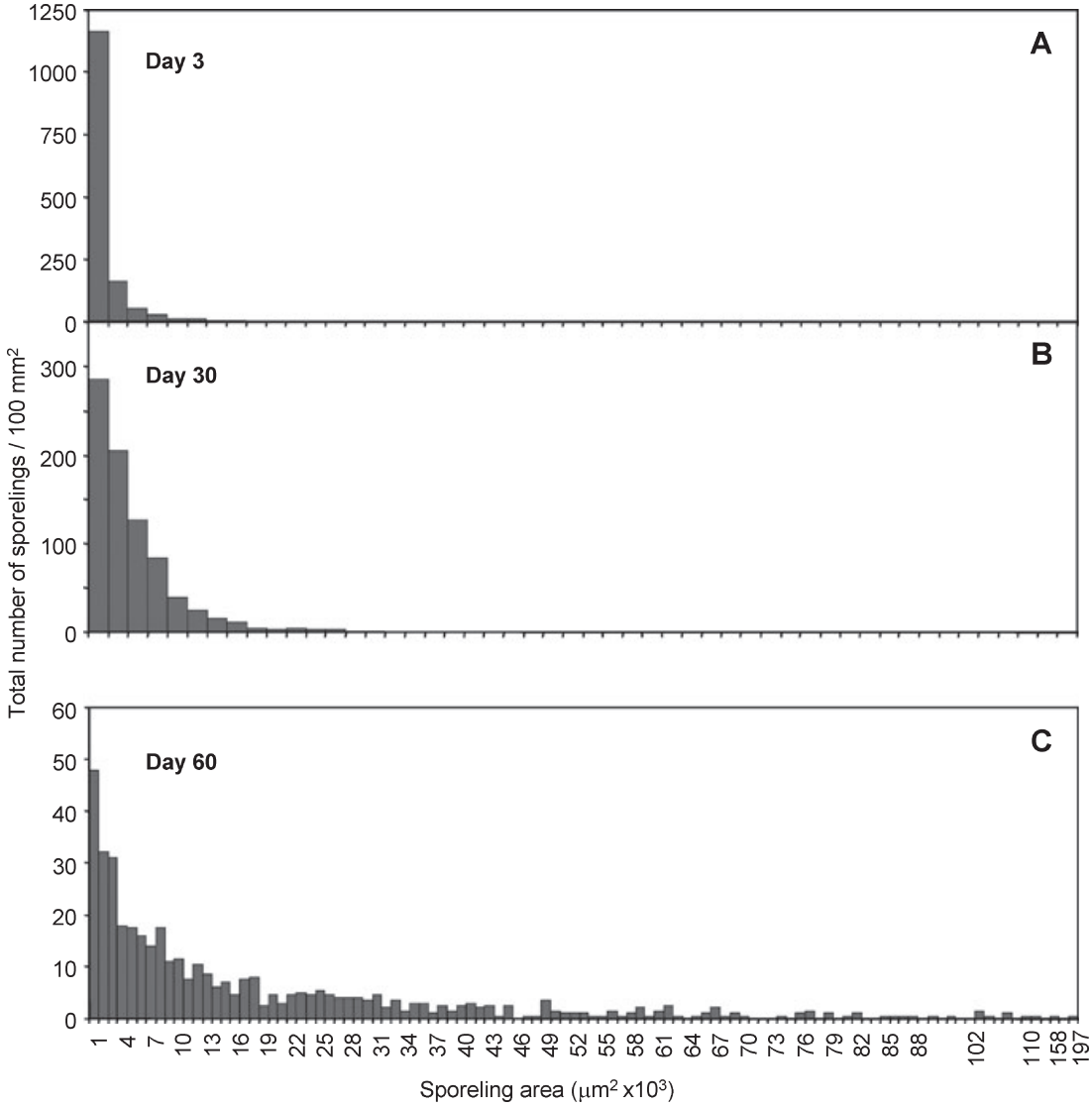

FIG. 5. Temporal distribution of sporelings incubated in the field after 3 (A), 30 (B), and 60 (C) d of field exposure. Sporelings have been arranged by size classes (based on area).

>50% higher (27.1 spores·mm^{-2}) than for the plates retained in the laboratory, and 71.89 ± 8.32% of them recruited forming groups of up to 60 spores. In these plates, there was also a reduction in the number of sporelings over time, from a total of 2,897 sporelings at day 3 (Fig. 5A) to 800 sporelings at day 60 (Fig. 5C). This reduction was also accompanied by a simultaneous increase in the average sporeling size, from 1,004 ± 62 μm^2 at day 3 to an average 18,230 ± 803 μm^2 at day 60, suggesting that mortality, growth, and coalescence are important factors determining size and numbers of sporelings in the field.

In contrast to the laboratory-incubated plates, however, the increases in sporeling size in the field-exposed plates over time were not accompanied by a reduction in the relative representation of the smaller size classes (compare Figs. 4C and 5C), which probably resulted from the continuous arrival of propagules to the field-exposed plates during the study period, underlining the importance of propagule settlement and recruitment. In addition, in the field-incubated plates, the sporeling sizes may exhibit reductions from one monitoring to another, and the maximum sporeling size reached in the field (197,050 μm^2) was about one-third the corresponding value in the laboratory-incubated plates.

Application of the Kruskal–Wallis test to the frequency distributions of sporeling sizes at day 3 and day 60 showed significant differences in the size distribution between dates ($\chi^2 = 1{,}357.71$, df = 1, $P < 0.0001$), despite the persistence and greater abundance of the smaller size classes at the end of the study period.

Survival, coalescence, and mortality in sporelings of different sizes: Mortality, coalescence, and survival occurred in different proportions in the three sporeling groups distinguished at day 15 (Fig. 6, A–C). By day 60, only 13.5% (69) of the 503 small-sized sporelings (Fig. 6A) survived without additional coalescence. About 1.8 times that number (107 sporelings; 21.1%) coalesced with other sporelings, while 65.4% (327 sporelings) disappeared from the plates. The overall pattern for the 1,369 intermediate-sized sporelings was similar to that for the small ones (Fig. 6B). About 14.2% (194 sporelings) survived without additional coalescence to day 60, another 422 sporelings (30.8%) coalesced, while 753 (55%) disappeared between day 15 and day 60. Despite the low number of large-sized sporelings (13; Fig. 6C), the pattern of response seems to be similar to that of the other two groups. In this case, two sporelings (15.4%) coalesced, four (30.8%) survived without additional coalescence, and seven (53.8%) disappeared between day 15 and day 60.

Application of the multinomial logistic regression to these results showed significant differences (Wald $\chi^2 = 22.2691$, df = 4, $P = 0.0002$) in the proportions of dead, alive, and coalescing sporelings separated by sizes and dates. Application of χ^2 test (equal proportions) to the proportions of dead and alive sporelings with and without coalescence at day 60 yields significant differences ($P < 0.0001$) in all comparisons (alive vs. dead, alive vs. coalescing, and dead vs. coalescing) for the three classes of sporeling sizes.

Survival as a function of size: In the detailed monitoring at day 60 of the 1,385 sporelings measured on day 45, 510 sporelings died, 276 sporelings survived with additional coalescence, and 599 survived without additional coalescence. The results of the multinomial logistic regression suggest that sporeling area is a significant variable (Wald $\chi^2 = 17.9564$, df = 2, $P < 0.0001$) in determining the proportions of sporelings that die or remain alive, with or without coalescence. Overall, the probability of surviving increases with increasing sporeling area (Fig. 7). The probability of coalescing also increases with sporeling area, but the slope of the corresponding regression increases with sporeling size more slowly than the slope of the surviving sporelings without coalescence.

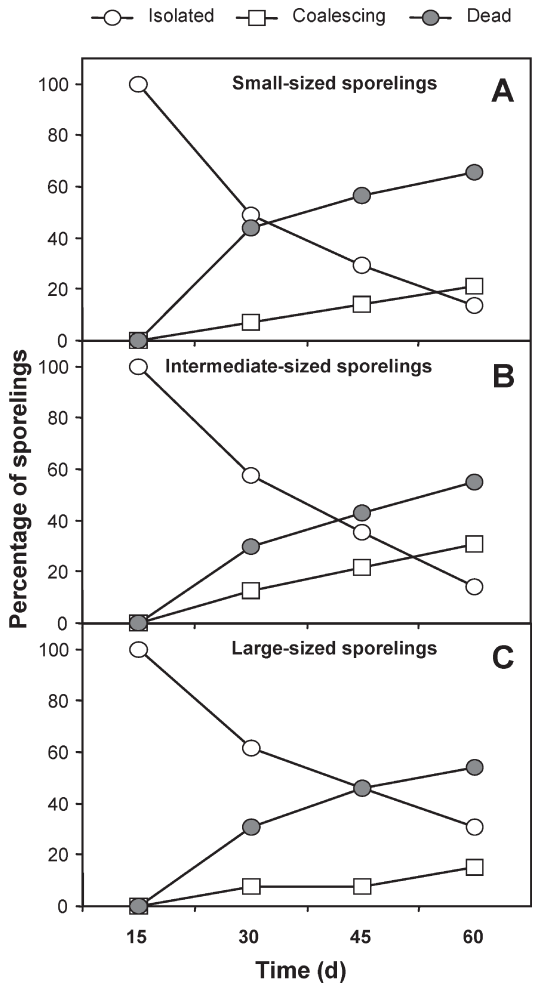

FIG. 6. Temporal changes in the proportions of sporelings dying and surviving with and without additional coalescence in the field-incubated plates. Sporelings have been separated into three size groups based on area, measured at day 15. They include small- (A), intermediate- (B), and large-sized (C) sporelings.

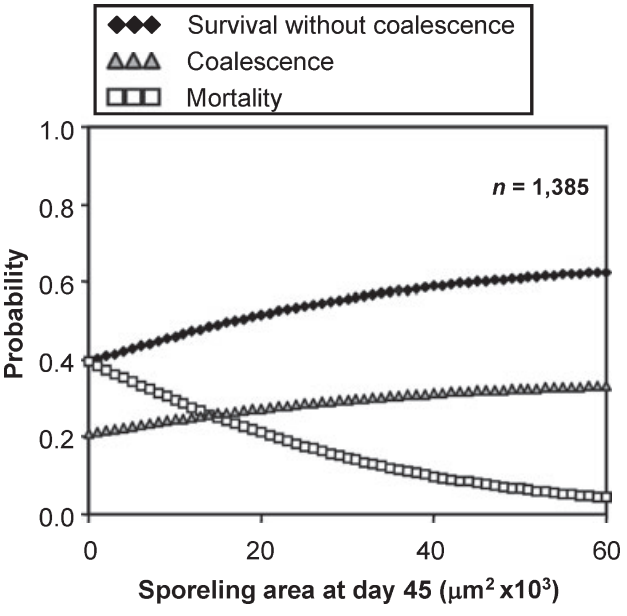

FIG. 7. Probability of surviving without additional coalescence, dying, or coalescing in the field as a function of sporeling size between day 45 and day 60. The model is based on the area of 1,385 sporelings measured at day 45 and monitored again at day 60. The corresponding equation for the survival probability is $1/(1 + e^{-4.46EXP-05x} + e^{-0.6316})$; that for coalescence is $e^{-4.46EXP-05x}/(1 + e^{-4.46EXP-0.5} + e^{-0.6316})$, while the probability equation for mortality is $e^{-0.6316}/(1 + e^{-4.46EXP-0.5x} + e^{-0.6316})$.

Crust fissions: Examination of the field-incubated plates revealed that crust fission was a rather infrequent event. Only one of the 2,897 field-incubated sporelings examined during this study exhibited fissions.

DISCUSSION

The dynamics of early recruitment of *M. laminarioides* are determined by three key processes: settlement, death, and coalescence. While the first two processes correspond to the classical concepts of birth and mortality applicable to population studies of all types of algae (see review in Chapman 1986), coalescence is a new component, only exhibited by organisms with the capacity to fuse during ontogeny.

Settler abundance is variable in space and time. Temporal patterns of spore production, their longevity before settlement, distances of a given site from the propagule sources, and effects of factors facilitating or reducing dispersal, settlement, and attachment are, among others, recognized as important sources of variability and are of widespread occurrence (see reviews and references in Santelices 1990, Fletcher and Callow 1992, Norton 1992, Johnson and Brawley 1998, Underwood and Keough 2001, Gaylord et al. 2002, Bobadilla and Santelices 2005). In our study, the significant differences in number and frequency distribution of settlers observed between field- and laboratory-reared experimental plates reflects this variability.

Settlement and recruitment directly influence sporeling population structure. In the absence of recruitment, as in our laboratory-incubated plates, sporelings gradually increase in size, with the smaller sporeling size classes tending to disappear over time. On the other hand, continuous recruitment, as in our field plates, created a sporeling population with representation of a wide range of sporeling sizes. The absence of demographic studies on recruitment of coalescing macroalgae prohibits conclusions about the frequency of these two alternative population structures in the field. However, since many coalescing species in cold and temperate latitudes exhibit seasonal spore production and recruitment (see Lobban and Harrison 1994 for examples), the two above sporeling population structures probably also occur in the field but in different seasons.

Although differing in their relative abundance, unisporic and multisporic recruits occurred in both field- and laboratory-incubated plates. The presence of multisporic recruits by the third day of recruitment is most important for coalescence and suggests the existence of groups of spores that settle and recruit in close proximity and coalesce shortly after settlement. In this study, group recruitment was observed on all plates, accounting for 30%–70% of the spores quantified at the first monitoring time. Group recruitment has also been observed in other coalescing seaweeds (Santelices et al. 1999, Morley et al. 2003), and it does not seem to be common in noncoalescing macroalgae. Previous results (Santelices and Aedo 2006) indicate that several factors affect the abundance of group recruitment in a given site, including spore abundance, dissolution rates of the mucilagous layer maintaining the released spores in close proximity, and the mode and periodicity of spore release.

Coalescence also appears as an important process determining sporeling population structure, and its relative importance increases with time. Thus, while at day 3, close to 25% of the sporelings on the laboratory plates were coalesced and multisporic, by day 60, their relative abundance had increased to >75%. Although we could not distinguish spore numbers in the field-exposed plates, the increases in sporeling areas well beyond the maximum size of unisporic sporelings suggest coalescence is also occurring in the field-exposed plates.

Mortality is the third process determining early recruitment dynamics of *M. laminarioides*. Its relative importance, however, varied over time and space, probably in direct relation with intensity of the mortality mechanisms (e.g., grazing, water movement, dessication, competition; Vadas et al. 1992, Johnson and Brawley 1998, Wright and Steinberg 2001). In our study, average mortality in the field was almost three times higher than in the laboratory-incubated plates. Despite this difference, however, in both kinds of plates the relative mortality decreases over time, and the probability of survival increases with sporeling area, as shown by the sporelings measured at day 45 and that survived to day 60.

Since coalescence increases sporeling size, it would be expected that most of the large-sized sporelings would correspond to coalescing individuals. Our results with laboratory-incubated plates (e.g., Fig. 4, A–C) indeed indicate that most of the larger individuals are multisporic. However, the growth rates of unisporic sporelings also showed a high degree of variability, with some individuals reaching larger sizes than some multisporic sporelings. Thus, although coalescence tends to increase average sporeling size and probabilities of survival, it is not a necessary condition for survival.

Both the environment and the number of spores coalescing to form the sporeling seem to influence growth rates in these individuals. Our results indicate field growth rates were approximately one-third of those reached under laboratory conditions, suggesting that the more stressful conditions of the field may not only affect sporeling survival but may also impose costs to growth. On the other hand, the slow growth rates and the sizes eventually reached by many multisporic sporelings, relative to values exhibited by some of the unisporic sporelings, suggest that coalescence may also impose a cost to growth. This possibility has not yet been considered

when evaluating the adaptive traits of coalescence (Santelices et al. 1999), and it should be considered in further detail to critically evaluate costs and benefits of coalescence.

Fissions and partial mortality are two additional processes that have been shown to be important determinants of the demography of colonial invertebrates (Hughes and Jackson 1980). Among macroalgae, fissions in the crustose bases of erect thalli have been observed in several members of the order Gigartinales and especially in the genus *Mazzaella* (Scrosati and De Wreede 1997, Scrosati 2001, Santelices et al. 2003a). Therefore, crust fissions were searched for, but their frequency proved to be too low to be of significance in the early recruitment of *M. laminarioides*. On the other hand, the quantification of partial mortality, as described for corals (Hughes and Jackson 1980), requires distinguishing dead parts of the organism in repeated measurements. Among corals, the exoskeleton of polyps is used for such estimations. However, that possibility does not exist in noncalcified macroalgae due to the lack of an exoskeleton or other permanent structures that may serve as marks between coalescing partners. In these organisms, reductions in size (e.g., diameter reductions in crustose holdfasts) could be used to temporarily estimate negative growth. However, such estimations would be easily confounded by positive growth. For this reason, partial mortality was not measured in these sporelings.

The diagram in Figure 8 illustrates the three processes determined as important in the dynamics of sporeling populations of this species (settlement, mortality, coalescence) and the flow of individual sporelings during population development. Also, the diagram illustrates the complexity of demographic processes in coalescing species. By adding components of different ages and sizes, these populations of coalescing taxa become a mixture of crusts of different ages and size classes. On the basis of our field and laboratory results, we predict that 45–60 d after settlement, any 2 cm^2 (=200 mm^2) of recruitment area could contain some 800–1,000 sporelings, ranging in size from recently recruited crusts, slightly larger than 50–100 μm^2, to grown sporelings, 0.2–0.6 mm in diameter. Some of these larger crusts (e.g., 300,000 μm^2) have incorporated between 60 and 75 coalescence events in their development and are formed by derivatives of as many as 65–70 initial spores. A projection of these results to field sizes suggests that small holdfasts of 1–2 cm of diameter, like those described by Scrosati (2001)

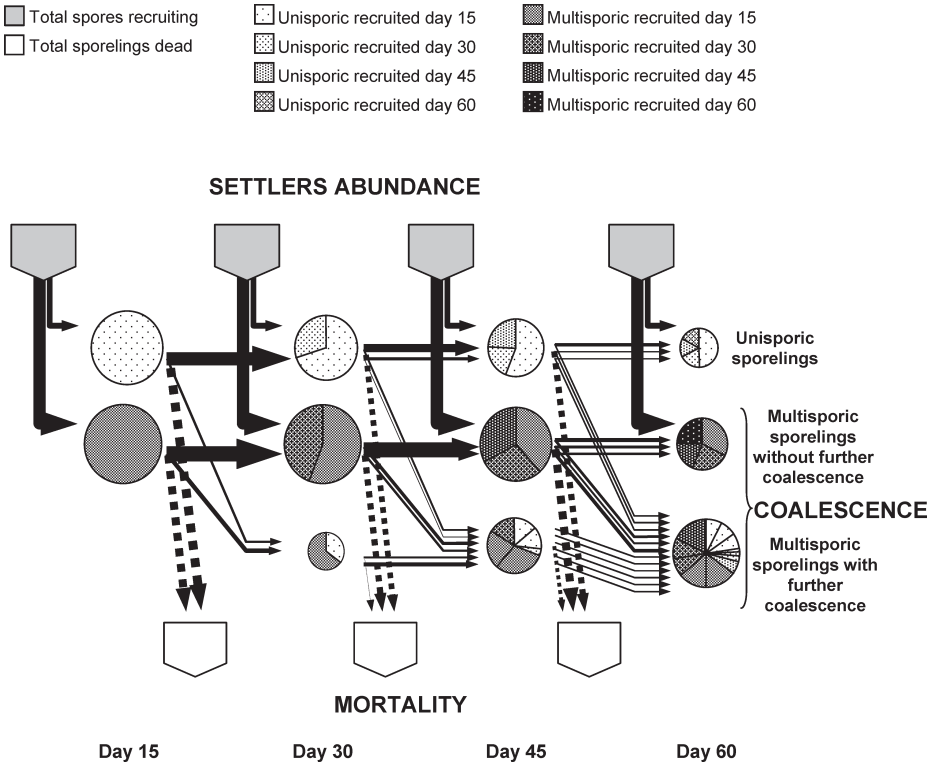

FIG. 8. Diagram of the dynamics of early recruitment in a coalescing species. Settler abundance, coalescence, and mortality appear as the key demographic factors. The population is composed of sporelings formed by one spore (unisporic) or by two or more spores (multisporic) of the same or different cohorts. As time progresses, both unisporic and multisporic sporelings may coalesce with other conspecific crusts, which, in turn, may either be unisporic or multisporic in origin. Arrows indicate flux of individual genets through the population compartments.

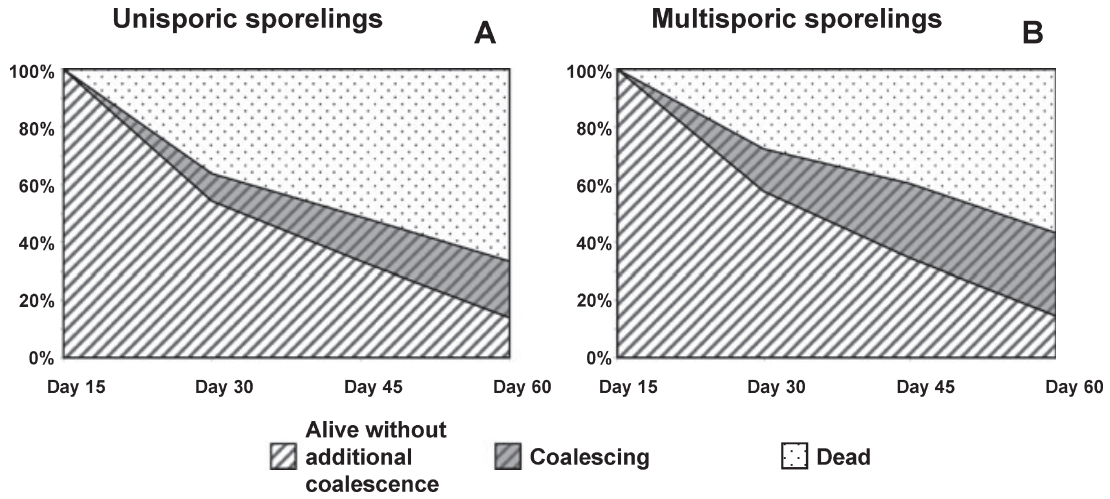

FIG. 9. Survivorship curve for a coalescing organism, such as *Mazzaella laminarioides*. Unisporic or multisporic sporelings in one cohort may survive without additional coalescence, die, or coalesce with other sporelings of the same or different cohorts. The proportions between alive without coalescing, coalescing, and dead sporelings may vary in different times and sporeling populations.

and Santelices et al. (2003a) for field populations of *Mazzaella,* might have already experienced hundreds of coalescing events before they become distinguishable as a field clump. Additional studies are required to evaluate if the relative importance of coalescence continues similarly in later recruitment stages of this species or if growth and thallus expansion becomes the principal factor. Field studies have already shown that coalescence and mortality may occur among field-established clumps (Santelices et al. 2003a) and that coalescence may occur at all ontogenic stages (Santelices et al. 2004).

Integration of results suggests that the type of survivorship curve for coalescing macroalgae is more complex than the one known for more conventional types of species (Fig. 9). Sporelings may not only die or survive, but they may also coalesce. These three possibilities are valid for unisporic as well as multisporic sporelings, which, after initial coalescence, may survive with the original number of spores that recruited (live without additional recruitment), coalesce again with other sporelings, or die. Previously, Tanner (2000, 2001) solved the demographic problems imposed by fissions in corals by increasing the recruitment values in the survivorship/mortality curves. However, coalescence or fusions not only modify the quantitative expression of some of the demographic parameters, such as number of sporelings, but also constitute an alternative outcome to survival and mortality. For that reason, the model proposed in Figure 9 incorporates qualitative rather than quantitative changes. Future research will need to indicate if this modified survivorship curve would apply to other species of coalescing macroalgae or modular invertebrates. As stated earlier, many common and ecologically important macroalgal species (e.g., *Chondrus*, *Gracilaria*, *Gigartina*, *Gymnogongrus*, *Ahnfeltia*, coralline crusts, *Grateloupia*, *Halymenia*, *Rhodymenia*) are coalescing entities, probably recruiting in a manner similar to *M. laminarioides.*

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