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QUANTIFICATION OF THE EFFECTS OF SPORELING COALESCENCE ON THE EARLY DEVELOPMENT OF *GRACILARIA CHILENSIS* (RHODOPHYTA)[1]

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ABSTRACT

Sporeling coalescence in species of Gracilariales and Gigartinales is predicted to result in larger basal areas of growing disks as well as earlier initiation, increased abundance, and faster growth rates of erect shoots as compared to noncoalescent sporelings. These responses have been interpreted as providing mutual benefits for organisms living in aggregation, counterbalancing disadvantages associated with crowding. Quantitative evaluations of sporelings of Gracilaria chilensis *failed to support several of these predictions. Sporelings were grown in the laboratory from a range of single sporelings to coalescent masses of 20 sporelings. Coalescent sporeling masses of* G. chilensis *exhibited larger basal areas than noncoalescent ones, but because the specific growth rates were inversely related to the original number of carpospores, no significant differences in actual area increments, during most of the experiment, were found among sporelings derived from one, two, or three to five coalescing sporelings. Initiation of erect shoots occurred at a similar time, regardless of their origin, i.e. coalescent or noncoalescent. Abundance of erect shoots was only loosely related to the number of coalescing sporelings. Even though by the end of the experiment (week 18), the total length of the longer erect shoots arising from coalescent sporeling masses was significantly greater than that of shoots arising from noncoalescent sporelings, total length was independent of the original number of coalescing sporelings. Furthermore, specific elongation rates between week 12 and week 18 were significantly greater for noncoalescent sporelings than for coalescent sporeling masses. Quantitative screening of other species seems necessary before generalizations on the ecological advantages of sporeling coalescence in seaweeds can be made.*

Key index words: coalescence; ecology; Gracilaria chilensis; *Rhodophyta; sporeling coalescence; sporeling growth*

The ability of sporelings and crusts of a single species to grow together forming a completely coalesced mass has been described in several studies of erect and crustose algae (see Maggs and Cheney 1990 for a review). Among the Rhodophyta, sporeling coalescence has been frequently reported in species of Gracilariales and Gigartinales (Rosenvinge 1931, Jones 1956, Chen and Taylor 1976, Tveter and Mathieson 1976, Rueness 1978, Maggs and Cheney 1990).

Sporeling coalescence often has been described as modifying the growth pattern of the coalesced mass. Thus, Jones (1956) noted an enhancement of sporeling growth in *Gracilaria verrucosa* as a result of crowding and coalescence. In addition, he observed that coalescent sporeling masses initiated erect shoots earlier and in larger numbers than uncoalesced sporelings. Tveter and Mathieson (1976) described similar findings with sporelings of *Chondrus crispus, Gracilaria verrucosa,* and *Mastocarpus stellatus* and reported, in addition, that the cells in the center of the coalesced sporelings of these species produced fronds that grew more rapidly than fronds of noncoalesced sporelings.

All the preceding responses have been interpreted as being advantageous for the seaweed. A larger basal area means a larger attachment surface for the growing sporeling. A faster growth rate can provide competitive advantages over other growing sporelings in environments with limited resources. Earlier emergence and faster growth of fronds would reduce the time required to grow beyond the boundary layer or to come out from under the sand and mud in the sandy-muddy bottoms where some of these species commonly occur (e.g. *Gracilaria*). Based on these assumptions and on ultrastructural evidence, Maggs and Cheney (1990) suggested that sporeling coalescence and intercellular connections should be viewed as a mechanism uniting separate individuals into a single "super-organism," an idea that Jones (1956) suggested earlier but without using the term. Aggregation would have sufficient mutual benefits to outweigh the disadvantages associated with crowding. Individual sporelings joined by secondary pit connections in coalesced masses would be expected to be under a single metabolic control, to behave as a single organism, and to cooperate in order to produce a shoot that would grow faster than those of uncoalesced sporelings.

The idea of the superorganism has the potential to integrate several isolated observations in a comprehensive framework and to emphasize intraspecific cooperation as an ecologically important interaction. However, there is a general lack of quantitative information attesting to the occurrence of these responses. For example, it is not clear what method Jones (1956) used to determine that sporeling coalescence leads to an enhancement of growth, if he was referring to specific or absolute growth, or if he was discussing basal disk growth or frond growth. Furthermore, coalescence may induce mul-

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tiple responses that differ in their supposed advantageous character. Thus, Maggs and Cheney (1990) regarded early sporeling coalescence as advantageous because it leads to large basal holdfasts. These larger holdfasts would form a continuous coverage of the substratum without gaps between individuals, thus reducing the ability of competing algae to gain a foothold. By contrast, Tveter and Mathieson (1976) suggested that early, as compared to late, coalescence limits the number of uprights produced, thus reducing the competing capacity of the erect axes.

To quantify the effects of sporeling coalescence on early development, we compared coalescent and noncoalescent sporelings of *Gracilaria chilensis* with respect to total basal area increments and growth rates of the sporeling disks as well as the time of initiation and number and elongation rates of erect shoots produced.

MATERIALS AND METHODS

The experiment was performed twice, once in March 1992 with cystocarpic thalli collected in the vicinity of Talcahuano Bay, Central Chile (36°46'S, 73°08'W), and once in September with cystocarpic thalli collected in Niebla, near Valdivia in Southern Chile (39°51'S, 73°20'W). Plants were transported by air in cool chambers to the laboratory in Santiago. They were washed with running tap water and maintained for 24 h in filtered seawater under controlled temperature (12 ± 2° C), irradiance (20 ± 10 μmol photons·m^{-2}·s^{-1}) and photoperiod (12:12 h LD). Sporulation of mature cystocarps was induced by immersion in microfiltered seawater after 24 h of dehydration at room temperature. Carpospores were inoculated into 24 small glass Petri dishes (50 mm diam, 10 mm deep) with filtered seawater and incubated in growth chambers under the conditions already described. Each Petri dish contained a square glass plate (35 × 35 × 5 mm) on which the carpospores settled.

During week 1 of incubation, the position of 51 isolated carpospores and 43 groups of carpospores (2–20) in the Petri dishes was marked and photographed to distinguish individual sporelings and sporeling masses during development. Patterns of cell division and early development were also followed during that week to eliminate carpospores with abnormal growth patterns (about 3% of the total number of carpospores). During week 2, developing sporelings were grouped into four classes. Class I included sporelings (n = 27 and 24 in the first and second experiments, respectively) developed from a single carpospore. Class II consisted of groups (n = 10 and 12, respectively) of two sporelings that had their boundaries touching within the first week of growth. Class III consisted of groups (n = 5 and 8, respectively) of three to five sporelings, and Class IV consisted of groups (n = 4 in each experiment) of 6–20 sporelings in close contact. All other sporelings on the glass plates were removed. Thus, the total number of developing sporelings on each glass plate was maintained between three to five to avoid density-dependent effects on growth. The sporelings in classes II–IV coalesced during the following 10 days such that original sporelings could no longer be distinguished. The resulting coalescent sporeling masses were used for growth measurements.

To promote growth, after 3 weeks culture dishes were transferred to a slightly higher temperature (16 ± 2° C) and irradiance regime (40–45 μmol photons·m^{-2}·s^{-1}), and the filtered seawater was replaced by SWM-3 (McLachlan 1973). To prevent diatom growth, 1 mL of a stock GeO_2 solution (6 mg·L^{-1}) was added to each liter of SWM-3. The glass plates were cleaned with a fine paint brush, and the culture medium was changed weekly. In the tenth week of growth, the glass plates with their thalli were transferred to larger Petri dishes (10 × 8 cm), each containing 300 mL of SWM-3 culture medium. Gentle air bubbling was also provided. Other abiotic conditions were as already described, including weekly changes in growth medium.

Between the second and tenth week of incubation, growing sporelings developed as dome-shaped, irregularly circular disks. The basal areas of the disks were measured each week. Area is not the best parameter for measuring growth of a three-dimensional organism. However, the small size of the sporelings and the need to keep them alive after repeated measurements did not allow measurement of changes in biomass or volume. In addition, even though the sporelings increased in size, their general shape remained constant during the experimental period. Results were used to calculate absolute increase in basal area. The absolute growth rate was calculated using the formula

$$K = \frac{\log_2 X_2 - \log_2 X_1}{t_2 - t_1}$$

(Brinkhuis 1985), where K is the absolute growth rate and X_1 and X_2 are the values of basal area at specific times (t_1 and t_2). The mean specific daily growth rate for each week of the experiment was calculated by dividing K by the average size during the time interval.

Elongation rates of the erect shoots in the four classes of sporelings were measured between the twelfth and eighteenth week of development using the materials from Talcahuano (March experiment). This was done by placing the glass plates on their sides and measuring the length of the erect shoots using an eyepiece in a stereomicroscope.

Growth rate data for the disks and shoots were subjected to arcsine transformation, and significant differences among the classes of sporelings were evaluated using ANOVA, followed by Fisher's PLSD *a posteriori* test (Snedecor and Cochrane 1967) or the Kruskall-Wallis one-way analysis of rank (Siegel and Castellan 1988). To avoid temporal pseudoreplication (Hulbert 1984), ANOVA was applied to comparisons among sporelings at a given time only. It did not include temporal comparisons of sporelings.

Results from both experiments were similar; thus, the data of only one of the experiments (either September or March) is used to show patterns of growth.

RESULTS

Carpospores first divided 2 days after their release. After 1 week, sporelings in close proximity began to coalesce. Coalesced and noncoalesced sporelings formed dome-shaped, irregularly circular disks that grew in a similar fashion. By the fifth week of development, there were no obvious morphological differences, other than size, between sporeling masses formed from a single carpospore or coalesced sporelings. Between the eighth and ninth weeks of growth, sporelings initiated erect shoots, which reached 2 mm in length by the eighteenth week.

All coalescent sporeling masses exhibited a larger basal area than noncoalescent sporelings. Furthermore, the greater the number of coalescent sporelings, the greater their basal area (Fig. 1). Nevertheless, sporeling masses derived from the coalescence of two sporelings did not have areas twice as large as those of noncoalescent sporelings. Actually, the relationship between the mean basal area that the sporeling masses achieved after 10 weeks of growth and the original number of car-

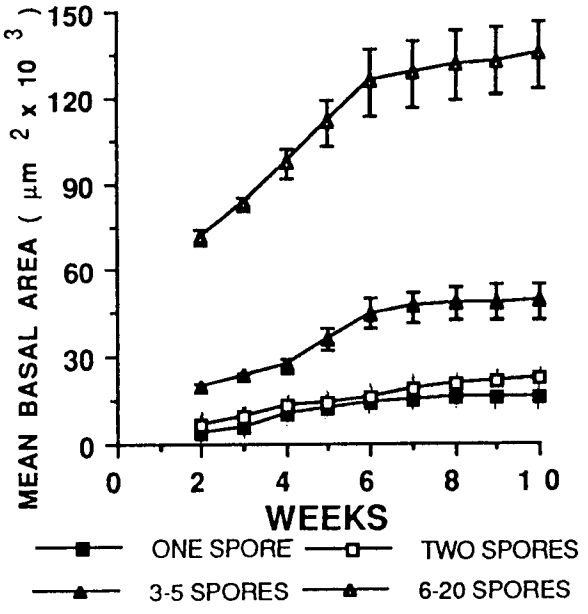

FIG. 1. Mean basal area (±SE) of coalescent and noncoalescent sporelings of *Gracilaria chilensis* during the first 10 weeks of growth under laboratory conditions (September experiment).

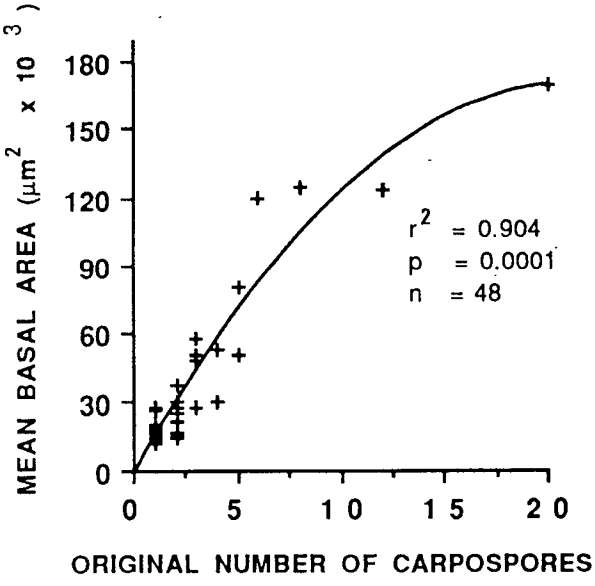

FIG. 2. Mean basal area of coalescent and noncoalescent sporelings of *Gracilaria chilensis* after 10 weeks of growth as a function of the original number of carpospores (September experiment). Equation for the polynomial fitting: $y = -1.57 + 16.1x - 0.379x^2$.

pospores that gave rise to them is not strictly additive (Fig. 2).

Area increments measured at biweekly intervals indicate (Fig. 3) that the coalescent masses of 6–20 sporelings exhibited significantly larger increments than any of the other treatments in the first 6 weeks of the experiment (Kruskal-Wallis analysis of rank, $P < 0.005$). No significant differences in area increments among sporelings derived from one, two, or three to five coalescent sporelings were found with the exception of class III sporelings that grew significantly more during weeks 4–6 (Kruskal-Wallis analysis of rank, $P < 0.005$). During the 4 weeks prior to the initiation of upright shoots, the actual area increment of all classes of sporelings was similar.

Calculated on an area-specific basis, the daily growth rates of sporelings and sporeling masses were inversely related to the original number of carpospores. The relationship was significant during the first 8 weeks of the experiment (Fig. 4).

Coalescent sporelings did not give rise to shoots earlier than noncoalescing sporelings. Erect shoots appeared between weeks 8 and 9 of growth regardless of sporeling origin.

Most noncoalesced sporelings and sporeling masses arising from the coalescence of up to five sporelings produced one erect shoot, although in all three classes a few sporelings initiated two erect shoots simultaneously (Table 1). The proportion of sporelings with simultaneous emergence of two erect shoots increased slightly as the number of coalescing sporelings increased. All sporelings derived from coalescence of 6–20 sporelings initiated three erect shoots. Whenever there were two or three erect shoots originating from a single sporeling, one shoot grew faster than the others.

By the twelfth week, the longest shoots arising from coalescent sporeling masses were close to 40% longer than those from noncoalescent sporelings (Fig. 5). Furthermore, by the end of the experiment (week 18), the total length of the longest erect shoots arising from coalescent sporeling masses was significantly greater than in shoots arising from noncoalescent sporelings. However, specific elongation

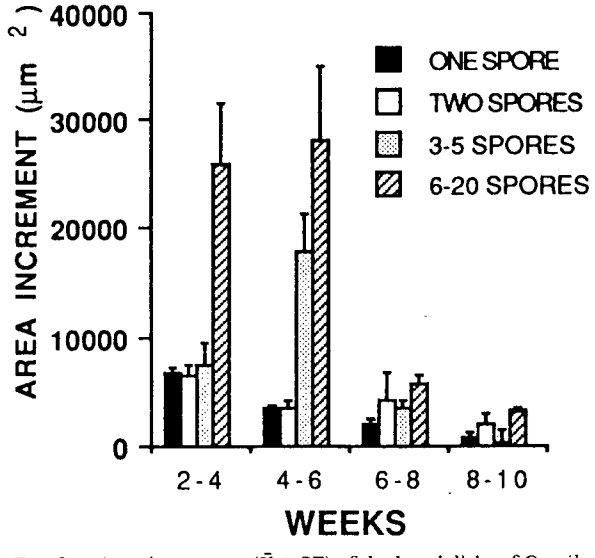

FIG. 3. Area increment ($\bar{X} \pm$ SE) of the basal disks of *Gracilaria chilensis* developing from one to several coalescing sporelings (September experiment).

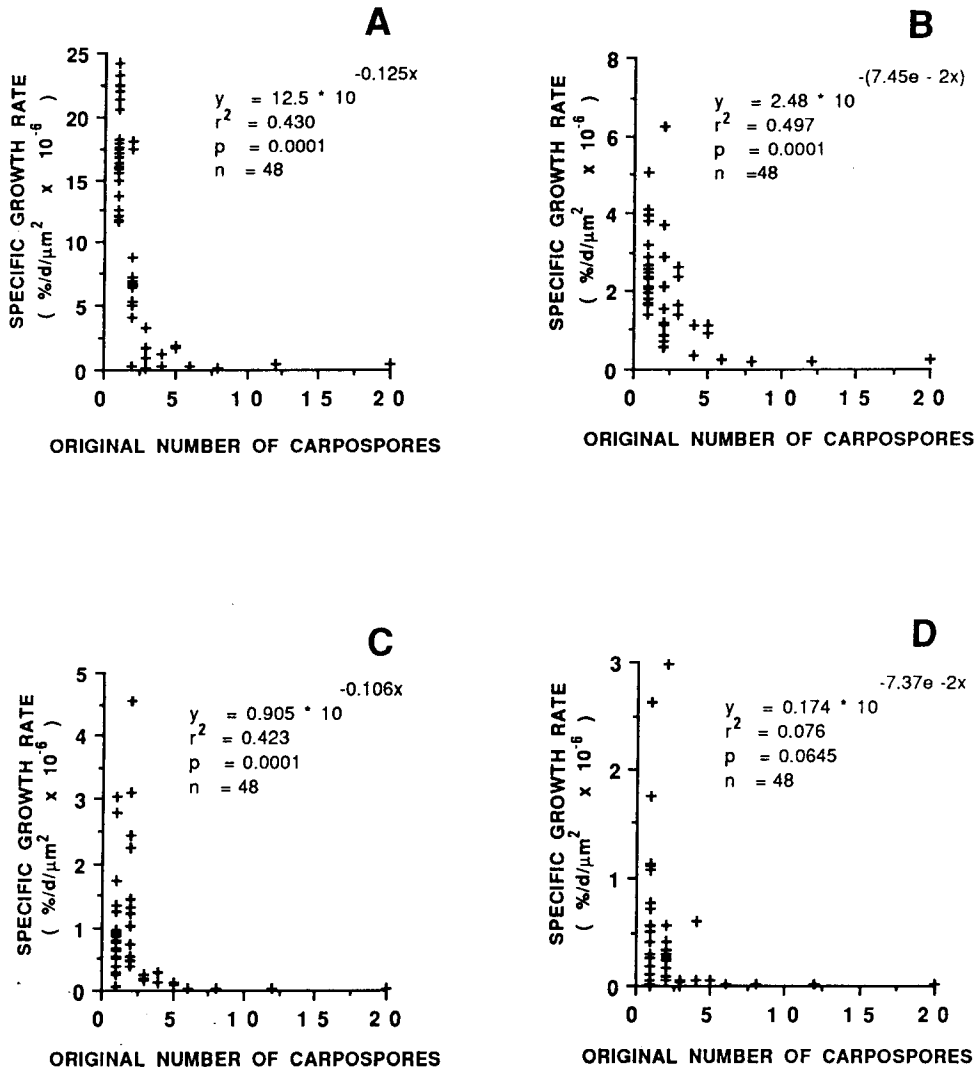

FIG. 4. Specific growth rates of basal areas of coalescent and noncoalescent sporelings of *Gracilaria chilensis* as a function of the original number of carpospores, measured at biweekly intervals (September experiment). A) Growth between weeks 2 and 4. B) Growth between weeks 4 and 6. C) Growth between weeks 6 and 8. D) Growth between weeks 8 and 10.

rates between weeks 12 and 18 were significantly greater for noncoalescent than for coalescent sporelings (Kruskall-Wallis analysis of rank, $P = 0.024$).

DISCUSSION

Sporeling coalescence, which has been previously reported in other species of Gracilariales and Gigartinales (Rosenvinge 1931, Jones 1956, Tveter and Mathieson 1976, Rueness 1978, Maggs and Cheney 1990), occurs extensively in *Gracilaria chilensis*.

TABLE 1. *Percentages of coalescent and noncoalescent sporelings of* Gracilaria chilensis *initiating one, two, or three upright shoots.*

Type of sporeling	Number of erect shoots		
	One	Two	Three
I Noncoalescent (n = 24)	93%	7%	0%
II Two coalescent sporelings (n = 12)	70%	30%	0%
III Three to five coalescent sporelings (n = 8)	65%	35%	0%
IV Six to 20 coalescent sporelings (n = 4)	0%	0%	100%

Coalesced sporeling masses of *Gracilaria chilensis* exhibited a larger basal area than noncoalesced sporelings in the first week of development. It is expected (Maggs and Cheney 1990) that, through coalescence and growth, the basal areas of coalescing sporelings would form a continuous coverage of the substratum, leaving no gaps between individuals and reducing the ability of competing algae to gain a foothold. However, under the culture conditions used in our experiments, coalescent sporeling masses of *G. chilensis* had specific growth rates inversely related to the original number of carpospores that gave rise to them. Therefore, the actual area increments shown by most classes of sporelings during most of the experiment were approximately similar. The exceptions were the area increments exhibited

by class IV sporelings during weeks 2–6 and by class III sporelings from weeks 4–6. Thus, if a larger basal area enhances attachment and competitive ability of the sporelings of *G. chilensis,* and that remains to be demonstrated, coalescence would be a significant advantage only when it involves a large number of sporelings.

The earlier initiation of erect shoots would be another adaptive trait of coalescent sporelings. Jones (1956) stated that erect shoots of *Gracilaria verrucosa* arose earlier from coalescent than from noncoalescent sporelings. Tveter and Mathieson (1976) also reported that shoots from coalesced sporeling masses of *Chondrus crispus* and *Mastocarpus stellatus* arose sooner than from uncoalesced sporelings. In *Gracilaria chilensis,* however, the initial appearance of erect shoots occurred at the same time, regardless of whether the sporelings were or were not the product of coalescence. This is an important departure from the prediction about the adaptive traits of coalescent sporelings because an early origin of erect shoots supposedly confers important competitive advantages in nutrients and light capture as well as the possibility of emerging earlier from under sandy or muddy bottoms.

Another adaptive trait would be an increased number of erect shoots formed in coalescent sporeling masses. Jones (1956) and Tveter and Mathieson (1976) have reported that even though there were always fewer shoots in coalescent sporeling masses than the total number of individual sporelings that coalesced, the greater the number of sporelings forming the coalesced sporeling mass, the greater the number of shoots produced. In the case of *Gracilaria chilensis,* most basal disks derived from noncoalesced and coalesced sporelings of up to five sporelings produced a single erect shoot. The percentage of sporelings producing two erect shoots slightly increased as the number of coalescing sporelings increased. This pattern was disrupted, however, in sporeling masses resulting from the coalescence of a larger number of spores (6–20), all of which produced three erect shoots. Thus, at least for *Gracilaria chilensis,* the number of erect shoots produced by sporeling masses does not seem to be a linear function of the original number of coalescent sporelings.

A faster growth rate of erect shoots arising from coalescent sporeling masses, as compared to noncoalescent sporelings, also has been interpreted as an adaptive trait resulting from sporeling coalescence. Our results reproduce this pattern when total growth (= total elongation of the longest erect shoots) is considered. However, when the specific growth rates are compared, erect shoots arising from noncoalescent sporelings can grow significantly faster than those of coalescent sporeling masses, as they did in our experiment during weeks 12–18. In addition, when only coalescent sporelings are considered, elongation rates of erect shoots do not keep

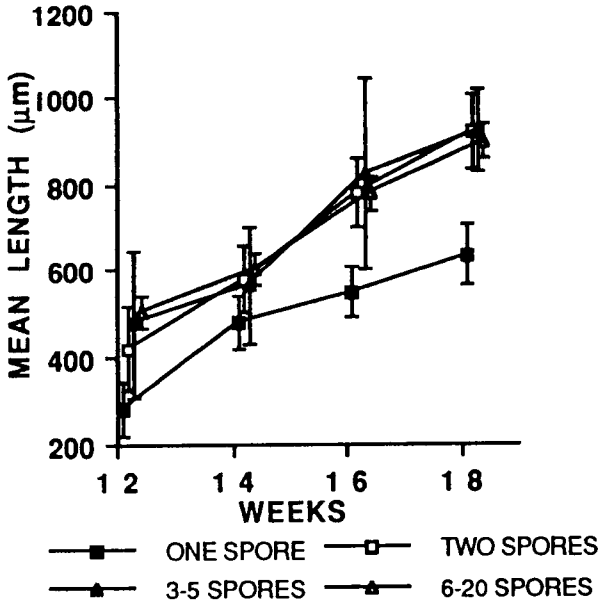

FIG. 5. Average length (±SD) of erect shoots arising from sporelings of *Gracilaria chilensis* as a function of number of coalescent sporelings and of time of growth (March experiment).

any numerical relationship with the number of coalescing sporelings. Again this is an important departure from previous findings. Furthermore, all sporelings with more than one erect shoot, regardless of their coalescent or noncoalescent origin, always exhibited one shoot elongating significantly more than the others, a fact that was also observed by Tveter and Mathieson (1976) and Maggs and Cheney (1990). Since multiple fronds are initiated at the same time, this might suggest growth inhibition of the other shoots in a given sporeling by the faster developing one.

The overall conclusion emerging from our data is that sporelings of *Gracilaria chilensis* exhibit only a few of the several adaptive traits expected to arise by sporeling coalescence. These traits are more likely to be shown when coalescence involves a larger number of spores. Quantitative screening of other species, including some of those previously studied, seems necessary before generalizations on the ecological advantages of sporeling coalescence can be made.

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ECOTYPIC VARIATION IN *PHYLLOPHORA PSEUDOCERANOIDES* (RHODOPHYTA) ENSURES WINTER REPRODUCTION THROUGHOUT ITS GEOGRAPHIC RANGE[1]

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ABSTRACT

Responses to temperature and daylength were determined in laboratory culture for isolates of the red alga Phyllophora pseudoceranoides *(Gmelin) Newroth et A.R.A. Taylor from Nova Scotia, Iceland, Roscoff (France), and Helgoland (Germany). All isolates grew from 3° to 25° C and survived from −2° or 0° C to 27° C but not 30° C. Reproductive requirements differed between life history phases and isolates. Isolates from Helgoland and Roscoff formed sporangial sori at 3°–20° C, tetraspores at 3°–12° C, and procarps at 10°–20° C, irrespective of daylength. Spermatangia developed at 10°–23° C but only in long days. As the other European isolates, the isolate from Iceland formed tetrasporangia at 3°–12° C, but it had an additional requirement for short days. The Nova Scotian isolate formed sori at 10°–20° C and sporulated at 10°–18° C. When grown plants were transferred from noninductive to inductive conditions, sori were formed after 4 months and tetraspores developed and were shed (1–)3 months later. Procarps formed 1(–3) months after transfer.*

The phenology of P. pseudoceranoides *was studied at Helgoland and Roscoff, where similar seasonal patterns were observed. Plants were perennial, forming new blades from October to June, which degenerated between August and February. In June, reproductive structures (sori, spermatangia, and procarps) started to appear on the new blades. From October to April, mature cystocarps were found. Mature tetrasporangia were observed only in February.*

The life history of P. pseudoceranoides *is regulated by temperature and daylength. Differential effects on the different life history phases all serve to confine the production of spores (both carpospores and tetraspores) to the winter season. Differences in response between isolates from different geographic regions bring about the same effect: spores are shed only in winter.*

The nature of the geographic boundaries of P. pseudoceranoides *is discussed.*

Key index words: biogeography; ecotypes; life history regulation; Phyllophora pseudoceranoides; *Rhodophyta; seasonality; temperature / daylength responses*

In most recent papers on the causation of biogeographic distribution limits of seaweeds, experimentally determined temperature (and photoperiodic) requirements for survival, growth, and reproduction are compared with environmental conditions at the geographic boundaries (e.g. Yarish et al. 1984, 1986, Breeman 1988, Cambridge et al. 1990, Orfanidis 1991, 1993). On the other hand, studies on the causation of reproductive seasonality in algae generally focus on detailed field observations (e.g. Kain and Bates 1993). In this article, we combine an experimental and field approach such as that previously employed by Novaczek et al. (1986a, b, c) and Novaczek and McLachlan (1987), for example. The purpose of this approach is, first, to determine whether or not the results of culture experiments adequately account for the seasonal course of events in the field and, second, to determine which factors are of primary importance when trying to explain biogeographic distributions.

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