

Morphogenesis in *Pterocladiaella capillacea* (Rhodophyta, Gelidiales): bud differentiation in relation to irradiance-temperature combinations

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ABSTRACT - In *Pterocladiaella capillacea* (Gmelin) Santelices et Hommersand, thallus morphogenesis is controlled by exogenous factors. At 14°C, the adventitious buds arising from the distal pole of frond explants developed into compressed axes, similar to the typical erect shoots of the species, under every irradiance tested. At 19°C and 24°C they developed into compressed axes when irradiance was 20-40 $\mu\text{mol m}^{-2}\text{s}^{-1}$, whereas they developed into terete axes, similar to the typical prostrate thallus, when irradiance was 2.5 to 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Buds regenerated from the proximal pole of explants differentiated, as a rule, into terete axes under all conditions, although they may become compressed under the maximum irradiance tested. A hypothesis based on apical dominance is suggested in order to explain the compressed or terete development of the thallus as a response to irradiance and temperature.

KEY WORDS - algal culture, *Gelidiaceae*, macroalgal ecophysiology, *Pterocladiaella*, regeneration, thallus morphogenesis

ABBREVIATIONS - PI = photon irradiance; LD = long day; SD = short day; LGB = lateral branches of limited growth

It is well known that the thallus of members of the family *Gelidiaceae* consists of erect fronds and prostrate axes. In *Pterocladiaella capillacea* (Gmelin) Santelices et Hommersand, the erect fronds have compressed and pinnately branched axes with spatulate apices, whilst creeping axes are terete with tapered apices, almost unbranched, and bear rhizoidal haptera (DIXON, 1958; FELICINI & PERRONE, 1994; SANTELICES & HOMMERSAND, 1997; SANTELICES, 1998).

In *P. capillacea* the development of the two kinds of thal-

lus can also be observed in culture. In a first study (FELICINI & ARRIGONI, 1967), carried out at room lighting and temperature, buds regenerated from both distal and proximal cut surfaces of intercalary explants of the frond all developed into terete axes similar to the typical prostrate thallus of the species. In the same way, the pre-existing spatulate apices of apical explants changed their morphogenetic trend, becoming tapered and growing as terete axes. Subsequent research (FELICINI, 1970) clarified that this effect was mainly caused by inadequate

lighting of cultures. In fact, using explants cultured at 20°C and different light intensity values, the following main results were observed: distal buds developed into compressed axes when light intensity was at least 800 lux (about 16 $\mu\text{mol m}^{-2}\text{s}^{-1}$) but into terete axes when cultured under lower irradiance; proximal buds grew as terete axes. Explants cultured under 850-1500 lux (about 17-30 $\mu\text{mol m}^{-2}\text{s}^{-1}$) showed morphological polarity due to the development of compressed axes at the distal end and terete axes at the proximal one (FELICINI, 1970; FELICINI & PERRONE, 1986, Figure 8; 1994, Figure 24). In such polarised explants ["adventitious embryos" according to BUGGELN (1981)], some anatomical differences between compressed and terete axes were also evident, such as the presence or absence of the inner rhizoidal filaments (hyphae) (FELICINI & PERRONE, 1986). However, in their natural environment, at the collection site, we observed new erect shoots of *P. capillacea* with spatulate apices rising from the prostrate system mainly in winter, when the population is subjected to low irradiance (10 to 20 $\mu\text{mol m}^{-2}\text{s}^{-1}$) and low temperature (11°C to 14°C). As this observation is in contrast with the above-mentioned experimental results, we re-examined the conditions of the previous cultures and realised that temperatures higher than those characteristic of the winter period had been used. Therefore, temperature could have an important role in bud differentiation. So, a bidimensional light-temperature experiment was performed (HALLDAL & FRENCH, 1958; EDWARDS, 1971), the results of which are described in the present paper. Moreover, considering that short-day is another environmental winter-factor at our latitude, a simple photoperiodic test was carried out in order to investigate the possible morphogenetic effect of daylength.

MATERIALS AND METHODS

Young fronds of *P. capillacea* were collected on the coast of Bari (southern Adriatic Sea, Italy), in winter-spring. Frond explants, consisting of 10-mm long intercalary segments of unbranched stipe, were cleaned under a stereo-microscope in order to remove as many epiphytic organisms present as possible. Explant cleaning was repeated every 2 weeks.

Culture experiments were performed four times; each test was in triplicate. The duration of cultures was 8 weeks. Culture medium consisted of filtered natural seawater, heated to boiling point and slowly cooled, enriched with 0.1 mM ammonium nitrate, changed weekly. Six mg l⁻¹ germanium dioxide was added to the medium during the first month of culture in order to reduce diatom contamination (LEWIN, 1966). Material

was grown in Pyrex crystallizing vessels (10-cm diameter). The plant material/medium ratio was 30 pieces/200 ml. Experiments were carried out simultaneously in three growth chambers under fluorescent light (40 W tubes, cool-white).

The highest irradiance used in our experiments was 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at vessel level. This is an intermediate value in the range (10-70 $\mu\text{mol m}^{-2}\text{s}^{-1}$) measured, throughout the year, at the collection site. Lower values were obtained using sheets of white paper as neutral filters. Photon irradiance was measured by a QRT1 light and temperature sensor (HANSATECH, U.K.). In the same way, the experimental temperatures were selected within their natural range from 11° to 30°C (FELICINI & PERRONE, 1994).

Therefore, the following conditions were set up in the irradiance-temperature tests: 2.5, 5, 10, 20 and 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI combined with 14°, 19° and 24°C ($\pm 1^\circ$); 12:12 h light/dark regime.

The following conditions were set up in the photoperiodism test: 8:16 h under 2.5 and 5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 14° and 24°C; 16:8 h under 2.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI at 14° and 24°C. Morphological observations and measurements were carried out at the end of experiments. An axis was considered "erect" (= compressed) when it had a spatulate apex and an evident ellipsoidal section (some lateral branch primordia could be present); it was considered "prostrate" (= terete) when it had a tapered apex and a circular or subcircular section (it could bear or not rhizoidal organs).

The number of explants bearing compressed or terete buds at the distal and proximal pole was counted. Bud morphology as a function of irradiance, temperature, test repetition and interactions among factors was analysed by a logistic regression model. The effects of irradiance and temperature on terete or compressed buds were also evaluated by the χ^2 test. Statistical analysis was carried out by SAS/STAT software.

RESULTS

One to two weeks elapsed between frond cutting and sprouting of bud primordia on 20-40% of the explants. This value reached a maximum (80-90%) at the end of the third week in culture. In some tests, a conspicuous number of explants (up to 40%) did not regenerate from the proximal pole under 20 and 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI. Bleaching and necrosis of a few axes only occurred at 14° and 24°C combined with 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ after 2 months in culture, immediately after the experimental period.

The length of distal buds after 8 weeks in culture ranged

between 4 and 14 mm. The length of proximal buds was 3 to 8 mm. Although growth seemed to be dependent on temperature and irradiance, an analysis of this parameter was not possible because it would be incorrect to compare the length of compressed and terete axes.

All the primordia of each cut surface differentiated into compressed or terete buds. The logistic regression model showed that distal bud differentiation into compressed or terete axes was not dependent on the collection time or repetition but only on irradiance and temperature and their interaction (Likelihood Ratio Test = 5944, $P < 0.0001$, $R^2 = 0.67$). In particular, probability of a distal bud becoming terete or compressed depends on irradiance, but in different ways at the different temperatures. Proximal buds were in prevalence terete and the logistic model was not able to detect any significant factors.

A contingency table for each type of bud was arranged and a χ^2 test was performed to show the effect of irradiance and temperature. Figures 1 and 2 show the distribution of compressed and terete buds regenerated from the distal and proximal cut surfaces of explants, as a combined effect of temperature and irradiance. The distal buds developed into compressed axes (orthotropic differentiation) at 14°C, regardless of irradiance, and at 19°C and 24°C combined with 20 and 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI ($\chi^2 = 1164.29$, $P < 0.0001$). They grew as terete axes (plagiotropic differentiation) both at 19°C and 24°C combined with 2.5 to 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI ($\chi^2 = 185.56$, $P < 0.0001$). At 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI, axes often had an intermediate form between a compressed and a terete thallus, however they were more similar to the prostrate form even though they rarely produced haptera. As a rule, all proximal buds underwent plagiotropic differentiation in the 2.5–20 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI range and regardless of temperature. Apices were tapered, and the terete axes did not produce lateral branches but rhizoidal organs were often present. Nevertheless, under 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PI, up to 60% (highly variable) of the explants exhibited buds with a spatulate apex which developed into erect type axes like the distal ones. At 14°C this flattening phenomenon seemed to be more constant and was also present under lower irradiance values (compressed buds: $\chi^2 = 76.61$, $P < 0.0001$; terete buds: $\chi^2 = 1.69$, $P = 0.99$).

Regarding the possible daylength effect, no morphogenetic responses were obtained within 8 weeks. As in the above-described tests, carried out under a 12:12 h photoperiod regime, at 14°C distal buds developed into erect type axes and, at 24°C, into prostrate type, both in SD and LD regimes.

DISCUSSION

DIXON (1958) first pointed out the morphological plasticity of the *Gelidiaceae* thallus under the influence of environmental factors. The irradiance-temperature combinations tested in the present study confirm the influence of light on distal bud development (FELICINI, 1970), and show that this effect is also controlled by temperature. The irradiance threshold for the orthotropic differentiation of distal shoots falls into the interval 10–20 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at 19° and 24°C.

These results are in accordance with our field observations. In fact, as mentioned in the introduction, the low irradiance-low temperature combination is, at our latitude, the winter condition in which most new erect shoots sprout from the prostrate system. Intermediate-high temperature combined with intermediate-high irradiance is the usual spring-summer condition in which the shoots grow as erect fronds.

The threshold for the orthotropic differentiation could be different for *P. capillacea* inhabiting tropical and subtropical seas, owing to adaptation to higher average values of irradiance and temperature. However, we should expect only a small shift of the critical value because it is well known that *P. capillacea* is a moderately sciophilous form (FELICINI & PERRONE, 1994). In Hawaii SANTELICES (1978) found this seaweed in shaded sites and assessed, by culture experiments, an optimum irradiance (as ft-candles) equivalent to 43–66 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Moreover, it is necessary to consider that the frond canopy of the same species, often thickened by macroepiphytes, shades the lower parts of the thallus (cf. SALINAS & VALDÉS, 1993 for *Gelidium*), so causing basal buds and branches to develop into creeping axes. This seems to be an excellent strategy for promoting substrate colonisation towards bare patches where new upright buds will have enough light to develop into erect fronds.

Daylength did not seem to affect bud morphogenesis in *P. capillacea*. Unfortunately, there is very little information in the literature regarding the morphogenetic effects of daylength in macroalgae (DRING, 1988; DRING & LÜNING, 1983; PERRONE & FELICINI, 1993). In particular, as regards *Gelidiaceae*, some results relative to photoperiodism in *Gelidium* species are rather contradictory (JUANES & PUENTE, 1993; SALINAS & VALDÉS, 1993; SANTELICES & VARELA, 1994). Further investigation is needed to draw more reliable conclusions.

Concerning the adventitious buds regenerated at the proximal pole of explants, their differentiation proved to be quite insensitive to exogenous conditions. These outgrowths seem to have a congenital plagiotropic

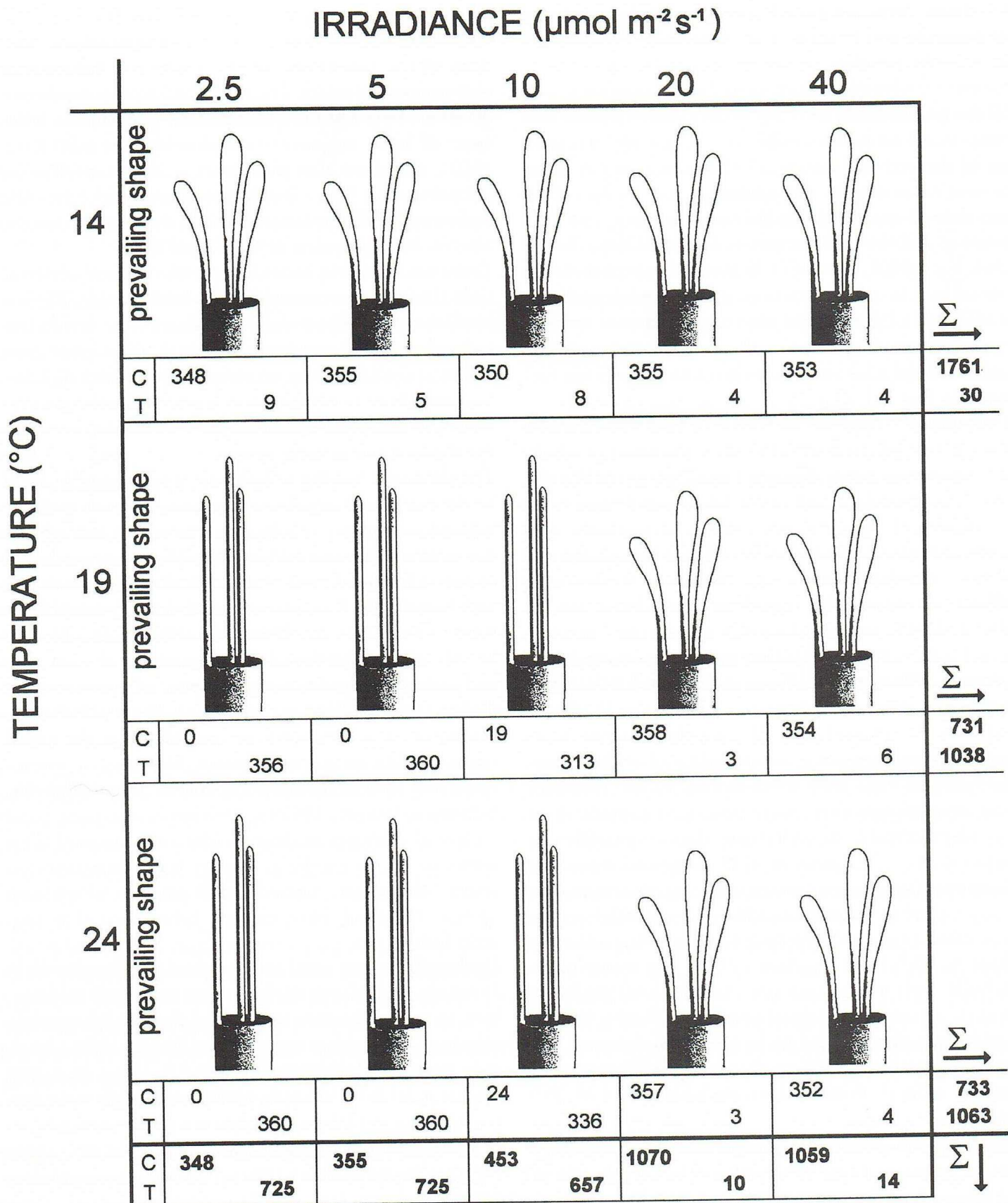


FIGURE 1 – Number of *P. capillacea* explants with compressed or terete shoots at the distal cut surface in relation to the combined effect of temperature and irradiance; $n_{\text{max}} = 360$ (30 x 3 x 4); C, compressed buds; $\chi^2 = 1164.29$, $P < 0.0001$; T, terete buds; $\chi^2 = 185.56$, $P < 0.0001$.

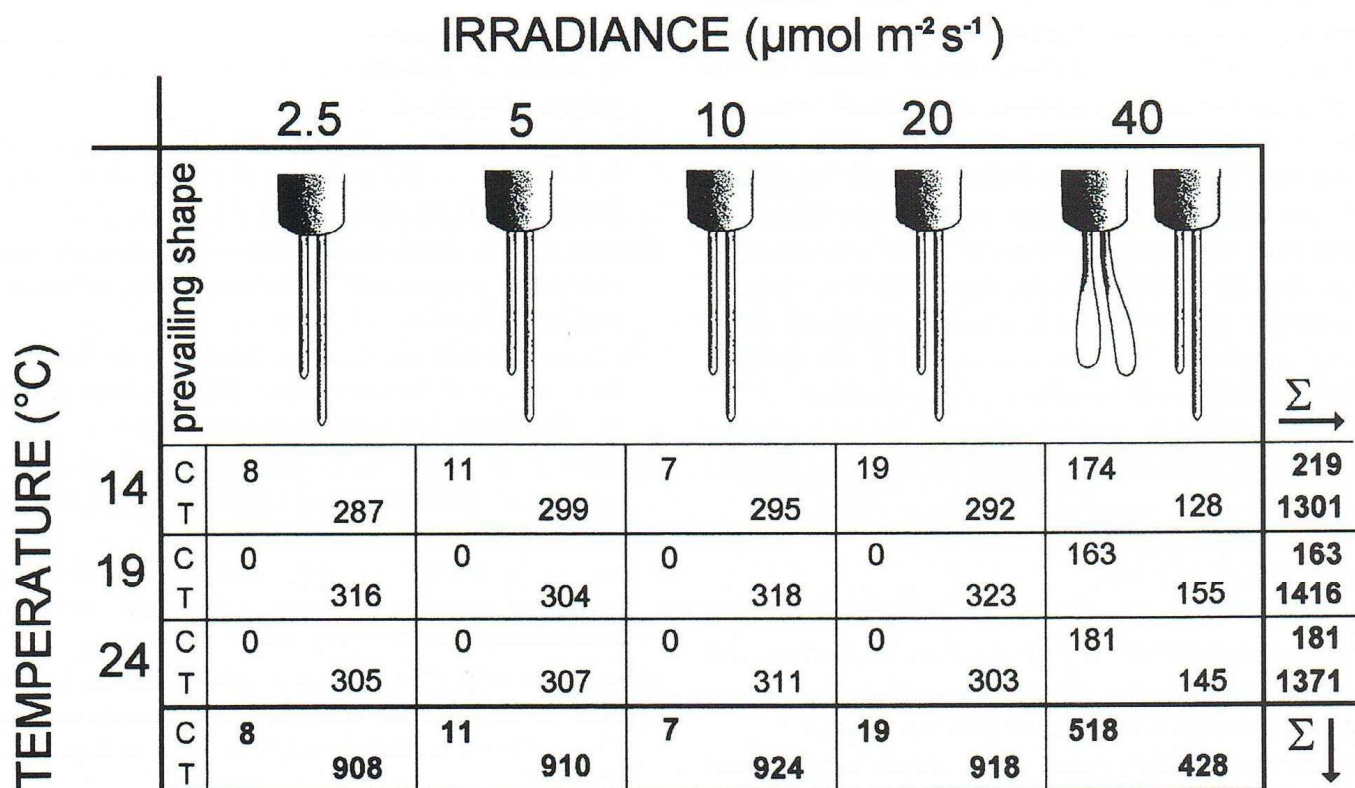


FIGURE 2 – Number of *P. capillacea* explants with compressed or terete shoots at the proximal cut surface in relation to the combined effect of temperature and irradiance; $n_{\text{max}} = 360$; C, compressed buds; $\chi^2 = 76.61$, $P < 0.0001$; T, terete buds; $\chi^2 = 1.69$, $P = 0.99$.

determinism which could be controlled by correlative endogenous mechanisms (cf. PERRONE & FELICINI, 1981 for *Schottera nicaeensis*). Such a determinism should prevent the flattening of the tapered primordia. Only the highest irradiance tested was able to promote their orthotropic differentiation, maybe because explants lose their original endogenous polarity under this condition. Apex shape, spatulate or tapered, of distal buds seems to be the morphological and functional key-point of bud differentiation. The compressed or terete shape of the derived axis is merely the consequence of the apical growth pattern. The spatial orientation of the axes, erect or prostrate, is certainly due to the different phototropic response of the two forms of thallus (cf. RICO & GUIRY, 1996).

The shape of an apex could be the result of mechanisms involving apical dominance. The tapering of a flattened apex could be interpreted as an enhancement of the dominance exerted by the initial cell(s) on the nearest branches of limited growth (LGB). Typically, *P. capillacea* frond apices show spatulate or retuse shape because the initial cell is not prominent (RODRIGUEZ & SANTELICES, 1987, 1988). Both the classical nutritional

theory and the more recent hormone-directed transport theory on apical dominance (SETH & WAREING, 1964; BOWEN & WAREING, 1971; MCINTYRE, 1972) seem to be suitable to support this hypothesis: the dominant apical cell(s) could attract and accumulate the available nutrients to the detriment of the LGB (L'HARDY-HALOS, 1971; FELICINI, 1993). N-starvation, for example, induced the frond apices of both *P. capillacea* (uniaxial) and *Schottera nicaeensis* (multiaxial) to taper and grow as terete creeping axes (FELICINI & PERRONE, 1972; PERRONE & FELICINI, 1974, 1988; FELICINI & PERRONE, 1994). This ability of the thallus to change its developmental trend - from compressed and erect to terete and prostrate - seems to be widespread among the gelidacean algae. It can be usually observed in the field especially in small-sized mat-forming species (PERRONE C., *pers. comm.*) and has been described for some species of *Gelidium* in laboratory cultures (SEOANE-CAMBA, 1989, 1991; SALINAS, 1991a,b; JUANES & PUENTE, 1993; SALINAS & VALDÉS, 1993; SANTELICES & VARELA, 1994). Irradiance and temperature can control the degree of apical dominance because these factors influence both meristematic activity and photosynthetic production.

In conclusion, these results further our understanding of the ecological significance of thallus morphogenesis in *P. capillacea*. Orthotropic differentiation usually occurs under adequate lighting necessary for frond photosynthesis in a temperature range which also permits a fast growth rate. However, it also occurs at lower irradiance levels on condition that a low temperature keeps the growth rate very slow; this seems to be a preparative phase for the spring development of new fronds. Plagiotropic differentiation is a survival strategy under adverse conditions but also a strategy for the renewal, thickening and clonal expansion of populations.

Experimental conditions inducing massive formation of self-attaching prostrate axes could be the basic method for mass culture of attached *P. capillacea* (KAIN, 1991).

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