Growth and reproductive phenology of *Pterocladiella capillacea* (Rhodophyta: Gelidiiales) from the southern Adriatic Sea

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Abstract

Growth, external morphology and reproductive phenology of a southern Adriatic population of *Pterocladiella capillacea* from a marine cave were investigated. Production of new uprights from creeping axes was limited to late autumn–winter; upright growth in length and branching order increase reached their maxima in late summer. Tetrasporic fronds were dominant from June to September, with a peak in July, while cystocarpic uprights were rather rare and recorded in August only. Two stable, distinct morphotypes occurred in this population at two sampling sites exposed to different daily photon irradiances. Uprights from the more illuminated site were regularly pinnate, with numerous closely packed lateral branches, representing the best-known phenotype of the species; in contrast, uprights from the more shaded inner site had very long and narrow axes, greater intervals between first-order branches and lower branching order. Statistical analysis demonstrated that upright growth in length and branching was significantly different at the two sites; upright biomass in the more illuminated site was generally higher because of the higher orders of branching.

Keywords: Adriatic Sea; phenology; *Pterocladiella capillacea*.

Introduction

External morphology is the most frequently described trait of the Gelidiæ (e.g., Akatsuka 1982, Santelices 1990, 1998, Hommersand and Fredericq 1996, Santelices and Hommersand 1997, Shimada and Masuda 2002, Xia et al. 2004, Millar and Freshwater 2005) and it is often used to separate genera and species. However, considerable morphological variability shown by most Gelidiæ renders this taxonomic criterion impractical in the absence of extensive field studies. Such variability is generally correlated with ecological, seasonal and geographical conditions. Both Dixon (1966) and Stewart (1968) suggested that external factors could not only affect the physiological activity of axial and cortical cells, but also modify both position and shape of previously formed branches, as well as the potential life span of the upright fronds. Moreover, it is common knowledge that clonal gelidiæan seaweeds are difficult to identify, especially when vegetative, because different species often occur in the same habitat with stolons that are sometimes entangled and anastomosed, and their uprights can be very similar in shape, size, color and branching. This can seriously flaw floristic and ecological work, even though morphology of the attachment system and the characteristics of rhizoid ontogeny have proven useful diagnostic and conservative taxonomic characters at the genus level (Perrone et al. 2006).

Among the Gelidiæ, *Pterocladiella capillacea* (Gmelin) Santelices et Hommersand (transferred from *Pterocladia* J. Agardh, Santelices and Hommersand 1997) has long suffered a chaotic taxonomy due to its great morphological variability. Morphological variants of *Pterocladiella capillacea* described by Dixon (1966) and Stewart (1968) (as *Pterocladiella*) are commonly found on Italian coasts, viz., (1) scarcely and sparsely branched thalli, (2) pinnate and bipinnate forms, and (3) older plants with repeating regenerations (cf. Felicini and Perrone 1994).

*Pterocladiella capillacea* from southeastern Italy has been subjected to numerous studies (Felicini and Antigno 1967, Calabrese and Felicini 1970, Felicini 1970, 1992, 1993, Felicini and Perrone 1972, 1986), some of which demonstrated that in lab-cultures thallus morphogenesis is strongly affected by light intensity, with irradiances lower than 10 μmol photons m⁻² s⁻¹ giving rise to long, narrow, scarcely branched thalli (Felicini et al. 2002).

Qualitative observations carried out throughout several years in a cave locally named Grotta della Regina (Adriatic Sea, southeastern Italy), where *Pterocladiella capillacea* is a component of the marine flora, highlighted the constant occurrence of two morphotypes of this species within the same population. The two forms characterize two distinct zones in the belt: a morphotype representing the species, as it is more often described, colonizes most parts of the rocky wall toward the cave opening; a second morphotype, corresponding to thalli experimentally cultured under low irradiance, lives in the inner part of the cave. In the intermediate zone, transition forms are present.

Our objective was to undertake a quantitative study on growth and reproductive phenology of this population, to investigate what environmental factors might affect thallus morphology and to make phenological comparisons.
of *Pterocladiella capillacea* from a temperate area with relevant studies from other latitudes.

**Materials and methods**

**Study site**

The Grotta della Regina is a small marine cave located at Torre a Mare, to the south of the city of Bari (southeastern Italy). Its width ranges from approximately 8 m (at the entrance) to approximately 13 m (at approximately 10 m from the entrance) and maximum height is approximately 5 m above sea level. The cave’s central basin is almost triangle shaped, with the apex towards the eastern Italy. Its width ranges from approximately 8 m at Torre a Mare, to the south of the city of Bari (south-eastern portion of 26°C in August) (Figure 2). Throughout the year, seawater temperature was quite similar at the two sites; the lowest value was 10°C in December, the highest, in August, was 28°C (Figure 2).

Tide tables for the last decade were consulted. On this coast, the highest tidal amplitude is approximately 30 cm. Relative intensities of water movement were measured at each sample site as erosion rates of plaster balls (Doty 1971) and were almost the same at the two sites.

Grazing pressure was not assessed; rare unidentified annelids (Polychaeta) live on *Pterocladiella* and are uniformly distributed in the belt. In our aquaria, the youngest parts of thallus could be cut into small pieces up to 1 mm long.

**Field sampling and laboratory procedure**

Sampling methodology was established on the basis of population type and species size (cf. Lobban et al. 1985). The smallest area for the estimation of biomass and density was a 25-cm² quadrat. Every month, 10 quadrats were randomly selected from each study site, sampled and marked out with stakes to avoid sampling them again in later months. In each quadrat, all uprights of *Pterocladiella capillacea* (erect fronds cut off, using a scalpels) were collected, placed in Teflon-lined vessels and taken to the laboratory. All uprights were counted and total wet biomass (to the nearest mg) was then determined for each quadrat. Freshly collected samples were used for biometrics and morphological observations and some of them were preserved in 4% formalin/seawater and some kept as herbarium specimens.

For 1 year (2005–2006), the following parameters were recorded every month for 50 randomly selected uprights from the two sites: length of the main axis, branching order, interval between first-order branches and width of the main axis measured 1 cm from the apex. Reproductive stage (vegetative, tetrasporic and cystocarpic) was also assessed by the presence/absence of reproductive organs observed under a stereomicroscope. Length of tetrasporic uprights and the interval between branches bearing cystocarps of female gametophytes were measured. The positions of tetrasporangia and cystocarps were also recorded.

**Statistical analysis**

All data collected were tested for normality and homogeneity prior to statistical treatment. The effects of sampling period as well as of sampling site on upright length, biomass and density were evaluated by two-way analysis of variance (ANOVA), followed by the Student-Newman-Keul’s (SNK) multiple comparisons. Biomass was plotted against upright density on a bilogarithmic scale for all of...
the quadrats. For both sites, the strength of the linear
association between log₁₀ (biomass) and log₁₀ (upright
density) was assessed using the Pearson correlation
coefficient (r). A t-test for unpaired samples was per-
formed to compare first-order branch mean interval, main
axis mean width and mean length between sterile and
fertile uprights. The association between variables, such
as sampling period, sampling site, branching order and
reproductive stage, were evaluated using contingency
tables and the χ²-test. When necessary, stratification by
time was made and the Cochran-Mantel-Haenszel test
(CMH χ²) was used. All data were analyzed using the
statistical software SAS (SAS Institute Inc. 1999–2000,
SAS Program for Windows Release 8.01. Cary, NC,
USA).

Results

Growth and external morphology

Site 2 uprights were regularly pinnate, with numerous,
short and closely packed lateral branches, representing
the best-known phenotype of the species. Occasionally,
approximately 50% of the uprights collected at this site
had pale, bleached or damaged apices, especially after
low spring tides, probably because of temporary expo-
sure to full sunlight. Due to this loss of apical parts, the
uprights were frequently characterized by many regen-
erated axes. Traces of regeneration also occurred at the
stipe level where several marks close to one another in
serial succession were often found.

Site 1 uprights, in contrast, had a more irregular mor-
phology, viz., longer and narrower main axes, greater
interval between first-order branches and lower branch-
ing order. Distal parts of both the main axis and lateral
branches very often grew into terete axes curving down-
ward and reattaching to the substratum, giving rise to
secondary prostrate axes (Figures 3–6).

A two-way ANOVA testing upright length variation by
month and site showed highly significant effects (Table
1); in particular, the interaction between sites and months
was significant and demonstrated that the effect of site
on plant length differed by month (Figure 7). During win-
ter, there was no site effect on length; in all other sea-
sons, site 1 plants were larger.

In the site 1 size class frequency distribution (Figure 8),
there was a shift towards the highest length classes
(9–12 cm) in the period from May to October. At site 2,
intermediate length classes (5–8 cm) were the most fre-
quent in all months of the year. At both sites, the smallest
length classes (1–4 cm) were present from November.

Monthly data on branching order for uprights at the
two sites were divided into four seasonal groups:
November–January, February–April, May–July and
August–October. In Figure 9, the variation of branching
order of uprights from both sites is depicted for each
season. At site 1, uprights with second- and third-order
branching progressively increased during the year. At site
2, uprights with third order branching were the most
abundant. The difference in the branching order between
uprights from sites 1 and 2 was significant over time
(CMH χ² = 179.21, p < 0.0001).

The interval between first-order branches was larger
for uprights from site 1 than for uprights from site 2
(mean value: 18.6 vs. 3.48 mm, t = 19.2, p < 0.001). Mini-
mum and maximum values ranged between 16 and
21 mm for uprights from site 1 and between 2 and 5 mm
for those from site 2.

The main axis of uprights from site 2 was wider than
from site 1 (mean width: 1.8 vs. 0.8 mm, t = 9.95,
p < 0.001). At site 2, width ranged between a minimum of
1.5 mm in late summer–early autumn and a maximum of
2 mm in the remaining months of the year. At site 1, width
varied between 0.5 and 1 mm in the same periods.

Biomass and density

A two-way ANOVA carried out to test biomass variation
with respect to months and sites showed a highly sig-
ificant interaction between sites and months, and sig-
nificant main effects (Table 1). The effect of site on
biomass varied by month (SNK test, Figure 10). Biomass
of uprights from site 2 was higher than that of uprights
from site 1 in each month (Figure 10), but the difference
was smallest in winter months.

There were significant effects of site and month as
main effects on density of uprights, but no interaction
between the two factors (Table 1). However, differences
in density were small, and SNK tests did not detect site
effects within any month of the year (Figure 11).
For both sites, logarithmically transformed values of biomass were positively correlated to equivalent values of upright density (site 1: $r=0.80$, $p<0.001$; site 2: $r=0.75$, $p<0.001$) (Figure 12). The $p$-values are relatively low owing to the large number of replicates. The coefficients of determination are around 60% for both sites, indicating that a relatively large proportion (~40%) of the variation in biomass is unexplained by variation in density.
Figure 7 *Pterocladiella capillacea*: mean upright length variation (±SD, n=50) at sites 1 and 2 throughout the year. Significant differences between site×month combinations are indicated by different letters (SNK, p<0.05).

Figure 8 *Pterocladiella capillacea*: size frequency distribution of sites 1 and 2 uprights throughout the year.

Figure 9 *Pterocladiella capillacea*: percentage frequency occurrence of different branch orders in different seasons for sites 1 and 2 uprights (n=150). \(\chi^2\)-test values for each seasonal group are reported.

Figure 10 *Pterocladiella capillacea*: mean upright biomass variation (±SD, n=10) at sites 1 and 2 throughout the year. Significant differences between months (p<0.05) are indicated by different letters (SNK tests).

Figure 11 *Pterocladiella capillacea*: mean upright density variation (±SD, n=10) at sites 1 and 2 throughout the year. Significant differences between months (p<0.05) are indicated by different letters (SNK tests).
Tetrasporic upright distribution for the two sites was significantly different among months ($x^2=35.68$, $p<0.001$). In both sites, tetrasporic uprights were present from June to September, with the highest percentage (83% at site 1 and 80% at site 2) in July (Figure 13). At site 1, tetrasporic uprights were longer (mean length: 14.2 cm vs. 10.7 cm, $t=6.1$, $p<0.001$) than sterile uprights. There was no difference in length between tetrasporic and sterile uprights (mean length: 7.1 cm vs. 7.4 cm) at site 2. Tetrasporangia were observed on all order branches and on stichidium-like ramuli directly inserted on the main axis.

Cystocarpic uprights were rather rare (4% at site 1 and 2% at site 2) and were recorded in August only. Therefore, they were not processed and only qualitative observations were made. Cystocarps were found exclusively on second-order branches. Branches bearing cystocarps were regularly spaced 2 mm apart. Development and maturation of cystocarps were acropetal. On the youngest branches, developing cystocarps were usually distal; on mature branches, they occupied an intermediate-proximal position. Branches with two cystocarps in series (aligned along the median line) or cystocarps with two ostioles were rarely observed.

Spermatangial uprights were never observed or not recognized under a stereomicroscope.

**Discussion**

In the Grotta della Regina, *Pterocladiella capillacea* populations follow a pattern of year-round growth in length. At both the selected sampling sites, very small uprights were present from November; therefore, production of new uprights from creeping axes was not continuous, but limited to late autumn–winter. In spring, uprights continued to grow, as shown by length and branching order increases, reaching their maximum size in late summer. Even though this growth trend was almost the same at the two sites, throughout the year, two stably distinct morphotypes occurred in this population of *P. capillacea*. Site 2 uprights were the well-known bushy, regularly pinnate, pyramidal fronds of the species; moreover, they also regenerated more frequently due to the loss of apical parts. Apex damage likely resulted from exposure to air and direct sunlight, especially during low spring tides. Obviously, the consequent reduction in apical dominance induces the formation of one or more often numerous apex-substitutive branches, strongly modifying branching and size of fronds (Buggeln 1981). The ability to regenerate in response to any injury is a widespread phenomenon in the Gelidiales, and in other red seaweeds; it has been widely studied in *P. capillacea* (Felicini and Arrigoni 1967, Felicini 1970, 1993, Felicini and Perrone 1986). For *P. capillacea* the occurrence of apical dominance had been suggested based on qualitative (Felicini and Perrone 1994) and quantitative (Scrosati 2002) observations. In *P. capillacea*, past regenerations can be easily detected by persistent marks on the thallus (Felicini and Perrone 1994).

Site 1 uprights had a different morphology, viz., longer and narrower main axes, scarce and longer “capillaceous” branches, some of which becoming new prostrate axes in late summer. Secondary formation of stoloniferous axes from upright distal parts has been termed “secondary heterotrichy” by Dixon (1973). According to Dixon (1958), plagiotropic differentiation of thallus in *Pterocladiella capillacea* may occur under unfavorable ecological conditions. In laboratory cultures, such a morphogenetic pattern has been triggered by irradiances lower than 10 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Felicini 1970, Felicini and Perrone 1994) and regulated by temperatures higher than 18°C (Felicini et al. 2002), conditions occurring at site 1 in summer. Under constant low light intensity, upright morphogenesis seemed to be controlled by a strong apical dominance, giving them the typical appearance of etiolated plants.

Previous work has shown that in the Gelidiales (as in many other red algae) light intensity interacts synergistically with temperature, water movement and nutrients (Santelices 1991).

Different morphotypes have been also described for *Pterocladia caerulescens* from Hawaii and interpreted as a consequence of season and depth (Santelices 1978a). Slightly distinct morphotypes of *P. capillacea* from Brazil were found along a gradient of water exposure (Oliveira et al. 1996).

Between our collection sites, seawater temperatures and water movements were quite similar; in contrast, daily irradiances were significantly different. Results from
the present study suggest that in the cave light, intensity may be the main physical factor influencing the distinct morphotypes of *Pterocladia capillacea*.

In the population of *Pterocladia capillacea* from Grotta della Regina, mean upright densities were comparable between sampling sites, and biomass values were higher at site 2 due to the greater abundance of lateral branching. Biomass at the two sites was higher than that found in Hawaiian and Californian populations of *Pterocladia capillacea*. In Hawaiian populations of *P. capillacea* and *P. caerulescens*, Santelices (1978b) found seasonal cycles of biomass with maxima in December and minima in May. For *P. capillacea* from Baja California, Mexico, the highest biomass values were generally observed in spring and summer and the lowest in autumn and winter (Scrosati and Serviere-Zaragoza 2000).

In the Grotta della Regina, the diploid phase of *Pterocladia capillacea* was the most abundant, though this cannot be proven, as it is impossible in the field to distinguish each individual thallus (genet) due to various factors, such as high frond densities and stolon intermingling. In addition, counts of reproductive fronds may not accurately represent the abundance of a given life-history phase (Serviere-Zaragoza and Scrosati 2002), because the haploid and diploid phases cannot be distinguished until mature reproductive organs are evident; at present, there is no quick method for the determination of the life-history phase of vegetative fronds for the Gelidiales, unlike the Gigartinales (Garbary and Dewreede 1988). On the other hand, tetrasporophyte dominance in our population of *P. capillacea* agrees with the observations of many researchers at different latitudes (Oliveira and Sazima 1973, Frllick and Andrade 1981, Neto 2000, Serviere-Zaragoza and Scrosati 2002). At our study sites, maximum abundance of tetrasporic uprights occurred at the time of maximum vegetative growth. This would suggest that growth and tetraspore production may be regulated by the same environmental parameters, as was shown for *P. caerulescens* in Hawaii (Santelices 1978a).

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**References**


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