Comparative ecophysiology of *Gelidium sesquipedale* (Rhodophyta) erect fronds and prostrate system

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Abstract

Even though there are studies on the ecophysiology of the erect fronds of the agarophyte *Gelidium sesquipedale* (Clem.) Bornet et Thuret, no work as been done on the ecophysiology of the prostrate axes. Vegetative growth of erect fronds from the prostrate system is the main process of population recovery from disturbances, particularly commercial harvesting. Erect fronds and axes of the prostrate system of *G. sesquipedale* were comparatively characterized in standard conditions and in two other experiments. In the first, algae were acclimated at 10, 15, 20 and 25°C to assess temperature effects on photosynthesis, pigments, carbon and nitrogen content. In the second, sun and shade thalli were compared for the same parameters and also for potential quantum yield. Apical portions of erect fronds had higher pigment content than prostrate axes, but identical nitrogen percentage, indicating the presence of additional nitrogen pools in the latter. Prostrate axes showed no photosynthetic activity after laboratory acclimation. Erect fronds photosynthesized after acclimation but at a reduced rate. Organic nitrogen increased during acclimation, most likely due to phycoerythrin synthesis, stimulated by low light conditions. Prostrate axes showed higher tolerance to light variations, presenting identical biochemical parameters in shade and sun exposed algae, while apical portions had more phycobilins and nitrogen in the shade. F_v/F_m values revealed photoinhibition in sun exposed thalli. Prostrate axes appear to store considerable amounts of carbon and especially nitrogen, probably as free aminoacids or proteins, besides being highly independent of environmental changes, which makes them crucial for the perennation of *Gelidium*.

Abbreviations: C:N - Carbon:Nitrogen; dw - dry weight; F_v/F_m - Variable fluorescence/Maximum fluorescence

Introduction

Gelidium sesquipedale (Rhodophyta, Gelidiaceae) is a commercially valued alga, harvested in the northeast Atlantic from France to Morocco and used as raw material for high quality agar (Santos & Duarte, 1991; Melo, 1998). The life cycle of Gelidiales is complex, with three different stages: carposporophyte, tetrasporophyte and gametophyte (Hawkes, 1990). Vegetative propagation, in which new erect fronds develop from a system of prostrate axes, attached to rocky substrates through rhizoids also occurs (Dixon, 1958). These axes are responsible for the renewal of fronds destroyed by storms and herbivory (Santelices, 1991), allowing the perennation of populations, particularly in commercially exploited areas (Santos, 1994).

The ecophysiology of *Gelidium* erect fronds has been studied by several authors, including the photosynthetic response to changing light fields (Torres *et al.*, 1991, 1995; Carmona, 1996; Rico & Fernández, 1996; Gómez & Figueroa, 1998; Silva *et al.*, 1998), differences between gametophytes and sporophytes (Sosa *et al.*, 1993) and nutrient concentration effects (Vergara *et al.*, 1993). In addition, some work has been done on the biology

and ecology of *G. sesquipedale* populations (Duarte & Ferreira, 1993, 1997; Gorostiaga, 1994, 1995; Santos, 1993a, b, 1994, 1995; Santos & Duarte, 1996; Santos & Nyman, 1998). All these studies focused only on the erect fronds, no information being available about the physiological behaviour of the prostrate system axes. This work deals, for the first time, with the ecophysiology of prostrate axes, comparing them with the erect fronds. The aim was to determine temperature and light effects on photosynthesis, respiration, pigments, carbon and nitrogen content of erect fronds and prostrate axes.

Materials & Methods

Sampling site and biological material

G. sesquipedale thalli used in all experiments were collected during the summer by SCUBA diving at a rocky shore near Albufeira (South Portugal: 37°00' N, 007° (58'W), at a depth of about 4 m, where tides are semidiurnal with a maximum amplitude of nearly 3 m.

Experimental design

For general characterisation purposes, thalli were collected and immediately frozen for further analysis. Both erect fronds (basal and apical portions) and prostrate axes were analysed for pigments, carbon and nitrogen content, to determine the variation of these parameters along the thalli.

An acclimation experiment was performed to determine temperature effects on photosynthesis, pigments, carbon and nitrogen content. Thalli were collected, cleaned of epiphytes and acclimated for seven days in 12 l plastic containers subdivided in 20 separate wells and filled with GF/F filtered seawater, inside a plant growth chamber (Fitoclima 750 E, Aralab, Lisboa, Portugal). Each of four containers was kept at a different temperature (10, 15, 20 and 25°C) and continuously aerated. The temperature range spans the minimum (12°C) and maximum (24°C) values recorded through the year at the sampling location. Photoperiod (14:10 day:night) and light intensity (75 µmol photons m^{-2} s^{-1}, white light, Osram Lumilux Plus L18W/21-840) were set to simulate the conditions measured at the sampling location. Gross photosynthesis, dark respiration, pigments, carbon and nitrogen were determined before and after the acclimation period in both the erect fronds and the prostrate axes.

In a second experiment, to test light effects on photosynthetic efficiency and biochemical composition, thalli were collected from two close locations at the same depth: a fully sun exposed shelf and a shaded crevice, where algae were naturally exposed to maximum photon irradiances of approximately 800 and 50 µmol photons m^{-2} s^{-1}, respectively. Photon irradiances were measured with a Li-193SA Underwater Quantum Sensor connected to a Li-1000 Data Logger (Li-Cor, Lincoln, Nebraska, USA). Erect fronds and prostrate axes from both sites were collected, shaded and brought ashore. Fluorescence measurements were performed on site and samples for pigments, carbon and nitrogen were frozen for further analysis.

Photosynthetic pigments

Samples of *G. sesquipedale* were frozen at -80°C for pigment analysis. Whereas erect fronds (basal and apical portions) could be used as individual samples (20 to 30 mg fresh weight) (5 replicates), prostrate axes had to be treated in bulk samples (*ca* 200 mg fresh weight) (3 replicates), due to low biomass and low pigment concentration. Samples were ground in liquid nitrogen, and then extracted in 100% acetone (chlorophyll *a* and carotenoids) or sodium phosphate buffer (pH = 6.88) (phycobiliproteins) (Rowan, 1989). Extracts were centrifuged at 13,000 rpm for five minutes in a bench centrifuge (Heraeus Biofuge A, Heraeus Sepatech, Germany). Pigment concentrations were determined spectrophotometrically (UV-160A, Shimadzu, Kyoto, Japan). The equations of Lichtenthaler (1987) were used for estimation of chlorophyll *a* and carotenoid content. Phycoerythrin and phycocyanin concentrations

were determined through the equations of Beer & Eshel (1985).

Carbon and nitrogen

Both erect fronds (basal and apical portions) and prostrate axes samples (0.5 to 1 mg dry weight) (5 replicates) were oven dried at 60°C for 48 hours for further analysis. Carbon and nitrogen contents were determined through elemental analysis (Macler, 1988), performed in a Carlo Erba CHNS-O EA1108 (Carlo Erba, Italy).

Photosynthesis measurements

Photosynthesis was measured with a Clark type oxygen electrode (DW3 measuring chamber, Hansatech Instruments, Norfolk, UK). Samples of 150-200 mg DW were incubated in 15 ml sterilised seawater enriched with $NaHCO_3$ (10 mM final concentration). Light was supplied by a Hansatech LS2 white light source (Osram Xenophot HLX 64610 12V 50W lamp). Photosynthetic rates (mg O_2 g $DW^{-1}h^{-1}$) were measured at increasing photon irradiance levels, from darkness (dark respiration) to 700 µmol photons m^{-2} s^{-1} (Silva & Santos, 1998), generating P-I curves. Hansatech A5 neutral density filters were used to obtain different photon irradiance levels. Measurements were made at the several acclimation temperatures and at 17°C for non-acclimated algae. Temperature was controlled by a water bath (Julabo HC, Julabo Labortechnik, Seelbach, Germany).

Fluorescence measurements

Chlorophyll *a* fluorescence was measured with a portable fluorometer (Plant Efficiency Analyser, Hansatech Instruments, Norfolk, UK). Samples (10 replicates) of *G. sesquipedale* were dark-adapted for 20 min, after which a saturation pulse (4,000 µmol photons m^{-2} s^{-1}, 0.4 s) was applied and F_v/F_m determined.

Statistical analysis

All results are presented as mean values ± standard error. When not stated otherwise, one or two way ANOVA or Student's t-test, were applied to test the significance of the results (α = 0.05) (Sokal & Rohlf, 1981). All variances were homogeneous, meeting the ANOVA assumptions. Maximum photosynthetic rate was estimated from P-I curves through non-linear regression using the Platt *et al.* (1980) model equation. All data treatment and statistical analysis was performed using the SigmaStat/SigmaPlot (SPSS Inc.) software package.

In the temperature acclimation experiment, there might be some concern about pseudoreplication due to lack of independence in acclimation (only one container per temperature was used for acclimation). However, we have no reasons to suspect that this will affect the experiment results. The photosynthetic production of each replicate was quantified in independent environments, i.e. in an oxygen electrode chamber with independent incubation media.

Results

The content of all photosynthetic pigments was higher in the apical portions of *G. sesquipedale* thalli, when compared with the prostrate system axes ($p<0.05$) (Fig. 1). The basal portions of erect fronds presented an intermediate value, evidencing an increase on photosynthetic pigments from the base to the tip of the thalli. On the other hand, nitrogen content was identical ($p>0.05$) in all the three considered portions of the algae, while carbon content was higher in the prostrate axes ($p<0.05$) (Fig. 2).

When the algae were acclimated at different temperatures, carbon content in the apical portions remained constant (35% dw), while nitrogen increased significantly ($p<0.05$) from (3% dw in the beginning of the experiment to (4 % dw at all temperatures in the end, which resulted in a decrease ($p<0.05$) in C:N ratio during acclimation (Fig. 3). Although carbon ((37% dw) and nitrogen ((3 % dw) content in the prostrate axes remained constant ($p<0.05$), a decrease in C:N ratio was also

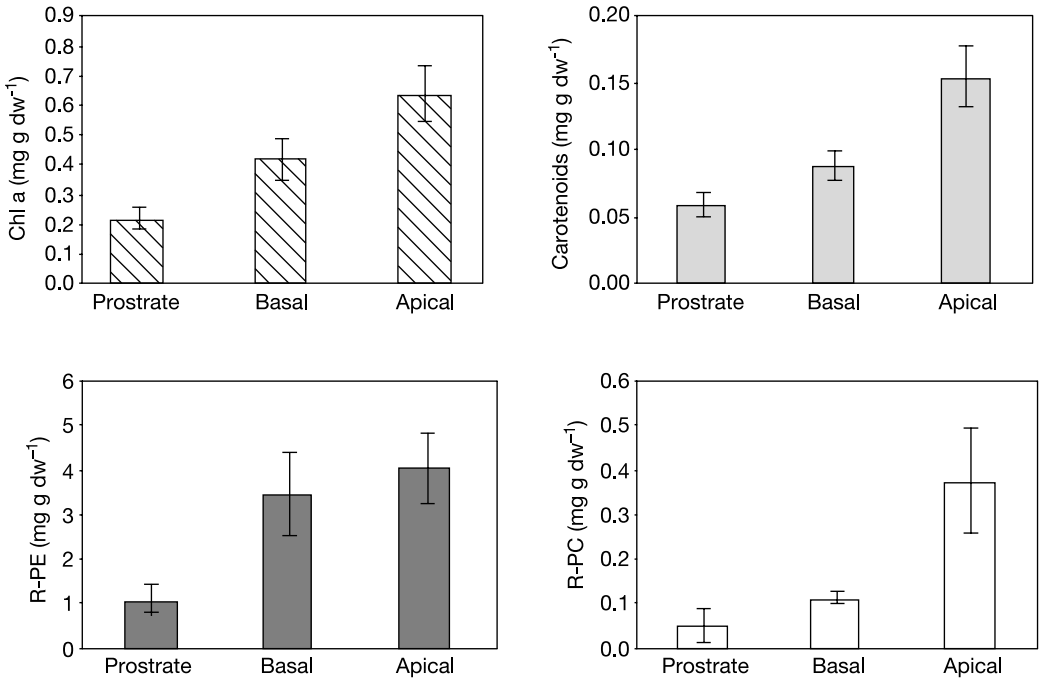

Figure 1. Concentrations of chlorophyll *a*, carotenoids, phycoerythrin and phycocyanin in *G. sesquipedale* prostrate, basal and apical parts of the thalli. Values represent means ±SE (n=5) for basal and apical portions; n=3 bulk samples for prostrate axis).

observed (Fig. 3).

The prostrate system showed no photosynthetic response when exposed to light in the oxygen electrode incubation chamber. The apical portions revealed a significant decrease in the maximum photosynthetic rate after being acclimated (Fig. 4) at all temperatures. Gross photosynthesis at 25°C was higher than at other temperatures ($p<0.05$), as was dark respiration.

When sun and shade algae were examined for potential quantum yield (Fig. 5), the apical portions showed higher ($p<0.05$) F_v/F_m values than the prostrate axes in both cases. Apical portions had higher ($p<0.05$) values in shade rather than in sun exposed algae. Prostrate axes had identical values in both types of exposure. Chlorophyll *a* and carotenoids from apical portions were identical either on shade and sun exposed algae, whereas phycoerythrin and phycocyanin were much higher ($p<0.05$) in shade thalli (Fig. 6).

Both nitrogen and carbon content in prostrate axes showed no difference between shade and sun exposed algae. However, shade thalli had much more nitrogen in the apical portions than sun exposed ones ($p<0.05$) (Fig. 7). Although not significantly, carbon content tended to be higher in prostrate axes than in apical portions, both in shade and sun fronds.

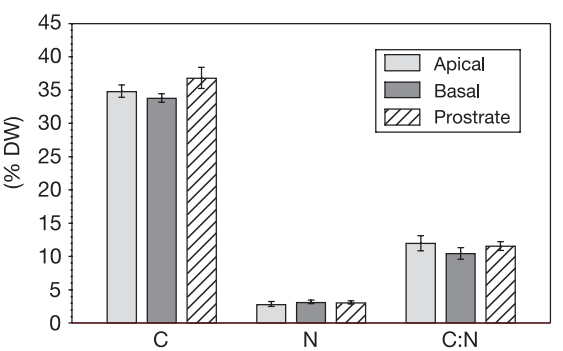

Figure 2. Carbon, nitrogen content and C:N ratio in *G. sesquipedale* prostrate, basal and apical parts of the thalli. Values represent means ±SE (n=5).

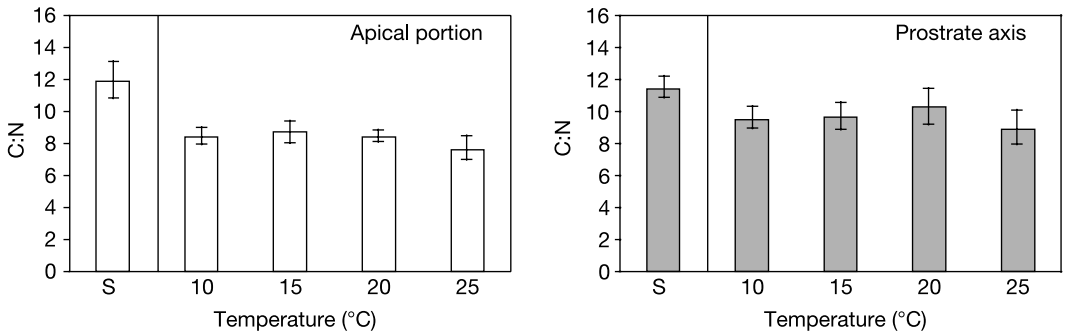

Figure 3. C:N ratio in apical portions and prostrate axis of *G. sesquipedale* before (S) and after acclimation at 10, 15, 20 and 25°C (C. Values represent means ±SE (n=5).

Discussion

The results indicate fundamental biochemical and metabolic differences between prostrate axes and erect fronds. The lack of photosynthetic response of the prostrate axes measured with the oxygen electrode, associated with the low pigment concentration and the reduced F_v/F_m values, clearly show that this part of the thallus does not play a significant role in light harvesting and carbon fixation. On the other hand, its carbon and nitrogen content suggests that the prostrate system, apart from being responsible by the fixation and perennation of *G. sesquipedale*, also acts as a storage site. Carbon was significantly higher in the prostrate system in one experiment and at least the same as in erect fronds in another sampling, indicating the likely existence of a carbon pool in this part of the thalli, probably starch and other polysaccharides, like rhizines, which tend to accumulate in the prostrate axes of *G. sesquipedale* (Nascimento, *unpublished data*).

Although in normal conditions the total nitrogen content was the same all over the fronds, the apical portions had always much more phycoerythrin, a major nitrogen storage form in red macroalgae (Naldi & Wheeler, 1999). Therefore, other nitrogen forms were stored in the prostrate axes in higher amount than in apical portions. These will most likely be either soluble proteins or free amino acids, two of the main forms of nitrogen storage in macroalgae (Naldi & Wheeler, 1999). The existence of important nitrogen pools in the prostrate system is further supported by two commonly accepted assumptions: nitrogen tends to be stored under low light conditions when metabolism is reduced and accumulation exceeds immediate requirements, which is the situation at the prostrate system level; on the other hand,

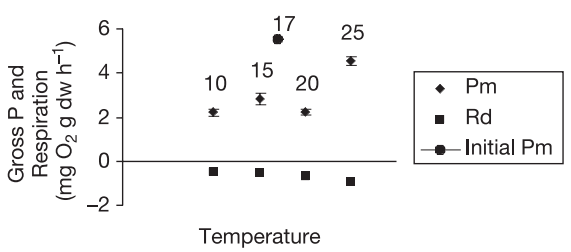

Figure 4. Maximum gross photosynthesis (P_m) and dark respiration (R_d) in apical portions of *G. sesquipedale* thalli before (initial) and after acclimation at 10, 15, 20 and 25°C. Values represent means ±SE (n=3).

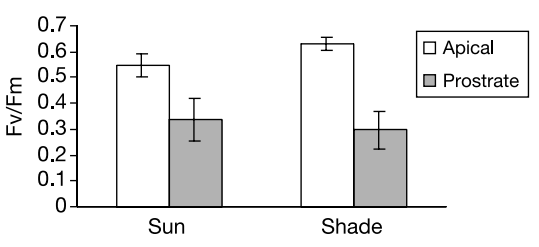

Figure 5. Potential quantum yield expressed as variable fluorescence/maximum fluorescence (F_v/F_m) in apical portions and prostrate axis of *G. sesquipedale* collected in sun and shade environments. Values represent means ±SE (n=10).

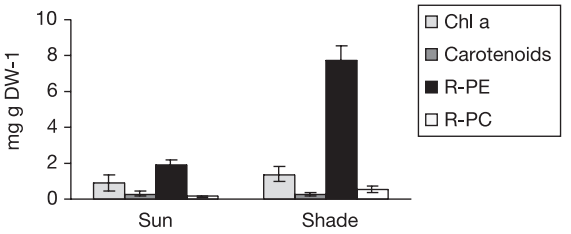

Figure 6. Chlorophyll *a*, carotenoid, phycoerythrin (R-PE) and phycocyanin (R-PC) in apical portions of *G. sesquipedale* collected in sun and shade environments. Values represent means ±SE (n=5).

perennial slow-growing species like *G. sesquipedale* usually store nitrogen to act as buffer when in shortage (Lobban & Harrison, 1997), a substantial advantage in temperate regions like Portugal, where nitrogen availability fluctuates seasonally, being lower in the summer. Since the prostrate system is responsible for the physical perennation of the algae, the accumulation of reserves would not only favour the regeneration of the thalli after breakage, but could also support growth under low nutrient supply (Pedersen & Borum, 1996).

The general decrease in maximum photosynthesis observed in the erect fronds after being acclimated was probably the result of a negative response to transplantation and manipulation. A significant increase in phycoerythrin was responsible for the rise of nitrogen in fronds during acclimation, leading to the observed drop in the C:N ratio. The prostrate axes showed less vulnerability to the acclimation conditions, with smaller variations in pigments, carbon and nitrogen contents.

Erect fronds revealed a 'sun' and 'shade' pattern, clearly expressed by phycobiliproteins, associated with nitrogen content. Algae growing in shade conditions tend to have higher concentrations of antenna pigments (Ramus, 1981). In *Gelidium* fronds, as in all red algae, phycoerythrin is the main light-harvesting pigment in the antennas of photosystem II. In our experiment, phycoerythrin is much higher in shade fronds, directly determining the nitrogen content values. These are also much higher in shade (≈3 % dw) than in sun exposed fronds (≈1 % dw). Nevertheless, this very

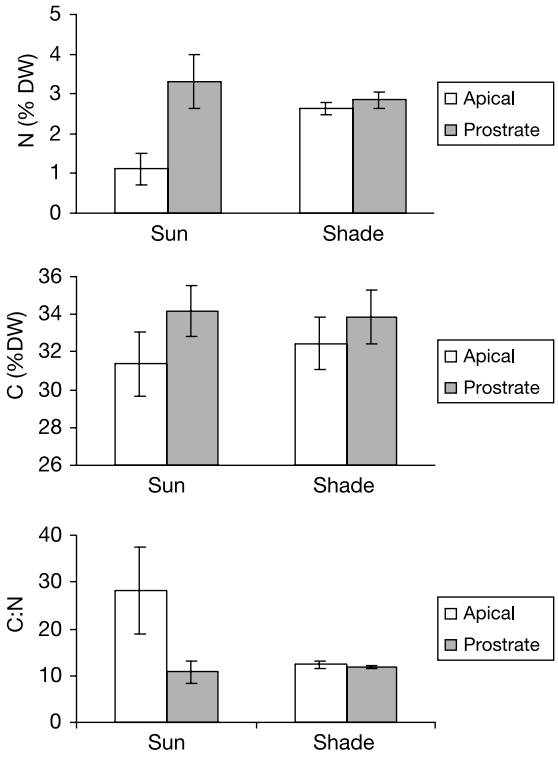

Figure 7. Carbon, nitrogen and C:N ratio in apical portions and prostrate axis of *G. sesquipedale* collected in sun and shade environments. Values represent means ±SE (n=5).

low phycoerythrin and nitrogen content is not only the result of sun exposure *per se*, but also relates to the characteristic 'bleaching' that many algae within the Gelidiales, including *Gelidium sesquipedale*, present during the summer (Santelices, 1988). This event results in a clear positive relationship observed between phycobilins and nitrogen content (Rico & Fernández, 1996). Potential quantum yield, expressed as F_v/F_m was lower ($p<0.05$) in sun than in shade fronds, revealing photoinhibition. 'Sun' and 'shade' patterns in *G. sesquipedale* were described previously by Duarte & Ferreira (1995), Carmona *et al.* (1996) and Silva et al. (1998) in different types of experiments.

Again, the prostrate axes showed no differences between sun and shade, which reinforces the idea of their low sensitivity to environmental changes.

Conclusions

The results of this work point to some important features of the prostrate system axes in *Gelidium sesquipedale* which are probably common to other members of the Gelidiales. In addition to its known key role in the species perennation and erect thalli regrowth, these axes also appear to store considerable amounts of carbon and particularly nitrogen, withstanding environmental changes in light and temperature without significant changes of those biological parameters. However, light appears to play a role in the production of erect axes, together with currents and substrate (Salinas, 1991). The physiology behind the development of the prostrate system and the rise of new upright axes is a complex process with many unknown aspects, requiring further work.

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