SEASONAL VARIATIONS OF PHOTOSYNTHETIC PIGMENTS, TOTAL C, N, AND P CONTENT, AND PHOTOSYNTHESIS IN PHYLARIOPSIS PURPURASCENS (PHAEOPHYTA) FROM THE STRAIT OF GIBRALTAR

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ABSTRACT

Photosynthetic pigments, C, N, and P tissue composition, and photosynthetic rate were measured from April to October in the brown alga Phylariopsis purpurascens (C. Agardh) Henry et South (Laminariales, Phaeophyta) growing at a 30-m depth in the Strait of Gibraltar. Irradiance reaching the population ranged from 13.5 to 27.5 mol·m⁻²·mo⁻¹. The available light for this species, expressed as a percentage of the irradiance above the water, was 1.8%. Dissolved inorganic nitrogen forms, NO₃⁻ and NH₄⁺, were constant from April to October, whereas phosphate was depleted in August. Chlorophyll a decreased from 520.0 ± 165.0 to 199.6 ± 159.9 µg·g⁻¹ dry weight; in contrast, chlorophyll c and carotenoids did not change until September but increased threefold in October. C:N and N:P ratios changed in the same way and in the same range. They were constant until July but increased from 15–17 up to 42 (C:N) and from 14 to 40 (N:P) in October, suggesting a severe P limitation of growth of this species. The dark respiration rate and the light compensation point were constant from April to October (0.5 ± 0.1 µmol O₂·m⁻²·s⁻¹ and 6.5 ± 0.2 µmol·m⁻²·s⁻¹, respectively), whereas the maximum rate of apparent photosynthesis, light onset saturation parameter, and half-saturation constant for light were maximum in April to May (3.7 µmol O₂·m⁻²·s⁻¹ and 40 and 41.5 µmol·m⁻²·s⁻¹, respectively) and October (3.6 µmol O₂·m⁻²·s⁻¹ and 50 and 53.7 µmol·m⁻²·s⁻¹, respectively). They were minimum in August (1.2 µmol O₂·m⁻²·s⁻¹ and 11.3 and 12 µmol·m⁻²·s⁻¹, respectively). These minimum figures yielded a negative carbon budget in August and 0 in September, whereas it was positive the rest of the year. Photosynthetic efficiency, estimated by the ratio between maximum apparent photosynthesis and light half saturation constant, showed a strong agreement with productivity measured by means of an independent method. These results indicate that lamina expansion in this species is controlled by photosynthetic efficiency.

Key index words: carbon budget; Phaeophyta; photosynthesis; Phylariopsis purpurascens; pigments; respiration; tissue composition

Phylariopsis purpurascens (C. Ag.) Henry et South is a small kelp that lives at more than a 25-m depth in the Western Mediterranean, Atlantic coast of the Iberian Peninsula and North Africa (Flores-Moya et al. 1993). In the Strait of Gibraltar, this species lives at a 30-m depth and is exposed to unusual strong currents, up to 2.5 m·s⁻¹ during spring tides. The
first visible sporophytes appear in April. Maximum growth takes place from May to September, and then growth ceases. At the end of October, the population disappears until the following April (Flores-Moya et al. 1993). To define the period of appearance of this species in the area, we will use the term production cycle throughout the text. The seasonal growth pattern has been described for different species of kelps; in general, the growing season is considered to be in the spring (Parke 1948, Sundene 1962, 1964, Kain 1971, Lüning 1971, Mann 1973). During this period, a steady increment in the amount of chlorophyll (chl) in *Laminaria hyperborea* (Gunn.) Foslie has been described, in agreement with the increment of photosynthetic potential (Drew 1983). The end of the growing season takes place in summer, when some species such as *L. hyperborea* (Lüning 1971) exhibit an enhancement of carbon assimilation. Surplus photosynthates are stored and are eventually used during the nongrowing period. The enhancement in assimilation coincides, in the same species, with a peak in activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) (Küppers and Weidner 1980).

Seasonal variations of photosynthetic performance have been described for some macroalgae (Hatcher et al. 1977, Drew 1983, Heine 1983, Smith et al. 1983, Levitt and Bolton 1990, Haroun et al. 1992, Weykam and Wiencek 1992). However, as pointed out by Drew (1983), a clear relationship between growth and photosynthetic rate is not always evident in some species of *Laminaria*.

Growth ceases in summer because nutrients are depleted (Chapman and Craigie 1977, Gagné et al. 1982, Drew 1983); however, a photoperiod-induced reduction of vegetative growth has been described for some species (Gagné et al. 1982, Guiry 1984, Conolly and Drew 1985a, b, Lüning 1986; for discussion, see also Lüning 1988, Lüning and tom Dieck 1989). Nitrogen has been reported to be the limiting factor for the growth of marine algae such as *Fucus spiralis* L. (Topinka and Robbins 1976), *Macrocystis pyriforme* (L.) C. Ag. (Jackson 1977), and *Codium fragile* (Sur.) Har. (Hanisak 1979). In contrast, there is little evidence of P-limited growth in seaweeds. To our knowledge, only Lapointe (1987) for *Gracilaria tikvahiae* McLachlan, O’Brien (1987) for *Enteromorpha prolifera* (Müll.) J. Ag., Lapointe and O’Connell (1989) for *Cladophora prolifera* (Roth) Kützing, Hernández et al. (1993a, b) for *Porphyra umbilicalis* (L.) Kützing, and Hurd and Dring (1990, 1991) for some intertidal species of *Fucus* have indicated P limitation of growth in the natural habitats of these species. Furthermore, Hurd et al. (1993) reported the appearance of hyaline hairs in some intertidal species of *Fucus* as a consequence of P deficiency. However, P-limited growth could be especially relevant for tropical macroalgae (Wheeler and Björnsäter 1992). Additionally, a recent reevaluation of the nutrititional status of a large list of seaweeds suggests that most macroalgae are exposed to phosphorus concentrations below the values that support maximum growth (Duarte 1992).

Because of storage capacity and the relative biomass enrichment of N with respect to P, the C:N:P ratio (Redfield et al. 1963) is so modified with respect to those defined for phytoplankton that it must be used for seaweeds with precaution (see Lobban et al. 1985). However, a good approach to determine which major nutrient limits growth in seaweeds is the study of the seasonal changes in elementary tissue composition C:N:P (Atkinson and Smith 1983, Wheeler and Björnsäter 1992, Hernández et al. 1993a).

Because we have described the reproductive physiology, growth and primary production of *P. purpurascens* in a previous paper (Flores-Moya et al. 1993), the aim of the present work was to investigate the variation in pigment content, apparent photosynthesis, dark respiration, and tissue composition during the life cycle of this species, in order to assess the major nutrient that limits growth, to explain the growth pattern, and eventually to compute an annual carbon budget.

## Materials and Methods

Sporophytes of *P. purpurascens* were collected monthly in Espona (36°26’N, 05°04’W; see map in Flores-Moya et al. 1993) during 1990, at a 30-m depth, by SCUBA diving. The first visible sporophytes were detected in April (<0.5 cm in length); the study finished after the first storm period in October (when cover was <0.1 plants m⁻², Flores-Moya et al. 1993). Seaweeds were maintained in a black bag during sampling in order to avoid photodamage of the light-harvesting antennae.

### Light Measurements

Daily photon flux density (PFD) curves at a 30-m depth were established every month from data collected at selected days. The PFD was measured every 15 min, from sunrise to sunset, by using a subsensible sensor Licor LI-192 connected to a data logger Licor-LI 1000.

### External Nutrient Concentrations

Replicate seawater samples (250 mL, n = 5) were collected at the same time as seaweeds. Samples were filtered through Whatman GF/F and stored frozen (−20°C) prior to analysis. Samples were analyzed with an autoanalyser Technicon AA-II. Nitrite, NO₂⁻, and NH₄⁺ concentrations were determined as described by Strickland and Parsons (1972). In the sampling of April and May, we measured NH₄⁺ in duplicate samples that were not frozen and not filtered or were filtered with alternative filters. Probably because of the high concentration of NH₄⁺ we measured, no significant differences between these handling procedures were found. Nitrite concentrations were always less than 3% of combined [NO₂⁻ + NO₃⁻] and are not reported separately, in agreement with Wheeler and Björnsäter (1992). Phosphates (P) were measured according to Fernández et al. (1985). Coefficients of variation for replicates (n = 5) in each nutrient analysis were lower than 5% of the mean.

### Photosynthetic Pigments

Samples for photosynthetic pigments were prepared immediately after collection. Disks of 1 cm in diameter were taken from two areas on the plant: at 5 cm from the stipe—blade junction and at the distal extreme of the blade, in three different plants. In April, only one sample was taken because plants were shorter than 5 cm in length. Disks were rinsed with distilled water, submerged in acetone neutralized with MgCO₃,
and kept at 4°C in the dark for 24 h. The disks were ground in a mortar using acid-washed sand as the abrasive. Homogenates were filtered with standard filter paper. Absorbances were measured in a Milton Roy Spectronic 501 spectrophotometer. Pigment concentrations were computed according to Talling and Driver (1963) for chl a, SCOR (1964) for chl c, and Strickland and Parsons (1963) for carotenoids.

**Tissue composition.** Samples for elementary analysis (1-cm-diameter disks) were also taken in situ, in triplicates, at 5 cm from the stipe-blade junction and in the distal extreme of the blade. Disks were transported to the laboratory in aluminium foil in an ice box and dried at 60°C for 24 h. Total C and N were measured with an elemental analyzer Perkin Elmer 240 (Niell 1976). For total P determinations, disks were ground in a mortar and digested in 5 mL of an acid mixture of concentrated nitric and perchloric acid (5:5 v/v) in a digestor block BD 40 from Technicon. Digestion was performed in two steps: the first one at 130°C for 90 min and the second at 204°C for 45 min (Sommers and Nelson 1972). Total phosphates in the digested extracts were measured according to Fernández et al. (1985). Coefficients of variation were lower than 5% of the mean for C, N, and P.

**Measurements of apparent photosynthetic rate.** Healthy thalli (5–10 g fresh weight), free of epiphytes, were maintained in the laboratory in an aquarium of 25 L filled with filtered (Whatman GF/C) seawater. Temperature was maintained at the same level as the sampling site. The PFD was 50 μmol m⁻² s⁻¹. Apparent photosynthesis rate (APS) was measured within 1 day of sampling.

A photosynthesis–irradiance (P-I) curve (Kirk 1983) was made monthly. Oxygen evolution was measured with a Clark-type aqueous-phase O₂ electrode from Hansatech, connected to a control box CB1D. Square samples (2 × 2 cm, about 0.4 g fresh weight) were taken out 15 cm from the stipe-blade junction and rinsed carefully three times with filtered seawater (Whatman GF/F) for 5 min. Oxygen evolution was measured in triplicate in natural filtered seawater (2 mL) taken from the seaweed beds. The different PFDs (from 2 to 550 μmol m⁻² s⁻¹) were obtained with a slide projector provided with neutral gray filters (Jiménez et al. 1990).

Photosynthesis–light curves were fitted to the Edwards and Walker (1983) equation. Curve fitting was made with the commercial software GraFit (Erithacus Software, London). The saturation constant (Iₛ) was obtained from the intersection between the saturated apparent photosynthesis (APSₛ) and the initial slope of the P-I curve (Kirk 1983); the initial slope was measured by the linear fit of the three initial values of the P-I curves. The ratio between APSₛ and the half-saturation constant (I₅₀) was used as an estimation of photosynthetic efficiency. To obtain the rate of dark respiration (DR), the reaction chamber was covered with aluminium foil. Temperature was maintained at constant 15 ± 0.1°C in all measurements.

**Carbon budget.** Gross photosynthesis was obtained from the sum of DR and APS. The APS at a 30-m depth, during a 24-h cycle, was computed every month considering the PFD values measured in situ and the parameters obtained from the P-I curves. Oxygen-based expression of APS and DR was converted to mass carbon units by using the photosynthetic quotient 1.17 O₂/CO₂, proposed for several red, green, and brown macroalgae by Axelsson (1988).

The conversion factor used to calculate the biomass from the surface area of the thallus was 107.8 g dry weight (DW) m⁻² (n = 35, r = 0.99). Surface values were obtained as described by Flores-Moya et al. (1993).

**Statistical analysis.** Normality of the data was checked by the Rankit method for small samples (Sokal and Rohlf 1986). Homogeneity of variances was assessed by the Fₚₚ test (P < 0.05) (Sokal and Rohlf 1986). Photosynthetic pigment concentrations and tissue composition of C, N, and P, at the two points on the blade, were compared by a Student’s t-test, and the mean concentrations obtained monthly were compared by analysis of variance.

**RESULTS**

**Light measurements.** PFD measured at a 30-m depth during the production cycle of *P. purpurascens* ranged from 13.5 in October to 27.5 mol m⁻² s⁻¹ in August (Fig. 1). The PFD reaching the population from April to October was 270 mol m⁻² s⁻¹. This figure represents 1.8% of the light measured above the water, a variable defined as light percentage depth by Lüning (1990).

**External nutrient concentrations.** Nitrates ranged from 7 to 8.5 μM, and NH₄⁺ from 7.0 to 10.2 μM over the production cycle. Phosphates were fairly constant from April to June (overall mean 4.2 ± 0.4 μM), decreased to undetectable amounts in August, and increased to 3.7 μM in October (Fig. 2).

**Photosynthetic pigments.** Monthly concentrations were computed with the values measured at 5 cm of the stipe–blade junction and at the distal extreme of the blade because the values were not significantly different (Student’s t-test, P < 0.05). Chl a concentration decreased from April to October (Fig. 3) from 520.6 ± 165.1 μg g⁻¹ DW in April to 199.6 ± 159.9 μg g⁻¹ DW in October. Chl a and carotenoid oscillations were small from April to September (mean concentration values of

![Fig. 1](image1.png) Seasonal variation of photon flux density (○) and carbon budget (histograms).

![Fig. 2](image2.png) Seasonal variations of external nutrients in seawater (○ = nitrates, O = ammonium, ■ = phosphates) from April to October in the *Phyllariopsis purpurascens* beds. Standard deviation (n = 5) smaller than symbol size.
381.6 ± 84.9 μg·g⁻¹ DW and 515.8 ± 94.4 μg·g⁻¹ DW, respectively) but increased threefold in October (Fig. 3).

Total C, N, and P content. Seasonal variation of total C, N, and P content was measured 5 cm from the stipe-blade junction and at the distal extreme of the blade. Total C, N, and P exhibited no significant differences between the extremes of the blade (Student's t-test, P < 0.05). The amount of total C increased dramatically from July to September, from 210 to 320 mg C·g⁻¹ DW (Fig. 4). Total N decreased steadily from 16.2 mg N·g⁻¹ DW in April to 9.2 mg N·g⁻¹ DW in October (Fig. 4). Total P was constant from April to June (3.0 mg P·g⁻¹ DW) but decreased dramatically in July and August to a minimum level of 0.5 mg P·g⁻¹ DW (Fig. 4).

C:N and N:P atomic ratios. The mean values of total C, N, and P at 5 cm from the stipe-blade junction and the distal extreme of the blade were used to

Fig. 3. Seasonal variations of photosynthetic pigments in the blade tissues. A) Chl a, B) chl c, and C) carotenoids. The mean monthly values were computed by the figures obtained at 5 cm from the stipe-blade junction and the distal extreme of the blade. Mean ± SD, n = 3.

Fig. 4. Seasonal variations of A) total carbon, B) total nitrogen, and C) total phosphorus contents at 5 cm from the stipe-blade junction (○) and in the distal extreme of the blade (●). Mean ± SD, n = 3.

Fig. 5. Seasonal variations of the C:N (○) and N:P (●) atomic ratios in the blade tissues. Mean ± SD, n = 3.
compute C:N and N:P atomic ratios. The C:N ratio was fairly constant (15–17) from April to July, but from this month to September the ratio increased up to 42 (Fig. 5). The N:P ratio followed the same pattern; it was around 14 from April to July but increased to 40 in September and October.

Redfield ratio. The C:N:P ratio can be graphically displayed by plotting C:P atomic ratio versus N:P atomic ratio (Fig. 6). Temporally consecutive points were joined with arbitrary lines. The Redfield ratio increased linearly from April to October, the initial figure being 187:13:1 in April to June and the final figure being 1600:40:1 in October.

Photosynthetic use of light and dark respiration. Saturated apparent photosynthesis, I_{0.5}, and I_{1} showed minimum values in August (1.2 \mu mol O_{2} m^{-2} s^{-1} and 11.3 and 12 \mu mol photons m^{-2} s^{-1}, respectively), whereas the maximum values were obtained in April (3.7 \mu mol O_{2} m^{-2} s^{-1} and 40 and 41.5 \mu mol m^{-2} s^{-1}) and in October (3.6 \mu mol O_{2} m^{-2} s^{-1} and 50 and 53.7 \mu mol m^{-2} s^{-1}, respectively) (Fig. 7). However, the light compensation point (I_{c}) did not change significantly (one-way ANOVA, \alpha = 0.05) during the production cycle, showing a mean value of 6.5 ± 0.2 \mu mol photons m^{-2} s^{-1} (Fig. 7). According to the values of I_{c} and PFD at a 30-m depth, photosynthesis was saturated from June to August but was not during other months. Photosynthetic efficiency was estimated as the ratio between APS_{max} and I_{0.5}; this ratio ranged from 0.06 in July to 0.11 \mu mol O_{2} \mu mol photons^{-1} in August (Fig. 8). Productivity, estimated as the ratio between monthly production (measured by the demographic method of Allen) and the initial monthly biomass (data redrawn from Flores-Moya et al. 1993) exhibited two peaks, one in June and the other in August (1.8 and 3.8 mo^{-1}, respectively), whereas minimum values were obtained in September to October (0.5 mo^{-1}) (Fig. 8).

Dark respiration rate exhibited no significant differences (one-way ANOVA, \alpha = 0.05) over the production cycle (mean 0.5 ± 0.1 \mu mol O_{2} m^{-2} s^{-1}) (Fig. 7).

Carbon budget. Net carbon assimilation increased from April (443.9 mg C g^{-1} DW mo^{-1}) to June (715.2 mg C g^{-1} DW mo^{-1}), decreased to a negative value in August (−419.2 mg C g^{-1} DW mo^{-1}), was close to 0 in September, and exhibited a second increase in October (271.3 mg C g^{-1} DW mo^{-1}) (Fig. 1). The carbon budget for the whole production cycle was 1.7 g C g^{-1} DW. Net carbon assimili-
loration increased in parallel with PFD from April to June, but from July to October there was no agreement between these variables.

**DISCUSSION**

*Ligh, pigments, and photosynthesis.* From April to October, PFD reaching the population of *P. purpurascens* in the Strait of Gibraltar is 270 mol·m⁻²·yr⁻¹. This value is lower than the irradiance reaching the deepest individuals of *Laminaria digitata* (L.) Lamour. and dense populations of *L. hyperborea* at this location but higher than PFD reaching the deepest individuals of these species in Helgoland (Lüning and Dring 1979). The computed light percentage depth, 1.8%, measured in the Strait of Gibraltar for the population of *P. purpurascens* was also lower than the 5% reaching a population of *Laminaria ochroleuca* La Pylaie in the Strait of Messina (Southern Italy) (Drew et al. 1982). Red algae from deep waters exhibited an I₄, overall value between 1 and 2 µmol·m⁻²·s⁻¹ (Lüning 1981), whereas *P. purpurascens* exhibited an I₄ between 6 and 7 µmol·m⁻²·s⁻¹. The diminution in the chl a:chl c ratio has been proposed by Geider et al. (1986) and Raven (1986) as the cause of the increase of I₄. Although the chl a:chl c ratio changed over the cycle in *P. purpurascens*, I₄ was constant. The morphology of the thallus, dark respiration rate, C:N:P ratio, and rheophic habitat of *P. purpurascens*, as in *L. ochroleuca* from the Strait of Messina (Drew et al. 1982), agree with the characteristics of a shade-adapted deep-water alga (Lüning 1990). They minimize dark respiration by reducing nonphotosynthetic tissues and slowing down the growth rate. These plants also enhance light-harvesting capacity, store a great amount of fixed carbon, and exhibit mechanisms to resist grazing (Lüning 1990).

Photosynthesis increases during the growing period in spring in several species of kelps parallel to the increment in photosynthetic pigments (Drew 1983, Smith et al. 1983, Wheeler et al. 1984, Gutkowski and Malezowski 1989). In contrast, *P. purpurascens* exhibited an increase in net oxygen production from April to June (Fig. 1) parallel to the increase in PFD, but during that period chl a decreased whereas chl c and carotenoids did not change significantly. This is also the pattern observed in *Sargassum muticum* (Yendo) Fensh. (Lewey and Gorham 1984). The amount of chl a was maximum in April, likely because the population was completely renewed every year (Flores-Moya et al. 1993). Afterward, as Lewey and Gorham (1984) suggested, pigments were diluted by the biomass increment during the growing period in spring. In *P. purpurascens*, APSₘₐₓ and chl a varied in the same way; there was a decrease in APSₘₐₓ in spring (Fig. 7), consistent with the decrease of the amount of chl a. However, during this interval, a maximum in the photosynthetic efficiency was detected (Fig. 8). This increment may be accounted for by the stronger diminution in I₄₃ than APSₘₐₓ during the same period. However, the pattern of chl a synthesis in overwintering dormant tissue should be different (Drew 1983, Smith et al. 1983, Wheeler et al. 1984, Gutkowski and Malezowski 1989). Young sporophytes of *P. purpurascens* are erect, even with strong currents, whereas mature sporophytes have erect stipes, but blades are horizontal, as in *L. ochroleuca* (Drew et al. 1982). Therefore, the greater amount of chl a at the beginning of the cycle might compensate for the low efficiency in light harvesting due to the position of the thalli.

There is no correlation between blade expansion (Flores-Moya et al. 1993) and photosynthetic parameters measured from April to October. However, population productivity, estimated by an independent method, agrees with photosynthetic efficiency (Fig. 8). This agreement is independent of external variables affecting growth or photosynthesis. Lapointe and Duke (1984) suggested that pigment level and the activity of Rubisco determine, respectively, the initial slope and the maximum apparent photosynthesis in the P-I curves. In *P. purpurascens*, the decrease in APSₘₐₓ from April to August, likely induced by nutrient limitation, was compensated by a parallel decrease in I₄ and I₄₃, yielding a maximum photosynthetic efficiency in August. According to Lapointe and Duke (1984), this behavior should be consistent with a decrease in the activity of Rubisco and a concomitant decrease in the amount of photosynthetic pigments, chl a in the case of *P. purpurascens* (Fig. 3). However, other brown algae such as *Macrocystis integrifolia* Bory exhibited a decrease in APSₘₐₓ in old individuals, being constant in I₄₃ (Smith et al. 1983).

Except in August, dark respiration was lower than gross photosynthesis, and it was almost constant over the cycle of *P. purpurascens*. This absence of variation in dark respiration has also been reported for *Ecklonia cava* Kjellman (Haroun et al. 1992), but Drew (1983) reported that annual carbon budget depends on dark respiration changes in *L. digita* and *Laminaria saccharina* (L.) Lamour., and Sand-Jensen (1988a, b) reported a strong relationship between growth rate and dark respiration in *Ulva lactuca* L. Significant temperature changes and extremely low irradiance (Sand-Jensen 1988a, b) are likely the cause for such dependence. In contrast, the constant temperature in the case of *P. purpurascens* beds (Flores-Moya et al. 1993) should be the cause of the lack of variation in dark respiration. As a consequence, growth and carbon budget follow the pattern of photosynthetic efficiency and photosynthetic capacity, respectively.

**Total C, N, and P content, and C:N, C:P, and Redfield ratios.** Total C content increased in *P. purpurascens* from July to October, whereas total N decreased steeply along the cycle. In *M. integrifolia* and *Nereocystis lutkeana* (Mert.) Post. et Rupr., total C content increased from April to September, whereas
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