CHANGES IN NUTRIENT CONTENT OF *PALMARIA PALMATA* IN RESPONSE TO VARIABLE LIGHT AND UPWELLING IN NORTHERN SPAIN[1]

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Light has been identified as one of the main factors affecting seaweed ecophysiology. We investigated the dependence of nutrient metabolism on sun and shade light conditions and whether episodes of upwelling of nutrient-rich subsuperficial water could reduce the summer nutrient limitation driving physiological changes in *Palmaria palmata* (L.) Kuntze. We measured the major nutrient pools, photosynthetic pigments, and light curves, under sun and shade conditions during a summer period when one upwelling was recorded. The redundancy analysis (RDA) produced two clear groups: sun- and shade-acclimated algae. Light was the major predictive factor. Sun-acclimated algae exhibited higher carbon (C) and lower nitrogen (N) and phosphorus (P) content in association with the storage of floridoside (main C reserve) to benefit from higher irradiance (under nutrient limitation). Among N pools, N reserves (phycoerythrin, nitrate) were a lower proportion of the total N in sun-acclimated algae, suggesting their degradation to fulfill the N demands of the cell. The orthophosphate content was also lower in sun-acclimated algae, indicating its utilization as a nutrient reserve. In contrast, N within cell walls and membranes and chl *a* contributed to a similar proportion of the total N in sun- and shade-acclimated algae, suggesting a response to sustain cell integrity. Transient high nutrient concentration due to the upwelling was unrelated to the nutrient content of the thallus. The storage of C as floridoside from high light exposure was shown to be the driving force for the metabolic adjustment of *P. palmata* at the end of summer before the onset of dormancy.

Key index words: carbon; light; nitrogen; nutrient; *Palmaria palmata*; phosphorus; shade; sun; upwelling

Abbreviations: DIVING-PAM, underwater portable pulse amplitude fluorometer; DMF, N, N-dimethylformamide; ETR, apparent photosynthetic electron transport rates; FAA, free amino acids; PC, phycocyanin; PCA, principal component analysis; PE, phycoerythrin; RDA, redundancy analysis; TIN, total insoluble nitrogen

Light environment has been identified as the key factor affecting the physiology of autotrophs (Lüning 1990, Dring 1992, López-Figueroa 1992, Kirk 1994, Lobban and Harrison 1997, Prescott et al. 1999, Buchanan et al. 2000). Plant canopies significantly decrease irradiance, reducing light penetration and causing major changes in light quality (i.e., green and far-red light are enriched compared to red or blue light; Smith 1982, Kirk 1994, Salles et al. 1996). Acclimation to such changes is common in macroalgae (López-Figueroa et al. 1990, Rüdiger and López-Figueroa 1992, Talarico 1996, Talarico and Maranzana 2000, Figueroa et al. 2003a) as well as phytoplankton and seagrasses (Kirk 1994). Typically, shade-acclimated plants synthesize photosynthetic pigments, such as chl and phycobilins, to enhance their efficiency in absorbing energy at subsaturating irradiances (Rüdiger and López-Figueroa 1992, Salles et al. 1996, Théry 2001, Figueroa et al. 2003a), and they show lower light-saturation values in light-response curves (Beach and Smith 1996, Sagert and Schubert 2000, Théry 2001, Figueroa et al. 2003b). Moreover, a wide range of metabolic responses, including enhancement of C fixation in sun conditions and reallocation of nutrient-rich constituents within the thallus, is also common (Young and Smith 1980, López-Figueroa and Rüdiger 1991, López-Figueroa 1992, Figueroa 1993, Vergara 1993, Figueroa 1996, Häder et al. 1997, McGlathery and Pedersen 1999, Figueroa et al. 2003b). These ecophysiological responses have been identified among the various factors that result in the efficient capture and utilization of incident radiant energy by macroalgae and thus influence the potential competitive ability of each species (see review in Carpenter 1990).

During the summer on temperate coasts, the highest irradiance values of the entire year typically coincide with the lowest nutrient availability (Hanisak 1979, Lobban and Harrison 1997). Nutrient starvation in summer is the most common reason for limitation of algal growth (Hanisak 1979, Conolly and Drew 1985a,b). The surplus of incoming radiation is managed by some macrophytes by converting C fixation products into reserve carbohydrates. These C reserves normally develop in perennial species in late summer and may be important in supporting growth the following winter, when irradiance is reduced but there is often higher nutrient availability (Davison et al. 1984, Rosenberg et al. 1984, Lüning 1990, Touchette and Burkholder 2002). In contrast, the synthesis of nutrient-rich compounds, such as proteins, during summer is limited by the low nutrient concentration in seawater; thus, N and P content of the thallus are usually minimal (Neish et al. 1977, Gerard 1982, Davison et al. 1984, Vergara 1993, Chopin et al. 1995, Rico and Fernández 1996, McGlathery and Pedersen 1999, Collén et al. 2004). This perennial strategy should give the species a significant growth advantage and has been suggested as a key factor for macroalgal competition (Carpenter 1990, Dring 1992).

In some coastal areas, winds blowing parallel to the shoreline drive surface water to flow offshore and result in the upwelling of subsurface water, which causes nutrient concentration to increase and temperature to decrease (Pikard and Emery 1990, Mann and Lazier 1991). Gerard (1982) suggested that *Macrocystis pyrifera* was able to exploit high pulses of nitrate associated with such upwellings to reduce N starvation in Southern California. Kelp decline and reduced productivity in response to nutrient starvation associated with reduction of upwelling events during El Niño have also been described in the same area (Gerard 1982, 1984, Dayton and Tegner 1984, Zimmerman and Robertson 1985, Dean and Jacobsen 1986). These responses are common in other geographic areas (Dieckmann 1980, Fujita et al. 1989). In reference to physiological changes, in particular, Gerard (1982), Probyn and McQuaid (1985), and Leichter et al. (2003) detected increased thallus N content in macroalgae during upwellings. In contrast, others have observed no effect of upwelling on macroalgal growth (Paine 1986, Levitt and Bolton 1990), recruitment and mortality (Paine 1986), or N content (Rico and Fernández 1996). The effect of this hydrodynamic feature on macroalgae is thus in need of further investigation.

We aimed to assess two main hypotheses concerning the metabolic adjustment of macroalgae to light environment and upwellings. We hypothesized that high incoming light would be exploited as C fixation, causing an increase in C content and a decrease of N and P (per unit weight), particularly in sun-acclimated algae. The second hypothesis was that coastal upwellings would result in higher N and P supply, which would allow macroalgae to take advantage of the favorable light conditions in summer, increasing these nutrients in the thallus and readjusting their metabolism accordingly.

To test these two hypotheses, we measured the major nutrient pools and photosynthetic pigments of individuals growing in sun and shade conditions in a field population of the perennial red seaweed *P. palmata*. Additionally, at certain dates during the experiment, we measured light-response curves with an underwater portable pulse amplitude fluorometer (DIVING-PAM). We did the sampling in late summer, and we also monitored the major environmental parameters (seawater temperature, N and P seawater concentration, and wind regimes). This species lives as an epiphyte of kelps; thus, sun-acclimated algae from above the kelp bed and shade-acclimated algae from underneath kelp canopies were available for the experiment. Several researchers have pointed out the importance of light in determining the growth and physiology of *P. palmata* (Hanelt and Nultsch 1995, Sagert and Schubert 2000, van de Poll et al. 2001). Moreover, we knew that low nutrient concentration limits growth of this species during summer in the studied site. Net growth rates close to zero values and low N and P thallus content were determined in intertidal and cultivated individuals in the area (Martínez and Rico 2002, Faes and Viejo 2003, Martínez et al. 2006). The study site is subject to episodic upwellings associated with north-east winds that could be alleviating this nutrient starvation (Botas et al. 1990, Marañón et al. 1995, Serret et al. 1999, González et al. 2003).

MATERIALS AND METHODS

Sun- and shade-acclimated material. We took the samples from a subtidal population (∼2.5 m depth) at Concha de Artedo on the northern Spanish coast (43°34′ N, 6°11′ W). In this population, *P. palmata* is epiphytic on *Cystoseira baccata* and *Laminaria hyperborea*. We collected six to eight individuals of *P. palmata* on nine different days between August 11 and September 7, 1999. We took half the individuals (3–4 replicates) from above the kelp bed, which were thus identified as "sun-acclimated algae"; the other half was taken from understory sites ∼50 cm underneath kelp canopies, consequently identified as "shade-acclimated algae." We measured irradiance on the surface of each *P. palmata* frond before collecting it and also just above the sea surface at the same time (between 11:00 and 17:30) to characterize light and shade conditions. For these measurements, we used a Fiber Quantum Sensor (Heinz Walz GmbH, Effeltrich, Germany). The mean irradiance value for sun conditions was 199 ± 21.7 (mean $\pm$ SE μmol photons $\cdot$ m$^{-2} \cdot$ s^{-1}, $n = 47$), which constituted $33 \pm 1.6\%$ of the light measured just above the sea surface (mean $\pm$ SE). The mean irradiance for shade conditions was $15 \pm 2.6\%$ (mean $\pm$ SE μmol photons $\cdot$ m$^{-2} \cdot$ s^{-1}, $n = 48$) and constituted $2 \pm 0.6\%$ of the incident light reaching the sea surface (mean $\pm$ SE). We avoided the collection of fronds from intermediate light conditions.

Environmental parameters. At least two seawater samples were collected for analyses of nutrient concentration per day of sampling. GF/F (Whatman International Ltd., Maidstone, UK) filtered seawater was collected with an acid-washed syringe.

Water samples were transported inside isotherm bags (< 4°C, in darkness) and kept at −20°C for <3 months prior to analysis. Seawater nitrate (NO_3^-), nitrite (NO_2^-), and orthophosphate (HPO_4^{2-}) were measured as described in Koroleff (1983), and ammonium (NH_4^+) as outlined in Álvarez (1993) using a Technicon II Autoanalyzer (Industrial Method no. 158-71 W/A, Dublin, Ireland). Temperature was measured using a continuous data logger (ONSET Computer Corp., Pocasset, MA, USA) fixed next to the population. Ekman transport values were kindly provided by the research group Grupo de Oceanografía (Universidad de Oviedo, Spain). This parameter was calculated each day from the wind regimes using the equation of Bakum (1973). Positive Ekman rates persisting during several days are indicative of wind regimes associated with upwellings.

Light-response curves. To characterize the overall photosynthetic performance of sun- and shade-acclimated algae, we measured on 3 d the in situ chl fluorescence of several samples just prior their collection using a DIVING-PAM (Heinz Walz GmbH, Effeltrich, Germany). We used algae acclimated to their natural light environment to investigate in the field the responses to sun and shade light conditions. We did not intend to give a precise description of the light response of the species. For this purpose, we calculated the relative apparent photosynthetic electron transport rates (ETR, μmol electrons $\cdot$ m^{-2} $\cdot$ s^{-1}) at eight increasing PAR pulses (μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) to construct the light response curve of each individual (DIVING-PAM Handbook of Operation, Heinz Walz GmbH 1998).

Biochemical analysis. We transported the samples from the field to the laboratory frozen inside a liquid-N container and kept them in this condition. Just before performing the analyses, we took *P. palmata* fronds out of the liquid N and removed the epiphytes. Total C and N thallus content were measured using a CHN Elemental Analyser (Perkin-Elmer Analytical Instruments, Shelton, CT, USA). Phosphorus thallus extraction was performed by alkaline persulfate digestion (Ameel et al. 1993), and orthophosphate for total P was measured in the digested sample as described for seawater samples. The protein fraction was extracted in SDS as in Naldi and Wheeler (1999), and protein content was measured by the Lowry's method using BSA (Fluka, Sigma-Aldrich, St. Louis, MO, USA) as standard (Harrison and Thomas 1988). The SDS insoluble fraction (the pellet from the centrifuged protein extraction solution) was recovered in a combusted GF/F filter, and its N content measured with a CHN analyzer to estimate the total insoluble N (TIN) according to Naldi and Wheeler (1999). Phycocyanin (PC) and phycoerythrin (PE) thallus contents were determined using trichromatic equations (Beer and Eshel 1985) following extraction in phosphate buffer (Evans 1988). Chl *a* was extracted overnight with N, N-dimethylformamide (DMF; Moran and Porath 1980). Calculations were based on the extinction coefficient of Inskeep and Bloom (1985). Carotenoid content was determined from the DMF extract following Wellburn (1994). For ammonium, nitrate, nitrite, and phosphate, and free amino acid (FAA) analyses, subsamples were ground with liquid N and boiled for 10 min in 100 mL bidistilled water (Hurd 1990, Naldi and Wheeler 1999). The extraction solution was then centrifuged (10,000*g*), and the supernatant was frozen for later analysis (Naldi and Wheeler 1999). Inorganic nutrients in this supernatant were analyzed as explained for seawater. The FAA content was measured with the *o*-phtaldialdehyde method (Roth 1971) using glycine as standard (Naldi and Wheeler 1999).

Expressions of compound contents. The proportion of fresh weight (fwt) to dry weight (dwt) was determined after drying a subsample at 60°C for 3 d to express the content of each constituent on a dwt basis. Additionally, all N pools were expressed in μmol N $\cdot$ g^{-1} dwt following the conversions in Naldi and Wheeler (1999). Similarly, P pools were expressed in μmol P $\cdot$ g^{-1} dwt. The percentage of total N or total P constituted by each pool was calculated (% total N, % total P for each compound). These percentage values would allow the investigation of the partitioning of N and P while removing the dilution effect caused by the hypothetic accumulation of N- and P-poor biomass (i.e., C reserves).

Statistical analysis. Analyses of RDAs were carried out on the physiological parameters (content of thallus constituents) for hypothesis testing (ter Braak 1999, ter Braak and Šmilauer 2002, Legendre and Legendre 2003). Such multivariate ordination was used because it allowed for investigating the variation of the content of all compounds at the same time by looking at the ordination plot. Univariate approaches were avoided to describe the metabolic adjustment of the alga as a whole. Each thallus constituent was represented by an arrow in the ordination plot pointing to the samples that showed the highest amount of that particular compound. Each sample (point in the plot) represented the mean content of all compounds calculated from the three or four fronds taken at one sampling date and light condition. The RDA is the constrained form of the more popular principal components analysis (PCA). The main advantage of using RDA is that the set of explanatory variables (the environmental parameters of concern to the hypotheses) could be included to construct the ordination plot from the beginning, and their variation is also represented by arrows in the plot. The resulting ordination plot summarized the physiological variation of *P. palmata* that was correlated to the environmental changes. The unexplained variance (not related to the environmental parameters considered) was not included in the RDA plot. Moreover, the small angles between the arrows are indicative of high correlation between the variables they represent; thus, the plot gave an idea of the relationships between the variables included. Nevertheless, one PCA was also performed to investigate if the ordination plot of the samples based solely on the physiological data was similar to that produced by the RDA.

To investigate *P. palmata* metabolic response to the environmental changes, the variables Light and Ekman were a priori considered as explanatory factors and included in a full model RDA. Light was included as a dummy variable (1 was assigned to samples collected in sun conditions, and 0 was assigned to samples collected in shade conditions), and Ekman corresponded to the Ekman transport rates calculated for each date. The RDA assumes a linear response of dependent variables (thallus constituents) in relation to the environmental parameters; this is the most logical functional response taking into account the nature of the parameters measured. However, linearity was further confirmed by performing preliminary detrended correspondence analysis (DCA; ter Braak and Šmilauer 2002). One DCA was performed detrending by segments. This method has the property that the extracted axes are scaled in units of average standard deviation (ter Braak and Šmilauer 2002). As the length of the extracted gradients was very short, models that assume linear relationships, such as RDA, are recommended (ter Braak and Šmilauer 2002).

A variance partitioning with RDA was performed to test the particular hypotheses concerning the metabolic response of *P. palmata* to Light and Ekman. Each of these factors was included as an explanatory variable to construct seldom RDAs in which the other was considered as covariable (Legendre and Legendre 2003). This procedure has been called partial RDA because it determines the variation explained by the explanatory variable after removing the variation accounted for by the covariable. To test the first hypothesis concerning the nutrient metabolic response of *P. palmata* to sun and shade conditions, the variable Light was a priori considered as the explanatory factor, and Ekman was included in a partial RDA as the covariable. In the same way, to test the effect of the upwelling

(i.e., the second hypothesis), a partial RDA was constructed including Ekman as the explanatory variable and Light as the covariable. The null hypothesis (H_0) was that the influence of the explanatory variable was not significantly different from random chance. All the partial models were tested for significance using Monte Carlo permutation tests with 10,000 randomizations (ter Braak and Šmilauer 2002, Legendre and Legendre 2003). All the analyses were conducted with CANO-CO (ter Braak and Šmilauer 2002).

RESULTS

Environmental parameters. The prevailing conditions at the studied site during summer were those typical for temperate areas and have already been published elsewhere (Botas et al. 1990, Marañón et al. 1995, Serret et al. 1999, Martínez and Rico 2002, González et al. 2003). The water column is thermally stratified. Correspondingly, high irradiances and temperatures occurred together with nitrate, nitrite, and orthophosphate seawater concentrations below detectable values, and calm sea conditions on most of the sampling dates (Fig. 1). Ammonium concentration did not show a clear trend, probably due to its rapid turnover in the water column and the variable nature of the zooplankton excretion and plankton decomposition processes that contributed to the ammonium production.

From August 30 until September 1, Ekman transport rates were well above zero values. These rates were within the range of values measured in upwelling conditions in the northern Spanish coast in previous research (González et al. 2003). These values were associated with a drastic decrease in temperature and an increase in seawater nitrate, nitrite, and orthophosphate (Fig. 1). These data suggested the upwelling of subsurface water to the surface. Downwelling conditions on the following 2 d caused by different a wind regime (Fig. 1, negative Ekman values) probably kept the upwelled mass of nutrient-rich water close to the coastline (Pikard and Emery 1990, Mann and Lazier 1991). Ammonium concentration did not change significantly during this event, probably because excretion and decomposition processes were similar in both the subsuperficial and surface layers, reducing the differences in this nutrient content between these two water masses (Mann and Lazier 1991). Ekman transport rates were also above zero values on August 20. However, values close to zero were measured on the following days, and there was no temperature drop. These data suggest that there was no upwelling, perhaps due to the short persistence of wind conditions that favor the occurrence of such events.

Light-response curves. Most of the sun-acclimated individuals (22 of 24) showed a saturation plateau in the light curve well above those observed for shade-acclimated algae (18 individuals, Fig. 2). These results, together with the accumulation of floridoside (see below), suggested higher C-fixation rates in sun conditions. In contrast, at low irradianc-
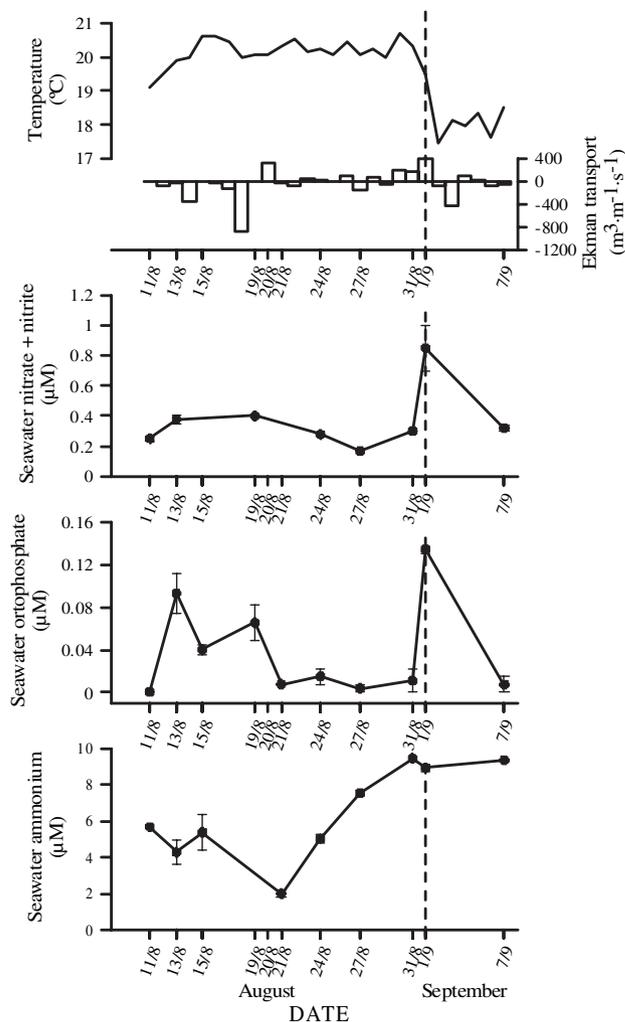

Fig. 1. Variation in main environmental parameters during the study. The dotted vertical line marks the occurrence of an upwelling of colder, nutrient-rich subsurface water.

es, there were no clear differences in the curves from sun- and shade-acclimated algae; thus at PAR levels similar to those that we measured at the understory (see ''Environmental parameters'' section above), both sun- and shade-acclimated algae showed similar photosynthetic efficiency.

Biochemical analyses. For both sun- and shade-acclimated algae, the N content and P content were very low when compared with data from other seasons that we measured in a previous annual survey in the same location (Table 1). In this study, we measured the maximum content of both nutrients in March: N content rose up to 4% dwt ($\sim$2,800 µmol N · g^{-1} dwt), and P content rose up to 0.45% dwt ($\sim$145 µmol P · g^{-1} dwt; see fig. 2 in Martínez and Rico 2002). These results support the low N conditions of the studied site during summer.

The thallus composition of sun- and shade-acclimated algae was very different (Table 1). In sun-acclimated algae, a higher portion of the dwt
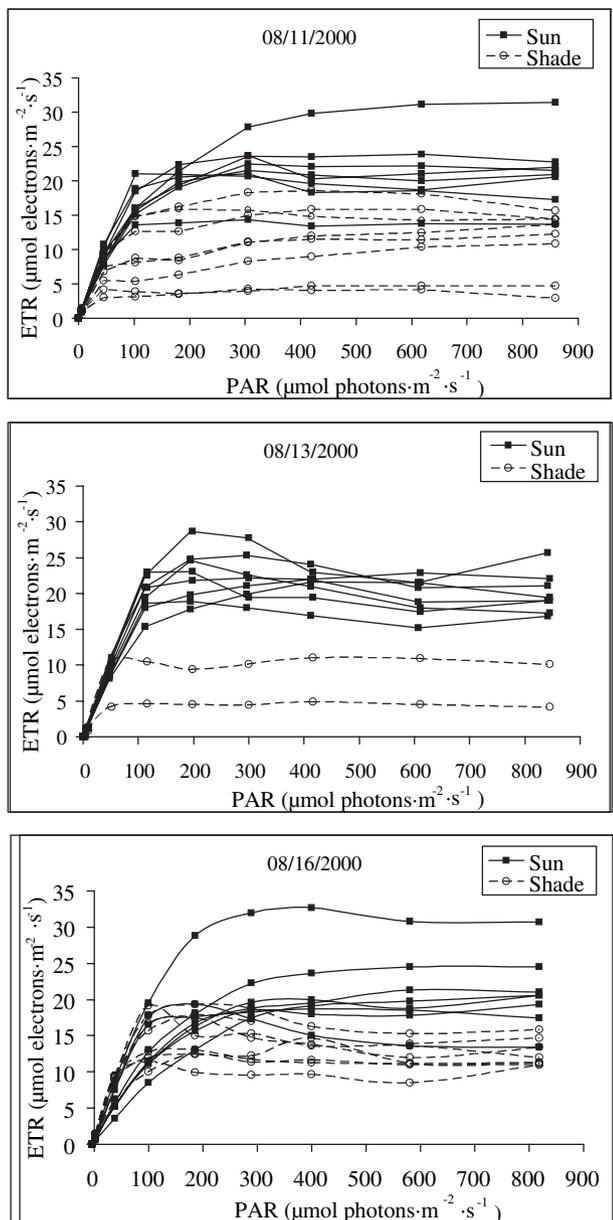
FIG. 2. Light-response curves of sun- and shade-acclimated algae collected on three dates. Relative apparent photosynthetic electron transport rates (ETR) were measured at eight increasing PAR pulses to construct a single light-response curve for each individual, using a DIVING-PAM.

Among the various N pools measured, soluble protein was the largest fraction of the dwt (Table 1). This pool also includes PE, which was a significant fraction of weight, and PC, which was a small fraction. The SDS-insoluble fraction (TIN) constituted the second largest N pool; it also contained proteins, but mostly within cells walls and membranes (Naldi and Wheeler 1999). The sum of extracted N accounted for $92.8 \pm 2.47\%$ of total thallus N (mean $\pm$ SE, $n = 53$; overlapping pools detected in more than one analysis were not summed twice). These findings indicated that the measurements provided a good assessment of major pools involved in N metabolism.

Among the N pools, PE showed the biggest difference between light conditions when expressed as percentage of total N (i.e., removing the dilution effect due to the floridoside storage). This pigment protein was a larger fraction of total N in shade- than in sun-acclimated algae (Table 1). Nitrate and PC, to a lesser extent, exhibited a parallel variation to PE. In contrast, other compounds did not show this trend, and differences were relatively minor.

Influence of environmental parameters on the physiological parameters. The RDA ordination constrained to Light and Ekman explained 51.3% of the variance of physiological data (full model, Table 2). This was a large fraction of the cumulative percent of variance explained by the corresponding PCA (75.2%), and the ordination plots for both analyses were rather similar (Fig. 3, PCA plot not shown). Therefore, the full-model RDA accounted for most of the physiological variation, which was clearly related to the environmental factors included. The first axis extracted by the partial RDA that included Light as the explanatory variable (and Ekman as covariable) explained 46.4% of the variance. This value was similar to the total variance explained by the full-model RDA, suggesting that Light was the major relevant environmental factor affecting *P. palmata* metabolism. In spite of the different locations of the two samples taken during the upwelling in the full-model RDA that suggested different physiological status, Ekman was not significant after partial RDA and was associated with a minor contribution to the explained variance (Table 2).

The ordination plot constrained to Light (not shown) was very similar to that constructed under the full model (Fig. 3). In both plots, two clear groups were shown: on the right-hand side, sun-acclimated plants were grouped together, while on the other side of the first axis, samples from shade conditions appeared together. Samples taken during the upwelling were located far away from these two groups in the full-model plot, but within their corresponding position relative to the first axis, and they were included in their corresponding group in the partial RDA constrained to Light (not shown). The different position of these two samples in the full-model plot was relative to the second axis,

was composed of C-rich compounds, as shown by the higher total C values. Accordingly, sun-acclimated algae had a higher content of the reserve carbohydrate floridoside. This C accumulation caused a dilution of the N and P content due to the addition of N- and P-poor carbohydrates such as floridoside. As a consequence, sun-acclimated algae were enriched with compounds containing C and lacking N and P. The very low concentration of these nutrients in the seawater probably prevented significant uptake by algae.

TABLE 1. Mean content of the thallus constituents.

Compound	Sun (mean ± SE)		Shade (mean ± SE)	
Total C[a]	36.7 ± 0.35		34.4 ± 0.31	
Floridoside	23.1 ± 1.17		16.6 ± 1.69	
P-total P[b]	35.1 ± 1.44		54.5 ± 1.78	
P-phosphate[b]	4.4 ± 0.32		10.7 ± 0.71	
Carotenoids[a]	0.2 ± 0.02		0.1 ± 0.02	
N-total N[c]	891 ± 32.2		1257 ± 43.0	
	$\mu mol\ N \cdot g^{-1}$ dwt	% total N	$\mu mol\ N \cdot g^{-1}$ dwt	% total N
N-total proteins	459 ± 26.7	52.0 ± 2.76	675 ± 29.1	54.4 ± 2.23
N-insoluble N	290.8 ± 21.11	33.0 ± 2.10	361.5 ± 25.00	28.9 ± 1.86
N-phycoerythrin	10.5 ± 3.15	1.1 ± 0.30	68.4 ± 4.23	5.6 ± 0.31
N-ammonium	16.1 ± 0.94	1.9 ± 0.11	20.0 ± 0.76	1.6 ± 0.07
N-phycocyanin	6.2 ± 0.82	0.7 ± 0.08	12.8 ± 1.01	1.0 ± 0.09
N-nitrate	2.6 ± 0.46	0.3 ± 0.05	9.4 ± 1.44	0.7 ± 0.10
N-chl *a*	3.4 ± 0.40	0.4 ± 0.04	5.1 ± 0.69	0.4 ± 0.05
N-nitrite	0.4 ± 0.03	0.045 ± 0.0094	0.6 ± 0.05	0.043 ± 0.0092
N-free amino acids[c]	0.07 ± 0.005	0.008 ± 0.0006	0.09 ± 0.005	0.008 ± 0.0005

[a]% dwt, [b]$\mu mol\ P \cdot g^{-1}$ dwt, [c]$\mu mol\ N \cdot g^{-1}$ dwt. Mean values for all the samples taken (including those taken during the upwelling event); $28 \le n \le 30$.

TABLE 2. Eigen values, cumulative explained variance (%) of species data, and species-environment correlation coefficients for the first two axes obtained by redundancy analysis (RDA) under the full model, and for the first axis extracted by partial RDAs under the reduced models.

Environmental predictors	Axis		F	P-value
	1	2		
Eigen values				
Full model (Light, Ekman)	0.49	0.02	7.89	0.0001
Light	0.46		14.22	0.0001
Ekman	0.05		1.57	0.1606
Cumulative explained variance				
Full model (Light, Ekman)	49.2	51.3		
Light	46.4			
Correlation coefficients				
Full model (Light, Ekman)	0.89	0.49		
Light	0.89			

The *F*-test value and significance (after 10,000 Monte-Carlo permutations) for each model are also shown.

suggesting a minor physiological response not related to the nutrient metabolism. These samples showed similar mean content of the major nutrient pools to the other sun and shade samples.

The most remarkable result was that nearly all of the thallus constituents were directly (floridoside, C, and carotenoids) or inversely (N- and P-rich compounds) related to Light (Fig. 3). Interestingly, the main photosynthetic pigments were related with less strength to the light conditions (as shown by the greater angles between the arrows representing them in Fig. 3). Sun-acclimated algae tend to have higher carotenoids (protection role) and lower chl *a*, PE, and PC (absorption role).

DISCUSSION

P. palmata exhibited a marked physiological adjustment to sun and shade conditions at the end of summer. The samples fell within two major groups: sun-acclimated individuals from the top of the kelp bed, and shade-acclimated algae from underneath kelp canopies. As predicted by the first hypothesis, sun-acclimated algae exhibited higher C and lower N and P content. This finding may be related to the accumulation of floridoside reserves (the main C reserve and photosynthate in this species, Morgan et al. 1980, Deniaud et al. 2003) to benefit from higher irradiance, when N and P have been shown to be limiting in the area (Rico and Fernández 1996, Martínez and Rico 2002). Faes and Viejo (2003) observed no net growth in a population of *P. palmata* close to our site. This species is perennial with increasing growth during spring and the beginning of summer; thus, the algae used in this study were not actively growing. However, Faes and Viejo (2003) measured the elongation of the fronds to estimate growth (i.e., length and dwt were correlated), and thus we cannot rule out an increase in the thickness of the algae that would result in increased weight. Nevertheless, we believe this process cannot be viewed as growth in the sense of maturation of the individuals, as used in population ecology, and thus the dilution phenomenon that we describe in this study was different from that associated with active growth periods of other species (McGlathery et al. 1996, McGlathery and Pedersen 1999). Rather, it is associated with a greater storage of C reserves in sun-acclimated algae. Correspondingly, the light-response curves suggested higher C-fixation rates in sun-acclimated algae. Higher light-saturation values in sun plants, and specifically in *P. palmata*, are commonly described and linked to a higher rate of the Calvin-cycle pathway, the limiting step at such saturating irradiances (Young and Smith 1980, Beach and Smith 1996, Sagert and Schubert 2000, Théry 2001,
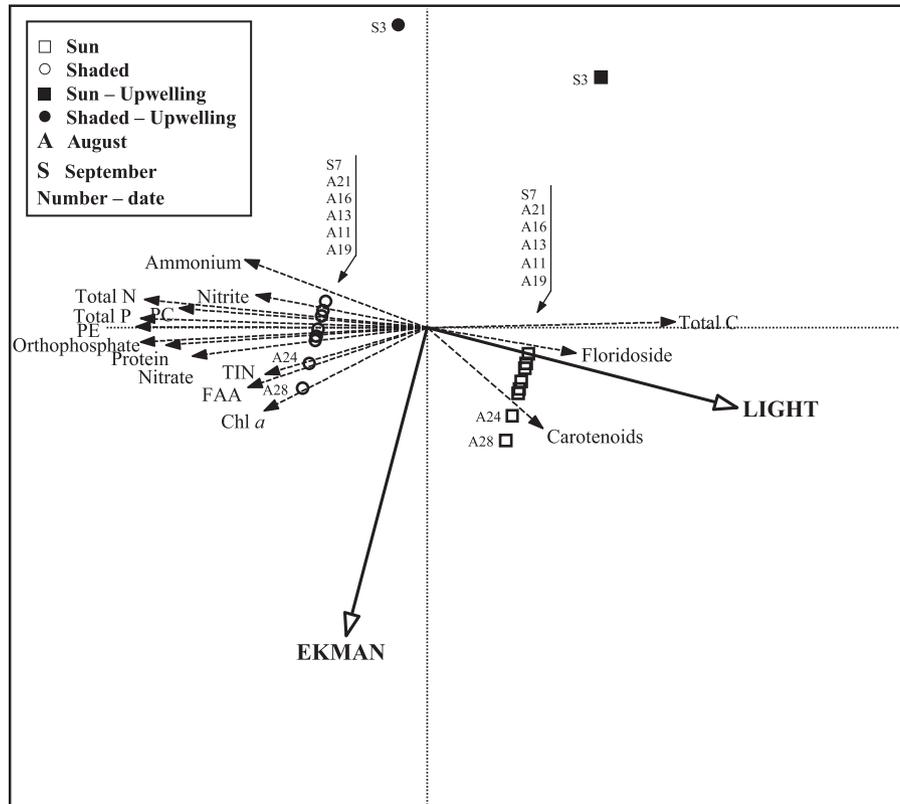
F<small>IG</small>. 3. Full-model redundancy analysis (RDA) carried out on the thallus components. Ekman and Light were included as explanatory variables (solid arrows). Each point in the RDA plot takes into account the mean content of all thallus compounds, calculated from the values measured in the algae collected on that particular date (labeled with a letter denoting the month, and number denoting the date), at one of the two light conditions (squares for sun conditions, and circles for shade conditions). Dashed arrows represent the variation of the thallus compounds measured. FAA, free amino acids; PC, phycocyanin; PE, phycoerythrin; TIN, total insoluble nitrogen.

Figueroa et al. 2003b). Particularly in *P. palmata*, Morgan and Simpson (1981) also described an increase in various soluble carbohydrates in response to high light in tank cultures, but these authors did not provide detailed information about the nature of these compounds. We observed similar C and floridoside responses in fronds cultured on ropes in comparison to those cultured shaded inside mesh bags in the same location during 2000 (Martinez et al. 2006). The content of the main structural carbohydrates (xylan; Lahaye et al. 1993, Deniaud et al. 2003) was not different between algae cultured on ropes or in bags, suggesting the fixation of C into reserves rather than into structural compounds (Martinez et al. 2006). All these results suggest a beneficial response from high light conditions in summer. Most of the small fronds that caused the first peak of high *P. palmata* density after the dormancy period during autumn and winter were grown from adults or from basal fragments of adults (Faes and Viejo 2003). This strategy is associated with the accumulation of C skeletons within the old thallus to allow vegetative growth in winter when irradiance is low and potentially limiting to photosynthesis (Davison et al. 1984, Rosenberg et al. 1984, Lobban and Harrison 1997). Therefore, the

development of carbohydrate reserves seems to be important for the vegetative propagation of *P. palmata*. Similar mechanisms have been shown within the life cycle of some marine perennial macrophytes in temperate areas (Davison et al. 1984, Dring 1992, Lobban and Harrison 1997, Touchette and Burkholder 2002).

As a result of this dilution process, all the nutrient-rich compounds represented a smaller fraction of the weight in sun-acclimated algae. However, the fraction of total N represented by each compound varied between sun and shade conditions, indicating different N partitioning. PE content exhibited the highest difference, constituting a small fraction of the total N in sun-acclimated algae and a large fraction in shaded algae. Nitrate showed a similar response. Both compounds have been associated with storage of N. PE was the main N reserve in *P. palmata* in a previous study (Martínez and Rico 2002). This compound exhibited the fastest response to seasonal N limitation and resupply in comparison to other N reserves (Martínez and Rico 2002). Talarico (1996) and Talarico and Maranzana (2000) recognized the storage role of PE in red seaweeds and the fact that PE is often not assembled into the antenna system, being photosynthetically

inactive. Nitrate is known to accumulate at concentrations as high as 18% of the total N in *P. palmata* (Morgan et al. 1980) and possibly represents a temporary storage pool in this species, as shown for other macroalgae (Vergara 1993, Lobban and Harrison 1997, Naldi and Wheeler 1999, Harrison and Hurd 2001). Both compounds were probably mobilized due to the higher demand of N for the synthesis of floridoside and cell metabolism in algae receiving higher radiant energy. In contrast to the response of N reserves, the other compounds were not mobilized. The soluble protein fraction is mainly composed of structural proteins and, to a lesser extent, PE and PC (Martínez and Rico 2002), explaining the minor differences between sun- and shade-acclimated algae. The second largest N pool was TIN, which is constituted by the N within cell walls and membranes and thus plays a key role in cell maintenance (Naldi and Wheeler 1999). A degradation of this pool was thus prevented, probably as a response to ensure cell integrity. Similarly, chl *a* constituted a similar fraction of total N in sun and shade conditions. This finding may be interpreted as a response to ensure the photosynthesis for C fixation. This evidence suggested a mobilization of N reserves and pools less critical for cell maintenance for N demands of C fixation and photosynthesis in sun-acclimated algae.

PC was slightly higher in shade-acclimated algae when expressed as percent of total N. Phycoerythrin rather than PC serves as an internal N source in *P. palmata* (Martínez and Rico 2002) and in many red algae (Fredriksen and Rueness 1989, Vergara 1993, Vergara and Niell 1993). Thus, PC preferential degradation for N requirements may not fully explain the degradation of this compound. Low irradiance and different spectral composition of light under canopies could be associated with photoacclimation of the photosynthesis apparatus by increasing PC and also PE (Häder and Figueroa 1997, Häder et al. 1997, Sagert and Schubert 2000, Talarico and Maranzana 2000, Figueroa et al. 2003b). However, we believe that such changes explained a minor part of the variation in phycobiliprotein content between sun- and shade-acclimated algae since the difference in PC content was small (differences between mean values: 0.3% total N) and differences in photosynthetic efficiency between sun- and shade-acclimated algae were not evident in the ETR versus PAR curves. From the overall results, we believe that an acclimation of the light antenna system was less important than the fixation of C and mobilization of N reserves.

As argued for N reserves, the lower percentage of P within the orthophosphate pool (i.e., lower % total P) in sun-acclimated algae suggested the storage and utilization of this compound. Similarly, some macroalgae store orthophosphate (Lobban and Harrison 1997), and *P. palmata* has been shown to store P when in excess (Martínez and Rico 2002).

This compound is expected to be mobilized for energy and P demands of C fixation in sun-acclimated algae, also contributing to their lower content (Cembella et al. 1984, Lobban and Harrison 1997).

In response to the upwelling, there was no marked difference in the N, P, and C metabolism, and Ekman was not included as an environmental predictor in the final model. In contrast to the clear response observed to light conditions, *P. palmata* was weakly affected by the upwelling of nutrient-rich water, contradicting our second hypothesis. The different locations of the samples suggested divergent physiological conditions of the algae, but this was not related to the nutrient metabolism response investigated in this study. A similar lack of response was observed in a close population of *Gelidium spinosum* during an upwelling in the summer of 1990. This event did not result in increased thallus N content (Rico and Fernández 1996). The prevailing nutrient-limiting conditions in the northern Spanish coast are well known (Rico and Fernández 1996, Martínez and Rico 2002), and there is no evidence supporting the idea that the upwellings alleviated this seaweed limitation. However, upwellings of longer duration, episodes of more frequent peaks, and pulses of higher intensity described in the studied area (Botas et al. 1990, González et al. 2003) may lead to different ecophysiological responses.

In conclusion, the results of this study support the importance of light as one of the main regulatory factors of N, P, and C metabolism. *P. palmata* showed a marked metabolic adjustment in response to sun and shade light conditions affecting the partitioning of N and P. The storage of C to benefit from high light availability in sun conditions, before the onset of dormancy, was shown to be the driving force for the adjustment of algal metabolism at the end of summer. The ability to store N and P in autumn and winter was previously identified as very important for the perennial strategy of this species (Martínez and Rico 2002). The development of major resource reserves allows *P. palmata* to cope with seasonal light and nutrient limitation periods. Nutrient storage responses have been suggested to be an advantage in perennial or long-lived species (Pfister and Van Alstyne 2003) and increase potential competitive ability (see review in Carpenter 1990).

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