SEASONAL VARIATION OF P CONTENT AND MAJOR N POOLS IN *PALMARIA PALMATA* (RHODOPHYTA)[1]

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The annual variation of major nitrogen pools, phosphorus, carbon, ash, and thallus water content in relation to seasonal environmental changes was studied in two northern Spanish populations of the edible seaweed *Palmaria palmata* (Linnaeus) Kuntze. Observed patterns were investigated using Spearman rank order correlation coefficients. There were significant relationships between thallus nutrient content and nitrate and orthophosphate seawater concentration, irradiance, temperature, and wave force. The highest levels of total N and P and nitrogenous compounds were observed during autumn and winter because the thallus stored N- and P-rich compounds in response to high nutrient seawater concentration when growth was limited by low light and temperature. Phycoerythrin and other proteins were the main N reserves. Thallus P content was higher in algae from the eutrophic site. During spring, reduced N and P thallus content and increased ash, water, and C content were observed in the growing fronds. N and P seawater concentrations were undetectable during summer when nutrient reserves were low and growth was reduced and eventually suppressed, suggesting nutrient limiting conditions. *Palmaria palmata* clearly could take advantage of elevated N and P concentrations to create storage reserves in winter to support early summer growth. This storage response reduced the dependence of algal nutrition on the external nutrient supply and supports the use of pulse fertilization to diminish summer nutrient limitation of cultured algae.

Key index words: ash; nitrogen; *Palmaria palmata*; phosphorus; phycoerythrin, phycocyanin; protein; seasonal; storage

Abbreviations: PC, phycocyanin; PE, phycoerythrin

INTRODUCTION

Nitrogen and phosphorus frequently have been suggested to limit marine macroalgal growth in temperate coastal environments (Hanisak 1979, Conolly and Drew 1985a,b). Nutrient concentrations in algal tissues have been widely used to indicate nutrient dep-

rivation (Duarte 1992, Hanisak 1979). Many seaweeds have evolved physiological responses to relieve this limitation by storing nutrient-rich compounds when nutrient supply is high. Rapid growth rates are sustained for some time by declining reserves when the nutrient availability is low. In this way, seawater N and P availability together with the storage capacity of the species determines the duration of its growth period (Rosenberg and Ramus 1982, Rosenberg et al. 1984, McGlathery et al. 1996, McGlathery and Pedersen 1999).

Besides nutrient seawater availability, the intertidal ecosystem is subjected to the seasonal variation of many environmental factors such as wave force or day length. Morgan and Simpson (1981a,c) suggested that apart from seawater N concentration, irradiance and temperature were also involved in N storage in *Palmaria palmata* (Linnaeus) Kuntze, but their research was restricted to greenhouse cultivation conditions. Our study is the first attempt to describe the seasonal variation of major nutrient pools of field populations and to relate them to the environmental variables that dominate the intertidal. Our aims are to single out which environmental variables are most important in driving *P. palmata* nutrient concentrations and which nutrient-rich compounds may be acting as nutrient reserves to supply growth demands when seawater nutrients become limited, determining the growth cycle of *P. palmata*.

In this species, protein is the major N pool and constitutes a large fraction of the dry weight of the algae (Morgan et al. 1980), but nothing is known about its storage role. Morgan and Simpson (1981a) predicted the existence of a phycobiliprotein-N storage pool in *P. palmata*, but they did not provide direct measurements of these compounds. Other macroalgae are able to store P (see Lobban and Harrison 1997, and references therein), but this is unknown for *P. palmata*. For mariculture purposes, the ability to store nutrients would reduce the need of fertilization of cultured algae and argues against continuous enrichment, which is more expensive than pulse inputs.

MATERIALS AND METHODS

Sample collection. The two populations studied were approximately 50 km apart along the northern coast of Spain: *Concha de Artedo* has relatively little human impact (43°34′N, 6°11′W), whereas *Playa de Estaño* is located less than 7 km from a large city (43°33′N, 5°35′W). *Palmaria palmata* is epiphytic on *Cystoseira baccata* (Gmelin) Silva at the intertidal fringe sampled. Eight fronds were collected monthly from June 1998 to July 1999, except in December 1998.

Biochemical analysis. Samples were rinsed in deionized water and epiphytes removed before the analysis. The protein fraction was extracted in alkali, and protein content was measured by Lowry's method using albumin solution from bovine serum (Fluka, Sigma-Aldrich, Seelze, Germany) as standard (Harrison and Thomas 1988). Phycocyanin (PC) and phycoerythrin (PE) contents were determined using trichromatic equations (Beer and Eshel 1985) after extraction in phosphate buffer (Evans 1988). P thallus extraction was done by alkaline persulfate digestion (Ameel et al. 1993), and orthophosphate was measured in the digested sample as described below. C and N thallus content were measured using a CNH Elemental Analyser (Perkin Elmer Analytical Instruments, Shelton, CT, USA). Ash and water contents were determined after drying at $60°$ C and incinerating for 2 h at $450°$ C. Biochemical contents were expressed as a fraction of the ash-free dry weight.

Growth data. Thallus length was measured on individuals from the Estaño intertidal population monthly from August 1999 to August 2000 except during April 2000. Recruits that settled on the tips of the *C. baccata* branches were not measured because the apexes were broken in short time, causing their loosing. Frond mortality and loss of tags were compensated for by tagging new individuals. From these data the specific growth rate (as $\%\cdot d^{-1}$) was calculated as described in Hunt (1990).

Reproductive phenology. Palmaria palmata exhibits a biphasic phenological cycle with haploid (male and microscopic female) and diploid (tetrasporophyte) phases (van der Meer and Todd 1980). Mature males were very rare (7%) in the studied area. No clear temporal trend was observed in the maturation of the tetrasporophytes, individuals forming or releasing spores, together with those that have released all the spores were collected from January 1999 to May 1999.

Environmental variables. Two seawater samples were collected monthly at three sites per shore, transported in an isotherm bag ($<4°$ C, in darkness), and kept at $-20°$ C less than 3 months before analyses. An additional sample was taken 2 weeks later in June, July, September, October, and December 1998 and February and April 1999 in Artedo and in February 1999 in Estaño. 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 (Technicon Instruments Corp., New York, USA, Industrial Method no. 158-71 W/A, Ireland). NO_2^- concentrations never exceeded 0.5 μM and were negligible in comparison with NO_3^-; therefore the concentration of both nutrients was summed. The Meteorological Station at Oviedo kindly provided the incident solar energy data (irradiance). This station is located between 30 and 35 km away from the shores. Theoretical surface day length (photoperiod) was calculated as described in Kirk (1983). Emersion time between tides was calculated as detailed in Arrontes (1987). Temperature was measured on the two shores by a continuous data logger (ONSET Computer Corp., Pocasset, MA, USA) fixed next to the populations. Only temperature values during high tide were used in the analyses. The maximum wave significant height was used to describe wave force. This variable is defined as the mean wave height of the third highest waves measured during a 20-min period. Wave height was measured by a scalar buoy (Mod WaveRider, DATAWELL, Heerhugowara, The Netherlands, depth 43 m) that was placed approximately 7 km away from Estaño. The wave force data were corrected for differences in exposure between the two shores by multiplying significant height values by an exposure coefficient inferred for each shore. These coefficients were calculated by propagating the most usual wind and wave regimes of the northern Spanish coast to the two shores using a wave propagation model (Oluca-MC) provided by the *Grupo de Ingeniería Oceanográfica y de Costas* from the University of Cantabria. This model takes into account shoaling, refraction, diffraction, and breaking processes.

Statistical analyses. To determine seasonal trends, irradiance, emersion, and wave force data were smoothed by the moving average method computed from three values (Legendre and Legendre 1998). Spearman rank order correlation coefficients (Spearman R) between the environmental factors and the algal tissue constituents were calculated to asses the strength of the relationships. This non-parametric correlation coefficient was used because it was not assumed that variables conform to the multinormal distribution (Legendre and Legendre 1998). The significance level for each correlation coefficient was calculated by sequential Bonferroni test applied to the relationship of each tissue constituent with all the environmental factors (Rice 1989); row-wide significance level was set at 0.05 (Table 1).

RESULTS

Seasonal environmental variation. Low levels of NO_3^- + NO_2^- and HPO_4^{2-} seawater concentrations were recorded during spring and summer, and maximum values were measured from November to February on the two shores (Fig. 1, A and B). Remarkably, HPO_4^{2-} concentration was significantly higher in Estaño than in Artedo (Fig. 1B). In contrast to other inorganic nutrients, NH_4^+ did not show a clear seasonal trend (Fig. 1A). High nutrient levels during winter coincided with minimum temperature and irradiance values, short day length, shorter emersion periods, and larger waves (Fig. 1, C–F). The summer conditions on the two shores were those typical for temperate zones: maximum temperatures and irradiances matched with nutrient seawater concentrations below detectable values and little wave action (Fig. 1).

Seasonal variation of Palmaria palmata *compounds.* The fronds had high pigment content from October/November to April. The pigment PE showed a more marked increase than PC toward its November maximum (higher slope in Fig. 2A). Total protein, N, and P thallus content displayed very similar patterns, reaching maximal values from January to April. Fronds from Estaño had higher P content (Fig. 2, B and C). All N pools and P content declined after May and showed lowest values during summer (Fig. 2, A–C) Phycobiliproteins showed a more pronounced decrease than total N and protein (higher slope in Fig. 2, A and B). The thallus C content displayed a minor seasonal trend, showing a slight increase and reaching its annual maximum in early spring (Fig. 2C). Ash and water thallus content both showed similar patterns with a marked increase up to March/April, high values during spring, and a clear reduction after June (Fig. 2D).

Spearman rank order correlation coefficients. All *P. palmata* compounds were related to at least one environmental variable except for the C content (Table 1). Seawater nutrient (NO_3^- + NO_2^- and HPO_4^{2-}) concentration, irradiance, temperature, day length, and wave force showed significant correlation coefficients with at least one compound. In contrast, NH_4^+ seawater concentration and emersion time between tides were not related to any measured algal constituent (Table 1). PE was the only N-pool that showed a significant positive correlation with NO_3^- + NO_2^- on both shores. PE was also higher with greater wave force and lower irradiance in Artedo. In contrast, PC, protein, and total N content were unrelated to seawater nutrient concentrations and were negatively related to temperature (Table 1). The thallus P content

TABLE 1. Spearman rank order correlation coefficients between algal compounds and environmental factors ($n = 12$).

	$NO_3^- + NO_2^-$	NH_4^+	HPO_4^{2-}	Temperature	Irradiance	Photoperiod	Emersion	Waves
Artedo								
PE	0.720[a]	−0.182	0.601	−0.629	−0.776[a]	−0.538	0.290	0.762[a]
PC	0.727	−0.182	0.671	−0.790[a]	−0.497	−0.210	0.022	0.685
Total protein	0.427	−0.168	0.469	−0.804[a]	−0.385	0.252	−0.232	0.329
N	0.601	−0.343	0.503	−0.895[a]	−0.175	0.098	−0.007	0.259
P	0.853[a]	−0.168	0.734[a]	−0.818[a]	−0.524	−0.336	0.029	0.622
Ash	−0.056	−0.189	−0.049	−0.441	0.336	0.790[a]	−0.261	−0.161
Water	−0.182	−0.308	−0.161	−0.161	0.266	0.804[a]	−0.363	−0.245
C	0.119	−0.119	0.175	−0.378	0.168	0.503	−0.131	0.231
Estaño								
PE	0.741[a]	0.448	0.580	−0.517	−0.650	−0.587	−0.157	0.678
PC	0.601	0.497	0.392	−0.727	−0.601	−0.098	0.045	0.517
Total protein	0.322	0.147	0.126	−0.741[a]	−0.385	0.287	0.097	0.259
N	0.622	0.287	0.441	−0.867[a]	−0.622	0.000	−0.030	0.476
P	0.524	0.266	0.294	−0.797[a]	−0.538	0.063	−0.153	0.434
Ash	−0.350	−0.245	−0.420	−0.308	0.182	0.909[a]	0.164	−0.427
Water	−0.510	−0.238	−0.566	−0.147	0.280	0.909[a]	0.082	−0.378
C	−0.042	−0.077	−0.273	−0.476	−0.105	0.594	0.007	−0.105

[a] Significant values.

was positively related to seawater HPO_4^{2-} and $NO_3^- + NO_2^-$ and inversely related to temperature in the Artedo population, but it was only related to temperature in Estaño. Ash and thallus water content were positively related to day length on both shores (Table 1).

Protein content and reproductive phenology. Total protein thallus content showed no clear trend in relation to reproductive status of the tetrasporophytes (one-way analysis of variance, $F_{2,42} = 1.32$, $P = 0.28$). Algae from each shore showed no distinct trend, and data were pooled. Protein content showed no clear relation with collection date during the fertile season (Spearman R = 0.283, $P = 0.06$, $n = 45$).

Growth cycle. Net growth rates were severely reduced during summer and finally suppressed during late summer and autumn corresponding to the frond decay and senescence of the algae (Table 2). A small proportion of the thallus persisted over winter but showed no evidence of growth. Recruits and new fronds produced from old thalli began growing in March, and growth rates peaked in June (Table 2).

DISCUSSION

The annual physiological cycle of *P. palmata* was regulated by environmental factors and thus exhibited strong synchronization with the seasons in the two populations studied. The most important environmental factors driving thallus nutrient content were seawater nutrient concentrations, temperature, irradiance, and wave force. As in most coastal waters, NO_3^- was the most abundant N source and the main inorganic nutrient affecting algal nutrition (Chapman and Craige 1977). In contrast, seawater NH_4^+ concentration was of little importance due to the reduced seasonal variation of this compound in seawater. Emersion showed a clear seasonal trend but did not affect algal compounds, probably because of the low intertidal nature of this species.

Summer environmental conditions on both shores largely affected the physiological status of *P. palmata*.

Thallus N and P content both exhibited a decrease during spring and remained low for most of the summer, suggesting N and P limitation. This agrees with the growth reduction of rope-cultured algae in Artedo during summer 2000. Fertilization (+N+P) of the cultured algae during late July and August resulted in increased growth rates similar to those observed during late May and June (growth rate as mg fresh weight·alga⁻¹·d⁻¹, mean ± SE, $n = 8$: May–June = 25.8 ± 8.12, July–August = 8.3 ± 5.91, July–August + N + P = 23.4 ± 12.64 [Rico et al., unpublished data]). Seawater NH_4^+ concentration in summer was generally lower than the rest of the year but remained relatively high, and NH_4^+ could be important to algal nutrition, contributing to the maintenance of growth at low tissue nutrient content. However, the decreasing growth rates and the strong dependence of *P. palmata* growth on nutrient enrichment in summer suggested that nutrient demands for growth were greater than external N supply. Under such limiting conditions the stored material was diverted to growth, contributing to the dilution of all algal nutrient pools by the addition of new biomass (McGlathery et al. 1996, McGlathery and Pedersen 1999). Summer nutrient limitation seemed a general feature to many red algae in northern Spanish coast. Other species such as *Gelidium latifolium* (Greville) Bornet were N limited due to the reduced N external supply and increased algal N demands by high irradiances during summer in a close locality (Rico and Fernández 1996).

Among the N pools, PE showed the fastest increase during autumn and remained high during winter as sufficient nutrients were available at both sites, suggesting that nutrient accumulation exceeded the metabolic requirements. Low light and temperature have been suggested to limit winter growth of various species and to promote the development of nutrient reserves (Rosenberg and Ramus 1982, Rosenberg et al. 1984, Lobban and Harrison 1997). The rise in thallus N content of *P. palmata* under high NO_3^- and low
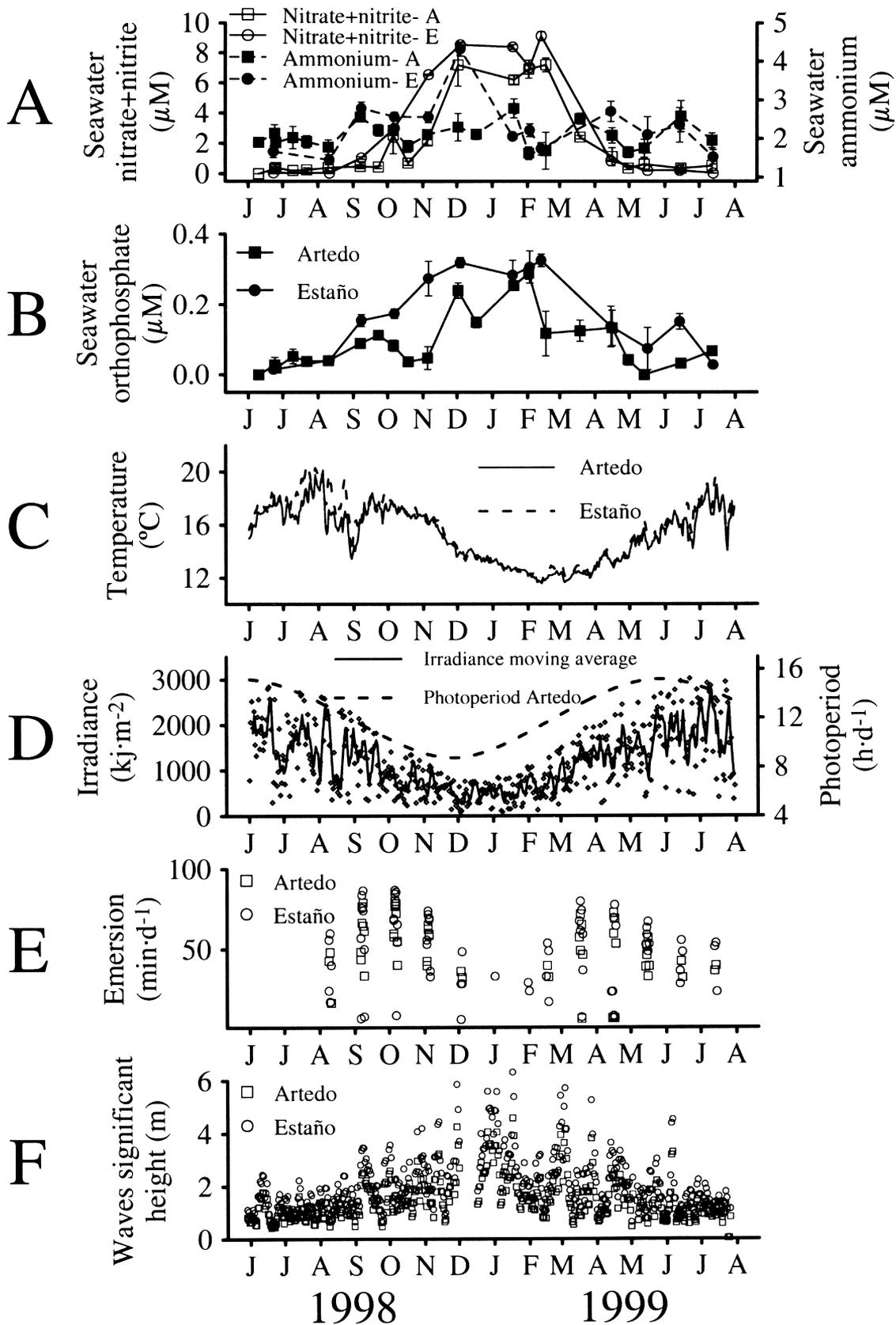
FIG. 1. Seasonal trend of environmental variables (mean of three replicates and SE when applicable). Ticks on the *x* axis correspond to the first day of each month. Data from the Artedo population are marked as Artedo or A and data from Estaño as Estaño or E.
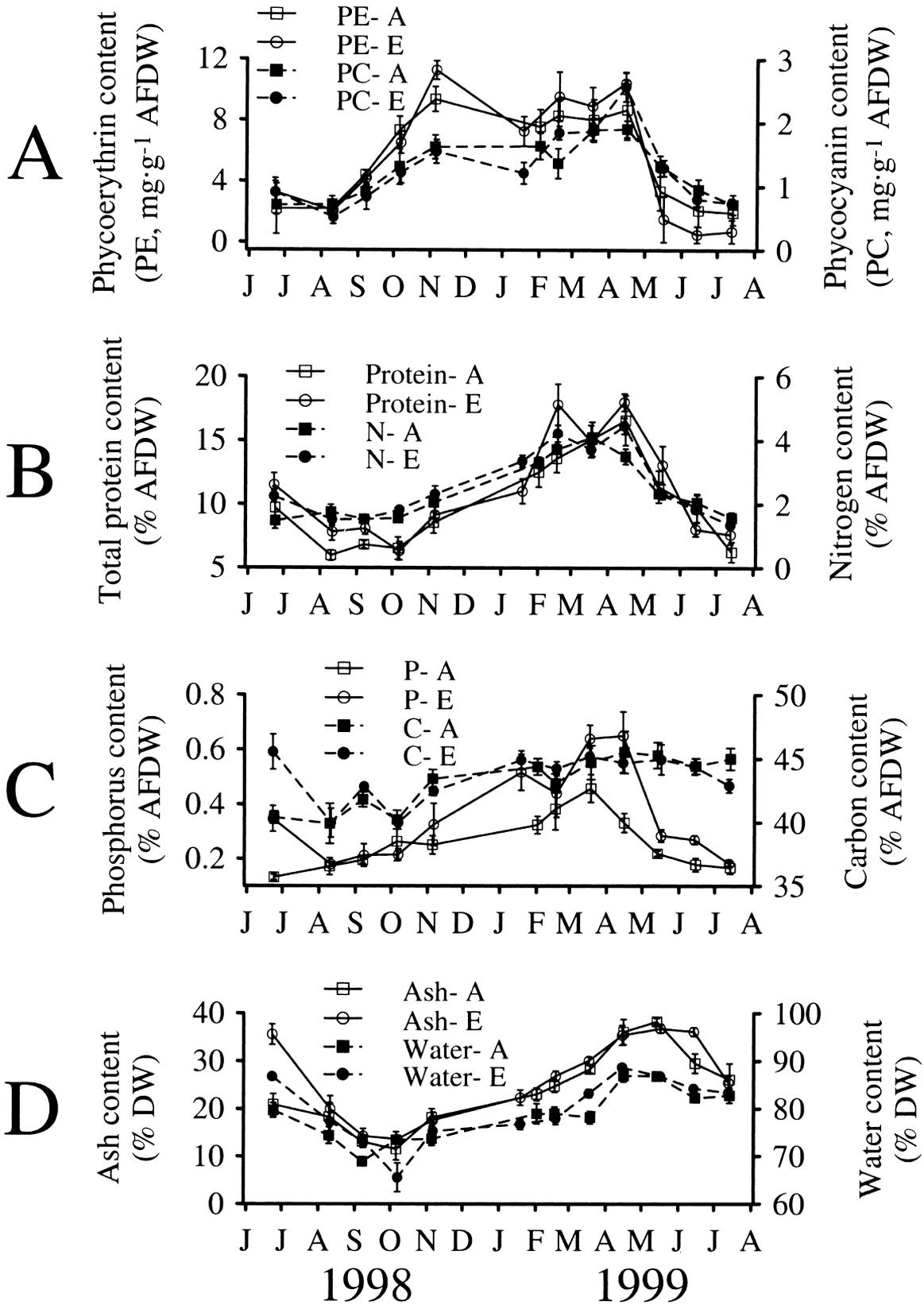
FIG. 2.   Seasonal trend of algal compounds (mean of eight replicates and SE). Ticks on the *x* axis correspond to the first day of each month. Data from the Artedo population are marked with A and data from Estaño with E.

TABLE 2. Growth cycle of the intertidal Estaño population.

Period	Specific growth rate		
	mean %·d^{-1}	SE	n
August 1999 to March 2000	−0.28	0.004	416
March–June 2000	0.70	0.009	165
June–August 2000	0.48	0.011	81

light and temperature conditions has been previously observed in culture by Morgan and Simpson (1981a,b). These authors pointed out that the deep red pigmentation of well-nourished fronds may be due to the accumulation of N as PE, which would support future growth under N deprivation conditions. The PE-N pool represented up to 8.3% (mean value of eight fronds) of the total thallus N, which constituted a significant portion (12.4%) of the protein-N pool (conversions as in Naldi and Wheeler 1999). PE accumulation was negatively related to irradiance in the Artedo population, and although not statistically significant, the same trend was observed in Estaño. This pigment protein is a light-harvesting antenna component involved in light absorption and photoacclimation to low irradiance (Fredriksen and Rueness 1989). Its accumulation would improve photosynthesis, and it is expected to enhance growth rate under nutrient sufficient conditions (Rosenberg and Ramus 1982). However, autumn algae showed signs of senescence and were not growing. The uncoupled trend of growth and PE accumulation supports Morgan and Simpson's (1981a,b,c) hypothesis that PE has a storage role. The relationship between wave force and PE suggests that increasing water turbulence further facilitated N uptake and accumulation due to the thinning of the boundary layer on the algal surface (Lobban and Harrison 1997).

PC is the second most important N-rich compound that accumulated during autumn and winter. It showed a weaker relationship with the environment and represented a small proportion of the total cell N (up to 1.3%, mean value) and of the protein-N (up to 2%, mean value). These findings suggests that PC is not a major N storage compound in *P. palmata*. Similarly, Fredriksen and Rueness (1989) concluded that PE rather than PC served as internal N source of the red seaweed *G. latifolium*.

Protein-N represented between 55% and 93% of the total cell N (mean value, conversions as in Naldi and Wheeler 1999) and accounted for as much as 20.5% of the ash-free dry weight. This explained the coupled N and protein seasonal variation and their similar response to environmental factors. Winter harvestable material had the highest nutritional quality in terms of protein content. As with PE, the uncoupling between the protein accumulation and growth suggests its storage role. In contrast to PE, the protein pool showed a longer time lag between seawater nutrient and turbulence increase, and pool en-

largement, and is thus expected to operate on a larger time scale. Correspondingly, protein depletion was less pronounced than that of PE during spring. Because phycobiliproteins never exceed 14.1% (mean value) of the total protein, the proportion of unknown protein in this study was large. This explains the divergent response between protein and PE. The existence of additional storage pools has been observed in other red seaweeds where PE was an important source of N during the early phase but other proteins and amino acids were the major N storage pools (Bird et al. 1982, Rico and Fernández 1996). In *P. palmata*, the storage role of the amino acid pool seems of minor importance over the annual cycle because it represents a small proportion of the N total content (0.25% in Morgan et al. 1980). Furthermore, winter protein accumulation coincided with the reproduction of *P. palmata* in the studied area. This would suggest the accumulation of protein in association with the development and maturation of reproductive structures. However, this was not the case as proteins did not accumulate differentially in the tetrasporophytes forming or bearing spores. In a similar way, Brenchley et al. (1997) found similar N content in receptacle tissue to that of vegetative tissue in the brown seaweed *Fucus serratus* Linnaeus.

Larger P storage pools developed under the eutrophic conditions causing reduced seaweed dependence on external supply and explaining the weak relationship between P tissue content and HPO_4^{2-} in Estaño. Similarly, increased P reserves during winter in response to eutrophication in the northeast coast of Fife (Scotland) were observed in two laminarian species (Conolly and Drew 1985a). Moreover, HPO_4^{2-} in Estaño was not related to any algal constituent, suggesting relaxed dependence of this population's physiology on P supply. Remarkably, temperature was the only environmental factor that was related to P content in both populations. As described for N pools, under low temperature and high nutrient regimes the rate of P accumulation exceeded growth requirements and P was stored.

The highest ash and water thallus content was observed during spring in the growing algae, suggesting their accumulation in association with algal growth. This agrees with the strong relationship between these compounds and day length, which determines the growth pattern. As observed in our study, Makarov et al. (1999) found *P. palmata* from the Barents Sea growing mainly in spring corresponding with increasing day length, and further related growth rate to different photoperiods in laboratory experiments. Morgan and Simpson (1981a) observed that growing tips of *P. palmata* displayed higher ash and water content than basal portions. However, these authors found N and ash content of the thallus positively related under saturating irradiance, low temperatures, and a variety of external NO_3^- conditions and suggested that ash increase was due to the accumulation of inorganic cat-

ions to ensure the ionic balance of the cell when NO_3^- reduction takes place, rather than directly associated to growth. In the studied populations, ash and total N content increased with a significant time lag: maximum ash was retarded in relation to the N content peak. Moreover, the response of these compounds to environmental factors were clearly different, suggesting that the accumulation of ash is not associated with NO_3^- reduction.

Thallus C content was slightly higher in spring, suggesting the synthesis of C-rich biomass in the growing fronds. Its limited seasonal variation and its independence from environmental factors further reflected the large amount of structural C in benthic macroalgae (Duarte 1992).

Palmaria palmata conforms within the "pseudoperennial life form type," following Sears and Wilce's (1975) classification. The small proportion of the thallus that persists over winter allows the species to take advantage of elevated N and P concentrations and to developing reserves that are later diverted into growth. PE and other proteins were the main N pools responsible for this storage response. Storage of P was greater in the area affected by urban waste. The storage capability has been pointed as a competitive advantage for utilizing nutrients in annual "opportunistic" algae (Rosenberg and Ramus 1982, Rosenberg et al. 1984). For mariculture purposes, *P. palmata* storage capacity supports the use of pulse fertilization to reduce summer nutrient limitation rather than more expensive continuous enrichment that has been proved to increase epiphyte growth in greenhouse cultures of this species (Morgan et al. 1980).

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