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Seasonal cycle of the gametophytic form of *Porphyra umbilicalis*: nitrogen and carbon

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ABSTRACT: Seasonal variations in environmental factors (irradiance, seawater concentration of NO_3^-, NO_2^- and NH_4^+, temperature) and in physiological variables of *Porphyra umbilicalis* (L.) Kützing (intracellular NO_3^-, NO_2^- and NH_4^+ concentrations, nitrate reductase activity, amino acids, soluble proteins, total C, total N, C:N:P ratio) were determined during winter 1991–92 at a site in Lagos (Málaga, southern coast of Spain). In the western Mediterranean Sea, the leafy thallus of *P. umbilicalis* occurs only during winter (mid-December to mid-March). Maximal % cover (80%) was reached in the first part of February. Conditions of low temperature (14.7 ± 0.6 °C), short days (10 h daylight) and low global solar irradiance (2.5 to 3.4 kWh m^{-2}) prevailed during the period of increase in % cover. The period of decrease in % cover was characterized by long days (12 h), higher irradiance (3.4 to 5 kWh m^{-2}), and slightly higher water temperature (15.5 ± 1.34 °C). NO_3^- concentration in seawater ($[NO_3^-]_e$) at the study site ranged from 1 to 6.7 µM, $[NO_2^-]_e$ was always lower than 1 µM, and $[NH_4^+]_e$, the largest source of inorganic nitrogen, ranged from 6.8 to 17.6 µM. Intracellular concentration of NO_3^- ($[NO_3^-]_i$) ranged from 2.8 to 51.3 mmol l^{-1} cell water (11.7 to 210.6 µmol g^{-1} dry wt), which represents a concentration factor of up to 4.2×10^4 with respect to the seawater. $[NO_2^-]_i$ ranged from 0.2 to 0.7 mmol l^{-1} cell water and $[NH_4^+]_i$ from 0.016 to 2.2 mmol l^{-1} cell water. Nitrate reductase (NR) activity varied widely during the winter cycle (between 1.2 and 9.4 µmol NO_2^- g^{-1} fresh wt h^{-1}). It was found to be a very sensitive parameter for indicating when NO_3^- is being used as the N source for growth. Therefore, low NR activity levels coincided with the highest $[NO_3^-]_i$ and were more or less independent of $[NO_3^-]_e$ when the alga was using NH_4^+ as N source. A large increase in NR activity was detected when $[NO_3^-]_i$ decreased sharply. Cysteine was the predominant amino acid, amounting to between 83.5 and 97.2% of total amino acid content (5 to 28.3 µmol g^{-1} fresh wt). No correlation was found between total amino acid and soluble protein content. Soluble protein content showed the same seasonal variation as total N and total C, and varied inversely to the C:N ratio. Winter changes in the Redfield ratio, from 258:20:1 (24 December 1991) to 495:38:1 (16 March 1992) (in atoms), suggest that primary production of *P. umbilicalis* is limited by P rather than N on this coast.

INTRODUCTION

Porphyra umbilicalis (Rhodophyta) occurs in 2 very easily distinguished phases, the leafy, haploid thallus, which is present in winter, and the so-called conchocelis stage, a filamentous form, present in summer (Drew 1949). This study focuses on the gametophyte, the leafy thallus. On the southern coast of Spain the gametophytic form of *P. umbilicalis* is usually present from December to March. In *Porphyra tenera*, the conchocelis stage develops conchosporangia under conditions of short daylength (Dring 1967), and in *Porphyra miniata* conchospores are released under low-temperature conditions (Chen et al. 1970). The germination of these conchospores produces the leafy thallus in the

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cold season. However, less information exists on the environmental factors determining the disappearance of the gametophytic phase at the end of winter. There is also little information on the circumstances under which the thallus produces monospores, which once released and fixed develop new leafy thalli, or α-spores and β-spores (carpospores), which are involved in the generation of the conchocelis stage. Besides identifying which environmental factors control the transition between both phases, it is also of interest to identify the environmental factor that controls *Porphyra* primary production.

Nitrogen and phosphorus have been reported to limit macroalgal primary production (Hanisak 1983, Lapointe & O'Connell 1989). Hence we focused our attention on both P and N metabolism. In this paper we concentrate on several relevant variables related to N metabolism; P metabolism is treated elsewhere (Hernández et al. 1993). Here we measure a number of environmental and physiological parameters for *Porphyra umbilicalis*: external and intracellular concentration of NO_3^-, NO_2^- and NH_4^+; nitrate reductase activity; protein and amino acid content; and C:N:P ratio. Our purposes were to: (1) study the annual cycle of *P. umbilicalis* in the Mediterranean Sea (south of Spain) from the standpoint of N metabolism; and (2) identify the relative importance of N and P in promoting seasonal growth of the leafy thallus, and ultimately investigate the environmental or biochemical factor controlling the seasonal disappearance of *P. umbilicalis*.

MATERIALS AND METHODS

Study site and sampling methods. *Porphyra umbilicalis* was studied and collected for experiments from a flat rock (upper intertidal zone) in Lagos (Málaga, south of Spain) between December 1991 and March 1992. Plants (ca 3 g fresh weight) were always collected at the same time (11:30 to 12:00 h, solar time) from the same area (1.5 × 0.5 m^2), selected at the beginning of the study. Immediately after collection, samples of fresh tissue were wrapped in aluminium foil and immersed in liquid nitrogen. Samples of seawater received the same treatment. Once in the laboratory all samples were maintained at -20 °C until analysis. The percentage of the sampling area covered by *P. umbilicalis* was used to estimate its abundance during the cycle. Percentage cover (% cover) was determined by a modification of the linear transect method: a ruler of 30 cm was thrown 10 times on the selected area, and the mean ratio between the length of the ruler on *P. umbilicalis* and the length without *P. umbilicalis* was considered a good estimation of percentage cover. Sea water and rock temperatures were measured with a liquid-in-glass thermometer.

Data presented in this study are the means of 2 to 3 replicates (see figure legends). Random plant samples were obtained from the entire selected area and therefore included many different individuals. Hence error bars represent the addition of 2 components, the variance due to the methodological procedure (chemical and biochemical analysis) plus the intraspecific variability of *P. umbilicalis* in the selected area. Since the selected area was small, it can be assumed that environmental factors were spatially homogeneous.

Determination of external and intracellular NO_3^-, NO_2^- and NH_4^+ concentrations. Nitrate (Wood et al. 1967), NO_2^- (Shinn 1941) and NH_4^+ (Slawyk & MacIsaac 1972) concentrations were determined in seawater samples by means of a continuous flow analytical system (BRAN+LUEBBE TRAACS 800). Intracellular NO_3^-, NO_2^- and NH_4^+ concentrations were determined as follows. Samples of frozen tissue were dried (70 °C, 24 h) and ground to a homogeneous powder, and 0.035 g of the dry powder was dissolved in 40 ml of deionized water. After 1 h of continuous shaking, the mixture was filtered (Whatman GF-C), and NO_3^-, NO_2^- and NH_4^+ determined in the filtrate by the same procedure referred to above (Corzo & Niell 1992a).

Amino acids. ***Preparation of samples:*** Samples of frozen tissue were homogenized in methanol (80 %). Precipitated proteins were removed by centrifugation (19 000 × *g*, 15 min). The pellet containing the proteins was resuspended in 80 % methanol to wash away possible remaining amino acid and centrifuged in the same conditions. To eliminate pigments, supernatants were passed through a solid phase extraction column (C18; Supelco, Bellefonte, USA). Finally, the extract was concentrated to 0.1 ml under reduced pressure (Speedvac concentrator, Salvant Instruments, USA).

Derivatization procedure and HPLC: To obtain the dansyl derivatives of the amino acid, 200 µl of 40 mM CO_3Li and 200 µl of 20 mM dansyl chloride were added to 0.1 ml of each extract and, after 1 h at room temperature (23 °C), the mixture was filtered (0.22 µm, Millex-GU13, Millipore) (modified from Márquez et al. 1986). A Hewlett-Packard (HP-1090) HPLC equipped with a Rheodyne injection valve, a 20 µl sample loop and a Supelcosil LC-18 column (4.6 × 15 cm) (Supelco, Bellafonte, USA) was used for the analysis. Derivatives were separated using the following mixture: 0.008 % triethylamine + 0.6 % acetic acid (polar phase) and methanol (nonpolar phase). Derivatives were measured at 254 nm. Individual amino acids were identified by comparison of elution time with an external standard (Sigma) and concentrations were calculated from the peak area. Two analyses were carried out for every sample.

Soluble proteins, pigments and C:N:P ratio. Soluble proteins and phycobiliproteins were extracted by grinding the samples of fresh tissue (frozen in liquid nitrogen; 0.15 g) in 5 ml of 0.1 M Na^+-phosphate, 4 mM EDTA, pH 6.5. Debris was removed by centrifugation (10 000 × *g*, 20 min, 4 °C). Phycoerythrin and phycocyanin were determined spectrophotometrically (Beckman DU-7) in the supernatant (Beer & Eshel 1985). Soluble proteins were determined in an aliquot of the same supernatant, as follows: 3 ml Coomassie Blue was added to 0.1 ml of sample, and extinction at 595 nm was measured (Beckman DU-7) after 5 min of incubation at room temperature (Bradford 1976). Concentrations were calculated by means of standards made with bovine serum albumin (Sigma).

Total C and total N were determined in triplicate on samples of dry ground tissue using a Perkin Elmer 240 CNH elemental analyser. Total phosphorus was determined by acid digestion in triplicate samples of dry ground tissue (Sommer & Nelson 1972).

Nitrate reductase activity. Determination of nitrate reductase (NR) activity requires a number of technical procedures difficult to implement in the field. Therefore the specimens have to be brought to the laboratory while ensuring that NR activity is affected as little as possible. In higher plants it has been shown that several cycles of freezing (liquid nitrogen) and thawing increased NR activity or left it unaffected when determined by the *in situ* method (Rhodes & Stewart 1974, Mauriño et al. 1985). Taking advantage of this fact, we collected samples of fresh tissue and immediately froze them in liquid nitrogen. Once in the laboratory the samples were thawed by immersion in seawater (2 min, 30 °C). After this point the protocol basically proceeded identically to the standard for fresh tissue (Corzo & Niell 1991a). Immediately after thawing, 0.15 g of tissue was introduced into test tubes containing 5 ml of assay medium (30 mM KNO_3, 0.01 mM glucose, 0.1 % 1-propanol, 0.5 mM Na-EDTA, 3 mM NADH, 0.1 M phosphate buffer, pH 8), which had previously been flushed with N_2 for 2 min. As soon as the alga was introduced, the test tubes were flushed with N_2 for an additional 2 min and then were immediately sealed and incubated in the dark for 30 min (10 °C). At the end of that time, 1 ml of assay medium was taken from the tubes. NADH was removed from samples by adding 0.5 ml of an activated charcoal suspension (0.83 % w/v). After strong stirring the samples were centrifuged (2000 × *g*, 10 min), and 1 ml of the supernatant was taken and assayed for NO_2^- (Snell & Snell 1949). Two independent NR assays were run for each sample.

The optimal assay temperature for frozen and thawed tissue was found to be 10 °C, while that for fresh tissue was 20 °C. NR activity at 10 °C was the same in both frozen/thawed plants and non-treated plants (results not shown).

Global solar irradiance. Global solar irradiance is defined as the total energy received through a particular band of wavelengths on a horizontal surface during a time interval. Data used in this paper are monthly means, based on data obtained daily for the period 1975 to 1983 by the Instituto Nacional de Meteorología. Data were collected at the station El Rompedizo (36° 40' N, 4° 29' W; 7 m above sea level; Málaga). The sampling site was located 30 km from this meteorological station but within the same interval (0.5 units) of global solar radiation (Font 1984). A thermoelectric pyranometer CN-5 (Kipp-Zonen) with a sensitive window of 300 to 2500 nm, connected to a continuous recorder (LS 144K; Camille Bauer), was used to measure global solar irradiance. Only about 45 % of the energy collected through the window can be used for photosynthesis.

RESULTS AND DISCUSSION

Cover and irradiance

The winter cycle of gametophytic *Porphyra umbilicalis* in the western Mediterranean Sea begins in December and extends to the middle of March. The cycle presented in this paper was considered typical, based on a visual assessment of cover relative to other years. The percentage cover in winter 1991–92 increased up to 80 % in the middle of the cycle (7 to 14 February), decreasing gradually after this date to only 8 % in the middle of March (Fig. 1A). Increase in biomass is a better indication of growth than increase in % cover, but determination of biomasss is destructive. Despite the limitations of the % cover method, it is still sufficiently reliable as a semiquantitative estimation of population growth. Water temperature and daylength have been reported to be the major environmental factors controlling the seasonal cycle of *Porphyra* spp. Conchospore production is stimulated by short days (Kurogi & Sato 1962, Dring 1967) and conchospore liberation by reduced temperature (Kurogi & Akiyama 1966). On the other hand, the blade-like thallus grows well in winter conditions (Zeng 1984), and the carpospores, which germinate into the conchocelis phase, are produced only during long days (Iwasaki 1961, Suto 1972). Along our coast the presence of *P. umbilicalis* thalli is limited to winter, and therefore conditions of low temperature (15 °C), short days (10 h daylight) and low irradiance (2.5 to 3.4 kWh m^{-2}) prevailed during the period in which % cover was steadily increasing (Fig. 1). Seawater temperature remained almost constant during the sampling period (Fig. 1B), which

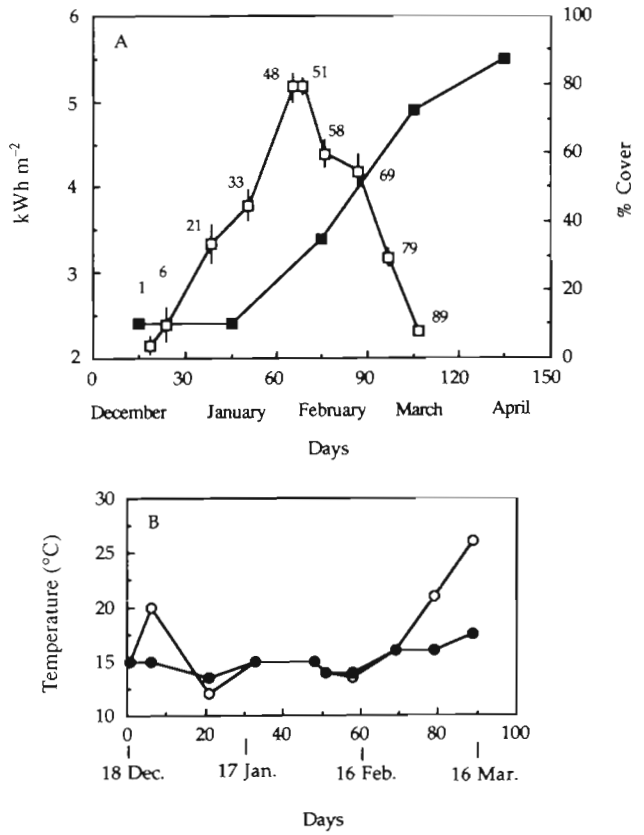

Fig. 1. (A) Percentage cover of *Porphyra umbilicalis* during winter 1991-92 in Lagos (Málaga, Spain) (□) and global solar irradiance (monthly means) (■) for the period 1975 to 1983 (Instituto Nacional de Meteorología). For % cover, bars representing standard error are shown when they exceed symbol size, and numerals represent the number of days from the beginning of the cycle, which was considered to be the first day of sampling (4 % cover). (B) Water temperature (●) and rock temperature (○) at the sampling site during the cycle. Measurements were made between 11:30 and 12:00 h (solar time)

suggests that temperature is not responsible for the seasonal changes in % cover, at least on the southern Spanish coast. However, the temperature on the rock (when emersed) at the time of sampling showed a clear increase coincident with the diminution in cover. Rock temperature measured as in this study only represents the particular temperature existing at the time of sampling (11:30 to 12:00 h, solar time), although it is obviously subject to a daily cycle. However, since the temperature of the earth's surface is closely related to solar irradiation, it is apparent from Fig.1A that an effective increase in temperature, parallel to that in global solar radiation over the last portion of the *P. umbilicalis* winter cycle, occurred when the alga was emersed. Therefore it remains unclear whether the decrease in growth after the first week of February is related to: (1) an increase in irradiance, (2) an increase in the temperature to which the population is subjected during emersion, or (3) both factors. This problem must be addressed in laboratory studies. In the case of *Laurentia pinnatifida*, which also disappears from this coast in summer, the increase in temperature was shown to be the essential factor instead of the increase in photon flux density (Flores-Moya et al. 1992).

Internal and external nitrate, nitrite and ammonium concentrations

Three ionic species of inorganic nitrogen are relevant for primary production in seawater: NO_3^-, NO_2^- and NH_4^+. At the study site, NO_3^- concentration in the seawater ($[NO_3^-]_e$) ranged between 1 and 6.7 µM, external nitrite concentration ($[NO_2^-]_e$) was always lower than 1 µM, and external ammonium concentration ($[NH_4^+]_e$) ranged between 6.8 and 17.6 µM, thus representing the largest source of inorganic N in the environment (Fig. 2). The 3 ionic species followed basically the same pattern pattern of evolution over time, i.e. they showed a peak in the second week of January and another near the end of the cycle (Fig. 2). Intracellular concentrations of each inorganic nitrogen species showed an almost total independence with respect to the external concentration (Fig. 3). In fact, $[NO_3^-]_i$ was considerably higher than $[NH_4^+]_i$, although intracellularly NO_2^- was still the least important ion quantitatively. This stresses the role of $[NO_3^-]_i$ as an internal storage pool of N, as claimed by others (Chapman & Craigie 1977, Wheeler & Weidner 1983, Corzo & Niell 1992a). Intracellular concentrations of NO_3^-, NO_2^- and NH_4^+, expressed in terms of µmol g^{-1} dry wt, may be

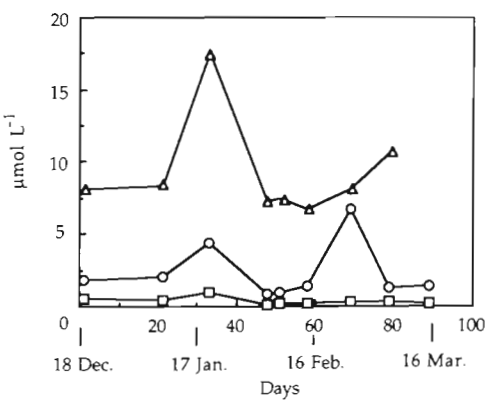

Fig. 2. External NO_3^- (○), NO_2^- (□), and NH_4^+ (△) concentration at the sampling site during winter 1991-92. Two samples were analysed for each day. Standard deviation smaller than symbol size

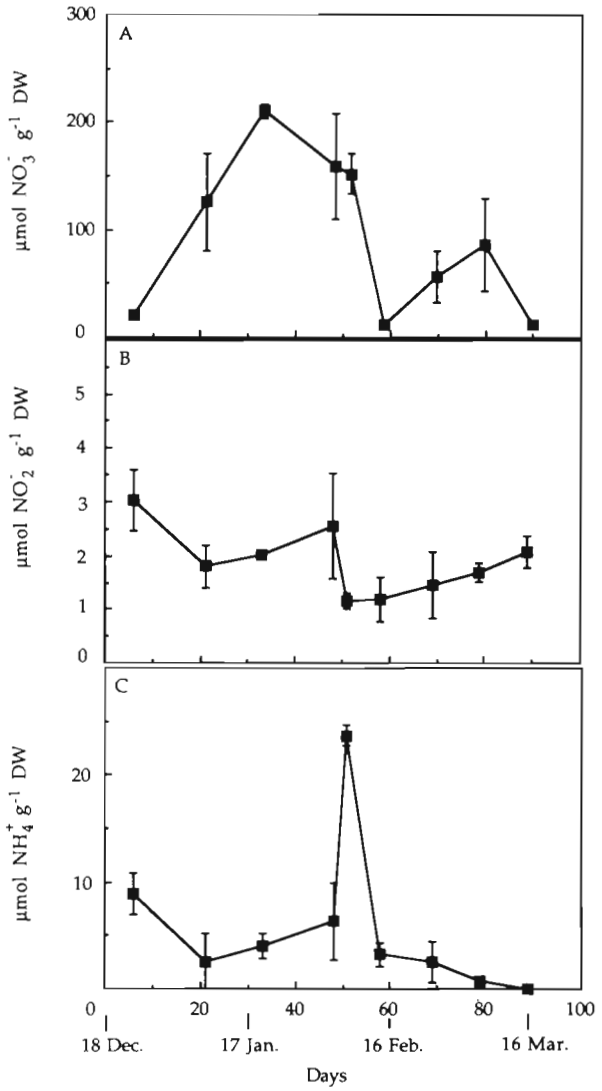

Fig. 3. *Porphyra umbilicalis*. Intracellular NO_3^- (A), NO_2^- (B) and NH_4^+ (C) concentration during the winter cycle. Data are means of 2 to 3 replicates. Standard deviation shown as a bar when it exceeds symbol size

expressed as µmol l^{-1} cell water by dividing by 4.1×10^{-3}. Using the mean concentrations for the entire period and taking NO_2^- concentration as the base unit, the ratio among the different ionic species (NO_3^-:NO_2^-:NH_4^+) in the environment was 6.2:1:25.1, whereas the intracellular ratio (of concentrations in µmol l^{-1} cell water) over the same period was 56.5:1:3.5. The ratio between the internal and external concentration of a particular nutrient (usually referred to as the concentration factor) reflects the capacity of an organism to concentrate that nutrient intracellularly. That is obviously a selective advantage in situations of low environmental nutrient concentration, since the resource is made unavailable for other competitors. The concentration factor for NO_3^- ($[NO_3^-]_i$:$[NO_3^-]_e$) ranged from 2×10^3 to 4.2×10^4, the largest concentration factor being coincident with the maximal % cover (Fig. 4). The case of NO_2^- is interesting, because the external concentration was very low (Fig. 2) and its concentration factor (1.3×10^3) was considerably higher than that for NH_4^+ (1.9×10^2). The comparably high internal concentration of NO_2^- may result either from uptake from the external medium despite the low $[NO_2^-]_e$ (Corzo & Niell 1992a) and/or from reduction of NO_3^-. When the reduction rate of NO_3^- exceeds that of NO_2^-, a net production of NO_2^- exists. However, in other species of marine macroalgae, the excess of NO_2^- is mainly released to the external medium (Corzo & Niell unpubl.), probably to avoid its toxic effects (Vennesland & Guerrero 1979). The most frequent cause of this imbalance is the limitation of NH_4^+ fixation due to an insufficient carbon skeleton production rate (Eisele & Ullrich 1977, Azuara & Aparicio 1985). The low concentration factor for NH_4^+ may be interpreted as the result of a large turnover of internal NH_4^+. When NH_4^+ is available it is preferred over NO_3^- and NO_2^- as N source (Syrett 1981, Ullrich 1983), which allows plants to save a considerable amount of free energy as reducing power (ΔG_o = 69 kcal mol^{-1} NH_4^+; Falkowski 1983). The large decrease in $[NO_3^-]_i$ from mid-January to mid-February (Figs. 3 & 4) was probably associated with the decrease of $[NH_4^+]_e$ (by ca 10 µmol l^{-1}) rather than with the decrease of $[NO_3^-]_e$ (by ca 3 µmol l^{-1}). Thus, in that period, the rate of growth as estimated by the increase in % cover did not change (Fig. 1) and was maintained by $[NO_3^-]_i$ as the main source of N.

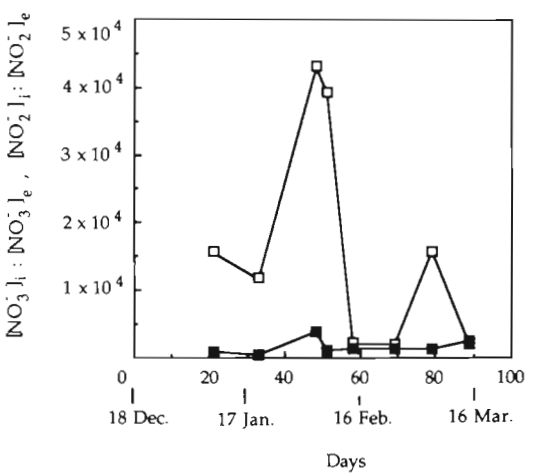

Fig. 4. *Porphyra umbilicalis*. Intracellular:extracellular concentration ratios for NO_3^- (□) and NO_2^- (■) during the winter cycle. Data are expressed as µmol l^{-1} for external concentration and as µmol l^{-1} cell water for intracellular concentration

Nitrate reductase activity

Very little information exists on the regulation of nitrate reductase in the environment, despite the extensive knowledge accumulated about the factors controlling both its activity and its biosynthesis under laboratory conditions. Nitrate and light are known to be major external factors controlling NR activity in higher plants (Deng et al. 1990) and microalgae (Velasco et al. 1989). Similar results were obtained for the marine green alga *Ulva rigida* (Corzo & Niell 1992b). Information on NR activity and its regulation in marine macroalgae is scarce, despite its acknowledged importance, since nitrate reduction is usually regarded as the limiting step in the assimilation of NO_3^-. From an ecological point of view, most of the work on NR activity has been done with phytoplanktonic species and communities (Packard 1979, Blasco et al. 1984, Hochman et al. 1986). In *Laminaria digitata*, NR activity was shown to undergo a daily cycle, displaying low activities in darkness and reaching its maximum value at the end of the light period (Davison & Stewart 1984b). NR also displayed a seasonal cycle, with maximum activities from late May to early June, coinciding with the period in which blade growth rate was maximum (Davison et al. 1984). NR activity in the leafy thallus of *Porphyra umbilicalis* varied considerably during the winter (Fig. 5). Maximal NR activity was measured at the beginning of the cycle, corresponding to a situation in which neither $[NO_3^-]_e$ nor $[NO_3^-]_i$ were at their maximal levels. Irradiance at this time of the year was also at its minimum value (Fig. 1A). Furthermore, external ammonium concentration was 8 µmol l^{-1}, similar to the level existing at the beginning of February which led to a decrease of $[NO_3^-]_i$. Therefore, it seems that *P. umbilicalis* cannot use external NH_4^+ as a relevant source of N when its concentration decreases below 8 µmol l^{-1}. This interpretation is supported by the fact that at this concentration, the level of NR activity was high both at the beginning of the cycle and in the middle of it, when the % cover was maximum. Ammonium has long been reported as an inhibitor of NR in laboratory experiments (Vennesland & Guerrero 1979). External NH_4^+ at levels higher than ca 8 µmol l^{-1} appears to be a sufficient source of N for *P. umbilicalis*, as it allows intracellular accumulation of large amounts of NO_3^-. Inhibition of NR activity is obviously key to the storage of NO_3^-. In *P. umbilicalis*, when $[NH_4^+]_e$ was about 17.6 µmol l^{-1} and $[NH_4^+]_i$ about 1.08 ± 0.3 mM l^{-1} cell water, NR activity was inhibited by 82 %. The decrease in $[NH_4^+]_e$ to levels below 8 µmol l^{-1} led to the cessation of intracellular NO_3^- accumulation, and $[NO_3^-]_i$ became the main source of N and was even able to maintain growth. The use of the internal NO_3^- reserves was associated with an increase in NR activity and with a concomitant increase in $[NH_4^+]_i$, probably due to the high rate of NO_3^- reduction (Fig. 3C). Similar results were obtained in laboratory studies with *Ulva rigida*: when NO_3^- reduction rate exceeded NH_4^+ fixation rate, NH_4^+ was accumulated intracellularly (Corzo & Niell unpubl.). A second, smaller increase in NR activity occurred in the last phase of the cycle, associated with a second maximum of $[NO_3^-]_e$ (Fig. 5).

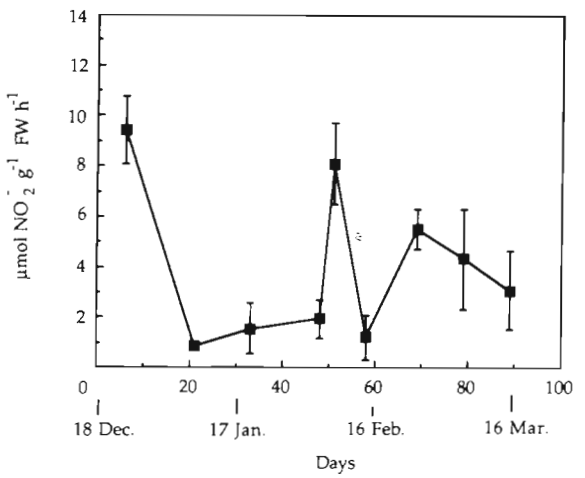

Fig. 5. *Porphyra umbilicalis*. Seasonal cycle of nitrate reductase activity in the leafy thallus. Three independent assays were performed for every sample. Standard deviation shown as a bar when it exceeds symbol size. FW: fresh weight

Previously reported values of *in situ* NR activity for different species of macroalgae are 1 (*Laminaria digitata*: Davison & Stewart 1984a, b, Davison et al. 1984; *Ulva rigida*: Corzo & Niell 1991a) to 2 (*Laminaria japonica*: Brinkhuis et al. 1989; *Petroglossum nicaense*: Dipierro et al. 1977) orders of magnitude lower than those reported here for *Porphyra umbilicalis*. Thomas & Harrison (1988) reported values of 60 (plants taken from the field) and 100 µmol NO_2^- g^{-1} protein h^{-1} (plants induced with 30 µM NO_3^- for 3 d) for *Porphyra perforata*. However, our values of NR activity, expressed in terms of soluble protein, attained a maximum of 1344 µmol NO_2^- g^{-1} protein h^{-1}. Some of the discrepancies may be due to real differences in NR activity between different species, and some may be due to methodological differences. In particular, none of the previous studies mentioned above used an external source of reducing power.

Amino acids and soluble proteins

Mean concentrations of the amino acids detected in *Porphyra umbilicalis* during the winter cycle are shown in Table 1. The time course of most individual amino acids (results not shown) was similar to that of

Table 1. *Porphyra umbilicalis*. Mean amino acid concentrations during the winter cycle. Since the majority of the amino acids were undetectable in several samples during the winter, n represents the number of seasonal samples used to obtain the mean. Each concentration value represents in turn the mean of 2 replicates (standard deviation lower than 1 %)

Amino acid	Concentration (µmol g^{-1} fresh wt)	n
Cysteic acid	0.18 ± 0.12	4
Taurine	0.04 ± 0.03	7
Asparagine	0.04 ± 0.01	2
Glutamine	0.21 ± 0.13	4
Serine	0.03 ± 0	3
Glutamate	0.4	1
Glycine	0.03 ± 0	3
β-Alanine	0.12 ± 0.1	3
Alanine	0.08 ± 0.06	4
Cysteine	9.49 ± 6.92	8
Norvaline	0.03 ± 0.02	7
Cystine	0.05 ± 0.04	4
Ornithine	0.04 ± 0.03	4
Tyrosine	0.02 ± 0.01	6

total amino acid content (Fig. 6); the main difference with respect to the general pattern was a large increase in β-alanine at the end of the cycle (not shown). Total amino acid content was maximal (28.3 µmol g^{-1} fresh wt) in the first part of the seasonal cycle, when % cover increased steadily. A second maximum appeared simultaneously with the second peak in $[NO_3^-]_e$ and $[NO_3^-]_i$. The discrepancy in the relationship of total amino acid content with both $[NO_3^-]_e$ and $[NO_3^-]_i$ between the 2 phases of the cycle (increase and decrease in % cover) may have been due to a large turnover rate in the amino acid pool in order to sustain protein synthesis during the first part of the cycle (increase in cover). The concentration of cysteine ranged from 83.5 to 97.2 % (mean 92.7 ± 4 %) of total amino acid content. Therefore, Fig. 6 may also be seen as the time course of cysteine in *P. umbilicalis* during the winter cycle. The amino acid which appeared at the elution time for cysteine was confirmed to be such by mass spectroscopy. We do not have any explanation so far for this large percentage of cysteine, and further experimental work has to be done in the laboratory to clarify this point. Cysteine was present at concentrations 1 to 2 orders of magnitude higher than those of the other amino acids during the winter cycle (Table 1).

Soluble protein content increased during the first part of the winter cycle, maximal content (14 mg protein g^{-1} fresh wt) being coincident with maximal % cover (beginning of February) (Fig. 7) and with minimal total amino acid content (Fig. 6). Later, soluble protein content decreased parallel to the decrease in % cover. In this period, at the end of the cycle, the amino acid pool increased in response to the increase in $[NO_3^-]_e$ and $[NO_3^-]_i$, probably as a consequence of a lower demand for protein synthesis. Alternatively, the increase in the amino acid pool could have been caused by the hydrolysis of proteins, which decreased considerably upon the second phase of the cycle. Phycobiliproteins (phycocyanin + phycoerythrin) represent 17.3 ± 2.6 % (n = 9) of soluble proteins. Phycocyanin constituted 30 % of the total biliprotein content. The changes in both phycocyanin and phycoerythrin during the winter cycle were similar to that of total soluble protein. Since a significant linear correlation (r = 0.91, n = 9) existed between seasonal varia-

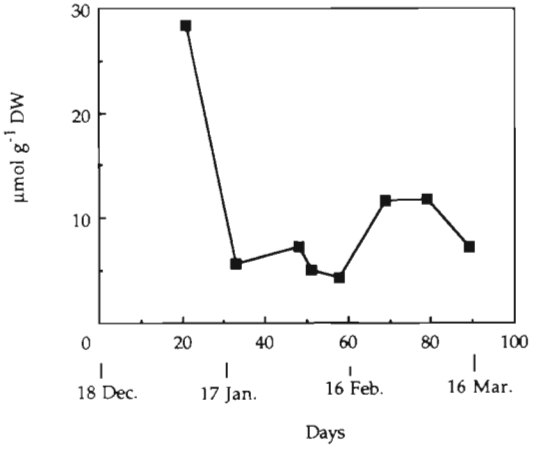

Fig. 6. *Porphyra umbilicalis*. Total amino acid content during the winter cycle. Data are means of 2 replicates, with standard deviation always being lower than 1 %. DW: dry weight

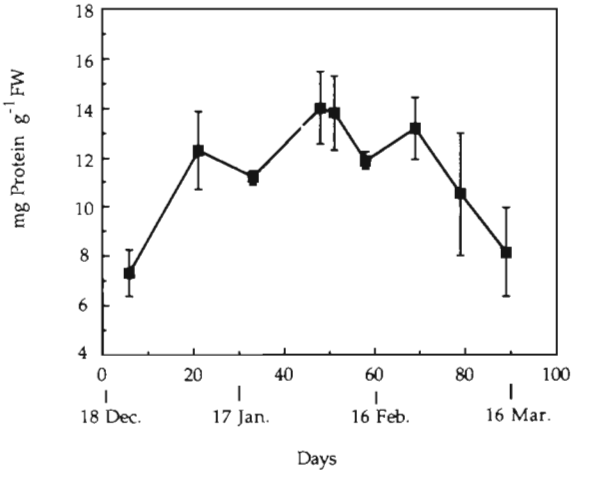

Fig. 7. *Porphyra umbilicalis*. Soluble protein content during the winter cycle. Data are means of 3 replicates. Bars indicate standard deviation

tions of total soluble proteins and phycobiliproteins in *Porphyra umbilicalis*, the biliproteins did not seem to play a special role as an N-storage compartment with respect to total soluble proteins, as reported by others for a number of species (Boussiba & Richmond 1980, Bird et al. 1982, Gantt 1990).

Senescence processes are largely unknown in macroalgae, although many species of algae have a life cycle characterized by alternation of generations, and therefore involving the substitution (death) of one form by the next one. The processes leading to death of one form and growth of the following generation are usually controlled by environmental factors such as temperature, photoperiod, irradiance, etc. In higher plants senescence is considered to be more an ordered 'programme of death' than a chaotic process (Sexton & Woolhouse 1984); this is probably also the case in seaweeds in general and in *Porphyra umbilicalis* in particular, since here the progressive disappearance of the alga is also associated with the production of carpospores. The general succession of events collectively called senescence in higher plants is very well established. A decline in chlorophyll content and soluble proteins is among the initial and most general features by which senescence is recognized (Beevers 1976, Sexton & Woolhouse 1984). We interpret as a sign of senescence the decline in chlorophyll (result not shown) and soluble protein contents in *P. umbilicalis* that appeared at the end of the winter cycle (after 25 February) (Fig. 7), the final consequence of which was the disappearance of the foliaceous form (Fig. 1). Alternatively, the drop in soluble proteins could have been a consequence of the N-sink that is created by the carpospore differentiation process, which necessarily involves protein synthesis. Since carpospores are released, this N is lost by the alga. Formation of carpospores occurs only during periods of long daylight in *Porphyra tenera* (Iwasaki 1961, Suto 1972), and in *P. umbilicalis* we detected the formation of carpospores in the last 2 wk of the cycle.

Total N content, total C content and C:N ratio

Total content of C and N generally followed the increase in biomass during the cycle (Fig. 8A). The ranges of variation for both parameters were quite different: while the maximal change in C content was 22%, the change in N content was up to 72% (with respect to the minimal measured contents). Both variables increased during the first part of the cycle, but while the increase in cover was sustained, a decrease in N and mainly C was observed from 8 January to 4 February. No other variable measured for this study showed this pattern.

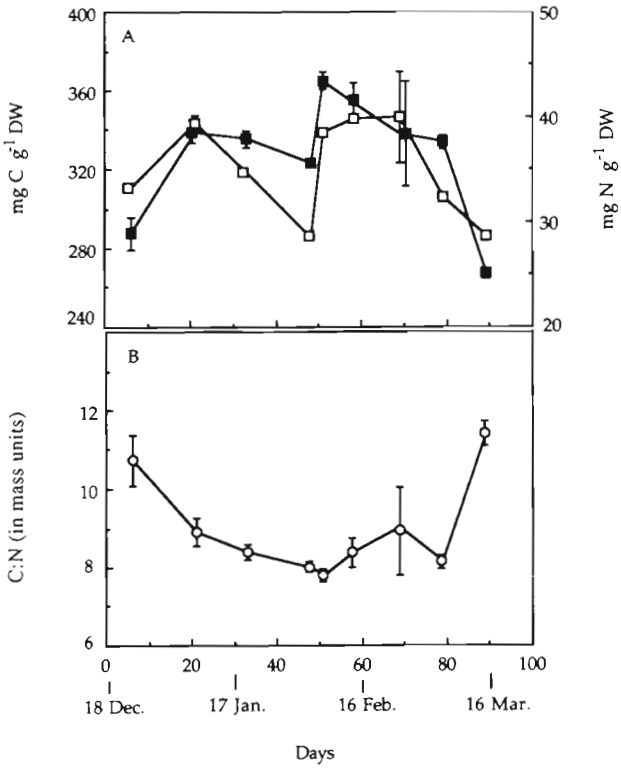

Fig. 8. *Porphyra umbilicalis*. (A) Total carbon (□) and total nitrogen (■) contents during the winter cycle. (B) C:N ratio. Data are means of 3 replicates. Standard deviation shown as a bar when it exceeds symbol size

The C:N ratio has been used in studies dealing with phytoplanktonic species or communities (Donaghay et al. 1978) and with macroalgae (Niell 1976, Hanisak 1983, Corzo & Niell 1991b), as a mean to assess the possible limitation of their growth by nitrogen. Duarte (1992) has reported N and C contents (% dry wt) of 1.9 ± 0.8 (n = 298) and 24.8 ± 6.3 (n = 296), respectively, for 46 species of macroalgae. Thus a typical C:N ratio for macroalgae is 13.05 (in mass units). However, the changes in C:N ratio for any single species may be considerable, depending on the N status of the alga. In *Ulva rigida*, under conditions of N-limited growth, the C:N ratio may rise transitorily up to 29, decreasing below 9 in the absence of N limitation (Corzo & Niell 1991b). The dynamic character of the C:N ratio must be taken into account, since it was found to change from 15 to nearly 8, 2 h after a NO_3^- pulse (0.3 mM) to N-starved *U. rigida* (Corzo & Niell 1991b). The C:N ratio in *Porphyra umbilicalis* ranged from ca 11 at the beginning and end of the cycle to 7.8 in the middle, the lower value coinciding with the maximal cover period (Fig. 8B). The seasonal evolution of C:N divided the cycle into 2 phases, one in which the ratio decreased, parallel with the increase in population biomass, and

one in which it increased, during the period when *P. umbilicalis* was disappearing from the intertidal. The C:N ratio decrease during the first phase suggests that N availability is not limiting, since the net rate of N increase is larger than the net rate of C increase, according to the preliminary model proposed by Corzo & Niell (1991b). Part of that nitrogen was accumulated as soluble proteins (Fig. 7). The second phase (decline in cover) was coincident with the increase in C:N ratio. This increase in C:N ratio corresponded with a decrease of total C and N contents, the N losses per unit of biomass being greater than the decrease of C. An increasing C:N ratio is currently believed to reveal a limitation of growth by N, but since in this phase growth is negative, with N and C levels decreasing, the difference in the relative rate at which N and C decrease seems to be key. Senescence processes were described in the previous section as being responsible for the decrease in soluble protein. The questions to be asked, then, are: Is the senescence process responsible for the larger relative decrease in total N with respect to C, senescence being triggered by environmental variables such as light (photoperiod, irradiance, etc.) or temperature? Or, is a limitation of N responsible for the senescence process, ultimately leading to the disappearance of the alga?

Seasonal variation in the Redfield ratio

A common way to determine whether growth is limited by N and/or P is to examine how the tissue composition of a particular species or community varies with respect to Redfield ratio (C:N:P) (Redfield et al. 1963). However, this is a rather static approach, since this ratio probably depends on species, as well as on physiological states other than limitation/non-limitation by N and P (Niell 1976, Atkinson & Smith 1983). Therefore it seems more useful to study the evolution of C:N:P than to compare any particular value. The Redfield ratio may be graphically displayed by plotting C:P against N:P. Each point represents a value for the Redfield ratio; the proportions of C and N may be read directly from the axes, and P is equal to 1. The Redfield ratio for the winter cycle of *Porphyra umbilicalis* is shown in Fig. 9. To obviate difficulties with a tridimensional plot, and to display the evolution of C:N:P with time, temporally consecutive points have been joined with arbitrary lines, so that Fig. 9 is in fact a phase space for the evolution of the Redfield ratio in *P. umbilicalis*. Measured values of C:N:P for *P. umbilicalis* displayed a well-defined relationship (dashed line in Fig. 9). It could be hypothesized that this relationship is species-dependent and that the interaction among carbon, nitrogen and phosphorus metabolism in *P. umbilicalis* tends to keep the C:N:P ratio close to the curve under various environmental circumstances. During the winter cycle, C:N:P evolved from 258:20:1 (24 December 1991) to 495:38:1 (16 March 1992); therefore, an enrichment in C (91.8%) and N (90%) occurred with respect to P in the algal tissue. The simplest interpretation is that there was a P deficiency, at least during the last part of the cycle. Thus the N deficiency which was apparent from the C:N ratio was accompanied by an even greater P deficiency. Therefore, the decrease in C:N ratio during the growth phase is a consequence of the storing of N. In the second phase this pool of N is consumed, but the main limiting compound is P. This view is supported by the fact that alkaline phosphatase activity increased considerably in the last part of the winter cycle (Hernández et al. 1993). Alkaline phosphatase activity has been shown to increase in response to P depletion in the external medium (Cembella et al. 1984) and to a decrease in the cellular P content (Taft et al. 1977, Pettersson 1980).

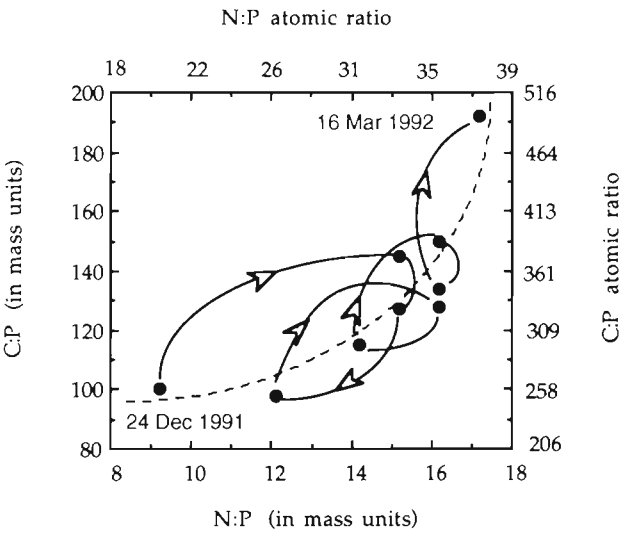

Fig. 9. *Porphyra umbilicalis*. C:P vs N:P, showing the evolution of the Redfield ratio during the winter cycle. Points have been linked with arbitrary lines to show the time course of the ratio. The dashed line represents the relationship that the relative contents of C, N and P tend to follow during the cycle

CONCLUSIONS

The measurement and analysis of N-metabolism-related variables suggested that *Porphyra umbilicalis* did not disappear from the environment as a consequence of N limitation, although % cover and obviously primary production are related to nutrient availability. NR activity was found to be at very low levels during most of the phase of increasing % cover, coin-

ciding with the highest $[NH_4^+]_e$ and $[NO_3^-]_i$. This observation suggests that the alga was using mainly NH_4^+ as the source of N (or other N-organic substrates, not measured in this work). Later, *P. umbilicalis* began using stored NO_3^- as the main N source, which was manifested by an increase in NR activity and in $[NH_4^+]_i$. However, there was a direct response of $[NO_3^-]_i$, NR activity and amino acid to the second and largest maximum of $[NO_3^-]_e$. This observation suggests that in the second part of the cycle $[NO_3^-]_e$ was the main source of N. The line of reasoning derived from the analysis of Redfield ratios suggests that the growth of *P. umbilicalis* could be P-limited at the end of the cycle. However, the decrease in tissue P content could be a consequence of 'programmed death' rather than a factor leading to the disappearance of the alga from the environment. Although larger, the decrease of total P occurred simultaneously with similar decreases in both total C and total N. The *P. umbilicalis* life cycle spanned a period in which water temperature increased by 2 °C, global solar irradiance increased from 2.8 to 5 kWh m^{-2} and photoperiod increased by 2 h. As has been done for the conchocelis phase, further experiments must be carried in the laboratory to identify which of these environmental factors provide the signal to: (1) suppress the differentiation and/or germination of monospores which produce new blade-like thalli, (2) initiate the cellular differentiation of carpospores, and (3) trigger the cellular mechanism leading to algal senescence at the end of the cycle.

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LITERATURE CITED

Atkinson, M. J., Smith, S. V. (1983). C:N:P ratios of benthic marine plants. Limnol. Oceanogr. 28: 568–574

Azuara, M. P., Aparicio, P. J. (1985). Spectral dependence of photoregulation of inorganic nitrogen metabolism in *Chlamydomonas reinhardii*. Plant Physiol. 75: 95–98

Beer, S., Eshel, A. (1985). Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. Aust. J. mar. Freshwat. Res. 36: 785–792

Beevers, L. (1976). Senescence. In: Bonner, J., Varner, J. E. (eds.) Plant biochemistry. Academic Press, New York, p. 771–794

Bird, K. T., Habig, C., De Busk, T (1982). Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J. Phycol. 18: 344–348

Blasco, D., MacIsaac, J. J., Packard, T T., Dugdale, R. C. (1984). Relationship between nitrate reductase and nitrate uptake in phytoplankton in the Peru upwelling region. Limnol. Oceanogr. 29: 275–286

Boussiba, S., Richmond, A. E. (1980). C-phycocyanine as a storage protein in the blue-green alga *Spirulina platensis*. Arch. Microbiol. 125: 143–147

Bradford, M. M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254

Brinkhuis, B. H., Renzhi, L., Chaoyuan, W., Xun-Sen, J. (1989). Nitrite uptake transients and consequences for *in vivo* algal nitrate reductase assays. J. Phycol. 25: 539–545

Cembella, A. D., Anyia, N. J., Harrison, P. J. (1984). The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: part 2. Crit. Rev. Microbiol. 11: 13–81

Chapman, A. R. O., Craigie, J. S. (1977). Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40: 197–205

Chen, L. C. M., Edelstein, T., Ogata, E., McLachlan, J. (1970). The life history of *Porphyra miniata*. Can. J. Bot. 48: 385–389

Corzo, A., Niell, F. X. (1991a). Determination of nitrate reductase activity in *Ulva rigida* C. Agardh by the *in situ* method. J. exp. mar. Biol. Ecol. 146: 181–191

Corzo, A., Niell, F. X. (1991b). C/N ratio in response to nitrogen supply and light quality in *Ulva rigida* C. Agardh (Chlorophyta: Ulvophyceae). Scient. mar. 55: 405–411

Corzo, A., Niell, F. X. (1992a). Inorganic nitrogen metabolism in *Ulva rigida* illuminated with blue light. Mar. Biol. 112: 223–228

Corzo, A., Niell, F. X. (1992b). Blue light induction of in situ nitrate reductase activity in the marine green alga *Ulva rigida*. Aust. J. Plant Physiol. 1992: 19: 625–635

Davison, I. R., Andrews, M., Stewart, W. D. P. (1984). Regulation of growth in *Laminaria digitata*: use of *in vivo* nitrate reductase activities as an indicator of nitrogen limitation in field populations. Mar. Biol. 84: 207–217

Davison, I. R., Stewart, W. D. P. (1984a). Studies on nitrate reductase activity in *Laminaria digitata* (Huds.) Lamour. I. Longitudinal and transverse profiles of nitrate reductase activity within the thallus. J. exp. mar. Biol. Ecol. 74: 201–210

Davison, I. R., Stewart, W.D.P. (1984b). Studies on nitrate reductase activity in *Laminaria digitata* (Huds.) Lamour II. The role of nitrate availability in the regulation of enzyme activity. J. exp. mar. Biol. Ecol. 79: 65–78

Deng, M.D., Moureaux, T., Leydecker, M.T., Caboche, M. (1990). Nitrate-reductase expression is under the control of a circadian rhythm and is light inducible in *Nicotiana tabacum* leaves. Planta 180: 257–261

Dipierro, S., Perrone, C., Felicine, G. P. (1977). *In vivo* nitrate reductase assay in *Petroglossum nicaense* (Duby) Schotter (Rhodophyta, Phyllophoraceae). Phycologia 16: 179–182

Donaghay, F. L., Demanche, J. M., Semall, L. F. (1978). On predicting phytoplankton growth rates from carbon:nitrogen ratios. Limnol. Oceanogr. 23: 359–362

Drew, K. M. (1949). Conchocelis phase in the life history of *P. umbilicalis* (L.) Kütz. Nature 164: 748–749

Dring, M. J. (1967). Effects of daylength on growth and reproduction of the conchocelis-phase of *Porphyra tenera*. J. mar. biol. Ass. U.K. 47: 501–510

Duarte, C. M. (1992). Nutrients concentration of aquatic

plants: patterns across species. Limnol. Oceanogr. 37: 882–889

Eisele, R., Ullrich, W. R. (1977). Effect of glucose and CO_2 on nitrate uptake and coupled OH^- flux in *Ankistrodesmus braunii*. Plant Physiol. 59: 18–21

Falkowski, P. G. (1983). Enzymology of nitrogen assimilation. In: Carpenter, E. J., Capone, D. G. (eds.) Nitrogen in the marine environment. Academic Press, New York, p. 839–868

Flores-Moya, A., Fernández, A., Niell, F.X. (1992). Influences of light intensity and temperature on the summer disappearance of *Laurentia pinnatifida* (Ceramiales, Rhodophyta). Cryp. Bot. 2: 345–350

Font, I. (1984). Atlas de la radiación solar en España. Instituto Nacional de Meteorología, Madrid

Gantt, E. (1990). Pigment and photoacclimation. In: Cole, K. H., Sheat, R. G. (eds.) Biology of red algae. Cambridge University Press, Cambridge, p. 203–219

Hanisak, M. D. (1983). The nitrogen relationships of marine macroalgae. In: Carpenter, E. J., Capone, D. G. (eds.) Nitrogen in the marine environment. Academic Press, New York, p. 699–730

Hernández, I., Fernández, J. A., Niell, F. X. (1993). Influence of phosphorus status on the seasonal variation of alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. J. exp. mar. Biol. Ecol. (in press)

Hochman, A., Nissany, A., Wynne, D., Kaplan, B., Berman, T (1986). Nitrate reductase: an improved assay method for phytoplankton. J. Plankton Res. 8: 385–392

Iwasaki, H. (1961). The life cycle of *Porphyra tenera in vitro*. Biol. Bull. 121: 173–187

Kurogi, M., Akiyama, K. (1966). Effects of water temperature on the growth and maturation of Conchocelis-thalli in several species of *Porphyra*. Bull. Tohoku reg. Fish. Res. Lab. 26: 77–85

Kurogi, M., Sato, S. (1962). Influences of light on the growth and maturation of conchocelis-thallus of *Porphyra*. II. Effect of different photoperiods on the growth and maturation of conchocelis-thallus of *P. tenera*. Bull. Tohoku reg. Fish. Res. Lab. 20: 127–137

Lapointe, B. E., O'Connell, J. (1989). Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: eutrophization of a confined, phosphorus-limited marine ecosystem. Estuar. coast. Shelf Sci. 28: 347–360

Márquez, F. J., Quesada, A. R., Sanchez-Jimenez, F., Nuñez de Castro, I. (1986). Determination of 27 dansyl amino acid derivatives in biological fluids by reversed-phase high-performance liquid chromatography. J. Chromatogr 380: 275–283

Mauriño, S. G., Echevarria, C., Vargas, M. A., Maldonado, J. M. (1985). Effect of freezing and thawing on *in situ* nitrate reductase activity in spinach and maize leaves. J. Plant Physiol. 120: 409–417

Niell, F. X. (1976). C:N ratio in some marine macrophytes and its possible ecological significance. Botanica mar. 19: 347–350

Packard, T. T. (1979). Half-saturation constants for nitrate reductase and nitrate translocation in marine phytoplankton. Deep Sea Res. 26: 321–326

Pettersson, K. (1980). Alkaline phosphatase activity and algal surplus phosphorus as phosphorus-deficiency indicators in Lake Erken. Arch. Hydrobiol. 89: 54–87

Redfield, A. C., Ketchum, B. A., Richards, F. A. (1963). The influence of organisms in the composition of sea-water. In: Hill, M. N. (ed.) The sea, Vol. 2. Wiley, New York, p. 26–77

Rhodes, D., Stewart, G. R. (1974). A procedure for the *in vivo* determination of enzyme activity in higher plants tissue. Planta 118: 133–144

Sexton, R., Woolhouse, H. W. (1984). Senescence and abscission. In: Wilkins, M. B. (ed.) Advanced plant physiology. Pitman Publishing, London, p. 469–497

Shinn, M. B. (1941). A colorimetric method for the determination of nitrite. Ind. Eng. Chem. (analyt. Edn) 13: 33–35

Slawyk, G., MacIsaac, J. J. (1972). Comparison of two automated ammonium methods in a region of coastal upwelling. Deep-Sea. Res. 19: 521–524

Snell, F. D., Snell C. T. (1949). Colorimetric methods of analysis, 3rd edn, Vol. 2. Van Nostrand, Princeton

Sommer, L. E., Nelson, D. W. (1972). Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. Soil Sci. Soc. Am. Proc. 53: 32–37

Suto, S. (1972). Variation in species characters of *Porphyra* under culture conditions. In: Abbott, I. A., Kurogi, M. (eds.) Contributions to the systematics of benthic marine algae of the North Pacific. Japanese Society of Phycology, Kobe, p. 193–201

Syrett, P. J. (1981). Nitrogen metabolism of microalgae. Can. Bull. Fish. Aquat. Sci. 210: 182–210

Taft, J. L., Loftus, M. E., Taylor, W. R. (1977). Phosphate uptake from phosphomonoesters by phytoplankton in the Chesapeake Bay. Limnol. Oceanogr. 22: 1012–1021

Thomas, T E., Harrison, P. J. (1988). A comparison of *in vitro* and *in vivo* nitrate reductase assays in three intertidal seaweeds. Botanica mar. 31. 101–107

Ullrich, W. R. (1983). Uptake and reduction of nitrate: algae and fungi. In: Laüchli, A., Bieleski R. L. (eds.) Inorganic plant nutrition. Encyclopedia of plant physiology, NS, Vol. 15a. Springer-Verlag, Berlin, p. 307–313

Velasco, P. J., Tischner, R., Huffaker, R. C., Whitaker, J. R. (1989). Synthesis and degradation of nitrate reductase during the cell cycle of *Chlorella sorokiniana*. Plant Physiol. 89: 220–224

Vennesland, B., Guerrero, M. G. (1979). Reduction of nitrate and nitrite. In: Gibbs, M., Latzko, E. (eds.) Photosynthesis. II. Photosynthetic carbon metabolism and related processes. Encyclopedia of plant physiology, NS, Vol. VI. Springer-Verlag, Berlin, p. 425–444

Wheeler, W. N., Weidner, M. (1983). Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. J. Phycol. 19: 92–96

Wood, E. D., Armstrong, F. A. J., Richards, F. A. (1967) Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. mar. biol. Ass. U.K. 47: 23–31

Zeng, C. K. (1984). Phycological research in the development of the Chinese seaweed industry. In: Bird, C. J., Ragan, M. A. (eds.) Proceedings of the Eleventh International Seaweed Symposium. Junk, Dordrecht, p. 7–18

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