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PHOSPHORUS AND NITROGEN NUTRITION IN *CHONDRUS CRISPUS* (RHODOPHYTA): EFFECTS ON TOTAL PHOSPHORUS AND NITROGEN CONTENT, CARRAGEENAN PRODUCTION, AND PHOTOSYNTHETIC PIGMENTS AND METABOLISM[1]

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ABSTRACT

The existence of a phenomenon in phosphorus (P) nutrition comparable to the "Neish effect" in nitrogen (N) nutrition (an inverse relation between seawater N enrichment and carrageenan content) was investigated in the temperate red alga Chondrus crispus *Stackhouse. Plants were preconditioned for 17 d and then cultured under varying enrichments of P (0, 3, 6, 10, 15 μM P·wk^{-1}) and a constant N enrichment (53.5 μM N·wk^{-1}) for 5 wk. Tissue total P, tissue total N, and carrageenan contents were then determined. Identical experiments were performed using* C. crispus *collected during the fall, winter, spring, and summer seasons. The procedure was repeated using material collected during the following fall season and cultured under constant P (6 μM P·wk^{-1}) and varying N enrichments (0, 3, 6, 10, 25 μM N·wk^{-1}). In the fall (P) experiment, carrageenan content was the highest [53.1 ± 0.3% DW (dry weight)], and tissue total P content was the lowest (1.71 ± 0.27 mg P·g DW^{-1}) in plants that received no P enrichment. Carrageenan content was stable (46.1 ± 1.8% DW) for plants given enrichments of 3 μM P·wk^{-1} and greater. Thus, a decrease in carrageenan content, concomitant with an increase in tissue total P content, was observed, but only at tissue total P levels below 2 mg P·g DW^{-1}. As these levels were always higher than 2 mg P·g DW^{-1} in the winter, spring, and summer experiments, carrageenan content remained constant within each season at 46.2 ± 1.3, 43.1 ± 0.7, and 44.5 ± 0.6% DW, respectively. Nitrogen enrichment of plants collected in the fall did not affect carrageenan content, which was stable at 49.3 ± 0.9% DW. When these plants were compared with those of the previous fall experiment (6 μM P·wk^{-1} and 53.5 μM N·wk^{-1}), a slight increase in carrageenan content was noted. Thus, at sufficiently high concentration, N also decreased carrageenan content in* C. crispus. *Phosphorus nutrition had no significant effect on photosynthesis versus irradiance parameters (P_{max}, α, R_d, I_c and I_k), the contents of the photosynthetic pigments chlorophyll-*a*, phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), and the ratios PE:APC and PC:APC. In contrast, N nutrition affected both P_{max} and the photosynthetic pigment contents. The data indicate that N limitation reduces the number of phycobilisomes but not their size. The greater reduction in phycobiliprotein than chlorophyll-*a *content corroborates the natural bleaching phenomenon regularly observed in* C. crispus *populations during summer when N levels are generally low in seawater. These results suggest that* C. crispus *in the temperate waters of the Bay of Fundy may experience N limitation, but P limitation is unlikely.*

Key index words: carrageenans; Chondrus crispus; *nutrient limitation; Neish effect; nitrogen; nutrients; phosphorus; photosynthesis; pigments; Rhodophyta*

The relationship between nitrogen (N) in seawater, N in algal tissue, growth, carrageenan production, and photosynthesis is well documented, particularly in the case of *Chondrus crispus* (Butler 1936, Neish and Shacklock 1971, Fuller and Mathieson 1972, Mathieson and Tveter 1975, Neish et al. 1977, Simpson et al. 1978). Generally, N limitation reduces growth, increases phycocolloid content, and decreases photosynthetic activities [for review, Chopin et al. (1990a)]. These studies led to the concept of the so-called "Neish effect" (reduced carrageenan content with increasing N availability).

In contrast to N, the effects of phosphorus (P) nutrition have been largely overlooked (Kornfeldt 1982, Chopin et al. 1990b), presumably because P was believed to be a secondary factor limiting algal growth (Ryther and Dunstan 1971). Recent evidence, however, suggests that P may limit seaweed growth in some coastal ecosystems. Most of the latter are subtropical or tropical (Birch et al. 1981, Smith 1984, Lapointe 1986, 1987, Chopin et al. 1990a), but a few are temperate (Manley and North 1984, Chopin 1985, Conolly and Drew 1985, Wheeler and Björnsäter 1992). Chopin et al. (1990a) demonstrated a P effect, comparable to the "Neish effect" in N nutrition, in a subtropical population of another red alga, *Agardhiella subulata* (C. Agardh) Kraft *et* Wynne, in Florida where P limitation in coastal waters has been reported (Lapointe 1985, 1987). Chopin et al. (1991) suggested that inorganic phosphate may be an important regulator of the path of carbon

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TABLE 1. *Schedule of the experiments and nutrient that was varied.*

Experiment	Season	Collection date	End date	Nutrient varied
1	Fall	7 Oct 91	2 Dec 91	Phosphorus (P)
2	Winter	19 Jan 92	19 Mar 92	Phosphorus (P)
3	Spring	16 May 92	10 July 92	Phosphorus (P)
4	Summer	14 July 92	7 Aug 92	Phosphorus (P)
5	Fall	19 Nov 92	13 Jan 93	Nitrogen (N)

flow in this species toward either glucose and starch or galactose and carrageenans.

The present work was designed principally to study the effects of P nutrition and secondarily of N nutrition on internal nutrient storage, carrageenan production, and photosynthetic parameters and pigments in *C. crispus* to determine if an effect similar to the "Neish effect" could also be observed with P nutrition in a temperate red algal species. Another goal was to evaluate the possible existence and impact of P and/or N limitation in the temperate waters of the Bay of Fundy.

MATERIALS AND METHODS

Chondrus crispus plants for all experiments were collected at Maces Bay in the Bay of Fundy, New Brunswick, Canada, where plants were taken from midlittoral tide pools. Dates for the beginning and end of each experiment, the seasonal designation assigned to each experiment, and the nutrient that was varied (P or N) are given in Table 1. Fresh material was transported immediately to the laboratory where it was sorted by life cycle phases, the female gametophytes of classes 4 and 5 [as defined by Chopin et al. (1988)] being retained. For experiment 5, the resorcinol method for distinguishing gametophytes from sporophytes in their vegetative state (Craigie and Leigh 1978, Shaughnessy and DeWreede 1991) had to be used because only a relatively small fraction of the plants was reproductively mature at that time of the year.

Approximately 1.8 ± 0.2 kg wet weight (WW) of female gametophytes were placed in a large holding tank [1.22 (depth) × 1.21 (width) × 3.62 (length) m, 5343 L] for a preconditioning period of 17 d. The preconditioning period served to generate both a reserve of seawater low in P and plants with the same low P content prehistory to be used for the remainder of each experiment. A photoperiod of 12:12 h LD per day was provided by 18 1.2-m-long 40-W cool white fluorescent tubes above the tank, giving a photon irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the center of the tank (Li-Cor Underwater Spherical Quantum Sensor). During the following 5-wk experimental period, the seawater was held under darkness in the large tank. Agitation and aeration of the seaweeds and seawater were accomplished by compressed air fed at the bottom center of the tank through holes drilled along a 2-cm (diameter) PVC pipe. The seawater was Freon-refrigerated to 13–14° C. A frame of 2.5-cm (diameter) PVC pipe enclosed in a nylon mesh was constructed inside the tank to prevent the seaweeds from becoming entangled in the Freon lines.

After the preconditioning period, 100 g WW seaweeds were transferred to each of 10 small tanks [common laundry tubs; 0.30 (depth) × 0.51 (width) × 0.56 (length) m] in a temperature-controlled room, where they were cultivated for 5 wk. The use of 10 culture tanks permitted the study of five sets of conditions, in duplicate, for each experiment. For experiments 1–4, each small tank was equipped with a single aeration line [1 cm (diameter) plastic tubing] placed at the bottom center of each tank and fed from the main compressed air line. The bubbling rate was set to achieve good water mixing but not to impose too vigorous a physical stress on the seaweeds. This agitation helped to suspend the negatively buoyant seaweed, promoted uniform light exposure, mixed nutrients, reduced the undisturbed boundary layer adjacent to the frond surface, and dispersed metabolic waste and byproducts (Craigie and Shacklock 1989). For experiment 5, the tanks were equipped with individual water cooling (Freon lines) and heating (Chromalox TPR-102 1000 W heater) systems controlled by a dual flip-flop thermostat with a 1.5° C temperature differential. Frames (43 × 48 × 25 cm) were constructed from 2.5-cm (diameter) PVC pipe and enclosed in nylon mesh. Two aeration lines (each placed one-third of the way across the width of the frame) were sewn into the bottom exterior of the enclosures. Seaweeds were placed in these enclosures to prevent them from becoming trapped in the cooling, heating, and aerating elements.

For experiments 1–4, N was supplied (as $NaNO_3$) as one pulse at 53.5 $\mu M \cdot tank^{-1} \cdot wk^{-1}$. P (as $NaH_2PO_4 \cdot H_2O$) was supplied to two tanks at each of the following concentrations: 0, 3, 6, 10, and 15 μM (all enrichments were supplied by one pulse per tank per week). In experiment 5, P was supplied at 6 $\mu M \cdot tank^{-1} \cdot wk^{-1}$, and N was given to two tanks at each of the following concentrations: 0, 3, 6, 10, and 25 μM. Seaweeds were grown in 50 L seawater with the appropriate nutrient regime and under a 12:12-h light:dark photoperiod. A photon irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the center of each tank was provided by two 45-cm-long 15-W cool white fluorescent tubes above each tank. Culture was carried out at 14 ± 1° C as this is the optimal growth temperature for the irradiance used (Craigie and Shacklock 1989). The pH varied between 8.1 and 8.3, with no manipulation required. The salinity ranged from 32 to 36‰ for all experiments. Both of these parameters were within acceptable limits for normal growth (Simpson et al. 1978, Bird et al. 1979, Shacklock and Craigie 1986).

At the end of each week, all seaweeds were removed, and the WW was recorded for each tank. The tanks were cleaned with freshwater. Fresh, nutrient-depleted seawater (50 L) from the large holding tank was added to each tank together with the appropriate amount of N and P. All fragments of seaweeds were discarded, and the WW biomass was adjusted to 100 g by discarding the appropriate mass of the least healthy plants (or the WW was simply recorded if it was less than 100 g). The culture procedure was continued for 5 wk. At the end of each experiment, the algae from each tank were dried in a forced-air oven at 60° C (72 h) before nutrient and carrageenan analyses. At the end of experiments 2–5, two plants from each tank were kept for photosynthetic measurements.

Nutrient analysis. At the beginning and end of each experiment, triplicate tissue samples per tank were taken to determine tissue total P and N contents. Only apices bearing no reproductive structures were used, because they show the most changes in nutrient content (Chopin et al. 1990b). Tissue total P content was measured by the method of Murphy and Riley (1962) after acidic mineralization (H_2SO_4 and HNO_3) in a Büchi 430 digester. Dissolved inorganic phosphorus (DIP) concentration in seawater in the large holding tank and in the small tanks was measured by the method of Murphy and Riley (1962). Tissue total N content was determined by the Kjeldahl method (with a Büchi 323 distillation unit) after acid/peroxide mineralization (H_2SO_4 and H_2O_2).

Extraction and content of total carrageenans. Duplicate samples per tank were extracted, and carrageenans were precipitated with hexadecyltrimethylammonium bromide (CTAB) (Craigie and Leigh 1978, Chopin et al. 1990a). The coagula were dried in a forced-air oven at 60° C for 72 h and weighed to determine the yield (= % DW).

Photosynthesis versus irradiance curves. The effect of photon flux density (PFD) on photosynthesis was studied by measuring oxygen

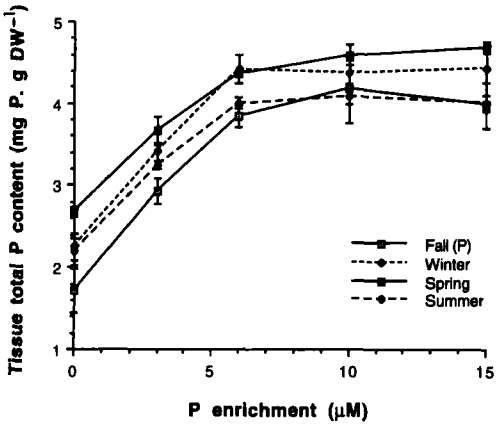

FIG. 1. Variations in tissue total P content (mg P·g DW^{-1}) of *C. crispus* cultured in different P enrichments at the end of the fall (P), winter, spring, and summer experiments. Values represent means (n = 6) ± SD.

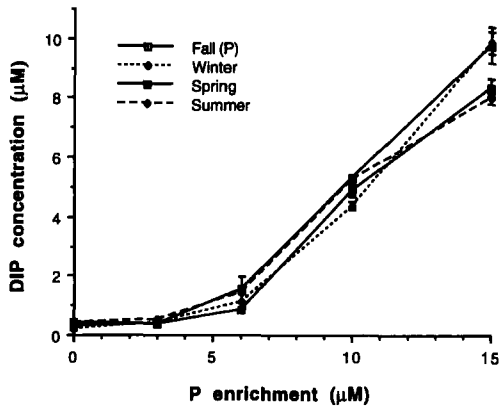

FIG. 2. Variations in residual DIP concentration (μM P) in seawater collected at the end of the fall (P), winter, spring, and summer experiments from tanks enriched with different levels of P. Values represent means (n = 6) ± SD.

flux with a Clark-type oxygen electrode as described previously (Davison et al. 1991). Vegetative apices (1 cm long) were cut from the experimental plants and placed in the chamber of a Hansatech DW-1 oxygen electrode (Hansatech, King's Lynn, England), together with 1.5 mL of 0.45-μm Millipore-filtered seawater from the corresponding experimental enrichment. Temperature was maintained at 14° C by means of a circulating water bath attached to the jacket of the electrode chamber. The output of the electrode was recorded on a chart recorder. The full-scale output of the electrode (with 100% air-saturated seawater) was set at 1 V. However, the back-off on the electrode control box allowed measurements to be made with the chart recorder set at 100 mV, increasing the sensitivity of the measurements, and reducing the time required to achieve a measurable rate of oxygen flux. All measurements of oxygen flux were corrected for O_2 consumption by the electrode. The surface of the apical section of tissue was placed at 90° to the incident light, provided by an EHL tungsten–halide lamp in a Kodak slide projector. Respiration was measured with the electrode chamber covered with black cloth, after which PFD was successively increased to approximately 3, 6, 12, 50, 150, 350, and 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$; PFD was adjusted by attenuating the light with Schott neutral density filters. The PFD was increased to the next value once a stable rate of oxygen consumption/production had been achieved (usually within 5 min). Because the electrode chamber was too small to admit the sensor of the light meter, the PFD values were measured at the position occupied by the plant, but with the electrode removed. The PFD values are, therefore, approximate because of the unknown effects of attenuation and/or focusing of the chamber and surrounding water jacket. PFD was measured with a Skye (Skye Instruments, UK) cosine (flat-plate) quantum meter.

After the rate of photosynthesis at the highest PFD had been determined, the plant tissue was removed from the electrode chamber, blotted dry with paper towels, weighed to determine WW, and then processed for chlorophyll determination (see below). Biomass-specific (WW) photosynthesis versus irradiance (PI) parameters were calculated as described previously (Davison et al. 1991). The light-harvesting efficiency (α; $\mu mol\ O_2 \cdot g^{-1} \cdot min^{-1}$/[$\mu mol$ photons$\cdot m^{-2} \cdot s^{-1}$]) was calculated from the slope of the initial light-limited region of the PI curve, and the rate of light-saturated photosynthesis (P_{max}; $\mu mol\ O_2 \cdot g^{-1} \cdot min^{-1}$) was determined from the mean of the two highest PFD values. The light-compensation point (I_c; μmol photons$\cdot m^{-2} \cdot s^{-1}$), which is the PFD at which respiratory O_2 consumption equals photosynthetic O_2 production, was calculated from dark respiration (R_d; $\mu mol\ O_2 \cdot g^{-1} \cdot min^{-1}$) and α as $-R_d/\alpha$, and the minimum PFD required to achieve P_{max} (I_k; μmol photons$\cdot m^{-2} \cdot s^{-1}$) was calculated as P_{max}/α.

Photosynthetic pigments. Chlorophyll-*a* was extracted from the intact apical section used to measure PI parameters with 2 mL of dimethyl sulfoxide (DMSO) essentially as described by Duncan and Harrison (1982). DMSO was added to the tissue, which was allowed to stand overnight in darkness at room temperature. Water (0.5 mL) was added to produce a 4:1 DMSO : H_2O solution, and absorbance was determined in a 1-cm path-length cell at 664 and 750 nm. The absorbance at 664 nm was corrected for turbidity by subtracting the absorbance at 750 nm and used to calculate the chlorophyll-*a* content (in mg·g WW^{-1}) as described by Seely et al. (1972).

A second set of apices was used to extract phycobiliproteins. The plant tissue was frozen in liquid nitrogen, ground to a fine powder in a mortar and pestle, and extracted by repeated freezing and thawing in 5 mL of pH 6.7 phosphate buffer. The extract was centrifuged in a bench centrifuge, and the absorbance of the supernatant was measured in a 1-cm path-length cuvette at 565, 615, 650, and 750 nm. The absorbance at 750 nm was subtracted from the other values to correct for turbidity, and the contents (in mg·g WW^{-1}) of allophycocyanin (APC), phycocyanin (PC), and phycoerythrin (PE) were determined according to the equations of Rosenberg (1981).

Statistical analysis of the photosynthetic data. Differences between treatments in the photosynthesis experiments were tested using Student's *t*-test (Zar 1984). Because the number of replicates in each treatment was small (n = 4), and because of the inevitable variability inherent in biological samples, it was decided a priori to only test two treatments, representing the lowest (0 μM P or N) and highest (15 μM P and 25 μM N) concentrations. Visual examination of the complete data set indicated that no differences occurred between any of the other concentrations when none were found between the lowest and highest values. As similar results were obtained with plants from the winter, spring, and summer (P) experiments, the only data shown in this paper are those comparing the summer (P) and fall (N) experiments.

RESULTS

Variations in tissue total P content. Variations in tissue total P content in *C. crispus* at the end of each experiment as a function of P enrichment were analyzed by pooling data from duplicate tanks (Fig. 1). In the fall (P), winter, spring, and summer experiments tissue total P levels increased with increasing

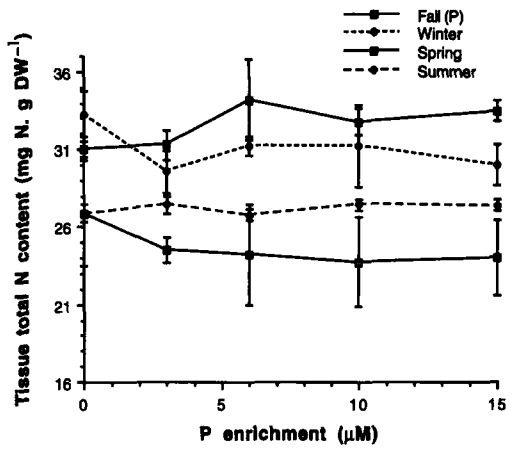

FIG. 3. Variations in tissue total N content (mg N·g DW^{-1}) of *C. crispus* cultured in different P enrichments at the end of the fall (P), winter, spring, and summer experiments. Values represent means (n = 6) ± SD.

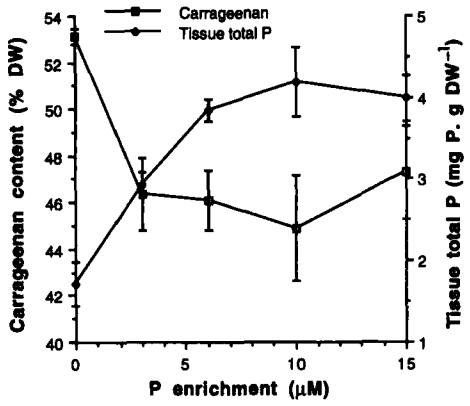

FIG. 5. Carrageenan content (% DW) and tissue total P content (mg P·g DW^{-1}) of *C. crispus* cultured in different P enrichments in the fall (P) experiment. Values represent means (n = 4) ± SD and means (n = 6) ± SD for carrageenan content and tissue total P content, respectively.

P enrichment up to 6 µM P·wk^{-1}. Further P enrichment did not affect the total P content of tissues, which were then saturated. Winter and spring plants reached a tissue saturation level of 4.42 ± 0.16 and 4.56 ± 0.17 mg P·g DW^{-1}, respectively. Fall (P) and summer plants reached saturation at 4.01 ± 0.32 and 4.03 ± 0.09 mg P·g DW^{-1}, respectively. In the fall (N) experiment, tissue total P content averaged 4.21 ± 0.25 mg P·g DW^{-1}, which corresponded to the saturation level resulting from an enrichment of 6 µM P·wk^{-1}, or higher, in the previous four experiments.

Variations in residual DIP concentration in seawater. In the fall (P), winter, spring, and summer experiments, tanks enriched with 0 and 3 µM P·wk^{-1} had a low level of residual DIP in seawater (Fig. 2). Between enrichments of 3 and 6 µM P·wk^{-1}, a slight increase in residual DIP in seawater was observed. Residual DIP in seawater drastically increased for enrichments higher than 6 µM P·wk^{-1}. This result is consistent with the saturation of tissue total P content at this level; further enrichment resulted in excess P remaining in the culture medium. DIP levels in the fall (N) experiment, in which all tanks were enriched with 6 µM P·wk^{-1}, were constant at about 1.87 ± 0.49 µM P.

Variations in tissue total N content. Plants collected from the highest nutrient environments (winter and spring) displayed the highest tissue total N content (31.06 ± 1.87 and 32.55 ± 1.76 mg N·g DW^{-1}, respectively; Fig. 3). Fall (P) plants contained the lowest amount (24.69 ± 1.92 mg N·g DW^{-1}), and summer plants had an intermediate level (27.19 ± 0.49 mg N·g DW^{-1}). In the fall (N) experiment, low enrichments of N (0–6 µM N·wk^{-1}) did not increase tissue total N levels (Fig. 4). At enrichments above 6 µM N·wk^{-1}, however, tissue total N content in-

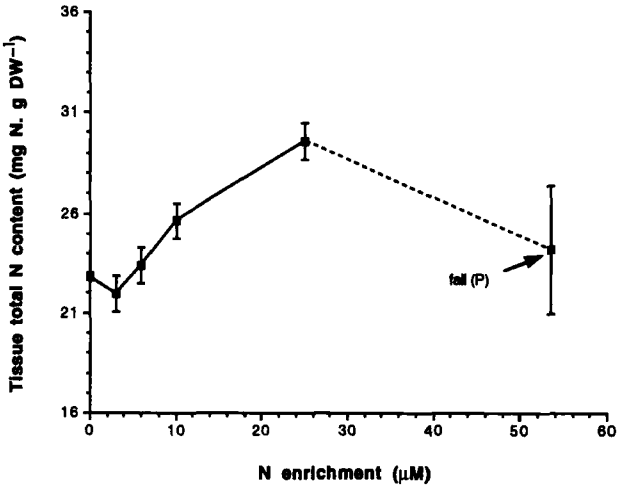

FIG. 4. Variations in tissue total N content (mg N·g DW^{-1}) of *C. crispus* cultured in different N enrichments at the end of the fall (N) experiment. The value from the fall (P) experiment (for 6 µM P·wk^{-1} and 53.5 µM N·wk^{-1}) has been included (dotted line). Values represent means (n = 6) ± SD.

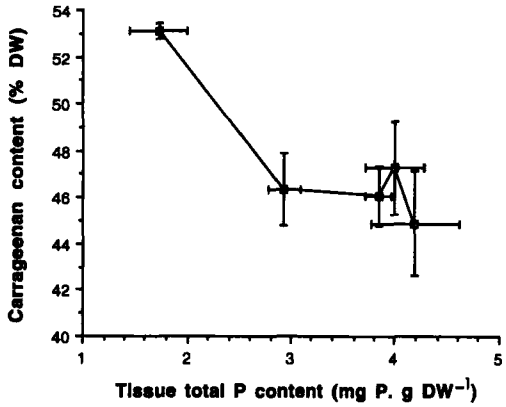

FIG. 6. Carrageenan content (% DW) of *C. crispus* as a function of tissue total P content (mg P·g DW^{-1}) at the end of the fall (P) experiment. Values represent means (n = 4) ± SD and means (n = 6) ± SD for carrageenan content and tissue total P content, respectively.

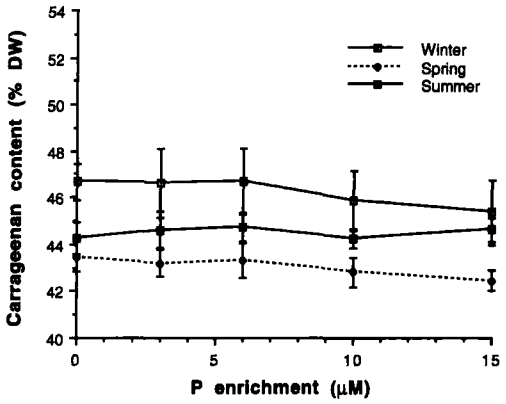

FIG. 7. Variations in carrageenan content (% DW) of *C. crispus* cultured in different P enrichments at the end of the winter, spring, and summer experiments. Values represent means (n = 4) ± SD.

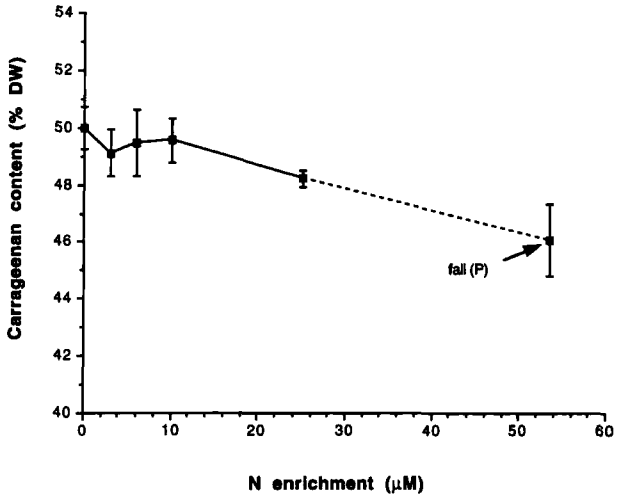

FIG. 8. Variations in carrageenan content (% DW) of *C. crispus* cultured in different N enrichments at the end of the fall (N) experiment. The value from the fall (P) experiment (for 6 μM P·wk^{-1} and 53.5 μM N·wk^{-1}) has been included (dotted line). Values represent means (n = 4) ± SD.

creased over the range tested. When the data from the fall (P) experiment (6 μM P·wk^{-1}, 53.5 μM N·wk^{-1}) were considered, a lower tissue total N content was found.

Variations in total carrageenan content. In the fall (P) experiment, carrageenan content decreased from 53.1 ± 0.3% DW to 46.3 ± 1.5% DW between the enrichments of 0 and 3 μM P·wk^{-1} (Fig. 5). Further P enrichments had no effect on carrageenan content, which remained stable at 46.1 ± 1.8% DW. When plotted as a function of total P content (Fig. 6), carrageenan content declined markedly when the tissue total P content increased from 1.71 ± 0.27 to 2.93 ± 0.16 mg P·g DW^{-1}. At higher tissue total P levels, carrageenan content remained low. The winter, spring, and summer experiments produced comparable results (Fig. 7). P enrichment had no effect on carrageenan content, which, in these experiments, differed only slightly (46.2 ± 1.3, 43.1 ± 0.7, and 44.5 ± 0.6% DW for winter, spring, and summer, respectively). These values were similar to the constant carrageenan content found in the fall (P) experiment at enrichments above 3 μM P·wk^{-1}. In the fall (N) experiment, increasing N enrichment up to 25 μM N·wk^{-1} produced no effect on carrageenan content, which averaged 49.3 ± 0.9% DW over this range (Fig. 8). When the data from the fall (P) experiment (6 μM P·wk^{-1}, 53.5 μM N·wk^{-1}) were considered, a lower carrageenan content was found.

Variations in photosynthetic parameters and pigments. Inorganic phosphate had no significant effect on any of the PI parameters and photosynthetic pigment contents and ratios (Tables 2, 3). In contrast, P_{max} and the photosynthetic pigment contents were significantly affected by nitrate availability: all increased with increasing N availability. The ratios PE:APC and PC:APC were unaffected by N supply, as were α, R_d, I_c, and I_k. Although α values were lower in the 0 μM N plants than in those cultivated at higher N concentrations, these differences were not significant because of the high variability among treatment replicates.

Overall, the PI parameters and pigment contents were similar between the P experiments, and plants grown under the highest N concentrations (Table 2). Those differences that did occur (R_d, I_c, total phycobilin, and APC contents) may be related to seasonal differences in environmental conditions, such as irradiance and temperature, that had long-

TABLE 2. *Effects of P and N on the PI parameters and photosynthetic pigment contents and ratios in* C. crispus. *Values represent means (n = 4) ± SD for summer (P) and fall (N) enrichment experiments. See text for abbreviations and units.*

Parameter	0 μM P	15 μM P	0 μM N	25 μM N
P_{max}	0.47 (0.16)	0.55 (0.10)	0.33 (0.04)	0.48 (0.09)
α ($\times 10^{-2}$)	1.11 (0.17)	0.91 (0.20)	0.75 (0.25)	0.89 (0.26)
R_d	0.13 (0.05)	0.11 (0.13)	0.06 (0.01)	0.05 (0.01)
I_c	12.10 (4.29)	12.40 (1.34)	9.33 (2.87)	5.86 (0.87)
I_k	42.00 (12.40)	61.90 (10.90)	46.80 (9.98)	55.32 (15.72)
Chlorophyll-*a*	0.75 (0.25)	0.61 (0.12)	0.43 (0.05)	0.60 (0.08)
Total phycobilin	2.52 (0.62)	2.71 (0.22)	0.29 (0.07)	0.93 (0.07)
Allophycocyanin	0.65 (0.18)	0.66 (0.10)	0.07 (0.02)	0.18 (0.06)
PE:APC	1.97 (0.24)	2.07 (0.27)	2.27 (0.65)	3.26 (1.87)
PC:APC	0.97 (0.12)	1.09 (0.06)	0.81 (0.12)	1.48 (0.86)

TABLE 3. *Effects of P and N on the PI parameters and photosynthetic pigment contents and ratios in* C. crispus. *Summary of Student's t-test of H_o = no significant differences between 0 and 15 μM P treatments or between 0 and 25 μM N treatments. n = 4 and t_{crit} = 2.45 in all cases. ns denotes not significant (accept H_0); *, **, and *** indicate rejection of H_0 at P < 0.05 to > 0.02, P < 0.02 to > 0.01, and P < 0.01 levels, respectively. See text for abbreviations and units.*

Nutrient	P			N		
Parameter	*t*	*P*	Level	*t*	*P*	Level
P_{max}	0.908	0.396	ns	2.910	0.027	*
α	1.150	0.295	ns	0.776	0.467	ns
R_d	0.659	0.534	ns	1.480	0.188	ns
I_c	0.116	0.911	ns	2.320	0.060	ns
I_k	0.159	0.877	ns	0.915	0.395	ns
Chlorophyll-*a*	0.929	0.389	ns	3.510	0.013	**
Total phycobilin	0.577	0.585	ns	12.000	<0.001	***
Allophycocyanin	0.119	0.909	ns	3.460	0.013	**
PE:APC	0.578	0.585	ns	0.994	0.358	ns
PC:APC	1.790	0.124	ns	1.560	0.170	ns

term effects on the physiology of *C. crispus* and persisted throughout the period of laboratory culture.

DISCUSSION

Tissue total P content. Tissue total P contents of fall (P), winter, spring, and summer plants increased with enrichments of P up to 6 $\mu M \cdot wk^{-1}$; further enrichment did not change this content, indicating uptake saturation (Chopin et al. 1990a). The season during which the plants were collected affected the saturation level attainable by the plants. Thus, the nutrient regime from which plants are collected influences the amount of P plants store in their tissues. Plants acclimated to a high nutrient environment (winter and first part of spring) store a greater amount of P, while plants collected from a low nutrient environment (second part of spring to fall) store lower levels of P. The fall (N) plants, exposed to a constant level of P enrichment, maintained a constant internal P level throughout the experiment. It can, therefore, be concluded that, in the range tested, ambient N concentration did not affect P uptake and storage.

Tissue total N content. Although there was some seasonal variation, tissue total N content was considered constant over the range of P enrichment tested in each of the fall (P), winter, spring, and summer experiments. There was also no visible increase in the degree of bleaching of these plants. We conclude, then, that the ambient P concentrations used in this study did not affect N uptake and storage. Lapointe (1985, 1987) reported, however, that in another red algal species, *Gracilaria tikvahiae* McLachlan, severe P limitation can induce N limitation. Fall (N) plants displayed no increase in tissue total N content in the range 0–6 $\mu M\ N \cdot wk^{-1}$ enrichment. At enrichment levels above 6 $\mu M\ N \cdot wk^{-1}$, there was an increase in tissue total N content, with no saturation reached over the range tested. If the value for an enrichment of 53.5 $\mu M\ N \cdot wk^{-1}$ [from the fall (P) experiment] is considered, it appears that further N enrichment would either not further increase tissue total N content of the plants, which should be saturated at a content of 26.48 ± 3.00 mg $N \cdot g\ DW^{-1}$, or, that after a period of increasing tissue total N content, excessive N enrichment could be detrimental to the plants, leading to a decrease of their content. From all these data, it appears that tissue total N contents higher than approximately 25 mg $N \cdot g\ DW^{-1}$ result from luxury uptake and accumulation of reserves, which can be used when insufficient N is available from ambient seawater.

DIP concentration in seawater. In the fall (P), winter, spring, and summer experiments, the increase of residual DIP in seawater at the end of the experimental period was pronounced for enrichments above 6 $\mu M\ P \cdot wk^{-1}$, which coincided with the total P saturation level of algal tissues. Thus, *C. crispus* absorbed all necessary DIP until its tissues reached their saturation level, after which any further P enrichment resulted in the nutrient remaining in seawater. From an aquaculture perspective, defining the necessary nutrient enrichment is of twofold importance. First, excessive enrichment would not be cost effective as accumulation of residual DIP would not benefit the algae and would be a waste of nutrients. Secondly, high levels of DIP would be released in the effluent(s) of the aquaculture system and be deposited in the natural environment. The latter is less and less tolerated in an increasing number of countries, in light of the evidence accumulating on the correlation between nutrient discharge into the coastal environment and phenomena such as green tides and toxic phytoplankton blooms (Fuhs 1969, Gordon et al. 1981, Gotham and Rhee 1981, Håkanson et al. 1988, Harrison et al. 1990). The amount of residual DIP in seawater for the fall (N) experiment was stable over all levels of N enrichment and corresponded to the level obtained for P enrichment of 6 $\mu M\ P \cdot wk^{-1}$ in the other four experiments. Thus, the level of N enrichment did not exert an effect on the accumulation of P.

Seasonal variations in carrageenan content. The seasonal variations in carrageenan content of *C. crispus* have been studied by several authors (Black et al. 1965, Fuller and Mathieson 1972, Rigney 1972,

McCandless and Craigie 1974, Mathieson and Tveter 1975, Chopin et al. 1987). These studies generally agree that there is an increase in carrageenan content during the summer and a decrease during the fall. A second increase occurs throughout the winter, followed by a sharp decrease in the spring. Neish and Shacklock (1971), Mathieson and Tveter (1975), Neish et al. (1977), and Simpson et al. (1978) have shown that, when other parameters are constant, carrageenan content is inversely related to the ambient seawater N concentration (the so-called "Neish effect"). It is, therefore, not surprising to find the lowest carrageenan contents in spring plants, as the tissue total N contents of these plants were the highest. Fall (P) plants had the lowest tissue total N contents and had the highest carrageenan contents. The tissue total N contents of summer plants were intermediate, and carrageenan contents were slightly lower than the fall (P) and winter levels.

Effect of P nutrition on carrageenan content. References to the possible importance of P in controlling carrageenan content in algae are rare (Chopin et al. 1990a, 1991). The seasonal profile of tissue total P content was similar to the one found for N. As tissue nutrient contents are correlated to seawater nutrient concentrations (Chopin et al. 1990b), the arguments which, until now, have credited seawater N concentration for the observed seasonal carrageenan variations could, therefore, be applied to DIP, particularly in the case of our culture systems where N was supplemented in a large amount (53.5 μM N·wk^{-1}) for all treatments and the only changing parameter was the DIP enrichment. The carrageenan content of fall (P) plants was stable (46.1 ± 1.8% DW) for P enrichments above 3 μM P·wk^{-1}. When the plants were cultivated without added P, carrageenan content increased to 53.1 ± 0.3% DW. Figures 5 and 9 summarize this relationship between carrageenan content and P nutrition, which is comparable to the "Neish effect" in N nutrition. Tissue total P level controls carrageenan content but only within a certain range. The only time a carrageenan content increase was recorded coincided with the only occasion when tissue total P content fell below 2 mg P·g DW^{-1} (1.71 ± 0.27). Control is lost when the tissue total P level exceeds this critical value. At higher tissue total P levels, carrageenan content is stable around the average respective seasonal values. From an aquaculture perspective, it would be more interesting to monitor the tissue total P content of plants than the enrichment level of seawater, even though the two are obviously related.

The question remains as to whether or not further tissue P depletion would have led to a further increase in carrageenan content. In culture, however, one has to reach a compromise between the desired yield of carrageenans and the general health of the plants. We observed, as previously reported by Neish et al. (1977), that severe P depletion induced pronounced fragmentation of the thalli. Natural pop-

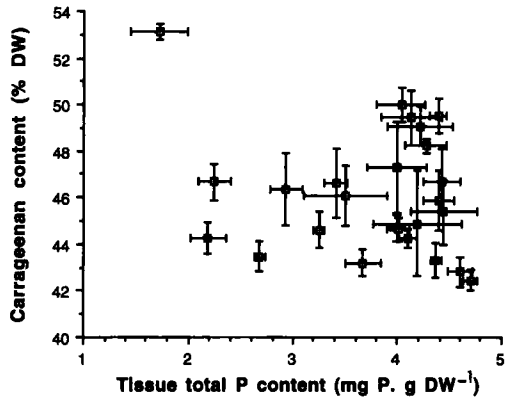

FIG. 9. Carrageenan content (% DW) of *C. crispus* as a function of tissue total P content (mg P·g DW^{-1}) at the end of each experiment. Values represent means (n = 4) ± SD and means (n = 6) ± SD for carrageenan content and tissue total P content, respectively.

ulations of *C. crispus* sampled in various locations on both sides of the Atlantic Ocean have shown large seasonal and geographical variations in carrageenan content ranging from 28 to 75% DW (Chopin 1986). These data should, however, be considered with caution, as some authors used an alcohol precipitation for the extraction of carrageenan, which often coprecipitates floridean starch, resulting in an overestimation of carrageenan content (Chopin et al. 1991). Still, these data do suggest the possibility that *C. crispus* could yield carrageenans at better than 53.1% DW. In an ongoing study of a natural population of *C. crispus* in Prince Edward Island, Canada, Chopin and Whalen (unpubl.) observed the lowest carrageenan content in the smallest plants [class 1, according to the classification of Chopin et al. (1988)] at 39.0% DW for a tissue total P content of 5.76 mg P·g DW^{-1}. The highest carrageenan contents were found in the largest plants (classes 4 and 5) at 69.2 and 66.6% DW for tissue total P contents of 2.10 and 1.96 mg P·g DW^{-1}, respectively. However, when P depletion in seawater was sustained for several summer months, a decrease of these high carrageenan contents resulted. Further study is obviously required before the above question can be answered, as all these data suggest 1) different nutritional requirements for plants at different levels of development; 2) different responses between plants in natural populations and plants under culture conditions for 7 wk; and 3) the possibility of differences between populations in different environments (i.e. the emergence of strains with different carrageenan yield characteristics).

Effect of N nutrition on carrageenan content. The carrageenan content of fall (N) plants was stable (49.3 ± 0.9% DW) up to an enrichment of 25 μM N·wk^{-1}. However, when the data collected during the fall (P) experiment (at 53.5 μM N·wk^{-1}, 6 μM P·wk^{-1}) were considered, a decrease in carrageenan content of 3.2% DW was recorded. Thus, a slight "Neish

effect" was indirectly demonstrated for these plants. The plants cultured without enrichment had a carrageenan content of 49.9% DW compared to 53.1% DW for the fall (P) plants. This difference reflects the fact that, while not N enriched, they were enriched with 6 μM P·wk^{-1}, thereby decreasing their carrageenan content.

Comparisons with a subtropical population of the red alga Agardhiella subulata. In all experiments, algal tissues reached total P saturation levels at a P enrichment of 6 μM P·wk^{-1}. This enrichment is lower than the required 15 μM P·wk^{-1} found by Chopin et al. (1990a) to cause saturation in the tissues of a subtropical population of the red alga *A. subulata*. Both species reached similar saturation contents: 4.25 ± 0.27 mg P·g DW^{-1} for *C. crispus* and 4.13 ± 0.45 mg P·g DW^{-1} for *A. subulata*. However, when both species were cultivated in seawater unenriched in P, *A. subulata* had a lower tissue total P content (0.51 ± 0.08 mg P·g DW^{-1}) than *C. crispus* (varying between 1.71 ± 0.27 and 2.68 ± 0.05 mg P·g DW^{-1}, according to seasonal variations). This could account for the tissue total P saturation being reached at a lower enrichment level (6 μM P·wk^{-1}) in *C. crispus* than in *A. subulata* (15 μM P·wk^{-1}). Interestingly, these tissue total P contents are in the same range as the estimated maximum cell quota (4.2 mg P·g DW^{-1}) and minimum cell quota (0.51 mg P·g DW^{-1}) found for the gametophytic phase of *Porphyra umbilicalis* (L.) Kützing by Hernandez et al. (1993). It is difficult to explain why *A. subulata* cultivated in P unenriched seawater had a lower tissue total P content than *C. crispus* as the preconditioning conditions for the two studies were different. Moreover, the specific growth rates of the two species differed (Chopin, unpubl. data). Interestingly, Chopin et al. (1990a) observed the same steep increase in residual DIP concentration in seawater at enrichments above 6 μM P·wk^{-1}.

The same authors found that the highest carrageenan content (43.9 ± 1.4% DW) in *A. subulata* was obtained at an enrichment of 3 μM P·wk^{-1}. P unenriched seawater gave the lowest content (25.7 ± 3.4% DW), while enrichments from 6 to 20 μM P·wk^{-1} gave intermediate values (around 34% DW). The reservoir DIP concentration in the study by Chopin et al. (1990a) was between 0.13 and 0.17 μM P. In the present study, it was higher for most weeks of the different experiments (as high as 1.23 μM P). This effectively increased the DIP concentration the algae were exposed to in each experiment. Thus, the temperate unenriched seawater in the present study was comparatively less P unenriched than the subtropical one in the study by Chopin et al. (1990a). This could, therefore, explain why the highest carrageenan content was observed in P unenriched seawater in the fall (P) experiment of this study, whereas it was recorded at an enrichment of 3 μM P·wk^{-1} for *A. subulata*. It could also explain why, concerning N nutrition, Neish and Shacklock (1971) recorded the highest carrageenan content in *C. crispus* cultured in unenriched temperate seawater, whereas DeBoer (1978) found that the highest carrageenan content in *A. subulata* occurred with a slight enrichment of subtropical seawater (0.5 μM N).

Chopin et al. (1990a) induced an increase of 9.9% DW (from 34 to 43.9% DW) in carrageenan content of *A. subulata* by decreasing P enrichment to 3 μM P·wk^{-1}. In the present study, the carrageenan content of the temperate red alga *C. crispus* increased from 46.1 to 53.1% DW by subjecting it to significant P starvation (tissue total P content lower than 2 mg P·g DW^{-1}) during a 7-wk period. Such a manipulation of the carrageenan content by 7% DW is similar to the manipulation by approximately 9% DW reported by Neish et al. (1977) when N fertilization level of their culture system was controlled. These results are of significance for algal aquaculture programs because it is critical to minimize fertilizer costs and optimize carrageenan production. An increase of 7% DW corresponds, in fact, to an increase of approximately 15% in carrageenan production, which is far from negligible at an industrial scale.

Effect of P nutrition on photosynthetic parameters and pigments. Unfortunately, the photosynthesis data do not provide an insight into the regulation of carrageenan production by P because photosynthesis was not measured at the end of the experiment (fall P) in which severe P limitation resulted in an increase in carrageenan content. However, despite the reduction in tissue total P content in *C. crispus* grown below 6 μM P·wk^{-1} enrichment, there was no significant effect of P nutrition on the PI parameters or contents of photosynthetic pigments in the plants from the winter, spring, and summer experiments. The PI parameters and pigment contents in all P enrichments and at 25 μM N were similar to those previously reported for *C. crispus* (Kübler and Davison 1993). These results suggest that P limitation is not an important factor controlling photosynthetic metabolism of *C. crispus* in the temperate waters of the Bay of Fundy. The rationale for this conclusion is that no impairment of photosynthetic metabolism was observed even after the plants were grown in the laboratory for 7 wk without P enrichment. Where P limitation of seaweed metabolism has been established, the addition of phosphate resulted in a rapid increase in both photosynthesis and growth (Lapointe 1986, 1987). The measurements of photosynthesis and pigment contents in this study were made on young, growing, apical sections of the thallus, and it is possible that these tissues may have been supplied with P redistributed from the older, nongrowing, regions of the plants.

Effect of N nutrition on photosynthetic parameters and pigments. In contrast to P nutrition, N nutrition had a significant effect on both P_{max} and the contents of photosynthetic pigments in *C. crispus*. These param-

eters were all significantly lower in plants grown at 0 μM N than in those grown at 25 μM N. The plants used in the N limitation experiment were collected in November when levels of inorganic N in coastal waters and cellular N reserves are generally high (Chapman and Craigie 1977, Lapointe and Duke 1984). Thus, the occurrence of N limitation in our culture study provides indirect evidence that, in common with other temperate seaweeds (Davison et al. 1984), the Bay of Fundy population of *C. crispus* may experience N limitation during the summer months.

The reduction in P_{max} presumably reflects the requirement of N for Rubisco and other Calvin cycle enzymes, which are believed to be the rate-limiting step in light-saturated photosynthesis (Wheeler and Weidner 1983, Lapointe and Duke 1984, Ekman et al. 1989, Davison 1991). Nitrogen is also a major constituent of photosynthetic pigments such as chlorophyll and phycobiliproteins, and the absence of N clearly reduced the ability of *C. crispus* to synthesize these compounds. A similar dependence of pigment contents on N availability has been noted in other red algae (Fredriksen and Rueness 1989). The reduction in pigment content reflects a decrease in either the number of photosynthetic units (PSI plus PSII) and/or a reduction in the size of the light-harvesting antenna associated with the reaction centers (Ramus 1981). In red algae, the primary antenna for PSI is chlorophyll-*a*, with PSII receiving energy from the phycobiliproteins (Gantt 1990). Phycobiliproteins are organized into structures called phycobilisomes (PBS) that have an invariant core of APC receiving energy from outer layers of PC and PE (Gantt 1990). The percentage reduction in APC in the 0 μM N plants compared with the 25 μM N ones (38%) was similar to the reduction in total phycobiliproteins (31%). This, together with the constant ratio of PC:APC and PE:APC between these treatments, suggests that N limitation reduces PBS number, but not size. It is consistent with the work of Levy and Gantt (1990) on the effect of N starvation in the unicellular red alga *Porphyridium purpureum*. The reduction in phycobiliprotein content in the 0 μM N plants compared with the 25 μM N ones was much greater than the reduction in chlorophyll-*a* (71%). This observation is consistent with the situation in nature where *C. crispus* and other red algae frequently become greenish during the summer (bleaching) because of reductions in phycobiliprotein contents relative to chlorophyll-*a*, presumably because of N limitation (Penniman and Mathieson 1987). Other laboratory studies have also demonstrated a greater reduction in PBS than chlorophyll content during N starvation, indicating that PSII is more susceptible to N limitation than PSI (Lapointe and Duke 1984, Levy and Gantt 1990). Differences did occur between the pigment contents of the nutrient-repleted plants from the N and P enrichment experiments. For example, allophycocyanin (and hence PBS) content was lower in the 25 μM N treatment than in any of the treatments in the P experiment. Because these experiments were performed with plants collected at different times of the year, it is probable that these differences either reflect persistent effects of seasonal changes in the natural environment, or endogenous rhythms in the plants.

Despite the relatively large changes in pigment content due to N limitation, no significant change was found in the light-harvesting efficiency (α). It contrasts to the situation in other red algae where N limitation reduces both pigment content and α (Levy and Gantt 1990). The most probable explanation for the lack of response of α to changes in pigment content in *C. crispus* is based upon the self-shading that occurs within the opaque thallus of this species, which is essentially optically black (Lüning and Dring 1985), combined with the fact that the light-field used to measure the PI parameters was unidirectional. In the highly pigmented plants grown under N-sufficient conditions, the majority of the incident light at the low light levels used to measure α is probably absorbed by the cells on the illuminated surface, with those on the opposite side receiving very little light. In contrast, in N-limited plants with less pigments, cells on both sides of the thallus may be able to participate in photosynthesis. Providing the percentage of incident light absorbed by N-repleted and N-limited plants was the same, and assuming moderate N limitation does not affect the quantum efficiency of photosynthesis (photosynthetic O_2 production per unit absorbed light), the photosynthetic O_2 production per unit incident light (α) would remain the same. Frost-Christensen and Sand-Jensen (1992) reported that the relationship between both light absorption and α and pigment content of aquatic macrophytes was hyperbolic, with absorption and α being independent of chlorophyll content in highly pigmented plants. The relationship between pigment content and photosynthetic performance has also been discussed extensively by Ramus (1990) in contrasting the photosynthetic responses of the chlorophytes *Ulva* and *Codium*.

In the range of tissue total P and N contents tested during the winter, spring, summer (P) experiments, and fall (N) experiments, no effect of P nutrition on the PI parameters and photosynthetic pigment contents and ratios in *C. crispus* was recorded; however, effects of N nutrition were observed. Further study should be conducted to investigate if severe P starvation, as the one induced in the fall (P) experiment and which increased the carrageenan production, would have an effect. Such a condition, if it can be created in culture, does not, however, appear to occur naturally in the temperate waters of the Bay of Fundy, where our data suggest that N, but probably not P limitation, can take place.

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Birch, P. B., Gordon, D. M. & McComb, A. J. 1981. Nitrogen and phosphorus nutrition of *Cladophora* in the Peel-Harvey estuarine system, Western Australia. *Bot. Mar.* 24:381–7.

Bird, N. L., Chen, L. C.-M. & McLachlan, J. 1979. Effects of temperature, light, and salinity on growth in culture of *Chondrus crispus, Furcellaria lumbricalis, Gracilaria tikvahiae* (Gigartinales, Rhodophyta), and *Fucus serratus* (Fucales, Phaeophyta). *Bot. Mar.* 22:521–7.

Black, W. A. P., Blakemore, W. R., Colquhoun, J. A. & Dewar, E. T. 1965. The evaluation of some red marine algae as a source of carrageenan and of its κ- and λ-components. *J. Sci. Food Agric.* 16:573–85.

Butler, M. R. 1936. Seasonal variations in *Chondrus crispus*. *Biochem. J.* 30:1338–44.

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. (Berl.)* 40: 197–205.

Chopin, T. 1985. Variations saisonnières de la nutrition phosphorée, des carraghénanes et de la croissance chez deux formes de l'algue rouge *Chondrus crispus* Stackhouse. Thèse 3ème cycle, Brest, 175 pp.

——— 1986. The red alga *Chondrus crispus* Stackhouse (Irish moss) and carrageenans—a review. *Can. Tech. Rep. Fish. Aquat. Sci.* 1514:1–69.

Chopin, T., Bodeau-Bellion, C., Floc'h, J.-Y., Guittet, E. & Lallemand, J.-Y. 1987. Seasonal study of carrageenan structures from female gametophytes of *Chondrus crispus* Stackhouse (Rhodophyta). *Hydrobiologia* 151/152:535–9.

Chopin, T., Hanisak, M. D. & Koehn, F. E. 1991. Effects of seawater phosphorus concentration on floridean starch content in *Agardhiella subulata* (C. Agardh) Kraft *et* Wynne (Rhodophyceae). *Bot. Mar.* 34:369–73.

Chopin, T., Hanisak, M. D., Koehn, F. E., Mollion, J. & Moreau, S. 1990a. Studies on carrageenans and effects of seawater phosphorus concentration on carrageenan content and growth of *Agardhiella subulata* (C. Agardh) Kraft and Wynne (Rhodophyceae, Solieriaceae). *J. Appl. Phycol.* 2:3–16.

Chopin, T., Hourmant, A., Floc'h, J. Y. & Penot, M. 1990b. Seasonal variations of growth in the red alga *Chondrus crispus* on the Atlantic French coasts. II. Relations with phosphorus concentration in seawater and internal phosphorylated fractions. *Can. J. Bot.* 68:512–7.

Chopin, T., Pringle, J. D. & Semple, R. E. 1988. Reproductive capacity of dragraked and non-dragraked Irish Moss (*Chondrus crispus* Stackhouse) beds in the southern gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* 45:758–66.

Conolly, N. J. & Drew, E. A. 1985. Physiology of *Laminaria*. IV. Nutrient supply and daylength, major factors affecting growth of *L. digitata* and *L. saccharina*. *Mar. Ecol.* 6:299–320.

Craigie, J. S. & Leigh, C. 1978. Carrageenans and agar. *In* Hellebust, J. A. & Craigie, J. S. [Eds.] *Handbook of Phycological Methods, Physiological and Biochemical Methods.* Cambridge University Press, Cambridge, pp. 109–31.

Craigie, J. S. & Shacklock, P. F. 1989. Culture of Irish Moss. *In* Boghen, A. D. [Ed.] *Cold Water Aquaculture in Atlantic Canada.* Canadian Institute for Research and Regional Development, Moncton, pp. 243–70.

Davison, I. R. 1991. Environmental effects on photosynthesis. I. Temperature. *J. Phycol.* 27:2–8.

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 of *Laminaria* spp. *Mar. Biol. (Berl.)* 84:207–17.

Davison, I. R., Greene, R. M. & Podolack, E. J. 1991. Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar. Biol. (Berl.)* 110:449–54.

DeBoer, J. A. 1978. Effects of nitrogen enrichment on growth rate and phycocolloid content in *Gracilaria foliifera* and *Neoagardhiella baileyi* (Florideophyceae). *Proc. Seaweed Symp.* 9: 263–71.

Duncan, M. J. & Harrison, P. J. 1982. Comparison of solvents for extracting chlorophylls from marine macroalgae. *Bot. Mar.* 25:445–7.

Ekman, P., Lignell, A. & Pedersen, M. 1989. Localization of ribulose-1,5-biphosphate carboxylase/oxygenase in *Gracilaria secundata* (Rhodophyta) and its role as a nitrogen storage pool. *Bot. Mar.* 32:527–34.

Fredriksen, S. & Rueness, J. 1989. Culture studies of *Gelidium latifolium* (Grev.) Born. *et* Thur. (Rhodophyta) from Norway. Growth and nitrogen storage in response to varying photon flux density, temperature and nitrogen availability. *Bot. Mar.* 32:539–46.

Frost-Christensen, H. & Sand-Jensen, K. 1992. The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. *Oecologia* 91:377–84.

Fuhs, G. W. 1969. Phosphorus content and rate of growth in the diatoms *Cyclotella nana* and *Thalassiosira fluviatilis*. *J. Phycol.* 5:312–21.

Fuller, S. W. & Mathieson, A. C. 1972. Ecological studies of economic red algae. IV. Variations of carrageenan concentration and properties in *Chondrus crispus* Stackhouse. *J. Exp. Mar. Biol. Ecol.* 10:49–58.

Gantt, E. 1990. Pigmentation and photoacclimation. *In* Cole, K. M. & Sheath, R. [Eds.] *Biology of Red Algae.* Cambridge University Press, New York, pp. 203–19.

Gordon, D. M., Birch, P. B. & McComb, A. J. 1981. Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Bot. Mar.* 24:93–106.

Gotham, I. J. & Rhee, G. Y. 1981. Comparative kinetic studies of phosphate limited growth and phosphate uptake in phytoplankton in continuous culture. *J. Phycol.* 17:257–65.

Håkanson, L., Ervik, A., Mäkinen, T. & Moller, B. 1988. The basic concepts concerning assessment of environmental effects of marine fish farms. *Nordic Council of Ministers* 90:1–103.

Harrison, P. J., Hu, M. H., Yang, Y. P. & Lu, X. 1990. Phosphate limitation in estuarine and coastal waters of China. *J. Exp. Mar. Biol. Ecol.* 140:79–87.

Hernandez, I., Fernandez, 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.* 173:181–96.

Kornfeldt, R. A. 1982. Relation between nitrogen and phosphorus content of macroalgae and the waters of Northern Öresund. *Bot. Mar.* 25:197–201.

Kübler, J. E. & Davison, I. R. 1993. High-temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. *Mar. Biol. (Berl.)* 117:327–35.

Lapointe, B. E. 1985. Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: interactions between concentration and frequency of nutrient pulses. *J. Exp. Mar. Biol. Ecol.* 93:211–22.

——— 1986. Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum gluitans* (Phaeophyceae) in the Western North Atlantic. *Deep Sea Res.* 33:391–9.

——— 1987. Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Mar. Biol. (Berl.)* 93:561–8.

Lapointe, B. E. & Duke, C. S. 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *J. Phycol.* 20:488–95.

Levy, I. & Gantt, E. 1990. Development of photosynthetic activity in *Porphyridium purpureum* (Rhodophyta) following nitrogen starvation. *J. Phycol.* 26:62–8.

Lüning, K. & Dring, M. J. 1985. Action spectra and spectral quantum yield of photosynthesis in marine macroalgae with thick and thin thalli. *Mar. Biol. (Berl.)* 87:119–29.

Manley, S. L. & North, W. J. 1984. Phosphorus and the growth of juvenile *Macrocystis pyrifera* (Phaeophyta) sporophytes. *J. Phycol.* 20:389–93.

Mathieson, A. C. & Tveter, E. 1975. Carrageenan ecology of *Chondrus crispus* Stackhouse. *Aquat. Bot.* 1:25–43.

McCandless, E. L. & Craigie, J. S. 1974. Reevaluation of seasonal factors involved in carrageenan production by *Chondrus crispus*: carrageenan of carposporic plants. *Bot. Mar.* 22:125–9.

Murphy, J. & Riley, J. P. 1962. A modified single solution approach for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31–6.

Neish, A. C. & Shacklock, P. F. 1971. Greenhouse experiments (1971) on the propagation of strain T4 of Irish Moss. *National Research Council of Canada, Atlantic Regional Laboratory, Technical Report* 14, 25 pp.

Neish, A. C., Shacklock, P. F., Fox, C. H. & Simpson, F. J. 1977. The cultivation of *Chondrus crispus*. Factors affecting growth under greenhouse conditions. *Can. J. Bot.* 55:2263–71.

Penniman, C. A. & Mathieson, A. C. 1987. Variation in chemical composition of *Gracilaria tikvahiae* McLachlan (Gigartinales, Rhodophyta) in the Great Bay Estuary, New Hampshire. *Bot. Mar.* 30:525–34.

Ramus, J. 1981. The capture and transduction of light energy. *In* Lobban, C. S. & Wynne, M. J. [Eds.] *The Biology of Seaweeds.* Blackwell Scientific Publ., Oxford, pp. 458–92.

——— 1990. A form–function analysis of photon capture for seaweeds. *Hydrobiologia* 204/205:65–71.

Rigney, J. A. 1972. Seasonal effects on the chemistry of *Chondrus crispus*. *In* Proceedings of the Meeting of the Canadian Atlantic Seaweed Industry, Environment Canada, Fisheries Service, Industrial Development Branch, Ottawa, pp. 95–106.

Rosenberg, G. 1981. Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae). Ph.D. thesis, Yale University, New Haven, 163 pp.

Ryther, J. H. & Dunstan, W. M. 1971. Nitrogen, phosphorus and eutrophication in the coastal marine environment. *Science (Wash. D.C.)* 171:1008–13.

Seely, R. G., Duncan, M. J. & Vidaver, W. E. 1972. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. *Mar. Biol. (Berl.)* 12:184–8.

Shacklock, P. F. & Craigie, J. S. 1986. Winter survival and growth of *Chondrus crispus* in onshore culture tanks. *Can. J. Fish. Aquat. Sci.* 43:263–8.

Shaughnessy, F. J. & DeWreede, R. E. 1991. Reliability of the resorcinol method for identifying isomorphic phases in the Gigartinaceae (Rhodophyta). *J. Appl. Phycol.* 3:121–7.

Simpson, F. J., Shacklock, P. F., Robson, D. & Neish, A. C. 1978. Factors affecting cultivation of *Chondrus crispus* (Florideophyceae). *Proc. Seaweed Symp.* 9:509–13.

Smith, S. V. 1984. Phosphorus versus nitrogen limitation in the marine environment. *Limnol. Oceanogr.* 29:1149–60.

Wheeler, P. A. & Björnsäter, B. R. 1992. Seasonal fluctuations in tissue nitrogen, phosphorus and N:P for five macroalgal species common to the Pacific Northwest Coast. *J. Phycol.* 28: 1–6.

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.* 19:92–6.

Zar, J. H. 1984. *Biostatistical Analysis,* 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, 718 pp.