Eco-physiological and biochemical study of two of the most contrasting forms of *Chondrus crispus* (Rhodophyta, Gigartinales)

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ABSTRACT: Seasonal variations of distribution of the gametophytic and tetrasporophytic generations, growth, carrageenans, phosphorus and nitrogen nutrition, and dry matter content were studied in 2 of the most contrasting forms of the red alga *Chondrus crispus* Stackhouse in Britanny, France. Seasonal variations of generations were more pronounced in the infralittoral than in the midlittoral form; the former had more reproductively mature plants and twice as many tetrasporophytes. Growth was greater in the infralittoral than in the midlittoral form. Contents of total carrageenans were comparable in the 2 forms; the κ-, ι-, and μ-carrageenan structures did not vary significantly (qualitatively, quantitatively, during the year, or between the 2 forms). The midlittoral form had a higher total phosphorus content, more marked seasonal variations and an earlier spring decline than the infralittoral form. Seasonal variations of total nitrogen content had different patterns in the 2 forms during summer The dry matter content of the infralittoral form was lower than that of the midlittoral form. These eco-physiological and biochemical differences and similarities are discussed in terms of the polymorphism of this alga.

INTRODUCTION

The extreme morphological plasticity of the red alga *Chondrus crispus* Stackhouse was recognized almost 2 centuries ago as attested by the denominations *Fucus polymorphus* Lamouroux (1805) and *Polymorpha* Stackhouse (1809). Different authors have proposed a wide range in the number of varieties, from 2 (Harvey 1846–1851) to 35 (Lamouroux 1813), and the status of the polymorphism of this alga still puzzles phycologists.

Many authors deemed morphological and coloration variations to be attributable to different ecological parameters: wave exposure and currents (Lilly 1968), salinity (Mathieson & Burns 1975), depth (Prince 1971), light intensity (MacFarlane 1956), and quantity of available nutrients in seawater (Neish & Fox 1971). Thomas (1938) and Newton et al. (1957) suggested that polymorphism was a multifactorial phenomenon resulting from several interacting environmental factors. The latter authors considered that some of the so-called varieties were different stages in development of the same plant and thus concluded that none of these forms were real varieties. MacFarlane (1968) used the term 'ecological types'. The transition between infralittoral forms and those of the upper part of the midlittoral zone has, however, not been clearly established. Between these extreme forms a whole series of intermediate forms exist which, moreover, can be observed within a few centimeters of each other in the same population. Therefore, environmental pressure alone cannot explain entirely the morphological heterogeneity of *Chondrus crispus*.

Genetic variability has been proposed by several authors. To this end, different kinds of experiments have been carried out: transplants (Floc'h 1969), chromosome analyses (Hanic 1973), growth of medullary tissues (Chen & Taylor 1978), structural and anatomical studies and hybridization trials (Chen & Taylor 1980a, b, Guiry 1991). None of them, however, allowed an unambiguous conclusion on the level of speciation of the different forms.

Over several years of investigations, encompassing the seasonal variations of distribution of the different generations of the life cycle, growth, carrageenan content, phosphorus and nitrogen nutrition, and dry matter content, a number of differences and similarities

between 2 of the most contrasting forms of *Chondrus crispus* have been observed and are reported in the present paper as an eco-physiological and biochemical contribution towards solving the polymorphism enigma of this alga.

MATERIALS AND METHODS

Materials. Algae were collected from Porz Liogan Cove in Brittany near Brest, France (Chopin & Floc'h 1987) at 2 different shore levels to allow all experiments to be conducted in parallel on two of the most contrasting forms of *Chondrus crispus* according to the Harvey (1846–1851) definition (Fig. 1).

The first form has its maximum of population density at a high level of the midlittoral zone, among *Ascophyllum nodosum*, and was therefore called the 'midlittoral' form. It is a large, thin, and membranous plant, with round tips, similar to the variety *typica* Turner described by Newton et al. (1957). The second form has its maximum of population density at very low tide among Laminariales and was consequently called 'infralittoral'. It is a narrow, subcylindrical, and cartilaginous plant, with sharp tips, resembling the variety *aequalis* Turner.

Gametophyte/sporophyte distribution. Approximately 3 kg fresh weight (FW) of plants of each form were collected monthly, between November 1982 and November 1983. In the laboratory, plants were sorted into 3 categories according to the presence or absence of cystocarps and sori on the surface of the fronds: (1) mature female gametophytes, (2) mature tetrasporophytes, and (3) immature plants (which, on occasion, included mature male gametophytes not detectable with the naked eye). Each set was washed with seawater, and epiphytes and epizoans were removed.
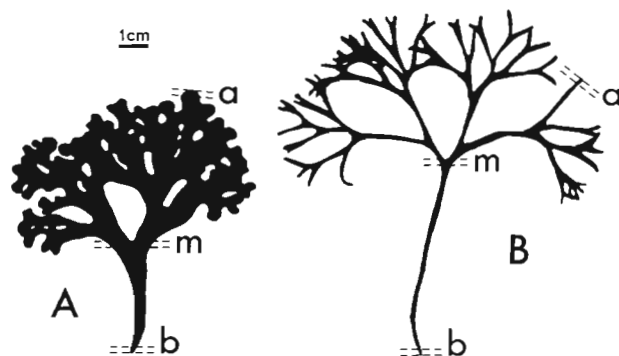

Fig. 1. *Chondrus crispus*. Two contrasting forms: (A) midlittoral form and (B) infralittoral form. Location of sections at 3 different parts of the thallus: apex (a), middle (m), and base (b)

Clean algae were then dried at 60 °C until constant dry weight was reached (minimum of 72 h). Each set was weighed and results were expressed in percentages of the total collection for each form and each month.

Measurement of growth by fluorescence labelling. To work with homogeneous material, this study, and the following experiments, were all performed on plants with 4 or more dichotomies and a frond length between 6 and 10 cm, corresponding to plants of Classes 3 and 4 in the classification system of Chopin et al. (1988). A fluorescent and vital stain, called Cellufluor (equivalent of Calcofluor White M2R), was used to localize and measure growth, from April 1984 to April 1985, based on the principle that newly-formed rows of cortical cells develop a dark area while cortical cells present at the time of the labelling pulse are still fluorescent. Labelling procedure in the field and transmission fluorescence microscopy observation in the laboratory have been described previously (Chopin & Floc'h 1987).

Extraction and content of total carrageenans. Samples of female gametophytes collected from November 1982 to November 1983 were extracted as described in Chopin et al. (1987). After centrifugation, the supernatant was precipitated in 2 volumes of ethanol. The coagulum was collected by filtration with a 34 μm Nytrel-TI filtration netting, dried for 24 h at 60 °C, weighed, and milled with a Dangoumau grinder. Second and third extractions were processed from the pellets of first and second extractions, respectively, and each time precipitates and coagula were collected in the same manner.

Infrared and ^{13}C-NMR carrageenan analysis. IR spectra from first, second, and third extraction samples were recorded with a Pye Unicam SP 2000 spectrometer. Sample films were prepared by evaporating 0.24 % carrageenan solutions on PVC plates.

Proton-composite pulse decoupled ^{13}C-NMR spectra of total extracts were recorded on a Bruker WM 400 spectrometer (100.57 MHz) in D_2O solution (20 mg ml^{-1}) at 80 °C. A spectral width of 12.5 kHz was used with a pulse angle of 65° (12 μs) and a repetition time of 0.655 s. Spectra of 16 K data points were accumulated. Chemical shifts (ppm) were measured relative to internal dimethylsulfoxide (DMSO, 39.6 ppm) and are reported as values downfield from external tetramethylsilane (TMS). Percentages of different carrageenan structures were calculated by integration of anomeric-carbon-atom peaks (Bellion et al. 1983, Usov 1984).

Determination of total P and total N content in the thallus. After drying at 105 °C to constant dry weight and acidic mineralization (H_2SO_4 + HNO_3), total P was measured monthly, between October 1982 and November 1983, by the method of Charlot (1966) on

parts, taken at 3 different locations (apex, middle, and base; Fig. 1), of approximately 60 thalli. Results from the 3 parts were then averaged to obtain data for the whole thallus. These samples were also used to determine the dry weight content, expressed in percentage of fresh weight.

Total N was measured on disks (total 250 mg FW) taken from approximately 25 thalli for each experiment, between November 1983 and August 1984. After extraction according to Hourmant et al. (1979), centrifugation, and mineralization (H_2SO_4 + H_2O_2), aliquots of the residue of the insoluble fraction pellet were distilled with a Parnas and Wagner apparatus and titrated with a sulfuric acid solution.

For all the studied parameters, comparison of the 2 forms was statistically tested with 2 nonparametric tests, the Kruskall-Wallis and the Mann-Whitney tests. Differences where $p < 0.1$ were taken as significant.

RESULTS

Distribution of mature female gametophytes and tetrasporophytes

Mature female gametophytes and tetrasporophytes represented, at some times of the year, very low percentages of the population; however they never disappeared totally (Fig. 2). Occurrence of matur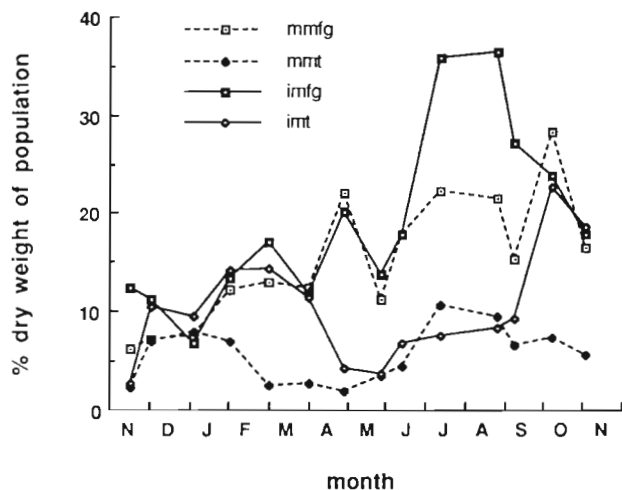e female gametophytes was maximal during summer in the infralittoral form and during summer/beginning of fall in the midlittoral one. For both forms it was minimal during winter. The distribution of the mature tetrasporophytes showed 2 peaks per year; they were recorded earlier (beginning of winter, summer) in the midlittoral than in the infralittoral form (winter, beginning of fall). Minima were observed in the middle of autumn and during spring.

Percentage variations were more pronounced in the infralittoral form, which contained more reproductive plants than the midlittoral form. The percentages of mature female gametophytes were always higher than those of mature tetrasporophytes except at the beginning of winter in both forms, and also in the middle of fall in the infralittoral form. When all the monthly collections were pooled, mature female gametophytes represented 18.9 % and 15.2 % of the infra- and midlittoral populations, respectively, whereas mature tetrasporophytes represented only 10.3 % and 5.6 %. The latter numbers also showed that there were almost twice as many mature tetrasporophytes in the infralittoral than in the midlittoral zone.

Variations of growth

As in both forms apical tissues were always actively growing regions (no fluorescence of the cell walls of the cortical cells) and no growth occurred at the level of the basal tissues (all cell walls of the cortical cells still labelled 1 mo after staining), tissues from the middle of the thallus were used to analyze the differences in growth pattern between the 2 forms. Counting the number of nonfluorescent cortical cell rows in transverse sections (Fig. 3) showed that growth was greater in the infralittoral form, except in November and April where it was similar in both forms. No growth was recorded for the whole winter in the midlittoral form, whereas there was still a slight growth during December in the infralittoral form. The measurement of the length of nonfluorescent tissue at the apex on longitudinal sections (Fig. 4) confirmed that growth of the infralittoral form was greater than or equal to that of the midlittoral form.

Carrageenan analyses

All IR spectra (Fig. 5) were characteristic of a dominant κ-carrageenan structure with a smaller amount of ι-carrageenan (peaks at 1240, 930, 845, and 805 cm^{-1}; Stancioff & Stanley 1969). No precursors (μ- or ν-carrageenans) could be detected by IR spectrometry. Spectra of the 2 forms were similar. When comparing spectra of first, second and third extractions, one can

Fig. 2. *Chondrus crispus.* Seasonal variations of mature female gametophytes and mature tetrasporophytes in the midlittoral and infralittoral forms. Results are expressed in % dry weight of the population. mmfg: midlittoral mature female gametophytes; mmt: midlittoral mature tetrasporophytes; imfg: infralittoral mature female gametophytes; imt: infralittoral mature tetrasporophytes
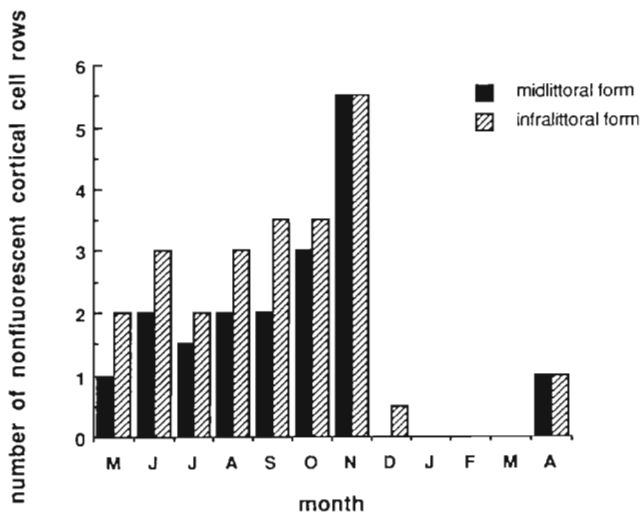
Fig. 3. *Chondrus crispus*. Seasonal variations of growth at the middle of the thallus in the midlittoral and infralittoral forms. Results are expressed in number of nonfluorescent cortical cell rows in transverse sections
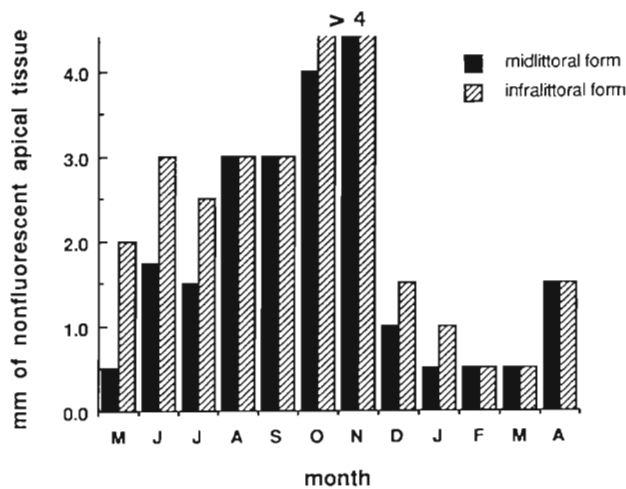

Fig. 4. *Chondrus crispus*. Seasonal variations of growth at the tip of the thallus in the midlittoral and infralittoral forms. Results are expressed in mm nonfluorescent apical tissue in longitudinal sections
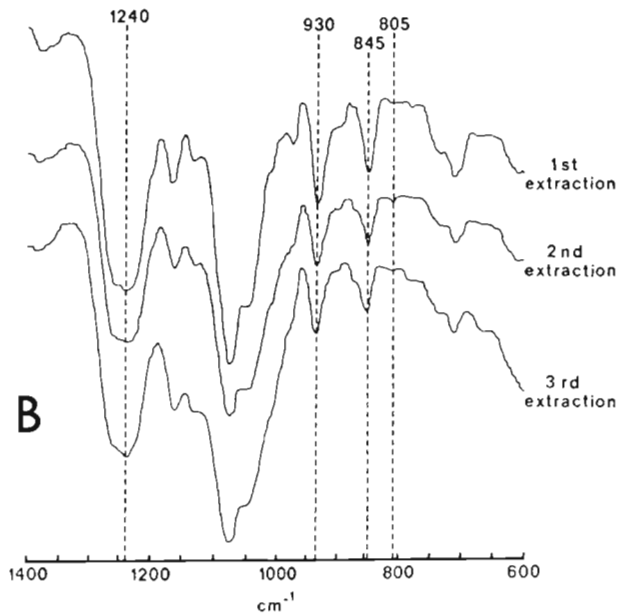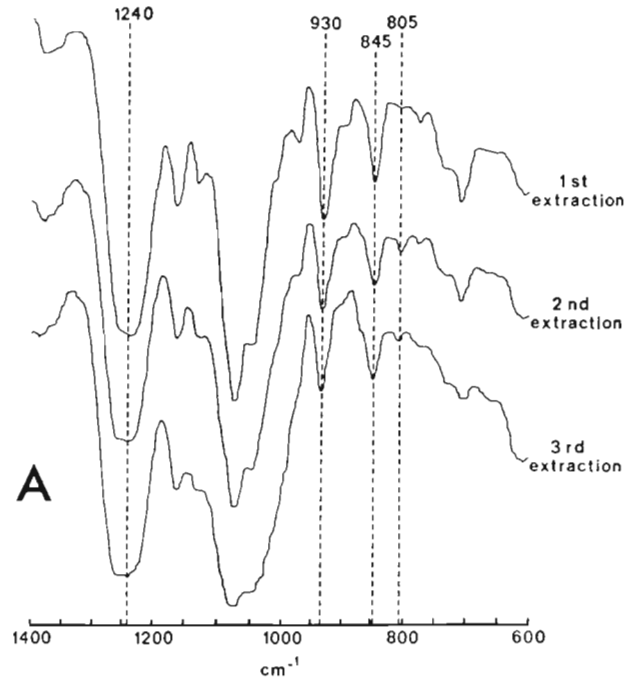

Fig. 5. *Chondrus crispus*. Dispersive IR spectra of the carrageenans extracted from female gametophytes of (A) the midlittoral form (1st extraction, 96.3 % of total extraction; 2nd extraction, 3.6 %; 3rd extraction, 0.1 %) and (B) the infralittoral form (1st extraction, 93.6 %; 2nd extraction, 6.2 %; 3rd extraction, 0.2 %) of *Chondrus crispus* collected on November 3, 1983

notice a general decrease in peak resolution as extractions progressed, and, in particular, the peak at 970 cm^{-1}, present in the first extraction, became a shoulder in the second, and completely disappeared in the third. The peak at 805 cm^{-1} (characteristic of the ι structure) slightly increased in the second and third extractions of the midlittoral form.

The study of the seasonal variations of total carrageenan content has been published previously (Cho-

pin et al. 1987): the pattern was similar in the 2 forms and vertical distribution had no significant influence on carrageenan content (Table 1). Only the periods of

Table 1. *Chondrus crispus*. Extreme contents of total carrageenans extracted from female gametophytes in the midlittoral and infralittoral forms. Results are expressed in % dry wt. Percentages in brackets indicate differences between maximal and minimal carrageenan contents

	Midlittoral form		Infralittoral form
Maximal content	49.9 (November)		48.4 (November)
Minimal content	37.9 (May)	37.4 (August)	35.9 (March)
Difference	12.0 (24.0 %)	12.5 (25.0 %)	12.5 (25.8 %)

lowest content were different: March for the infralittoral form, and May and August for the midlittoral form.

IR spectra of the 3 extractions for each form were obtained monthly in the aim of studying potential qualitative and quantitative seasonal variations of the different structures of carrageenans in the total extracts. The peak ratios 930/845 and 1240/845 were calculated; however, due to the lack of precision of this spectrometric method and the difficulty in defining the base-line for the different spectra, no conclusion could be reached. ^{13}C-NMR spectrometry was then used.

All ^{13}C-NMR spectra (Fig. 6) showed 12 major peaks with chemical shifts characteristic of the κ-carrageenan structure, which is dominant in all extracts. The ι-carrageenan structure, in lesser quantity, was also detected by the presence of the peaks Ia_1 (91.7 ppm) and Ig_4 (71.8 ppm). The signals X (104.5 ppm) and Y' (97.5 ppm) indicated the presence of the precursor μ-carrageenan structure.

The seasonal study of carrageenan structures was conducted on total extracts of plants of both forms collected in February, May, and August. Spectrum comparison (Fig. 6) did not show any qualitative variations of the different carrageenan structures according either to seasons or forms. One can, however, notice the occurrence of several signals in the region of the Ig_4 peak, in the 2 forms, especially in February and, to a lesser extent, in August. The significance of the presence of these signals remains unknown as they are presently unassigned. The methods of obtaining the

spectra allowed the calculation of the relative composition in κ-, ι-, and μ-carrageenan structures of the extracts by integration of the peaks corresponding to the signals Ia_1 (91.7 ppm), Ka_1 (95.0 ppm), and Y' (97.5 ppm). Table 2 showed no marked quantitative seasonal variations of the different carrageenan structures. Moreover, the slightly higher content in κ-carrageenan (72 %) and lower content in μ-carrageenan (5 %) of the midlittoral form compared to the infralittoral form (68 % and 8 %, respectively) represented variations at the limit of the detection threshold of the ^{13}C-NMR spectrometry. It is, therefore, better to conclude that there was no significant difference between the 2 forms, which had an average dominant κ-carrageenan feature (70 %) with less ι-carrageenan (23 %) and a small amount of μ-carrageenan (7 %).

Variations of total P and total N contents

Data on seasonal variations of total P content for the whole thallus have been published previously (Chopin et al. 1990b). They showed that the total P content was always higher in the midlittoral than in the infralittoral form, except at the end of May when the 2 forms had similar contents. Moreover, the drop in total P content during spring and summer was in 3 steps in the midlittoral form (abrupt drop starting at the end of April, levelling off until mid-July, and abrupt drop again to the lowest value at the end of August), whereas it started 1 mo later (end of May) and was progressive

Table 2. *Chondrus crispus*. κ-, ι-, and μ-carrageenan contents of total extracts from female gametophytes in the midlittoral and infralittoral forms. Results are expressed in % of total extracts

Structure	Form	Month			Average	
		February	May	August		
κ	Midlittoral	71	70	73	72	70
	Infralittoral	68	66	69	68	
ι	Midlittoral	23	25	22	23	23
	Infralittoral	25	24	22	24	
μ	Midlittoral	6	5	5	5	7
	Infralittoral	7	10	9	8	

Fig. 7 *Chondrus crispus*. Seasonal variations of the P concentration factor in the midlittoral and infralittoral forms
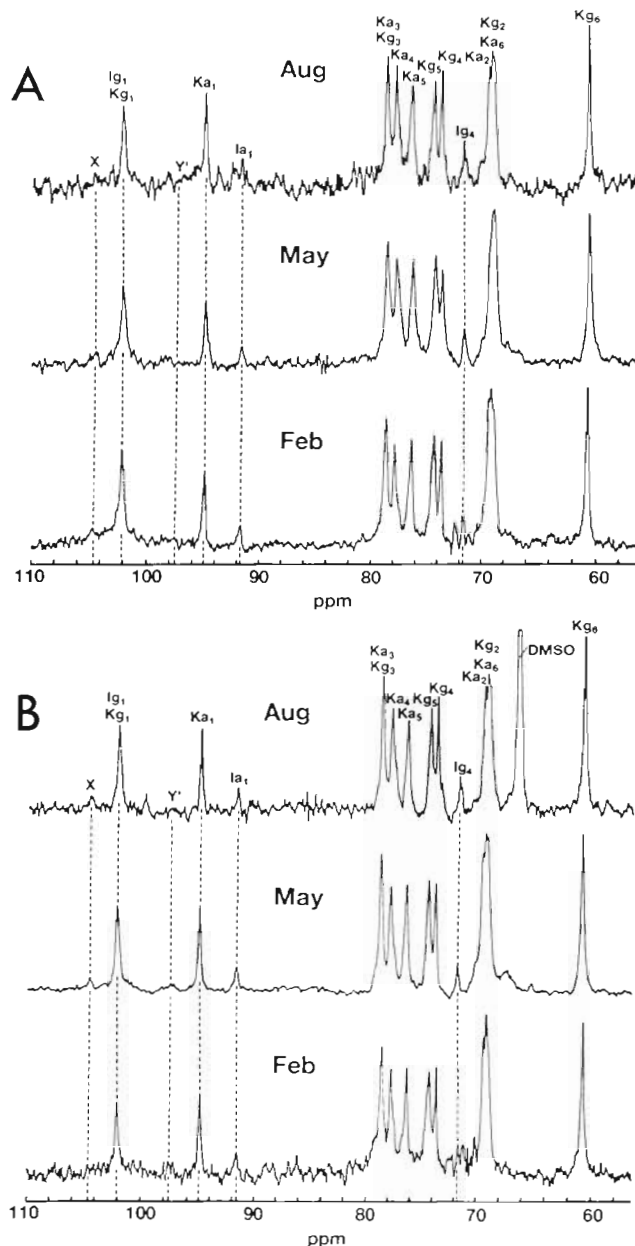
Fig. 6. *Chondrus crispus*. ^{13}C-NMR spectra of the carrageenans extracted from female gametophytes of (A) the midlittoral form (February 1983, 83 000 scans; May, 25 000 scans; August, 45 000 scans) and (B) the infralittoral form (February, 20 000 scans; May, 77 000 scans; August, 74 000 scans). Chemical shifts (ppm), measured relative to TMS, are assigned to carbon atoms of the repeating disaccharide of κ- and ι-carrageenans (g = 1,3-linked β-D-galactose-4-sulfate; a = 1,4-linked 3,6-anhydro-α-D-galactose or 1,4 linked 3,6-anhydro-α-D-galactose-2-sulfate). X and Y' refer to the anomeric carbon atoms of 1,3-linked β-D-galactose-4-sulfate and 1,4-linked α-D-galactose-6-sulfate, respectively, of μ-carrageenan
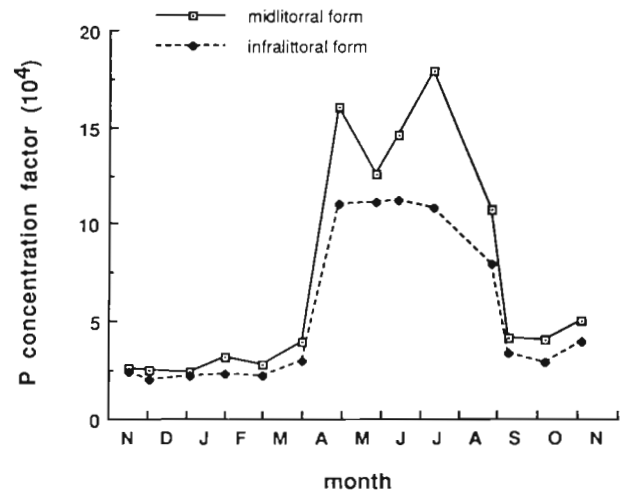
through the end of August in the infralittoral form. The total P content varied from 1.9 to 3.3 mg P g^{-1} (dry wt) in the midlittoral form and from 1.5 to 2.5 mg P g^{-1} (dry wt) in the infralittoral form. The calculation of the concentration factor, between the total P content in the thallus and the mineral P content in seawater (Chopin et al. 1990b), and the pattern of its seasonal variations (Fig. 7) showed that it was similar in the 2 forms from the end of summer to the beginning of spring but different during the second part of the spring and most of the summer. It was always higher in the midlittoral form (between 2.4×10^4 and 17.9×10^4) than in the infralittoral form (between 2.0×10^4 and 11.2×10^4).

Total N content was high in November and dropped significantly until June in both forms (Fig. 8). From June to August, the 2 forms evolved differently: the total N content increased to the level recorded in March in the midlittoral form, whereas it continued to decrease in the infralittoral one.

Variations of the dry matter content

The seasonal pattern of the dry matter was comparable in the 2 forms (Fig. 9). The infralittoral form, however, had a statistically significantly lower dry matter content than the midlittoral form, and did not show as marked winter and summer maxima.

DISCUSSION

Comparison of studies on the distribution of the different generations in the isomorphic alga *Chondrus crispus*
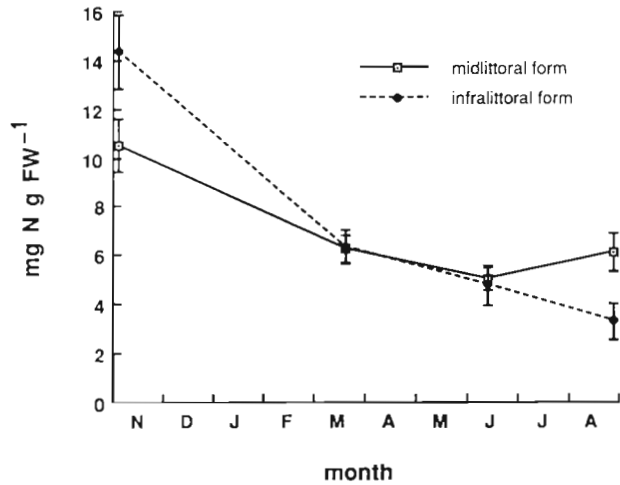
Fig. 8. *Chondrus crispus.* Seasonal variations of total N content in the midlittoral and infralittoral forms. Results are expressed in mg N g^{-1} fresh wt. Means ± standard deviation (n = 3)
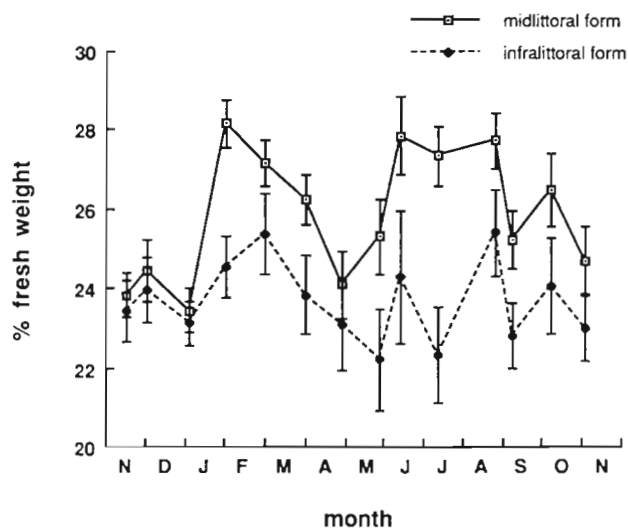

Fig. 9. *Chondrus crispus.* Seasonal variations of dry matter content in the midlittoral and infralittoral forms. Results are expressed in % fresh weight. Means ± standard deviation (n = 5)

is difficult as some take into consideration only mature plants (most studies, including ours) and others consider both vegetative and mature plants and differentiate gametophytes and tetrasporophytes on the basis of the type of carrageenans they contain (resorcinol test of Craigie & Leigh 1978). At Porz Liogan, the unimodal distribution of gametophytes, and bimodal distribution of tetrasporophytes, were similar to those observed in many locations (Chopin 1986). The reverse type of distribution (bimodal for gametophytes and unimodal

for tetrasporophytes) has, however, been reported in Spain (Fernandez & Menendez 1991), and bimodal distribution for both generations has been shown in Canada (Chopin et al. 1988). These changes in mode of distribution and in the ratio gametophytes/tetrasporophytes have been attributed, so far, to wave exposure, depth, differential reproductive capacity, harvesting pressure, and substratum disturbance (Prince & Kingsbury 1973, Mathieson & Burns 1975, Craigie & Pringle 1978, Bhattacharya 1985, Chopin et al. 1988, Lazo et al. 1989); however further investigations, especially over a longer period of time, are needed to check if other parameters could be responsible.

In the infralittoral form, the summer maximum of the gametophytes was between the 2 tetrasporophyte maxima (winter, beginning of fall) which could be explained by the alternation of generations in the life cycle of this species. This pattern was, however, not observed in the midlittoral form in which both generations were significantly represented during summer. Contrary to what Tveter-Gallagher et al. (1980) observed in New Hampshire, USA, the seasonal variations of the 2 generations at Porz Liogan were less marked in the midlittoral than in the infralittoral form. This could be explained by the fact that environmental conditions changed more in the midlittoral than in the infralittoral zone and created a less stable habitat favourable to the preferential development of one generation over the other. The population at Porz Liogan was also different from that of New Hampshire (Mathieson & Burns 1975) in that, except at the beginning of winter and middle of fall, tetrasporophytes were less abundant than gametophytes whatever the level on the shore. They were, however, twice as frequent in the infralittoral than in the midlittoral zone, which corroborates the observations of Mathieson & Burns (1975) and Craigie & Pringle (1978). When gametophytes and tetrasporophytes were considered together, the 2 forms showed a different reproductive phenology as mature plants represented 29.2 % in the infralittoral form and 20.8 % in the midlittoral form.

The generally observed greater size of the adult plants of the infralittoral form over those of the midlittoral form could be explained by the difference in growth rate, both transverse and longitudinal, between the 2 forms, as displayed by fluorescence labelling. The growth of *Chondrus crispus* has been studied in different locations on both sides of the Atlantic Ocean (Chopin 1986); generally, it is significant during spring, maximal in summer, and minimal during winter. In Britanny, it was maximal during fall. Causes of shifts in the optima seem to be essentially related to the environmental conditions particular to each study site and to the units chosen to measure the growth (length, surface, biomass, number of newly formed rows of cortical cells).

Contents of total carrageenans were comparable in the 2 forms. There was only a trend for the infralittoral form to have a higher content in summer and a lower content at the end of winter and the beginning of spring. The most-quoted biological roles of the cell-wall polysaccharides of algae are mechanical, hydration, and electro-chemical regulation (Rees 1962, Kloareg & Quatrano 1988). A role in hydration regulation has been promoted by authors who established a positive correlation between the vertical distribution on the shore of the Phaeophyceae and their cell-wall thickness or content (Black 1954, Zaneveld 1969, de Lestang-Laisne & Quillet 1972). Other authors, however, indicated no correlation between thallus water retention, thickness of cell walls and shore location (Dromgoole 1980, Schönbeck & Norton 1980, Norton et al. 1982). Nevertheless, resistance to desiccation is closely related to bathymetric level and appears to depend on physiological mechanisms preventing dehydration. These involve not only internal metabolic adaptations (Kremer & Schmitz 1973, Quadir et al. 1979) but also the buffer capacity of the cell wall at the cellular level and the osmotic pressure regulation by gel elasticity of the matrix compounds at the plasmalemma level (Kloareg & Quatrano 1988). The hydration regulating role of carrageenans in Rhodophyceae is, however, questionable. Fuller & Mathieson (1972), and Mathieson & Tveter (1975) showed that carrageenan content of *Chondrus crispus* was lower in midlittoral than in infralittoral plants in New Hampshire. In Britanny, as the seasonal pattern of carrageenan content was not statistically different between the 2 forms, which are partly identified by their location on the shore, no correlation between vertical distribution and carrageenan content could be established.

The contrasting morphologies of these 2 forms could not be explained by cell-wall differences, either at the total carrageenan content level or at the level of the carrageenan structures, which were remarkably stable both qualitatively (the κ-, ι-, and μ-structures occurred all year in both forms) and quantitatively (the slight variations in κ- and μ-structures were at the threshold of significance using the ^{13}C-NMR technique).

Concerning P nutrition, seasonal variations of total P content were more pronounced, and the spring decline started 1 mo earlier, in the midlittoral than in the infralittoral form. The lower total P content in the infralittoral form could be explained by its higher growth rate and consequently greater dilution of P in growing tissues. The seasonal variations of P content in seawater have a greater range than those in the thallus (Chopin et al. 1990b). These variations governed, at least partially, seasonal variations in the concentration factor, which however had an inverse pattern (highest values during late spring and summer). Moreover, as the total P content was higher, except at the end of

May, in the midlittoral than in the infralittoral form, the concentration factor was always higher in the former, especially during summer. Such high values for the P concentration factor are comparable to those recorded in other algae such as *Laminaria hyperborea* (Floc'h 1982). They cannot be explained by simple passive ionic exchanges but only by active metabolic absorption (Chopin 1985).

The seasonal variations of total N content in this study are similar to those reported in other studies for *Chondrus crispus* (Mathieson & Tveter 1975, Kornfeldt 1982) and other red algae (Lapointe & Ryther 1979, Rosenberg & Ramus 1982, Bird 1984), brown algae (Chapman & Craigie 1977, Gagné et al. 1982), and green algae (Hanisak 1979, Birch et al. 1981, Gordon et al. 1981). Seasonal variations of irradiance could be one of the main factors responsible for those of N content (Lapointe & Tenore 1981, Morgan & Simpson 1981, Kornfeldt 1982). According to Durako & Dawes (1980), high irradiance and temperature during summer induce protein degradation and bleaching of red algae which then turn greenish. The latter phenomenon is well known in *C. crispus* (Chopin 1986) and affected the infralittoral more than the midlittoral form (unpubl. data). This was confirmed by the different pattern of total N content from June to August in the 2 forms: increase in the midlittoral form and decrease in the infralittoral one. Mathieson & Tveter (1975), who also observed this difference between the 2 forms during summer, attributed it to the midlittoral plants having a broader tolerance to environmental factors compared to the infralittoral ones. Another explanation could be an interaction between P and N nutrition. Lapointe (1985, 1987) suggested that severe P limitation can induce N limitation in *Gracilaria tikvahiae*. In our case, the total P content of the infralittoral form in August reached 0.15 % (dry wt), which is in the range of the critical P content for growth as defined for other red and brown algae (Manley & North 1984, Lapointe 1985, Chopin et al. 1990a), N depletion continued and bleaching occurred. In the midlittoral form, the total P content in August was barely below 0.2 % dry wt, which is the upper limit of the critical P content for growth, while, instead of decreasing, total N content increased to the March level and bleaching was not as severe as in the infralittoral form.

Vertical location on the shore had a statistically significant influence on the dry matter content, the infralittoral form having a lower content than the midlittoral form all year long. Summer and winter maxima of dry matter content have also been reported for other algae (Wort 1955); however, these results partially disagreed with those of Vinogradov (1953), who found dry matter content of cold and temperate Rhodophyceae to be also high during summer and fall but low during winter. These differences in periods of maximal or minimal dry

matter content seem due to geographical variations, as indicated by Morgan et al. (1980) in *Palmaria palmata* and by Munda (1972) in several algae including *Chondrus crispus*. Neish et al. (1977) showed that nutrient enrichment of seawater led to a decrease of dry matter content of *C. crispus* in culture. This is not corroborated by our results in which levels of dry matter were high in winter, a period of high nutrient concentration in seawater in Brittany (Chopin et al. 1990b).

This study has demonstrated differences (at the level of the distribution of mature female gametophytes and tetrasporophytes, growth, phosphorus and nitrogen nutrition, and dry matter content) and similarities (at the level of the carrageenans) in the eco-physiology and biochemistry of these 2 most contrasting forms of *Chondrus crispus*. However they do not allow the question of their significance to be settled. Are they the reflection of environmental variations relative to their location on the shore or the expression of the genetic component of the polymorphism of this species? To test the first hypothesis, studies similar to the present one should be conducted on algae with as extended a vertical distribution as *C. crispus* but without presenting polymorphitic plants (gametophytes of *Mastocarpus stellatus*, for example). For the second hypothesis, it would be desirable to conduct studies of transplants, tissue cultures, formation of reproductive organs, structure, and hybridization, in a more thorough fashion than previously (Floc'h 1969, Chen & Taylor 1978, 1980a, b, Guiry 1979), to be able to define the degree of speciation represented by the presently observed polymorphism in what is today designated as the single entity *C. crispus*. The short duration of previous studies (between 5 and 13 mo), the fact that they were conducted on plants that already had well-channeled morphogenetic potentialities, and the questionable choice of the parental stocks should prompt one to be cautious in reaching any conclusion. Guiry (1991) showed interfertility between different populations of *C. crispus* with different morphologies, and between different species of the genus *Chondrus*, emphasizing that in the plant kingdom crossability is not an absolute criterion of speciation. Analyses of enzymatic systems by electrophoresis, already initiated by Cheney & Mathieson (1979), and DNA/RNA sequencing techniques should provide new insights on the presence, or absence, of genetic divergence between the 2 forms, and, therefore, on their level of speciation.

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