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Seasonal variation in growth and carrageenan content of *Calliblepharis jubata* (Rhodophyceae, Gigartinales) from the Normandy coast, France

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Abstract

Study of the seasonal variation in the quality and content of iota carrageenan in *Calliblepharis jubata* from the Normandy coast of France shows that seasonal fluctuation of the environment affects the growth and chemical composition of this red alga. Growth increases during winter, when there is little synthesis of carrageenan and floridean starch is accumulated. When inorganic nitrogen content decreases, growth also decreases and stops (May to August); with high light intensity, the metabolism is oriented towards a synthesis of parietal carrageenans to the detriment of the reserve products such as floridean starch.

Introduction

Carrageenans are sulphated polysaccharides extracted from red algae of the Gigartinales and Cryptonemiales. These polymers are used as gelling agents and thickeners in the food industry. From the numerous species of carrageenan-containing algae that grow around the French coast, only two species, *Chondrus crispus* Stackhouse and *Mastocarpus stellatus* (Stackhouse) Guiry, are exploited for their kappa-carrageenans, whereas the industrial production of iota-carrageenans relies on exotic seaweeds, belonging to the genus *Eucheuma*. It is important to look for new sources of iota-carrageenan from the French coasts. Four species growing along the English Channel have been found to contain iota-carrageenan: *Calliblepharis ciliata* (Hudson) Kütz., *Calliblepharis jubata* (Goodward & Woodward) Kütz., *Cystoclonium purpureum* (Hudson) Batters and *Gymnogongrus crenulatus* (Turner) J. Agardh. (Deslandes et al., 1985, Cosson et al., 1990).

On the Normandy coast, *C. jubata* has a biomass potentially suitable for exploitation. It is now appropriate to investigate the ecophysiology of the species and to study the seasonal variation in the quality of carrageenan and its properties.

Material and methods

A representative population of *C. jubata* situated at Cap Levy in the Barfleur region of Normandy, France, was investigated. The plants were growing on a granite substrate, which had been eroded to form numerous basins more or less around the zone below the midlittoral. These basins had a rich, varied flora with *C. jubata* especially abundant. The study was carried out for a period of one year (October 1989–October 1990), with monthly sampling. For comparison, occasional samples were obtained from Barneville (western coast of Cotentin) and Gatteville, a more exposed area close to Cap Levy. At each sampling time, surface water temperature was recorded and the concentrations of nitrate and nitrite measured using the method of Bendschneider and Robinson (1952). The size of the thalli was evaluated by measuring the branch lengths and widths of each individual plant. An average was obtained from sampling 100 individuals collected at random.

The samples were rinsed in freshwater to eliminate salt on the thalli surfaces; 100 g fresh alga was dried to constant weight at 60 °C. The dried seaweed was ground to a powder and total N determined using the Kjeldahl method. For polysaccharide extraction, 4 g dry seaweed was rehydrated and pretreated in ace-

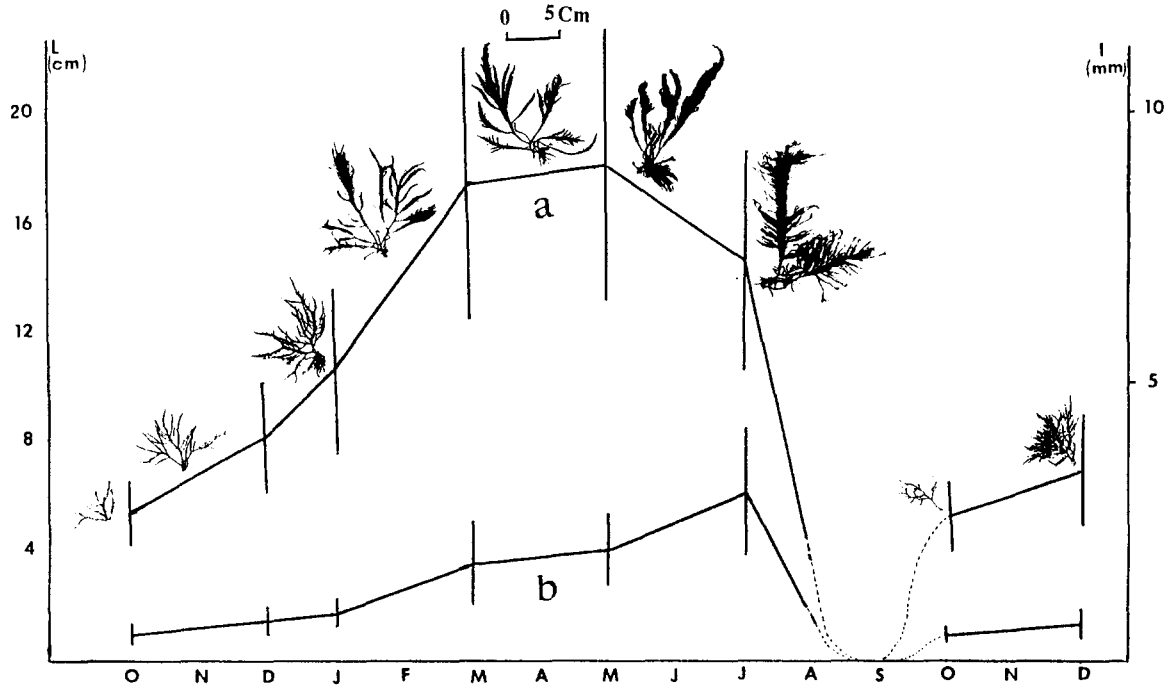

Figure 1. Growth in (a) length and in (b) width with morphological aspects of *C. jubata in situ.*

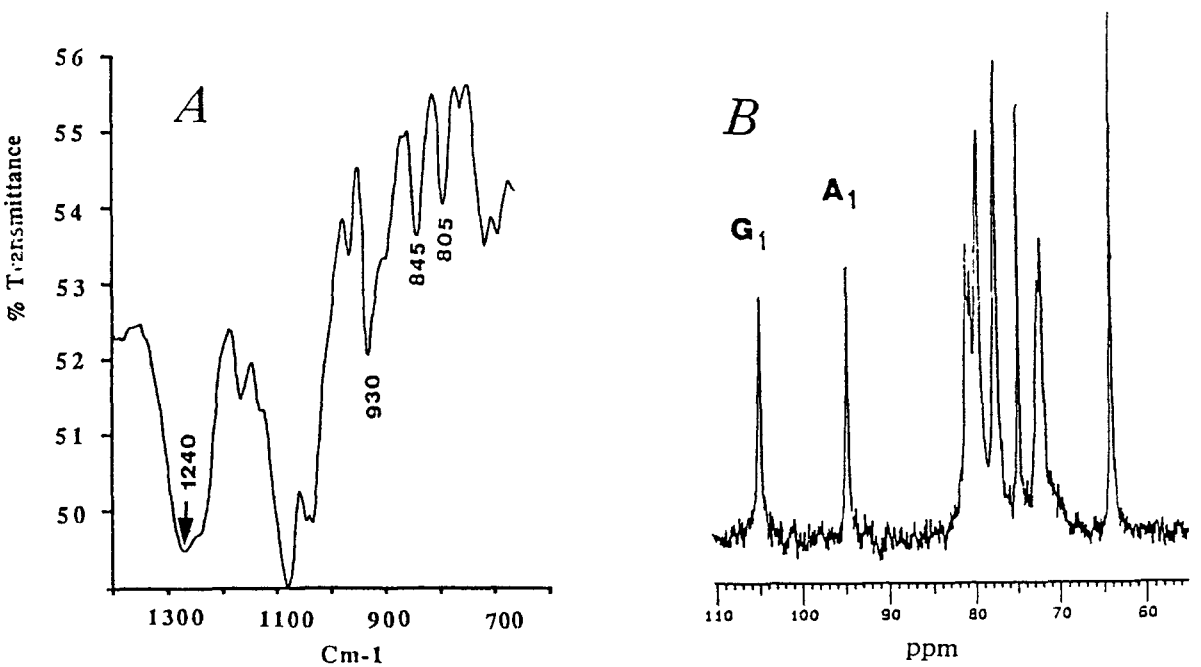

Figure 2. Spectroscopic methods for analysis of carrageenans from *C. jubata*: (A: IR spectrum; B: ^{13}C NMR spectrum)

tone followed by ethanol to eliminate the organosoluble fraction. The residue was placed in 400 ml 0.1 M KOH for 30 min at 85 °C. After grinding by an ultraturrax, the suspension was maintained at 85 °C for 5 h before being filtered under pressure using a CF/B glass fibre filter to remove the cellulose. The warm filtrate was then neutralized by the addition of 0.5 N HCl and concentrated to 150 ml. The polysaccharide was

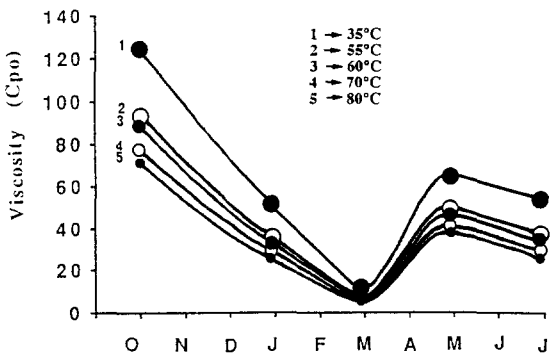

Figure 3. Influence of temperature on viscosity of a 1% solution of carrageenan extracted from *C. jubata* (October 1989 to July 1990).

precipitated by adding the warm solution into twice its volume of ethanol 95% (V/V). The precipitate was dried under vacuum at 60 °C and ground to powder using a Dangoumeau type ball mill. The neutral sugars obtained after hydrolysis of the polysaccharides were reduced with $NaBH_4$ and the extract analysed by gas liquid chromatography. Inositol was chosen as the internal standard since its retention time is close to that of galactose. The apparatus used was a Perkin-Elmer chromatograph equipped with a flame ionisation detector (FID). The column was filled with 3% SP2340. The vector gas was helium.

Total sugars were analysed by the method of Dubois et al. (1956), the 3–6 anhydrogalactose by resorcinol reagent (Yaphe & Arsenault, 1965) and sulphate by the turbidometric method of Jackson and McCandless (1978), after hydrolysis in 1 N HCl for 6 h at 100 °C.

The infrared spectra were obtained by Fourier transformation (FTIR) using a Nicolet MX60 machine. Films were prepared from 0.25% carrageenan solution on polyvinyl chloride plates. H-Decoupled, ^{13}C NMR spectra were recorded with a Brucker AC 300 spectrometer at 85.32 MHz. Samples (40 mg ml^{-1}) were dissolved in D_20 (2 ml) and spectra recorded at 80 °C. Viscosity was estimated using a Brookfield VII 80 machine with 1% polymer.

Results

Growth and morphological aspects of Calliblepharis jubata in situ

Figure 1 shows the development of the length and width of thalli during the year, with representative morphological aspects of the thalli. The first observation of *C. jubata* thalli on the shore was recorded at the end of September and they were about 4 cm in length and 1 mm in width. Because of its small size, it was sometimes difficult to distinguish *C. jubata* from other species like *Gigartina acicularis* (Roth) Lamouroux and *Gelidium* sp., which grew in the same environment.

An active growth phase was observed from October to March, the average size of thalli reaching up to 17 cm in length and 7 mm in width. The algae had a cartilaginous and dark red aspect. The individualization of the thalli was sometimes difficult, because of the numerous fusions (anastomoses) between adjacent plants leading to the formation of thick tufts. After March, the growth length practically stopped, while the growth in width continued until July. With the beginning of the summer the algae started to decolourize and to decay and finally disappeared in August.

At the beginning of July prominent cystocarps were observed on approximately 10% of female gametophytes. However, we also occasionally observed these cystocarps in March. Thus, it was difficult to determinate exactly the reproduction period. Tetrasporocystes on tetrasporophytes have never been observed at Cap Levy.

Variation in dry weight and carrageenan content

The study of polysaccharides extracted from *C. jubata* by FTIR (Figure 2) showed the presence of three characteristic bands typical of iota-carrageenan. This identification was confirmed by ^{13}C NMR spectra (Figure 2), characterized by two peaks G1 (103 ppm) and A1 (92.8 ppm) in the anomeric zone, characteristic of iota-carrageenan (Zinoun, 1993).

However, carrageenans were considered to be homogeneous and the possibility of a low quantity of kappa-carrageenan being present was excluded. However, the carrageenan nature seemed to stay the same (iota dominant) throughout the year, even though some physical properties were not conserved throughout the seasons, such as a decrease in the apparent viscosity of carrageenan during the active phase of growth (Figure 3). Dry matter, expressed as % fresh weight, varied between 9% in March and 22% in July (Figure 4); phycocolloid content expressed as % of dry weight was minimum (15%) in February and maximum (45%) during June–July.

Active growth corresponded to a decrease in % dry/fresh weight, probably related to a reduced synthesis of carrageenan (cf Figures 1, 4). In the growth

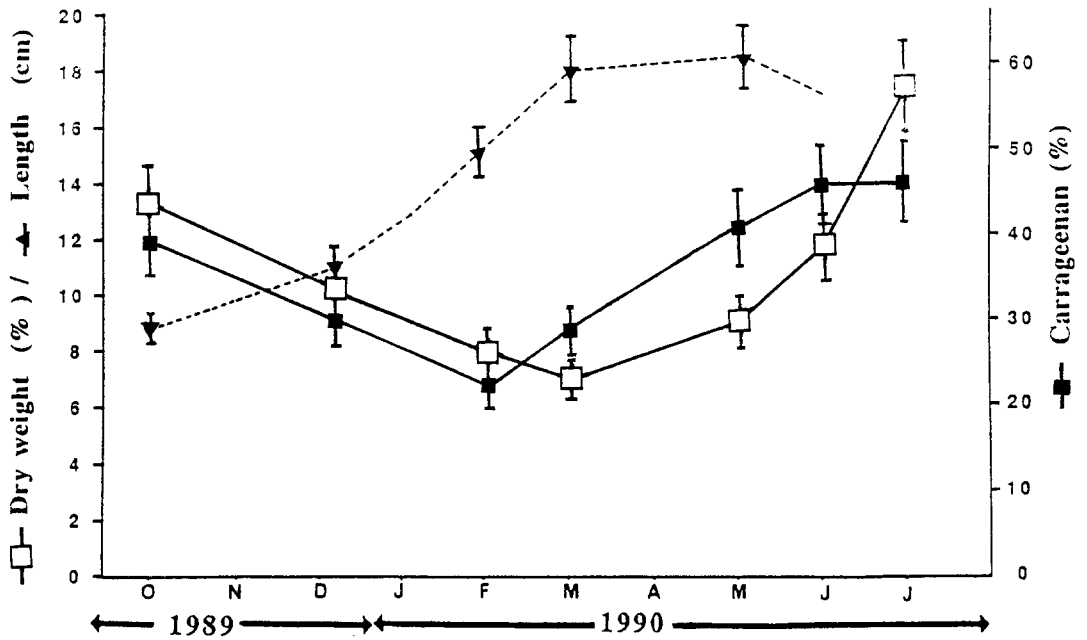

Figure 4. Variations of thalli length (▲), dry weight (◊) and carrageenan content (♦) from *C. jubata* between October 1989 and July 1990.

Table 1. Seasonal evolution of carrageenan yield and total nitrogen in *C. jubata* in comparison with sea temperature and nitrate content of seawater at Cap Levy (October 1989 to July 1990)

Month	Seaweed sample *Calliblepharis jubata*	Surface sea water			Temperature °C
	% N	% Carrageenan	NO_3-N mg l^{-1}	NO_2-N mg l^{-1}	
October	3.19	38.0	34.6	0.279	15
December	4.11	30.0	19.7	0.087	12
February	3.05	29.0	7.70	0.157	12
May	3.02	40.0	3.60	0.037	15
July	2.71	45.0	1.60	0.033	17

phase, the young thalli had a thin cell wall essentially composed of cellulose and had relatively prominent vacuoles. After the growth phase, the walls became thick with the synthesis of carrageenan, which led to an increase in the dry weight. Carrageenan content and % dry weight varied approximately in parallel throughout the year (Figure 4).

Total nitrogen in *C. jubata* followed the inorganic nitrogen ratio in the environment, and was approximately opposite to carrageenan content (Table 1).

Moreover, the nitrogen and carrageenan contents of *C. jubata* varied with the sampling sites (Table 2). The thalli at Gatteville were filamentous (winter form), whereas at the two other stations they were broad (aestival form). For that reason there was a higher dry matter content in Cap Levy and Barneville samples than in those from Gatteville. Carrageenan and total nitrogen contents differed for the algae from North Cotentin (36–33% carrageenan and 3.3–3.8% N) West Cotentin (52% carrageenan 1.1% N), corresponding to a geographical zone characterized by different temperature and turbidity of the water.

A causal relationship between ambient inorganic nitrogen and chemical composition was confirmed in nitrogen-starved laboratory cultures. Percent dry weight, % carrageenan and % starch increased markedly and % N and pigments (not shown) decreased during the first 15 days without nitrogen (Table 3).

Table 2. Comparison of chemical composition of *C. jubata* collected at three stations in June 1990

	% Dry weight	% Carrageenan	% N	% Starch
Gatteville	14.5	36.0	3.78	29.0
Cap Levy	22.6	39.3	3.27	6.3
Barneville	21.5	52.2	1.15	6.6

Table 3. Changes in biomass (dry weight), carrageenan, total N and floridean starch contents in *C. jubata* grown in culture medium. Growth conditions: 16°C, 16/8, 200 μmol photon m^{-2} s^{-1}, without nitrate

time (day)	% Dry weight	% Carrageen	% N	% Starch
0	9.3	37.0	3.36	3.7
15	18.0	42.6	1.43	32.0
25	25.0	46.0	1.12	38.0

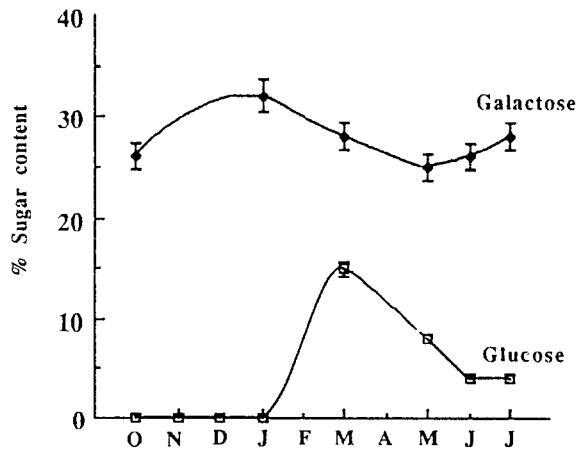

Figure 5. Seasonal variation in glucose and galactose content in phycocolloids of *C. jubata*. (% dry weight).

Discussion

The seasonal fluctuations in environment appear to influence both the growth and the chemical composition of thalli of *C. jubata*. This is especially so for aqueous inorganic nitrogen, which increased during the growth period of the thalli (December to May) at the same time that the temperature rises (Table 1).

During active growth, the carrageenan content decreases. However, there is an accumulation of floridean starch during March in the middle of this growth period, marked by high glucose contents (Figure 5). However, from May to August, even though the inorganic nitrogen content is decreasing rapidly in the sea water and the temperature and irradiance are increasing, growth slows down and then stops. The decreasing sea water nitrate concentration during spring is probably the factor leading to visible loss of thallus pigmentation, while dry weight and carrageenan increase from March onward. Less nitrogen and more light apparently direct cellular metabolism towards the synthesis of parietal products to the detriment of reserve products such as floridean starch.

Two phases characterise the development of *C. jubata*. First, growth from December to March, corresponding to increasing seawater temperatures (10 to 16 °C), moderate light intensity, non-limiting nitrogen availability, and preferential synthesis of starch. Secondly, a stop in the growth phase (from March to September) corresponds to high temperatures, strong irradiance and decreasing aqueous nitrogen content. The metabolism of the alga slows and shifts towards synthesis of parietal carrageenans.

The analysis of polysaccharides of *C. jubata* (Figure 5) shows the presence of galactose as the dominant sugar. The glucose content varies seasonally, with a spring maximum. High contents of starch-associated glucose in spring suggest that actively growing thalli synthesize this cytoplasmic storage polymer. Following cessation of growth, starch decreases to 3% dry weight, while carrageenan increases. These observations suggest that floridean starch, synthesised during the growth phase, is utilised as a reserve for carrageenan synthesis during the period of cell wall synthesis (Zinoun, 1993).

These data collected *in situ* were confirmed by laboratory culture of *C. jubata* to conditions of N limitation. By reducing nitrogen availability, it is possible to increase the carrageenan production of thalli in *Chondrus crispus* (Neish et al., 1977) and *Hypnea musciformis* (Gordon-Guist et al., 1982). On the other hand, the nature of the carrageenan did not vary during the year, unlike in *C. crispus* (Chopin et al., 1987). The only recorded modifications concern the quantity of colloids and some rheological properties. The harvested samples included mostly non-fertile thalli. It is possible that there is a biochemical difference in the carrageenan linked to the alternation of generations, as in *C. crispus*.

However, we have shown that carrageenans are of the iota type in the female gametophytes collected in summer.

We propose two hypotheses: (1) only gametophytes exist on the Normandy coast, so there is only one type

of carrageenan; (2) in populations of gametophytes and tetrasporophytes, carrageenan biochemistry is independent of generation as in *Cystoclonium purpureum* (Bert et al., 1989). However, we did not observe any tetrasporocystes on thalli during this year of study. It seems that there were only gametophytes containing iota-carrageenans in the studied population.

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