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Population studies and carrageenan properties of *Chondracanthus teedei* var. *lusitanicus* (Gigartinaceae, Rhodophyta)

Leonel Pereira* & José F. Mesquita
Laboratory of E.M. and Phycology, Department of Botany, University of Coimbra, 3000 Coimbra, Portugal

*Author for correspondence; e-mail: leonel@ci.uc.pt

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Abstract

Features of an intertidal population of *Chondracanthus teedei* var. *lusitanicus*, which occurs in sandy basins on rocky shores of part of the Portuguese coast (Buarcos, Figueira da Foz), were studied over one year. Biomass and plant size showed a small increase in early spring (April), a marked increase in early summer (June/July) and were at a minimum in late summer. There was generally more tetrasporophytes (4–32.5%) than female gametophytes (3–29%), which contrasts with other geographical regions where *C. teedei* populations have been studied, such as Brazil and France. However, non-fructified thalli predominated throughout the year. Phycocolloid extracts were compared for the various stages using spectroscopic methods (FTIR, FT-Raman, ^{1}H- and ^{13}C-NMR). These showed a hybrid carrageenan belonging to the lambda family in the tetrasporophyte and a hybrid kappa-iota-mu-nu carrageenan in the female gametophyte and non-fructified thalli. The average phycocolloid content was 34.9% dry weight, with a maximum of 43.6% in July. The combination of high available biomass and phycocolloid content makes this species a potentially important source of kappa/iota hybrid carrageenan in Portugal additional to the traditionally harvested carrageenophytes.

Introduction

The selection of species or strains with high, good quality carrageenan content, together with biological, ecophysiological and biochemical study is a valuable contribution particularly considering the increasing industrial importance of these phycocolloids. In this context, a research was conducted on a population of *Chondracanthus teedei* var. *lusitanicus* (Rodrigues) Bárbara and Cremades in Buarcos Bay (Figueira da Foz, Portugal). Other population studies on this species have been made in Brazil (Braga, 1985, 1990) and France (Zinoun, 1993).

Hommersand et al. (1993) transferred this species to the genus *Chondracanthus*; *C. teedei* (Mertens ex Roth) Kützing (basionym: *Ceramium teedei* Mertens ex Roth; synonym: *Gigartina teedei* (Mertens ex Roth) J.V. Lamouroux), originally described from Portugal (Guiry, 1984), is widespread in the northeastern Atlantic, Mediterranean and the Black Sea (Ardré, 1970; Orfandis, 1993). It has also been reported from Japan (Mikami, 1965), the Indian Ocean (Silva et al., 1996) and Brazil (Ugadim, 1975). In France, Spain and Portugal, the species is frequent in the lower intertidal zone on sheltered habitats. Rodrigues (1958) described it as new variety, *C. teedei* var. *lusitanicus* (Rodrigues) Bárbara and Cremades (basionym: *Gigartina teedei* (Roth) J.V. Lamouroux var. *lusitanica* Rodrigues). Ardré (1970) and Bárbara and Cremades (1996) reported this variety in several Portuguese localities and the North West coast of Spain (Ría de A Coruña), respectively.

C. teedei has an alternation of isomorphic gametophytes and tetrasporophytes. The life cycle and the reproductive morphology were described by Guiry (1984) and Braga (1985, 1990) and, according the latter author, there always seems to be a higher proportion of gametophytes than tetrasporophytes. However, a population of *C. teedei* from Buarcos Bay (Figueira da Foz, Portugal) has shown to have a larger percentage of

fertile tetrasporophytes than fertile female gametophytes (Pereira and Mesquita, 1994; Pereira, 1996).

Carrageenans are sulphated polysaccharides present in the cell walls of members of the Gigartinales, which have been used extensively as gels and thickeners in food and industrial preparations. There are about 15 idealized carrageenan structures identified by Greek letters (Chopin et al., 1999). However, a more versatile binomial version, incorporating the substitution pattern of each sugar unit, was developed by Knutsen et al. (1994). Chemical differences are known to exist among carrageenans from different generations of the life cycle of some carrageenophytes. The tetrasporophyte thalli of members of the Gigartinaceae produce lambda-family carrageenan and the gametophyte thalli produce a kappa or kappa/iota-hybrid type of carrageenan (Craigie, 1990; Chopin et al., 1999; Van de Velde et al., 2001). In commerce, kappa/iota hybrid carrageenan, known as "kappa-2" carrageenan (Bixler, 1996) or "weak-gelling" kappa (Falshaw et al., 2001, 2003), is extracted primarily from gametophytes of *Gigartina* and *Chondracanthus* species (Chopin et al., 1999; Bixler et al., 2001). Copolymers of kappa and iota carrageenan produce gels under certain conditions (Falshaw et al., 2001, 2003). The precursor (mu and nu) carrageenans both contain a sulphate ester group at position-6 of a 4-linked α-D-galactose unit. This type of structure reduces the ability of the carrageenan to form a gel by interrupting the sequences of carrabiose repeat units that form double helical structures during gel phase. Most of these 6-sulphated units are converted to the corresponding 3,6-anhydride during alkaline, industrial extraction ($Ca(OH)_2$ or NaOH) of carrageenans (Falshaw et al., 2001, 2003). The rheological implication of the hybridization is the weakening of the hard, brittle gel of kappa carrageenan by the soft, elastic gel character of iota carrageenan. Kappa-2, while weaker in water gelling properties than kappa, retains reasonably high milk reactivity as required for dairy applications (Bixler et al., 2001).

The purpose of this study was to compaer native and alkaline-extracted carrageenan from different generations of the life history of *C. teedei* var. *lusitanicus* (tetrasporophyte, female gametophyte and non-fructified thalli) using FTIR, FT-Raman, ^{1}H- and ^{13}C-NMR. The comparison was planned such that no extraction was involved. Carrageenans present in an alga can be identified rapidly by FTIR (Chopin and Whalen, 1997; Chopin et al., 1999) and FT-Raman directly on only a few milligrams of ground dried seaweed. NMR spectroscopy (Van de Velde et al., 2002a) was included in order to quantify the different carrageenan fractions.

Materials and methods

Ecological studies

A representative population of *C. teedei* var. *lusitanicus* situated at Buarcos Bay, central west coast of Portugal, was studied for about one year. The plants were growing on both a rock substratum and in sand basins, where *C. teedei* var. *lusitanicus* can be prolific. All samples were collected from an area of 100×100 m of the rocky shore. For determination of length, life cycle phase and biomass of the thalli, eight quadrates (10×10 cm) where randomly positioned in the extensive beds of *C. teedei* and destructively sampled (Braga, 1990; Chopin, 1997). At each sampling time, air temperature, surface water temperature, salinity and pH were recorded *in situ*. In laboratory the samples were washed in filtered seawater and the plant measured before drying (60°C, 48 h) for the biomass calculation. The separation of the female gametophytes, tetrasporophytes and non-fructified thalli was made with a stereomicroscope.

Seasonal variation in phycocolloid content

For evaluation of dry weight and carrageenan content a minimum of 100 plants, greater than 3 cm in length, were collected randomly each month. The material was divided into three lots following examination under a stereomicroscope: tetrasporophytes, female gametophytes bearing cystocarps and non-fructified thalli. The samples were rinsed in distillated freshwater to eliminate salt and debris from the surface and dried to constant weight at 60 °C. The dried seaweed was ground in order to render the samples uniform.

With the objective to evaluate the potential of this seaweed as source of copolymers of kappa and iota carrageenan, with increasing importance in the food industry (Falshaw et al., 2001; Van de Velde and De Ruiter, 2002; Pereira and Mesquita, 2003), the selected method was the alkali-extraction (which resembles the industrial carrageenan extraction).

Phycocolloid extraction methods

Before extraction, the ground dry material was rehydrated and subsequently treated with a moisture of

methanol 100% and acetone 100% (V/V) to eliminate the organosoluble fraction (Zinoun and Cosson, 1996).

For extraction of native carrageenan the seaweed samples were placed in bi-distillated water (50 mL g^{-1}), pH 7 at 85 °C for 3 h.

For alkaline-extraction (industrial method), the samples were placed in a solution (150 mL g^{-1}) of NaOH (1 M) at 80 °C for 3 h, according to Pereira et al. (2003) and neutralised to pH 8 with HCl (0.3 M).

In both cases, the solutions were filtered hot, under suction, twice, through cloth and glass fibre filter. The extract was evaporated in a vacuum to one-third of the initial volume. The carrageenans were precipitated by adding the warm solution to twice its volume of ethanol (96%). Coagula were dried in an oven for 48 h at 60 °C, and then weighed to determine the carrageenan content (% of dry weight).

Carrageenan analysis by FTIR and FT-Raman

Samples of ground, dried algal material were analysed by FTIR (Chopin and Whalen, 1993; Chopin et al., 1999) and FT-Raman for determination of natural phycocolloids composition. The FTIR spectra of ground dried seaweed, native and alkali-modified carrageenan, were recorded on an IFS 55 spectrometer, using a Golden Gate single reflection diamond ATR system. All spectra were the average of two counts, with 128 scans each and a resolution of 2 cm^{-1}. The room temperature FT-Raman spectra were recorded on a RFS-100 Bruker FT-spectrometer using a Nd:YAG laser with excitation wavelength of 1064 nm. Each spectrum was the average of two repeated measurements of 150 scans each and a resolution of 2 cm^{-1}.

Carrageenan analysis by ^{1}H and ^{13}C-NMR

^{1}H-NMR spectra were made on a Bruker AMX600 spectrometer operating at 500.13 MHz at 65 C. Typically 64 scans were recorded with an interpulse delay of 5 s (T_1 values for the resonance of the anomeric protons of κ- and ι-carrageenan are shorter than 1.5 s). Sample preparation for the ^{1}H-NMR experiments involved dissolving the carrageenan sample (5 mg mL^{-1}) at 80 °C in D_2O containing 1 mM TSP (3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt) and 20 mM Na_2HPO_4, followed by sonication for three periods of 1 h in a sonicator bath (Branson 2510). Chemical shifts (δ) are referred to internal TSP standard ($\delta = 0$ ppm for ^{1}H) according to Knutsen and Grasdalen (1987). Assignments of the ^{1}H-NMR spectra were based on the chemical shift data summarised by Van de Velde et al. (2002a).

^{13}C-NMR spectra of female gametophytes and non-fructified thalli alkaline-extracted carrageenans were recorded on a Varian Unity 500 spectrometer at 125.69 MHz. Samples (15/20 mg mL^{-1}) were dissolved in D_2O and spectra recorded at 80 °C, 10.000 accumulations, pulse 15 μs, acquisition time 3 s and relaxation delay 5 s. ^{13}C-NMR spectra of tetrasporic carrageenans were recorded on a Bruker AMX500 spectrometer operating at 125.76 MHz essentially as described in literature (Van de Velde et al., 2002a, b). The sample preparation was as follows: a solution of 5 mg mL^{-1} carrageenan in H_2O was prepared at 80 °C. This solution was sonicated for three periods of 30 min in melting ice (Heat Systems XL 2020 sonicator, 12 mm tip, power 475 W, frequency 20 kHz); the solution was centrifuged at elevated temperature to remove insoluble material. The sonicated material was dialysed against phosphate buffer (20 mM Na_2HPO_4; 3 times 2 L), water (1 time 2 L) and lyophilised. The material was dissolved at a concentration of 70–100 mg mL^{-1} in D_2O containing 20 mM Na_2HPO_4 and 30 mM TSP. Chemical shifts (δ) were referred to an external TSP/DMSO standard ($\delta_{DMSO} = 39.45$ ppm for ^{13}C) according to Usov et al. (1980). Assignments of the ^{13}C-NMR spectra were based on the chemical shift data summarised by Van de Velde et al. (2002a).

Results

Physical-chemical data

Air and water temperatures, salinity and pH were taken from June 2000 to August 2001 (Figures 1A and 1B). The water temperature varied from 11.9 °C (January 2001) to 22 °C (June 2001). The air temperature oscillated between 10.2 °C (January 2001) and 23 °C (August 2000 and June 2001). The salinity ranged from 26 to 34‰. The maximum pH value was 8.5 (June 2000) and the minimum 7.6 (August 2000).

Plant size and biomass

The length measurements (Figure 1C) were made from June 2000 to August 2001. The maximum average thalli length was 7.7 ± 0.4 cm ($n = 100$) and the minimum 2.9 ± 0.2 cm ($n = 100$). The average biomass

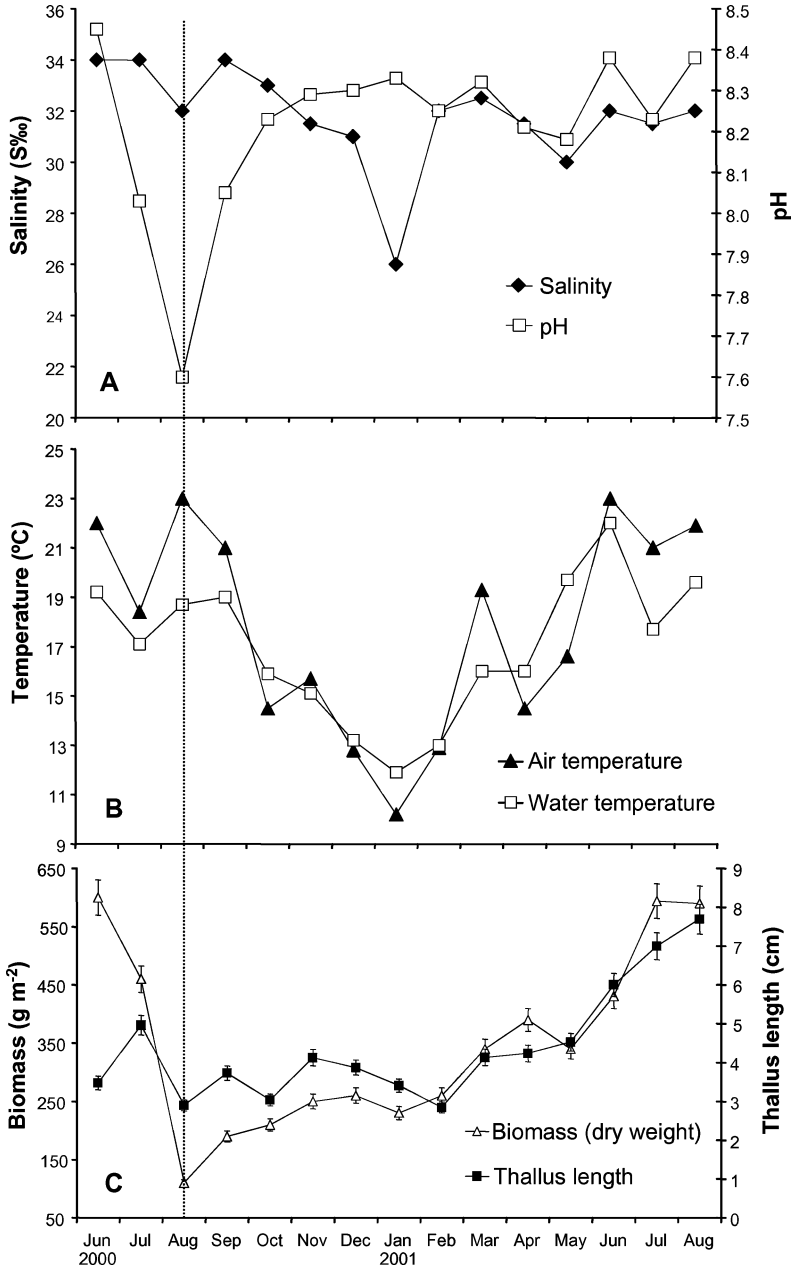

Figure 1. (A) Air and water temperature and (B) salinity and pH in Buarcos Bay. (C) Changes in thallus length (mean ± SE, $n = 100$) and biomass (mean ± SE, $n = 8$) of *C. teedei* var. *lusitanicus*.

data (dry weight) are shown in Figure 1C. These values varied between 110 ± 1.9 g m^{-2} ($n = 8$) and 594 ± 10.5 g m^{-2} ($n = 8$). The biomass and plant size had low values in autumn and winter; a small increase occurred in early spring (April 2001) and the greatest values of these parameters were recorded in early summer (June/July 2001).

Life history phase

The percentage of individuals of each generation is represented in Figure 2. Non-fructified thalli were dominant in all samples, its percentage changed from 43% (September 2001) to 82.5% (July 2001). Female gametophytes were found in all samples, from 3%

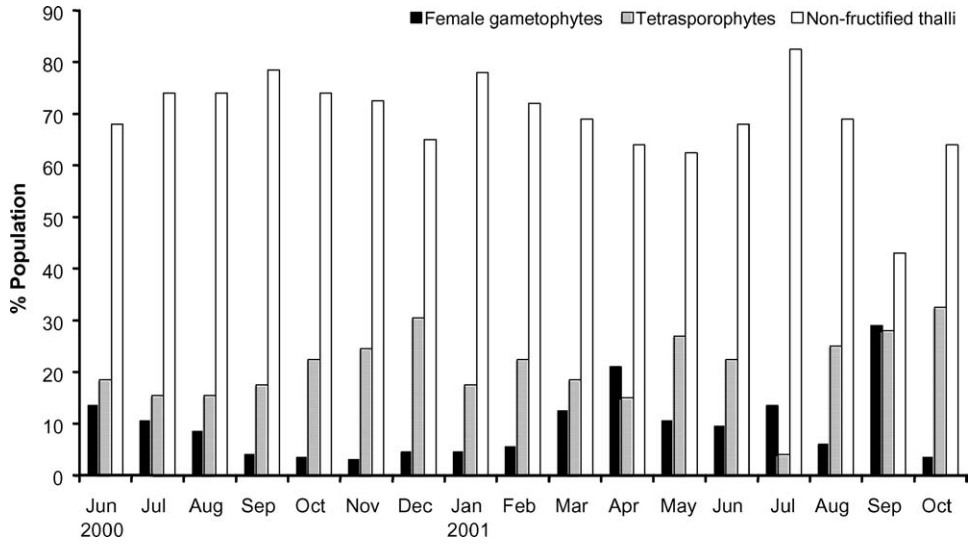

Figure 2. Structure of the population studied.

(November 2000) to 29% (September 2001). Thalli with tetrasporocystes (fertile tetrasporophytes) were also found in all samples, with a maximum percentage of 32.5% (October 2001) and minimum of 4% (July 2001). As compared to fructified thalli, namely the female gametophytes bearing cystocarps, the tetrasporophytes are, generally, more abundant.

Variation in dry weight and carrageenan content

Monthly carrageenan content (% dry weight) of female gametophytes, tetrasporophytes and non-fructified thalli are represented in Figure 3A. The maximum carrageenan content was found in a tetrasporophyte sample, with 58% dry mater in July 2001. The minimum content was found in a non-fructified sample, with 23% in February 2000.

The annual average of the content in carrageenan was of $34.9 \pm 1.0\%$ ($n = 15$), with an average of $37.9 \pm 1.5\%$ ($n = 15$) in the female gametophytes, $35.4 \pm 2.1\%$ ($n = 15$) in the tetrasporophytes and $31.4 \pm 4.4\%$ in the non-fructified thalli.

Dry matter (Figure 3B), expressed as percentage fresh weight, varied between $12.1 \pm 1.5\%$ ($n = 3$) in November 2001 and $17.5 \pm 0.8\%$ ($n = 3$) in December 2000; overall phycocolloid content, expressed as percentage dry weight, was minimum ($26.4 \pm 2.5\%$, $n = 3$) in December 2001 and maximum ($43.6 \pm 12.5\%$, $n = 3$) in July 2001.

Monthly total carrageenan content and percentage dry weight were negatively correlated ($r = -0.5589$) throughout the year (Figure 3B). The phycocolloid yield was low in autumn and winter; a small increase occurred in early spring (April 2001); the greatest values of carrageenan content were registered in summer (July 2001).

Carrageenan analysis by FTIR and FT-Raman

Figure 4 shows the FTIR and FT-Raman spectra of ground seaweed samples (A, D), native carrageenan (B, E) and alkali-extracted carrageenan (C, F) of *C. teedei* var. *lusitanicus* female gametophytes. All spectra show a band at 845 cm^{-1}, which is assigned to D-galactose-4-sulphate (**G4S**) and a band at 805 cm^{-1}, which indicates the presence of 3,6-anhydro-D-gactose-2-sulphate (**DA2S**). The presence of a strong band a 930 cm^{-1} in the FTIR spectra, weak in FT-Raman spectra, indicates the presence of 3,6-anhydo-D-galactose (**DA**) in all samples (Chopin et al., 1999; Pereira et al., 2003). The broad signal in FTIR, between 1210 and 1260 cm^{-1} was characteristic of sulphate esters in general (Chopin et al., 1999). Additional peaks at 867 cm^{-1} (Galactose and D-galactose-6-sulfate = **G/D6S**), 825 cm^{-1} (Galactose and D-galactose-2-sulphate = **G/D2S**) and 820 cm^{-1} (**G/D6S**), with little intensity, they correspond to the presence of carrageenan precursors (mu and nu) in the samples of ground seaweed (A) and native carrageenan (B) (Chopin et al., 1999). The presence of bands at 825 and 867 cm^{-1}, corresponding to the existence of precursors, is more evident in the FT-Raman spectra (D, E).

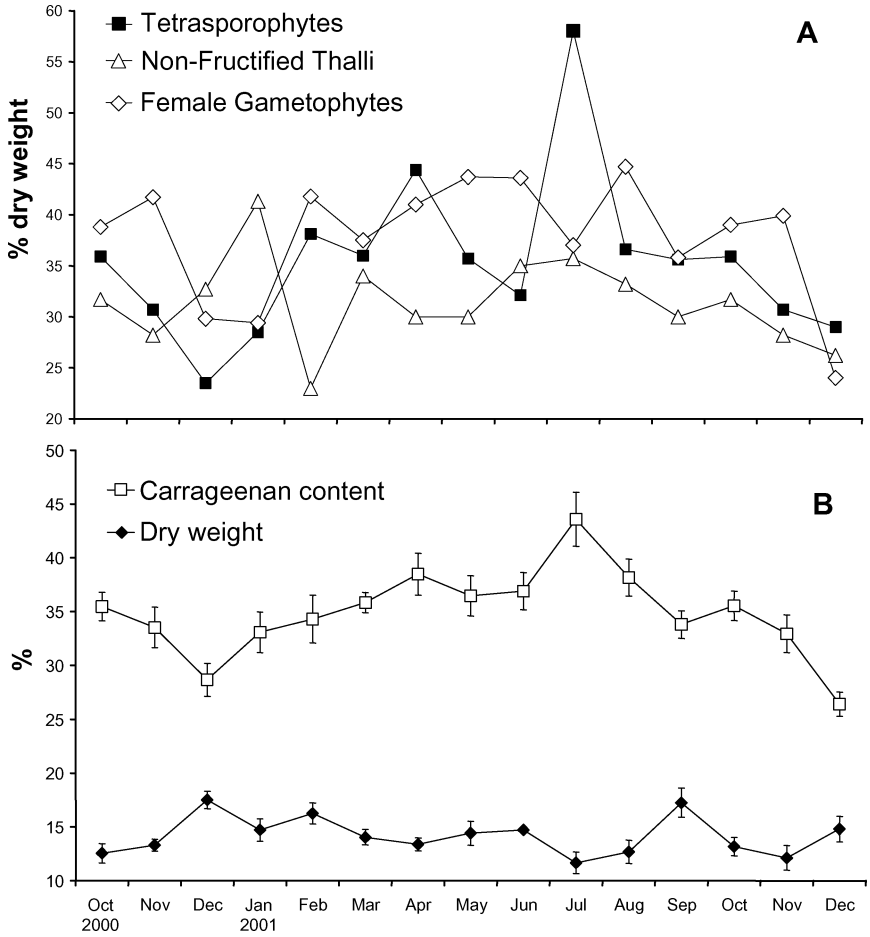

Figure 3. (A) Monthly changes in carrageenan content of different life-stages of *C. teedei* var. *lusitanicus*. (B) Changes in dry weight and overall carrageenan content (mean ± SE, $n = 3$).

Figure 5 shows the FTIR and FT-Raman spectra of ground seaweed samples (A, D), native carrageenan (B, E) and alkali-extracted carrageenan (C, F), from the non-fructified thalli. These spectra are very similar to the ones presented for female gametophytes, which showed a strong absorbance at 1240 cm^{-1} for sulphate esters and three other bands at 805 cm^{-1} (**DA2S**), at 845 cm^{-1} (**G4S**) and 930 cm^{-1} (**DA**). The ground seaweed (G) and native carrageenan (H) showed additional peaks at 820 cm^{-1} (**G/D6S**), 825 cm^{-1} (**G/D2S**) and 867 cm^{-1} (**G/D6S**), the last ones, more evident in the FT-Raman spectra (D, E), corresponded to the precursors.

The ratio between 805 and 845 cm^{-1} absorption bands in FTIR spectra was calculated (Correa-Díaz et al., 1990) and used as a parameter to determine the degree of the iota/kappa hybridisation (Figures 4 and 5). The ground seaweed and native carrageenan presented a similar ratio 805/845, with values between 0.69 and 0.87 (Figures 4A, 4B, 5A and 5B). The increment in the ratio 805/845 in the alkali-treated carrageenan (Figures 4C and 5C) corresponded to an increment of the iota fraction relatively to kappa fraction.

The FTIR and FT-Raman spectra of tetrasporophytes samples of *C. teedei* var. *lusitanicus* are shown in Figure 6: ground samples (A and D), native carrageenan (B and E) and alkali-extracted carrageenan (C and F). The infrared spectra of all samples showed a strong absorbance at 1240 cm^{-1} for sulphate esters and a narrow peak between 825 and 830 cm^{-1} (**G/D2S**). In addition to these signals of larger intensity, appear in all the FTIR spectra three shoulder peaks at 905 cm^{-1} (**DA2S**), 930 cm^{-1} (**DA**) and 1070 cm^{-1} (**DA**), related to the presence of tetha-carrageenan.

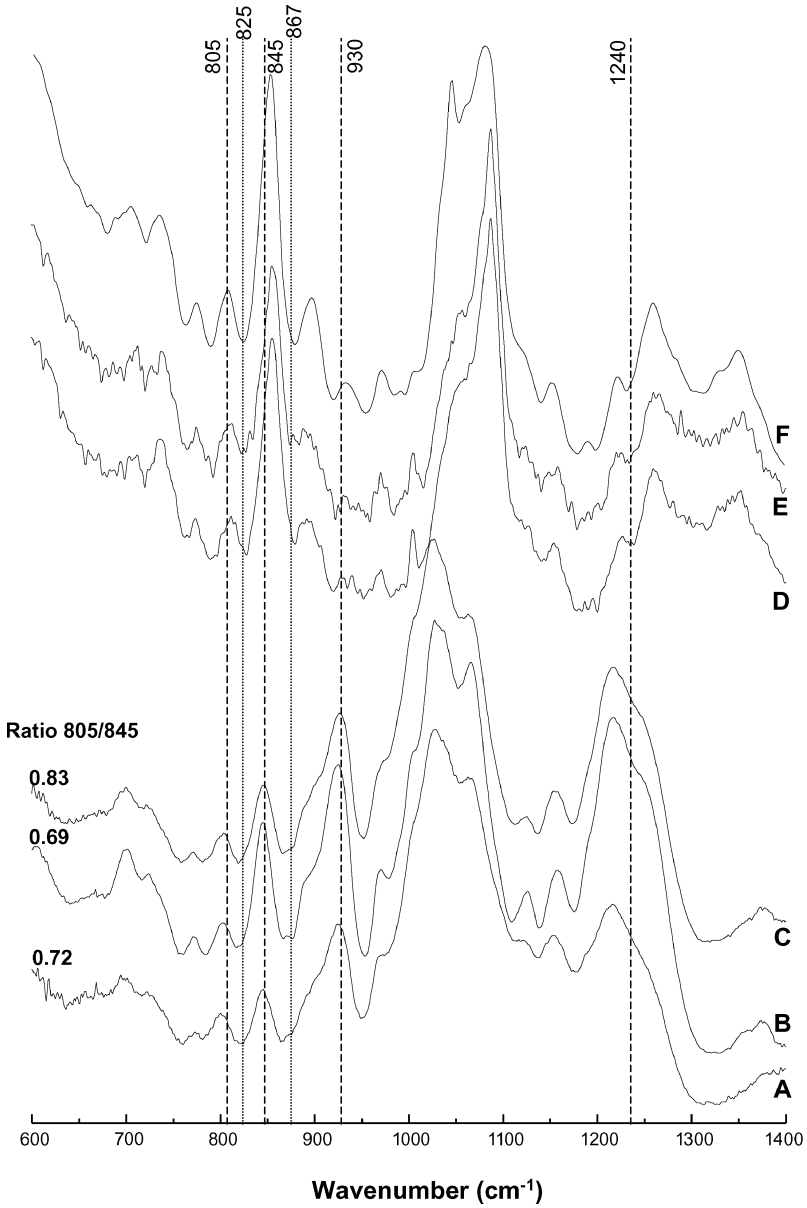

Figure 4. FTIR and FT-Raman spectra of ground seaweed samples (A, D), native carrageenan (B, E) and alkali-extracted carrageenan (C, F) of *C. teedei* var. *lusitanicus* female gametophytes.

All Raman spectra (Figures 6D–6F) present strong absorbance at 1070 cm^{-1}, which corresponds to C-O of 3,6-anhydrogalactose (**DA**), and three other close-lying bands in the spectral region of 815-850 cm^{-1}, related with the high sulphate contents (Pereira et al., 2003). The presence of 815 and 850 cm^{-1} peaks (Figures 6D–6F) allows us to conclude that the main constituent fraction is a ksi-carrageenan (Pereira et al., 2003). The 825 cm^{-1} and 905 cm^{-1} peak, present in all Raman spectra (Figures 6D–6F), correspond to the presence of tetha-carrageenan.

Carrageenan analysis by ^{1}H- and ^{13}C-NMR spectroscopy

The ^{1}H-NMR spectra of native and alkali-modified carrageenans from *C. teedei* var. *lusitanicus* female gametophytes and non-fructified thalli revealed, in the

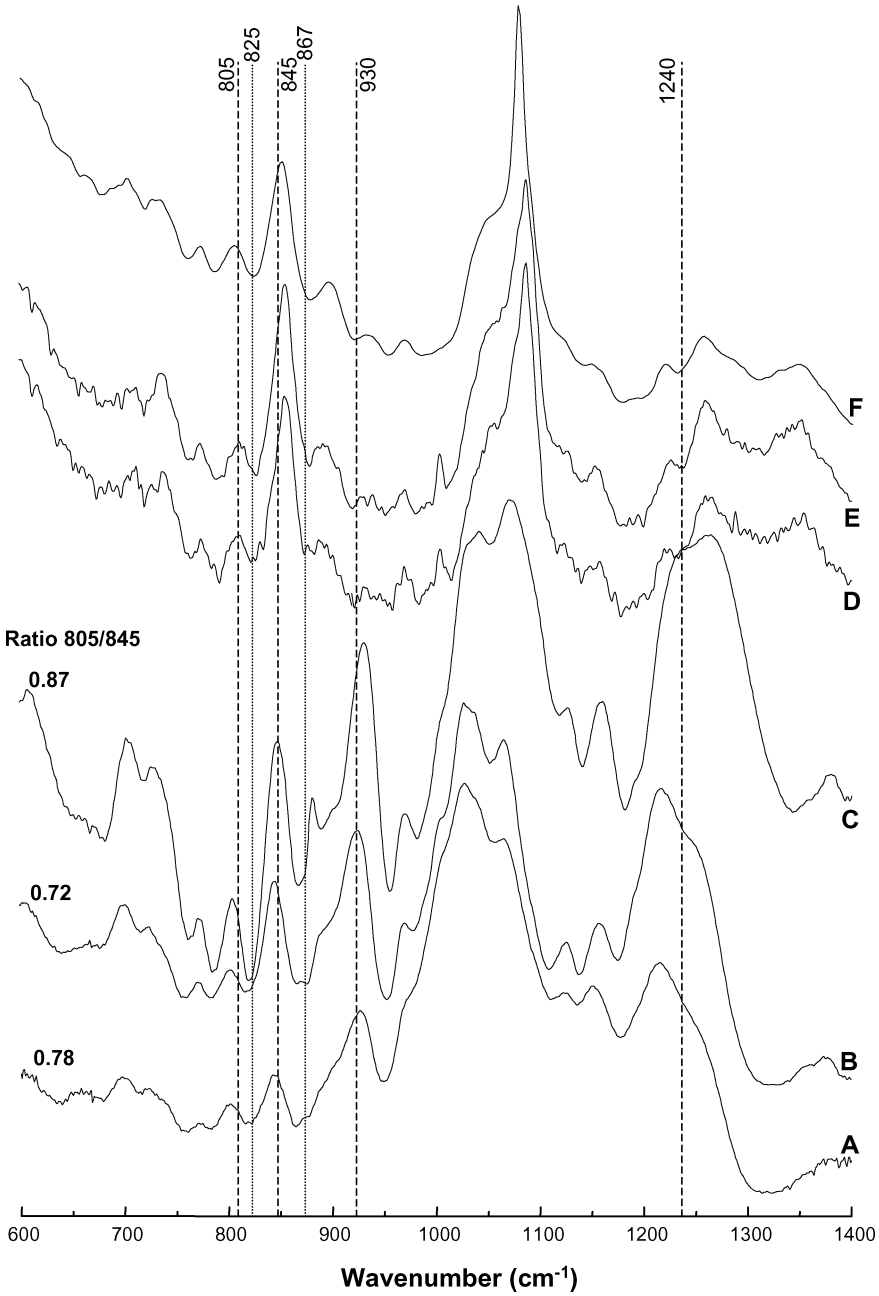

Figure 5. FTIR and FT-Raman spectra of ground seaweed samples (A, D), native carrageenan (B, E) and alkali-extracted carrageenan (C, F) of *C. teedei* var. *lusitanicus* non-fructified thalli.

anomeric protons zone, two major signals at 5.11 ppm and 5.32 ppm (Figures 8c and 8d). These signals correspond to the anomeric protons of the 3,6-anhydro-α-D-galactose (**DA**; kappa-carrageenan) and the 2-sulphated 3,6-anhydro-α-D-galactose (**DA2S**; iota-carrageenan), respectively. Minor components detected in the spectra of the native carrageenans (Figures 8a and 8b) gave a signal at 5.52 ppm and were assigned to the anomeric proton of the α-D-galactose-2,6-disulphate (**D2S, 6S**; nu-carrageenan) and 5.25, assigned to the α-D-galactose-6-sulphate (**D6S**; mu-carrageenan) (Ciancia et al., 1993; Stortz et al., 1994;

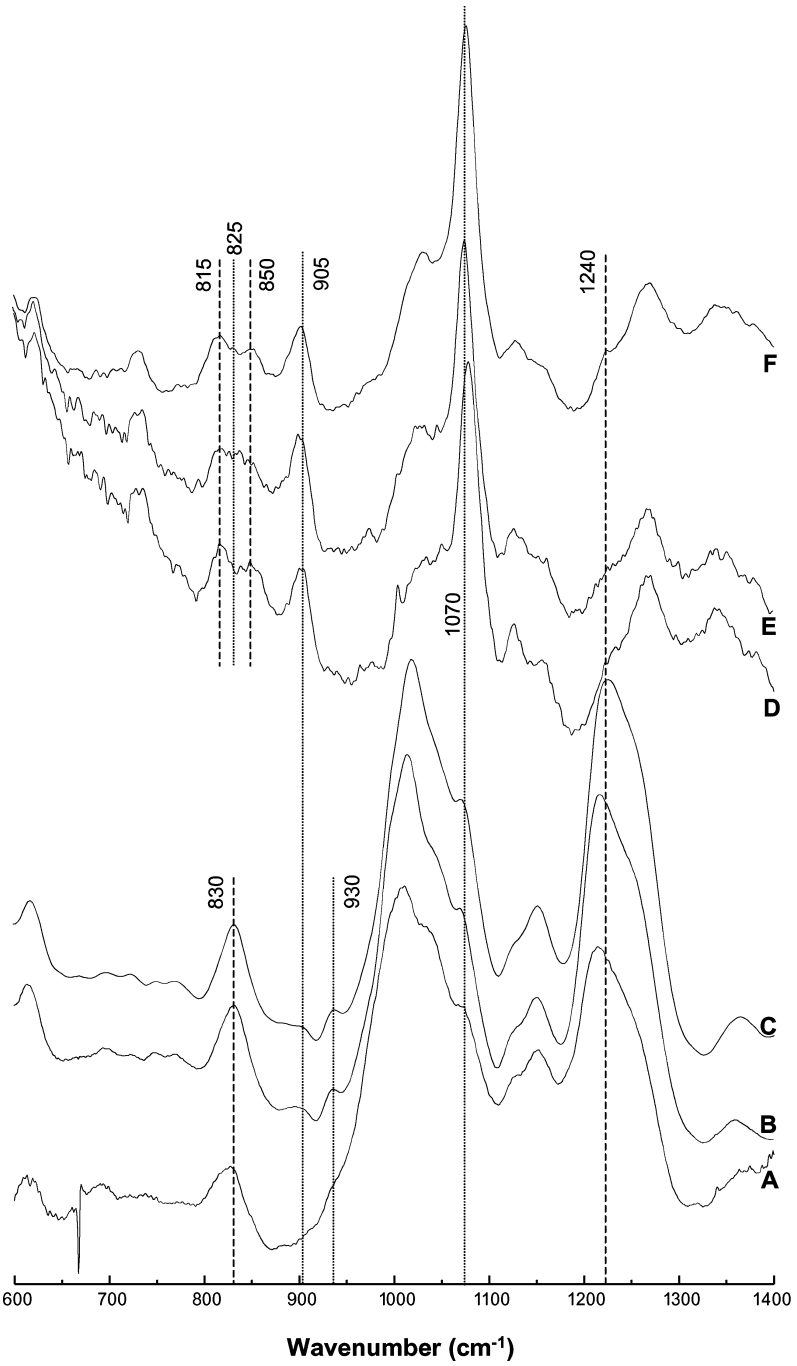

Figure 6. FTIR and FT-Raman spectra of ground seaweed samples (A, D), native carrageenan (B, E) and alkali-extracted carrageenan (C, F) of *C. teedei* var. *lusitanicus* tetrasporophytes.

Van de Velde et al., 2002a). In addition to the carrageenan signals, the spectrum of the alkaline-extracted female gametophyte sample showed a minor signal with a chemical shift of 5.37 ppm. This signal is assigned to floridean starch, which is a usual and natural contaminant of carrageenan samples (Knutsen and Grasdalen, 1987; Van de Velde et al., 2002a, b). The intensity of the above mentioned resonance is used to

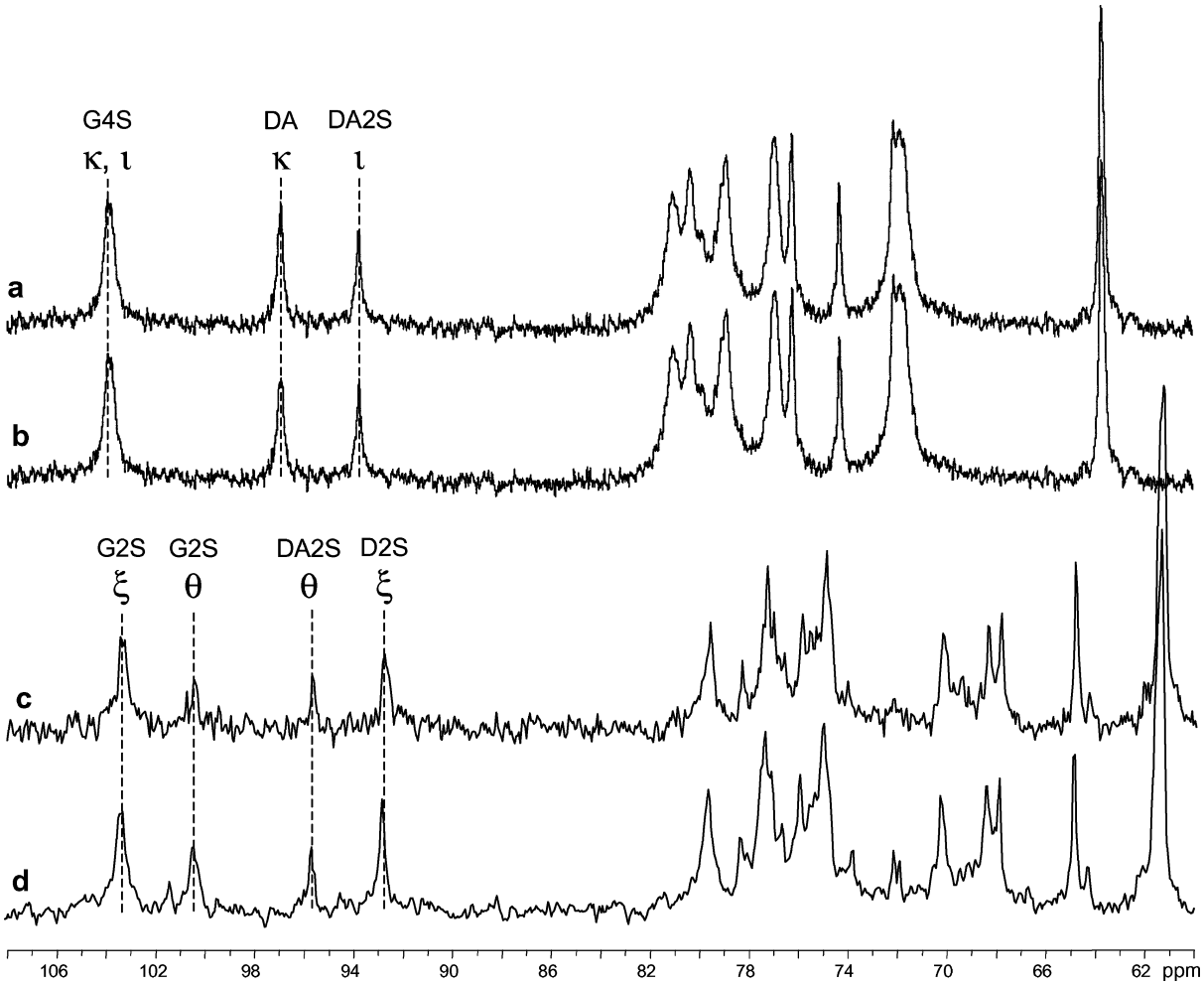

Figure 7. ^{13}C-NMR spectra of carrageenans of *C. teedei* var. *lusitanicus* female gametophytes (a), non-fructified thalli (b) and tetrasporophytes (c – native, d – alkali-extracted). Letter codes refer to nomenclature of Knutsen et al. (1994).

quantify the composition of the different carrageenans (Table 1).

The ^{13}C-NMR spectra of the alkaline-extracted carrageenan (female gametophytes and non-fructified thalli) present, in the anomeric region, three major peaks (Figures 7a and 7b): 102.5 ppm, referring to anomeric carbon of the β-D-galactose-4-sulphate residues (**G4S**), common for the kappa- and iota-carrageenans; 95.3 ppm, correspondent to anomeric carbon of 3-6-anhydro-galactose (**DA**) of kappa-carrageenan and 92.1 ppm relative to anomeric carbon of anhydro-galactose-2-sulphate (**DA2S**) of iota-carrageenan (Usov et al., 1980; Van de Velde et al., 2002a).

The ^{13}C spectrum of the native and alkaline-extracted carrageenan of tetrasporophytes (Figures 7c–7d) showed signals at 103.3 and 92.8 ppm, 100.4 and 95.7 ppm that could be assigned to the anomeric carbons of ksi and tetha-carrageenan, respectively (Falshaw and Furneaux, 1994, 1995).

Discussion

There were obvious regular patterns over the experimental periods in most of the population parameters. Average biomass and length of thalli generally show a seasonal pattern, with high values in early summer and low values in late summer, autumn and winter. An exception was the decline in August 2000, which coincided with an abnormal period of high temperature and low pH (dotted line in Figure 1).

Table 1. Composition of carrageenans based on NMR analysis.

Carrageenan composition	Kappa - κ (%mol)	Iota - ι (%mol)	Mu - μ (%mol)	Nu - ν (%mol)	Floridean starch
C. teedei var. lusitanicus					
Female gametophytes					
Native	52.7	37.5	4.6	5.2	–
Alkali-extracted	58.1	41.9	–	–	+
Non-fructified thalli					
Native	47.2	45.8	0.7	6.3	+
Alkali-extracted	49.7	50.2	–	–	
	Ksi-ξ (%mol)	Tetha-θ (%mol)			–
Tetrasporophytes					
Native	67	33			–
Alkali-extracted	67	33			–

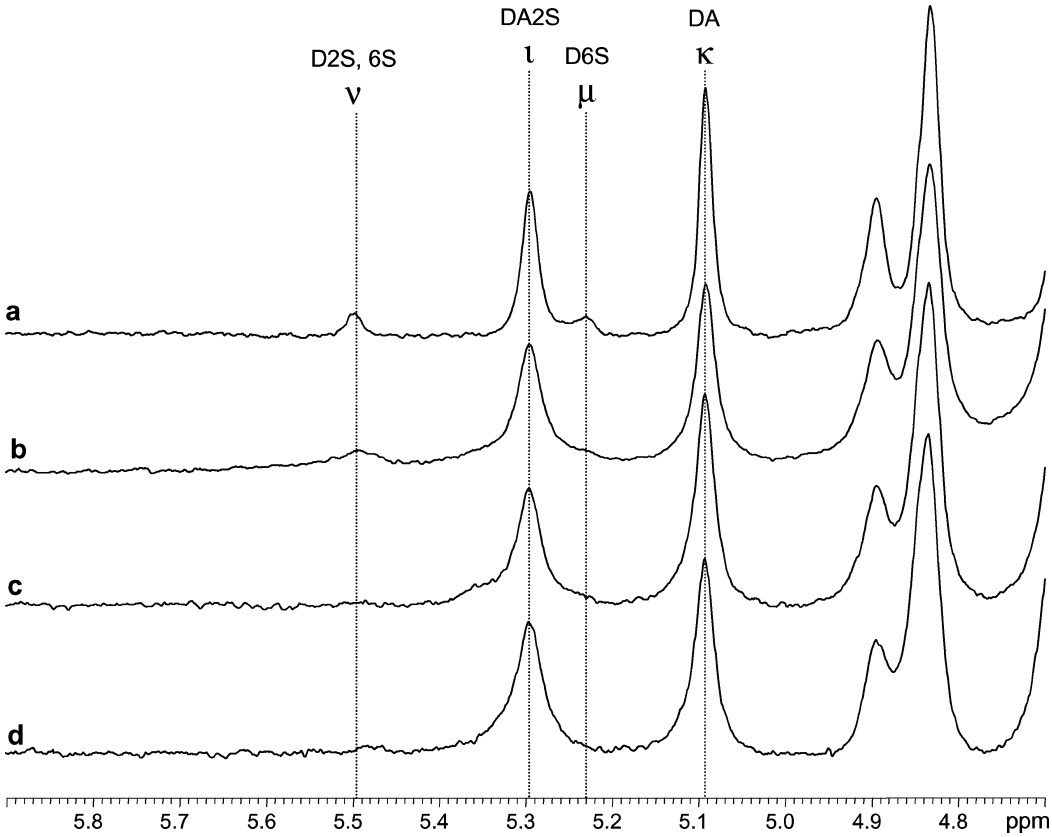

Figure 8. ^{1}H-NMR spectra of native (a, b) and alkali-extracted (c, d) carrageenans of *C. teedei* var. *lusitanicus* female gametophytes (a, c) and non-fructified thalli (b, d). Letter codes refer to nomenclature of Knutsen et al. (1994).

In this population there was a predominance of non-fructified plants (average $= 69.4 \pm 2.2\%$, $n = 17$). In relation to the fructified thalli, tetrasporic plants were predominant (total average $= 21.0 \pm 1.7\%$, $n = 17$), as compared to fertilized female gametophytes (average $= 9.6 \pm 1.7\%$, $n = 17$). This is surprising because in other geographical regions where *Chondracanthus teedei* populations have been studied,

the situation has shown to be the reverse, i.e., the tetrasporophyte appears as a very rare generation (0.5–2%, in Brazil) (Braga 1985, 1990) or, apparently, it does not exist, as is the case of French coast (Zinoun et al., 1993). The presence of female gametophytes in spring/summer and tetrasporophytes in autumn/winter is related to the temperature and day length. In contrast to the description of Braga (1990), the formation of cystocarps is probably conditioned by high temperature and long day length, an opposing correlation existing in development of the tetrasporocysts.

The dry weight and carrageenan content in tetrasporophytes, female gametophytes and non-fructified plants of *C. teedei* var. *lusitanicus* (Figure 3B) were slightly different. Cystocarpic plants had the highest yield, and non-fructified plants the lowest. Overall, the spatial patterns were similar for each of the reproductive phases. The maximum content in carrageenan (see Figure 3A) was found in a tetrasporophyte sample (58% dry mater, July 2001) and the total average carrageenan content in this species was $34.9 \pm 1.0\%$ ($n = 15$) dry matter. These values, although raised, are below reports for samples harvested in Brazil (76%, Saito and Oliveira, 1990) and France (70%, Zinoun, 1993; Zinoun et al., 1993a, b).

The factors responsible for seasonal patterns of phycocolloid content in carrageenophytes include photoperiod, life history phases, growth stage, air and water physical-chemical parameters, nutrients, and, as they are interrelated, their effects are difficult to separate (Chopin, 1986; Bolton and Joska, 1993). However it is possible that the low colloid content (see Figure 3A and 3B) in December and the high colloid content in July 2001 can be related to high and low nutrient content of the water, respectively. Buarcos Bay is subjected to a considerable seasonal variation in water nutrients due to the permanent discharges of the Mondego River, which annually discharges 8.5×10 m^3 yr^{-1} of water, 120 tonnes of nitrogen and 15 tonnes of phosphorus into the bay (Flindt et al., 1997). However, great variations in dissolved inorganic nitrogen, with high values in the winter and low values in the summer, are present in the discharged water (Flindt et al., 1997; Martins et al., 2001). In effect, the inverse correlation between carrageenan yields and nitrogen concentration was demonstrated in the culture experiments with the *C. teedei* (Zinoun, 1993; Zinoun et al., 1993a).

Relative to the phycocolloid nature, our spectroscopic analysis showed that the two phases in the life history of *C. teedei* var. *lusitanicus* seem to present a variation similar to that existing in other species of *Chondracanthus* genus (see Chopin et al., 1999): the gametophyte stages produce carrageenans of the kappa family (hybrid kappa/iota/mu/nu carrageenan), while the tetrasporophyte stages produce carrageenans of the lambda family (hybrid ksi/tetha-carrageenan). The alkaline-extracted carrageenan from female gametophytes showed lower sulphate content and a decrease in a galactose to the benefit of 3,6-anhydrogalactose. The presence of some 4-linked galactose 6-sulphate in native samples was converted to anhydro-galactose upon alkali-treatment, in sequence of the precursors conversion in kappa and iota carrageenan. Within the experimental error of the ^{1}H-NMR analysis the precursor units are stoiciometrically transformed to the gelling carrageenans (Table 1).

The *C. teedei* var. *lusitanicus* non-fructified thalli appeared to contain a similar phycocolloid to the female gametophytes, on the basis that the FTIR, FT-Raman, ^{1}H- and ^{13}C-NMR spectra of their carrageenans were identical. However, although both thalli produced a kappa/iota/mu/nu hybrid carrageenan (Table 1), the non-fructified thalli possessed identical amounts of kappa and iota-fraction, while in female gametophytes the kappa-fraction was slightly dominant (in alkaline extraction).

Our results, in respect to the female gametophytes and non-fructified thalli alkaline-extracted carragenanas, are in accordance with those presented for Zinoun (1993) and Zinoun et al. (1993b). However these authors reported that *C. teedei*, which produced kappa/iota hybrid carrageenan, contains only nu-carrageenan as the biological precursor in native extract. The analysis of the FTIR, FT-Raman and ^{1}H-NMR spectra allowed us to conclude that the native carrageenan presents both precursors: mu and nu. Since the mu-carrageenan is only 4.6% mol (see Table 1) and repeating units with a concentration above 5%mol shoed resonance above the noise within ^{13}C spectra, ^{13}C-NMR will not give additional information. Moreover, mu and nu-carrageenan show comparable chemical shifts within the anomeric region (Van de Velde et al., 2002a). However, the studies made by Zinoun (1993) and Zinoun et al. (1993b) were based on ^{13}C-NMR spectra and, therefore, probably the presence of mu-carrageenan in the samples of native carrageenan was not detectable. But, to confirm such hypothesis it would be necessary to analyze samples of *C. teedei* obtained from the French coast.

The FTIR spectra of tetrasporic samples of *C. teedei* show sharper peaks at 830 cm^{-1}, but little absorption at 820 cm^{-1}, which indicates the presence of ksi carrageenan (Figures 6A–6C). The presence of 815 and 850 cm^{-1} peaks in FT-Raman spectra confirmed the presence of the mentioned carrageenan (Pereira and Mesquita, 2003). For beyond this main fraction, the tetrasporic colloid presents tetha-carrageenan in both native and alkaline-extracted samples. Despite ^{1}H-NMR spectra not to be conclusive (results not presented), the ^{13}C-NMR spectra confirmed the presence of a hybrid ksi/tetha-carrageenan. Integration of the anomeric carbon resonances revealed a ksi/tetha-ratio of 68% mol ksi-carrageenan and 32% mol tetha-carrageenan.

The presence of tetha carrageenan in native extracts has been found in other tetrasporic *Chondracanthus* and *Gigartina* species (see Chopin et al., 1999). Tetha-carrageenan is also formed by alkaline treatment of lambda-carrageenan (Falshaw and Furneaux, 1994; Falshaw et al., 2003).

In respect to the methodology used in this study, the Raman spectroscopy is considered a complementary technique of the IR spectroscopy by some authors, presenting similar spectra to the ones of infrared. However, certain bands of weak intensity in FTIR appear clear in FT-Raman, what increases the information and allows the correct interpretation of the vibrational spectra. In comparative studies of carrageenan types, the FTIR spectra provide enough information. However, FT-Raman allows an accurate identification of diverse colloids, with particular reference to the variants of the lambda-family (Pereira et al. 2003) and to the carrageenan precursors (mu and nu). Important characteristic bands, such those at 825 and 867 cm^{-1}, necessary to the identification of the precursors (mu and nu), are more easily observed in the FT-Raman. Nevertheless, in the infrared (Figures 4 and 5) the absorption of these bands is extremely weak. Discrimination between kappa and iota carrageenan is based in the 805 cm^{-1} peak, which has a stronger signal in FT-Raman spectra than in FTIR (Pereira et al., 2003). The FT-Raman spectra have an 815–900 cm^{-1} band with additional information to distinguish the lambda-family carrageenan variants when compared with FTIR spectra. The Raman spectra show typical peaks at 815 and 859 cm^{-1} for ksi-variant (Pereira et al., 2003) and 825 cm^{-1} for tetha-variant (Figure 6). Relative to infrared spectra, laser Raman spectroscopy provides, in certain bands, an improved resolution.

However, the FTIR and the FT-Raman are habitually used in the qualitative determination of phycocolloids, without quantification. For this purpose proton NMR spectroscopy is the technique currently used (Van de Velde et al., 2002a). This technique can be used, in general, for two different purposes: for fast analysis of small samples (only 5 mg of sample is necessary) and for quantification of different carrageenan types. Quantitative determination of the composition of different carrageenan repeating units is based on the intensity of the resonance of the anomeric protons (Van de Velde et al., 2002a). The resonance of kappa, iota, nu, mu and lambda carrageenans are well resolved in the anomeric region, however some overlap may occur when ksi or tetha-carrageenan is present. In the case of such overlaps, additional ^{13}C-NMR spectroscopy is necessary to identify the different carrageenan repeating units present.

In conclusion, the combination of readily available biomass and colloid content of this seaweed makes it a potentially important source of co-polymer of kappa and iota-carrageenan, in addition to the traditionally harvested Portuguese carrageenophytes (Santos and Duarte, 1991; Pereira and Mesquita, 2003) *Chondrus crispus* and *Mastocarpus stellatus*. However, collection of seaweeds from the rocky shore has its limitations, always contains the risk of over-collection and subsequent loss of the population. With respect to farming *C. teedei* as a source of carrageenan, Zinoun (1993) and Zinoun et al. (1993a) showed that it is possible to manipulate the metabolism of this species in way to produce high carrageenan yields. We think that the next step will be to develop studies in order to introduce this species in to aquaculture integrated systems, combining fish and seaweed aquaculture for water bioremediation in intensive fish farming.

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