Journal of Applied Phycology (2021) 33:2379–2390
https://doi.org/10.1007/s10811-021-02457-5

Strain selection in *Chondracanthus teedei* (Gigartinaceae, Rhodophyta) using tetraspore and carpospore progeny: growth rates, tolerance to temperature and carrageenan yield

Eduardo de Oliveira Bastos[1] · Paulo Antunes Horta[1] · Leila Hayashi[2]

Received: 25 November 2020 / Revised and accepted: 16 March 2021 / Published online: 17 May 2021

Abstract

The feasibility of strain selection using tetraspore and carpospore progeny from wild fronds of *Chondracanthus teedei* to obtain strains with high growth rates, tolerance to temperature, and high yield of native carrageenan was investigated. A total of 34 strains were isolated, 14 from gametophytic progenitors and 20 from tetrasporophytic progenitors. The strains showed a heterogeneity of morphologies, colors, sizes, and growth. These differences were observed right from the early stages of development, resulting in individuals with more than one characteristic different from the others. Ninety days after selection, the strains differed in their growth rate, ranging from 0.57 to 5.13% day^{-1} in strains from gametophytic progenitors and 2.16 to 6.79% day^{-1} in strains from tetrasporophytic progenitors. When exposed to the temperature gradient, most strains (24 of 34) showed optimal growth rate values at the intermediate temperature used (25 °C) or without significant differences between 25 °C and one of the other temperatures used. However, four strains showed optimal growth rate values only at the extreme temperatures used and two gametophytic strains maintained their growth rate values without significant differences, irrespective of temperatures used, with a higher mean value for strain S27I (6.46 ± 0.07% day^{-1}). The yields of carrageenan also varied among strains and temperatures with maximum value for strain S27I (74% DW for 15 °C and 25 °C; 73% DW for 30 °C). Thus, we believe that a combination of strains in different seasons could provide greater stability in biomass production and native carrageenan to, in turn, provide economically viable production.

Keywords *Chondracanthus teedei* · Rhodophyta · Strain selection · Carrageenan · Tolerance to temperatur

Introduction

The world macroalgae market is dominated by only a few species. For carrageenophytes, production is largely concentrated in *Eucheuma* spp. and *Kappaphycus* spp., based on marine farming, with the exception of *Chondrus crispus* Stackhouse and *Gigartina skottsbergii* Setchell *et* Gardner harvested from natural populations (Hurtado et al. 2017; FAO 2020a). However, the demand for species diversification has increased in the last decade, driven mainly by the development of the phycocolloid industry, an increase in direct consumption, and the use of nutraceutical and pharmaceutical products (Shannon and Abu-Ghannam 2019).

According to the FAO (2020a), 91.7% of the world's macroalgae production in 2018 was obtained through aquaculture. In Brazil, although phycology is relatively well advanced and has a wide sea coast and good diversity of macroalgae, commercial aquaculture is embryonic with an estimated production of 730 t in 2018 (FAO 2020b). As a consequence, Brazil still depends on the import of fresh biomass and several of its products (Simioni et al. 2019). Even if Brazil does not have good floristic and climatic conditions for the production and processing of brown macroalgae for food and/or alginates, the country could become a producer of red algae to obtain agar and carrageenan or even for in natura consumption (Hayashi et al. 2014). In view of this, several studies have been carried out with native macroalgae showing promising results (Pereira and Silva 2021; Carneiro et al. 2021); however, these have never reached a significant commercial level so far (Simioni et al. 2019). As regards the

✉ Eduardo de Oliveira Bastos
bastos_e@hotmail.com

[1] Phycology Laboratory, Department of Botany, Biological Sciences Center, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil

[2] Department of Aquaculture, Federal University of Santa Catarina, Florianópolis, Brazil

carrageenophytes production, promising native species include *Hypnea pseudomusciformis* Nauer, Cassano & M.C.Oliveira and *Chondracanthus teedei* (Mertens ex Roth) Kützing (de Oliveira 1997; Simioni et al. 2019). For *H. pseudomusciformis*, the studies carried out to date have not shown results that would stimulate the sector by coming up against technical difficulties (Reis et al. 2006). With *C. teedei*, only studies related to taxonomy, reproduction, and ecology have been carried out (Braga 1985, 1990).

Although seaweed aquaculture technologies have developed dramatically in recent decades, there is a growing need for predictability and control over production processes and, hence, calls for intensified search for strains with the desired characteristics and high yields (Levy et al. 1990). Genetics and the development of new production techniques have led to improvements in obtaining strains in terrestrial agricultural plants in recent decades. However, strain selection of macroalgae is still an embryonic field (Robinson et al. 2013). Some programs for selecting macroalgae strains have been implemented over the past few years. The main target characteristics have been growth rate (Paula et al. 1999), more attractive morphology (Li et al. 1999), more resistance to breakage (Mollion and Braud 1993), tolerance to abiotic variation (Yan et al. 2010), tolerance to disease (Park and Hwang 2014), and high phycocolloid production (Hayashi et al. 2007).

Studies related to strain selection usually involve a pool of individuals from one or more populations. Experiments are then carried out in natural and/or laboratory conditions in order to express the desired phenotypes (Bulboa et al. 2008). Recently, new techniques have been used to improve strains, including protoplast fusion (Reddy et al. 2008), mutagenesis (Charrier et al. 2015), and genetic modification (Robinson et al. 2013). However, these techniques are difficult to perform and costly, in addition to the challenges of public acceptance owing to genetically modified organisms and environmental risks (Robinson et al. 2013). However, spore selection, although little used, has shown good results allowing high genetic variability and continuous selective breeding (Paula et al. 1999; Yan et al. 2010).

Macroalgae release an enormous number of spores that become part of the plankton. This reproductive effort is necessary due to losses during the development of germlings. Among the numerous sources of failure in recruiting spores are grazing, displacement to aphotic zones or to unfavorable surfaces, and limiting abiotic conditions (Maggs and Callow 2006). Temperature plays an important role as it regulates spore fixation, germination, and germling growth determining the patterns of biogeographic distribution (Yokoya and Oliveira 1993). This results in the introduction of complex reproductive mechanisms that often involve syngamy, mitospores, meiospores, and parthenogenetic development of unfertilized gametes (Liu et al. 2017). This complexity, in turn, results in high genetic diversity and contributes to the maintenance of populations in relation to environmental pressures (Coleman et al. 2020). Thus, strains with stress-tolerant genotypes are selected owing to environmental pressures, replacing less resistant individuals in the population or expanding the biogeographic distribution (Coleman et al. 2020). Some studies have been carried out to test the possibility of selecting strains from spores. Paula et al. (1999) and Bulboa et al. (2007), for instance, obtained more robust strains with high morphological differences and satisfactory growth rates using tetraspore progeny from *Kappaphycus alvarezii* (Doty) Doty ex P. C. Silva and *K. striatus* (Schmitz) Liao, respectively. Yan et al. (2010) obtained temperature-tolerant strains of *Neoporphyra haitanensis* (T.J. Chang & B.F. Zheng) J. Brodie & L.-E.Yang (formerly *Porphyra haitanensis*) by exposing germlings from conchospores at elevated temperatures.

In recent years, interest in macroalgae of the genus *Chondracanthus* has grown as a result of their economic potential for extraction of carrageenan, use in cosmetics, and direct consumption (Contador et al. 2020; Pereira and Silva 2021). *Chondracanthus teedei* is a cosmopolitan species found in Brazil from the state of Ceará (3° 42′ 24.30″ S and 38° 28′ 7.75″ W) to Rio Grande do Sul (31° 54′ 42.27″ S and 51° 35′ 23.09″ W) with Uruguay as its southern limit in the western Atlantic (34° 57′ 53.54″ S and 54° 56′ 22.98″ W) (Coutinho 1982; Joventino et al. 1998; Coll and Oliveira 1999) and is considered promising for carrageenan production (Pereira and van de Velde 2011). The life cycle is isomorphic triphasic (Pereira and Silva 2021) and differences in carrageenan composition are observed between the stages with gametophytes producing kappa/iota hybrid carrageenan and tetrasporophytes produces xi-theta hybrid carrageenan (Pereira and Mesquita 2003, 2004; Pereira et al. 2009). Zinoun et al. (1993b) show that it is possible to manipulate the metabolism of *C. teedei* by varying the temperature of the medium which results in changing the composition of carrageenan and its yield. The first studies with *C. teedei* in Brazil were carried out by Braga (1985) who demonstrated a tendency of biomass increase in the winter and decrease in the summer for southeast populations. In the laboratory, the author also observed that the species survives at temperatures between 18 and 26 °C, with greater growth rate at 22 and 26 °C without influence of the photoperiod. These results indicate that the species could be used for biomass production and carrageenan extraction in places where the amplitude of annual temperatures becomes a problem in cultivation, especially for tropical species such as *K. alvarezii* introduced in the south of Brazil. Thus, the present work aimed to evaluate the potential for strain selection using tetraspore and carpospore progeny from wild fronds of *C. teedei* to obtain strains with high growth rates, tolerance to temperature, and high yield of native carrageenan in view of the economic potential of this

species for the phycocolloid production, functional food, and nutraceuticals.

Material and methods

Progenitors and spore release

Fronds of *Chondracanthus teedei* were collected in the northern part of Praia das Caravelas (27° 21′ 26.57″ S and 48° 32′ 12.69″ W) in Santa Catarina State, Brazil. After collection fronds were stored in plastic bags with seawater and transported inside coolers to the Laboratory of Phycology at Universidad de Santa Catarina (LAFIC-UFSC). In the laboratory, the fronds were separated by life cycle phase by visual inspection looking for cystocarps and tetrasporangial sori. Five female gametophytes with cystocarps and five sporophytes with tetrasporangial sori were selected at random. These progenitors were represented with the letters being A, B, C, D, and E for female gametophytes with cystocarps, and F, G, H, I, and J for sporophytes with tetrasporangial sori (Fig. S1 in the on-line Supplementary material). From each progenitor, four frond fragments containing reproductive structure (cystocarp or tetrasporangial sori) were removed and placed individually in Petri dishes with slides for microscopic observation and 50 mL of culture medium (Fig. 1). This totaled four Petri dishes for each progenitor (Fig. 1). After 24 h, the frond fragments were removed, and spores cultured for 30 days at 25 °C, photosynthetically active radiation (PAR) of 20 μmol photons m^{-2} s^{-1} with 2 fluorescent lamps (Osram 30 W Daylight), 12-h photoperiod, and salinity of 35. The culture medium consisted of sterilized seawater and von Stosch solution (8 mL L^{-1}). Seawater was sterilized with a mechanical filter (5 and 10 μm) and exposed to UVC light in a slow continuous flow. In the first 7 days, 6 mL of GeO_2 (3 μg L^{-1}) was added to control possible contamination by diatoms, and potassium penicillin (0.2 mg mL^{-1}) was added to inhibit cyanobacterial growth (Oliveira et al. 1995; Garcia-Jimenez et al. 1999). Culture medium was renewed weekly.

Initial isolation of strains

For strain selection one germling per Petri dish was randomly removed and cultivated in isolation for 60 days (Fig. 1). This totaled four germlings for each progenitor, resulting in 40 selected strains (20 from gametophytic progenitors and 20 from tetrasporophytic progenitors). After selection germlings were kept in Erlenmeyers with 50 mL of culture medium for 15 days, and then moved to Erlenmeyers with 250 mL of culture medium under constant aeration and 80 μmol photons m^{-2} s^{-1} (Fig. 1). One Erlenmeyer was used for each selected strain. Culture medium was renewed weekly and every 15 days the fronds were weighed with an analytical balance (BioPrecisa FA-2104N) and photographed. The strains are represented with the letter S of strain followed by a strain number and letter that indicates their progenitor. Example: S23H—Strain 23 originated from tetrasporophytic progenitor H. After 60 days of isolated cultivation, crosses were performed in the gametophytic strains to identify the female gametophyte by the formation of cystocarps (Fig. 1). To this end, the gametophytic strains were labeled and kept in the same container (aquarium with 3 L of culture medium) for 15 days. After this period, the strains were cultivated again isolated to increase biomass for subsequent experiments. The parameters were kept the same as previously described.

Effects of temperature on growth rate and native carrageenan yield in selected strains

To evaluate the effect of temperature on the growth rate and production of native carrageenan of the selected strains, a gradient table as described by Oliveira et al. (1995) was used. The conditions were those described above, varying only temperature (15, 25, and 30 °C). For this, 2 g of each strain selected previously was placed in Erlenmeyer with 500 mL of culture medium under constant aeration for 28 days. The culture medium was renewed weekly and the fronds were weighed. The experiment was carried out using three Erlenmeyers for each strain. The growth rate was calculated using the initial and final fresh biomass. Growth rate was calculated as Gr (% day^{-1}) = $[(W_t/W_i)^{1/t} - 1] \times 100$, where W_i = initial fresh biomass, W_t = fresh biomass after 28 days, and t = experimental time in days.

Native carrageenan yield in selected strains was extracted according to Pereira and Mesquita (2004) with modifications. Fronds were washed with distilled water, dried at 60 °C until constant weight, and ground with liquid nitrogen. The dry material was rehydrated with distilled water (50 mL g^{-1}) and the solution was kept at 85 °C for 3 h under constant agitation. Afterwards, the solution was centrifuged (3500× g for 15 min) and the supernatant separated. Native carrageenan was precipitated by adding twice the volume of ethanol (98° GL) in the warm supernatant, then centrifuging at 3500× g for 15 min. The precipitate was dried at 60 °C until constant weight and weighed. Native carrageenan yield is given as % of dry weight.

Statistical analysis

The data obtained in the strain selection were analysed with descriptive statistics while the data of the growth rate and native carrageenan yield resulting from the temperature experiment were analysed using parametric statistics. After evaluating normality, growth rate and native carrageenan yield data were tested for homogeneity of variances by the Cochran test. Since assumptions were obtained, two-way ANOVA was

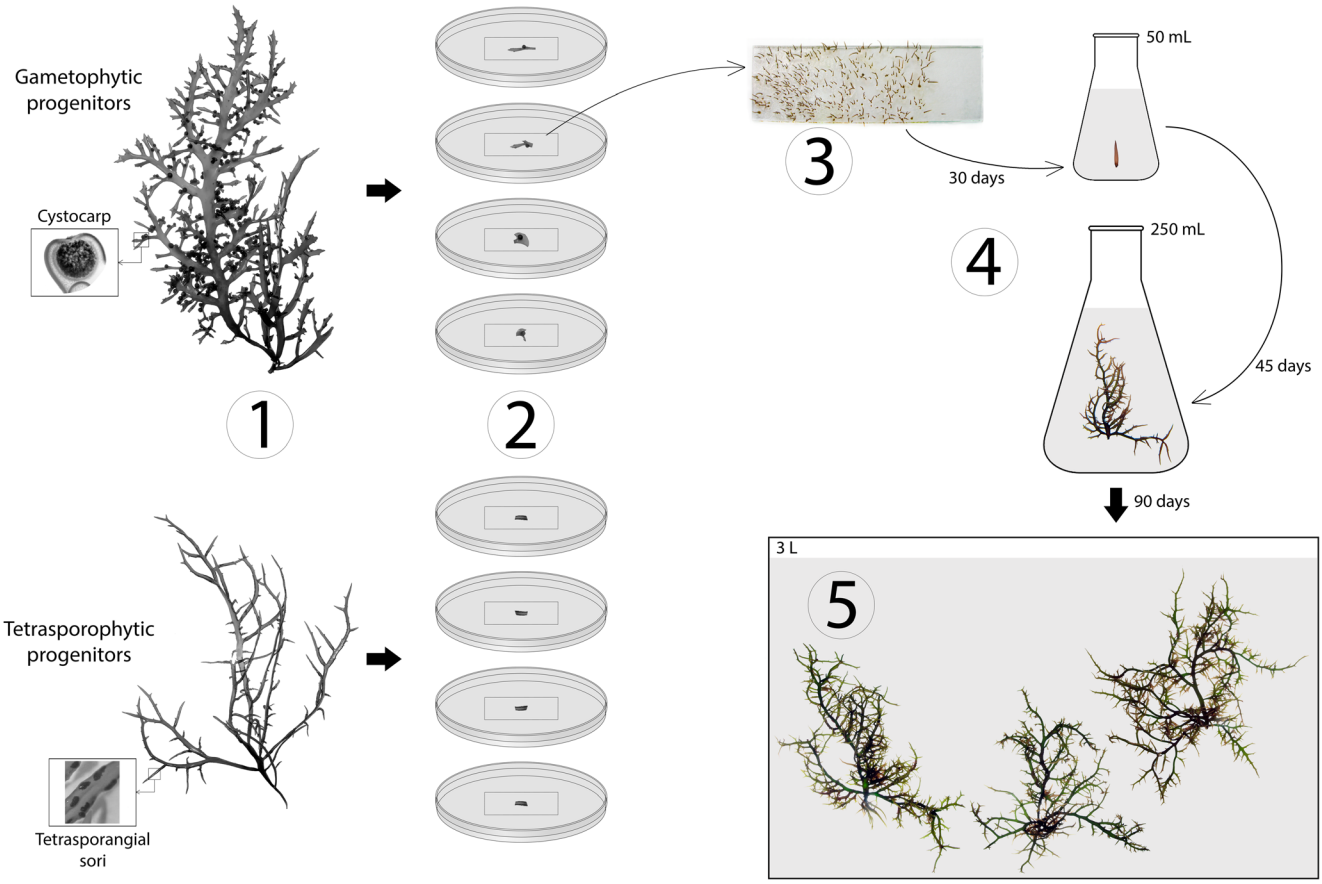


Fig. 1 Sequence of strain selection process. (1) Gametophytic or tetrasporophytic progenitors with reproductive structures. (2) Petri dishes containing a frond fragment with reproductive structure. (3) Germlings after 30 days. (4) Selected strains grown in isolation. (5) Cross between strains from sporophytic progenitors to identify female gametophytes

performed to evaluate the performance of the strains submitted to different temperatures. For that, temperatures and strains were used as independent factors, and growth rate and native carrageenan yield as dependent factors. The analyses were carried out between the strains referring to the progenitors of each life cycle stage. Analyses were not carried out between the life cycle stages. When significant differences were observed ($p < 0.05$), the Student-Newman-Keuls (SNK) multiple post hoc comparison test was applied. Statistical analyses were performed using STATISTICA 12 software (StatSoft, Inc. 2011).

Results

Strain selection

Tetraspores and carpospores in large numbers were obtained from fronds enabling the selection of 20 strains of female gametophytes and 20 strains of tetrasporophytes. However, six strains (two from progenitor A, two from progenitor B, and two from progenitor E) did not survive the first 15 days after selection. On the other hand, 34 strains survived, 14 from gametophytic progenitors and 20 from tetrasporophytic progenitors, and these were kept in the laboratory for at least 2 years.

Heterogeneity of morphologies, colors, sizes, and growth was observed in these strains. These differences were observed right from the early stages of development, resulting in individuals with more than one characteristic different from the others, even though they came from the same progenitor (Figs. 2 and 3; Fig. S2 to S11 in the on-line Supplementary material). The color ranged from dark crimson and red black to greenish throughout strain development (Fig. S2 to S11 in the on-line Supplementary material), with greenish being more prominent at 90 days (Figs. 2 and 3). Strains from gametophytic progenitors showed few ramifications (S06C, S10D, and S11D) (Fig. 2), while strains from tetrasporophytic progenitors showed dense ramifications (S17F, S27I, and S30I) (Fig. 3).

Reproductive structures were observed in some strains. Tetrasporangial sori were observed releasing viable spores in practically all strains from gametophytic progenitors 30 days after selection. S04B, S05C, and S08C did not show reproductive structures, even after 90 days (Fig. 2). Strain S07C showed cystocarps 45 days after selection and release of viable carpospores after 90 days (Fig. 2). Reproductive structures were observed in

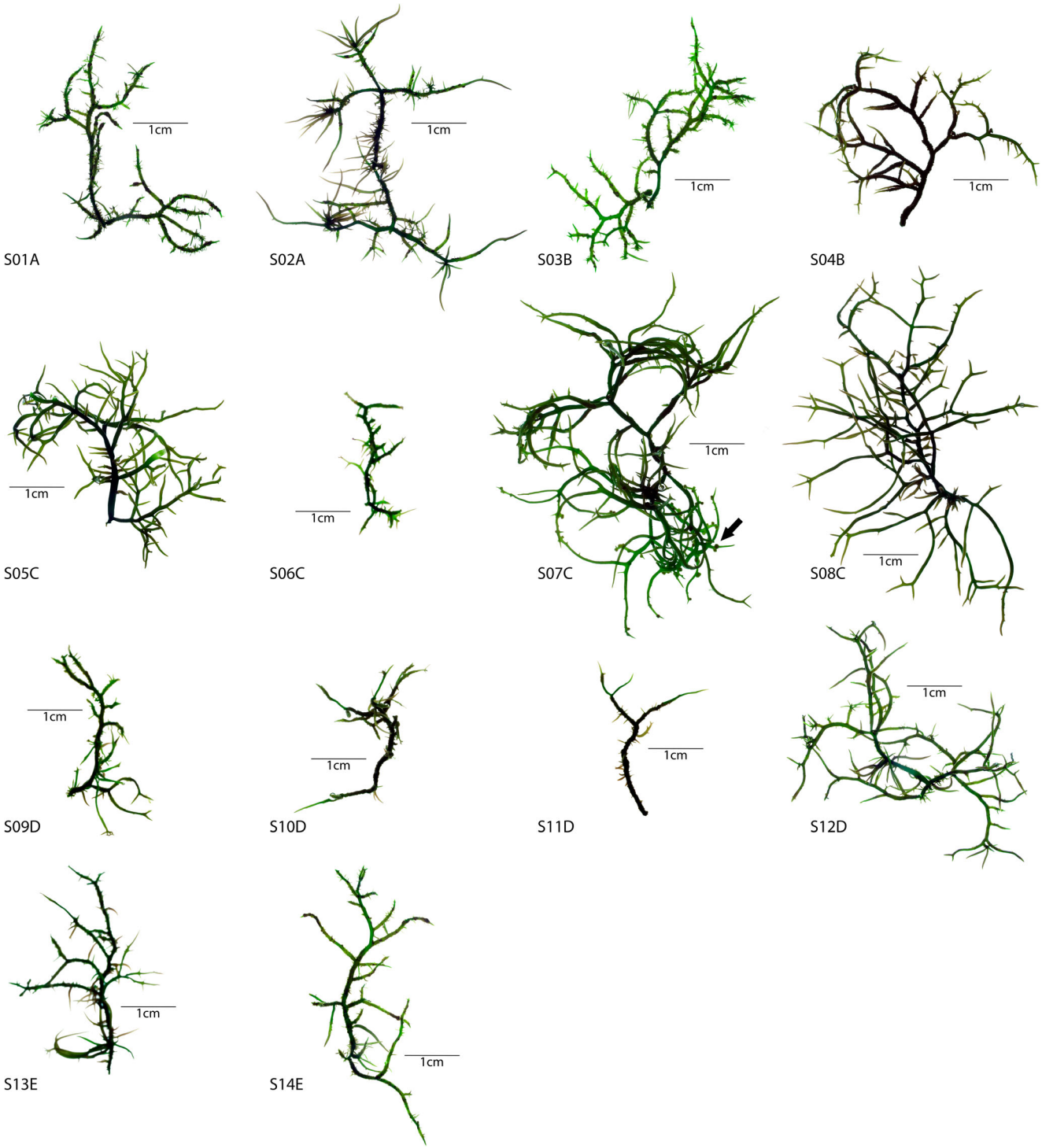
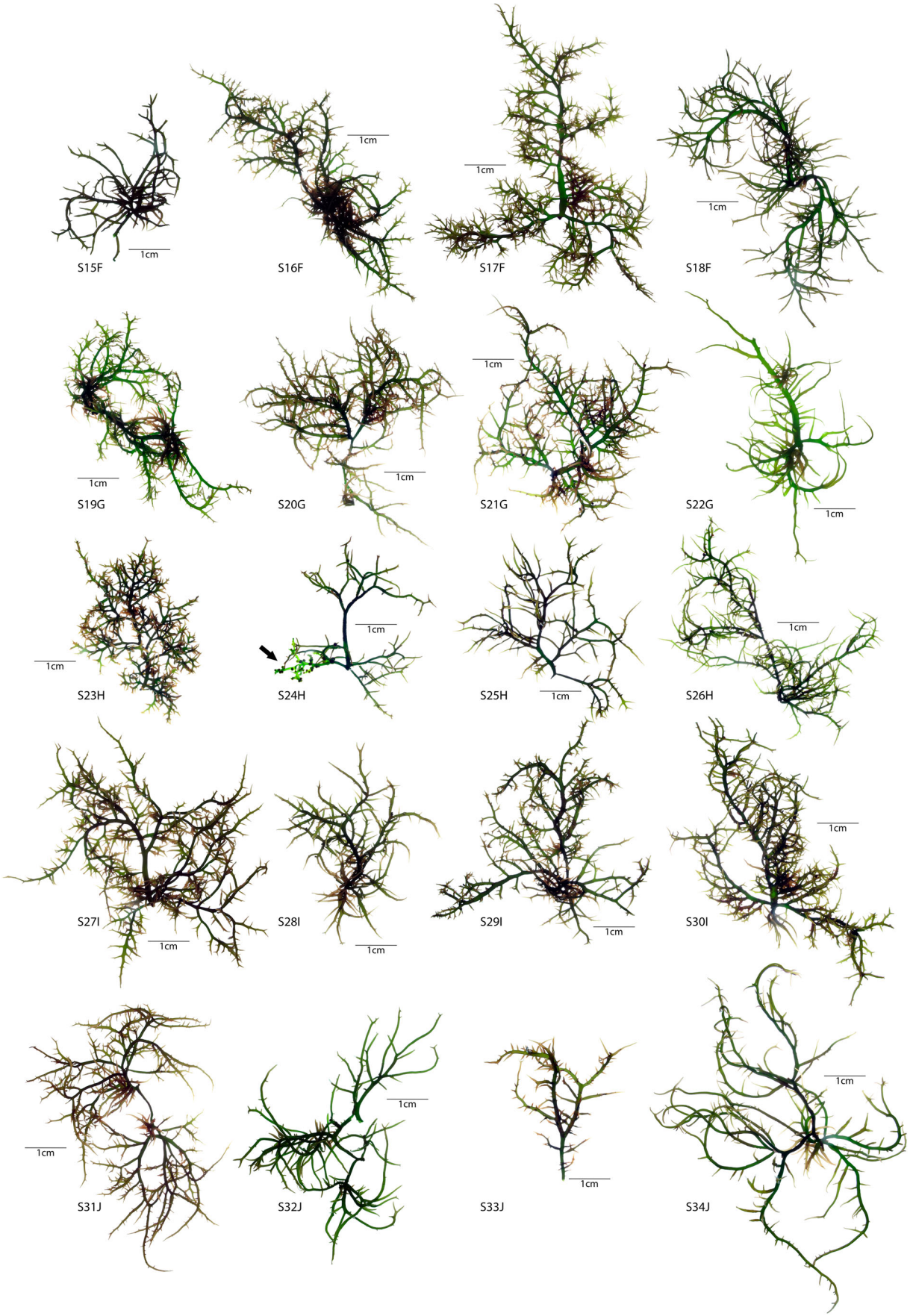


Fig. 2 Strains obtained from carpospore progeny of *Chondracanthus teedei* after 90 days in laboratory culture. Arrow in S07C indicates cystocarps

only one strain from tetrasporophytic progenitors while kept isolated. Cystocarps were observed in S24H after 30 days, and release of viable carpospores was noted 45 days after selection (Fig. 3). Fifteen days after contact between gametophytic strains, cystocarps were observed in 12 strains (Table 1). Ninety days after selection, the strains differed in their final fresh weight, ranging from 0.02 to 0.27 g in strains from gametophytic progenitors and 0.05 to 0.74 g in strains from tetrasporophytic progenitors (Table 1). Moreover, GR ranged from 0.57 to 5.13% day^{-1} in strains from gametophytic progenitors with the highest value recorded for S07C, which presented cystocarps (Table 1). For strains from tetrasporophytic progenitors, GR values ranged from 2.16 to 6.79% day^{-1} with the highest value recorded for S17F (Table 1).


S15F
S16F
S17F
S18F
S19G
S20G
S21G
S22G
S23H
S24H
S25H
S26H
S27I
S28I
S29I
S30I
S31J
S32J
S33J
S34J
1cm

◀ **Fig. 3** Strains obtained from tetraspore progeny of *Chondracanthus teedei* after 90 days in laboratory culture. Arrow in S24H indicates cystocarps

Effects of temperature on growth rate and native carrageenan yield in selected strains

Growth rate (GR) results of the selected strains exposed to different temperatures are shown in Figs. 4 and 5. All strains demonstrated positive growth throughout the experiment with significant differences between temperatures and strains selected from gametophytic (two-way ANOVA: $F(26) = 40.307$, $p < 0.001$) and tetrasporophytic (two-way ANOVA: $F(38) = 35.240$, $p < 0.001$) progenitors. A heterogeneous response was observed at different temperatures between strains, including those belonging to the same progenitor (Figs. 4 and 5). Higher GR values were observed, on average, at 25 °C for both stages of the life cycle. In comparing the GR of strains from the gametophytic progenitors, we observed the highest values of GR for S07C ($5.61 \pm 0.20\%$ day^{-1}) at 25 °C, followed by S12D ($4.76 \pm 0.21\%$ day^{-1}) at 15 °C and S05C and S07C ($4.42 \pm 0.16\%$ day^{-1} and $4.54 \pm 0.75\%$ day^{-1}, respectively) at 30 °C (Fig. 4). For these GR values, only S07C at 25 °C is significantly different (two-way ANOVA, SNK; $p < 0.007$) (Fig. 4 and Tab S1 in the on-line Supplementary material). On the other hand, strains from tetrasporophytic progenitors demonstrated the highest GR values for S21G and S27I ($7.11 \pm 0.36\%$ day^{-1}

Table 1 Final fresh weight, growth rate, and reproductive structure observed in carpospore and tetraspore progeny of *Chondracanthus teedei* in laboratory culture for 90 days

Progenitors	Strain	Final fresh weight (g)	Growth rate (% day^{-1})	Reproductive structure
Gametophytic	S01A	0.10	3.36	Tetrasporangial sorus *
	S02A	0.15	3.94	Tetrasporangial sorus *
	S03B	0.12	3.61	Tetrasporangial sorus *
	S04B	0.11	3.52	Infertile
	S05C	0.18	4.32	Infertile
	S06C	0.02	0.57	Tetrasporangial sorus *
	S07C	0.27	5.13	Cystocarp*
	S08C	0.23	4.70	Infertile
	S09D	0.03	1.07	Tetrasporangial sorus *
	S10D	0.03	1.16	Tetrasporangial sorus *
	S11D	0.03	1.04	Tetrasporangial sorus *
	S12D	0.12	3.48	Tetrasporangial sorus *
	S13E	0.07	3.04	Tetrasporangial sorus *
	S14E	0.08	2.85	Tetrasporangial sorus *
Tetrasporophytic	S15F	0.14	3.90	Cystocarp**
	S16F	0.36	5.71	Cystocarp**
	S17F	0.74	6.79	Cystocarp**
	S18F	0.41	5.90	Cystocarp**
	S19G	0.31	5.44	Cystocarp**
	S20G	0.40	5.63	Cystocarp**
	S21G	0.40	5.77	Infertile or male gametophyte
	S22G	0.18	4.58	Cystocarp**
	S23H	0.10	3.36	Cystocarp**
	S24H	0.07	2.79	Cystocarp*
	S25H	0.10	3.18	Infertile or male gametophyte
	S26H	0.11	3.63	Infertile or male gametophyte
	S27I	0.54	6.32	Cystocarp**
	S28I	0.18	4.23	Infertile or male gametophyte
	S29I	0.33	5.44	Cystocarp**
	S30I	0.54	6.49	Cystocarp**
	S31J	0.37	5.59	Infertile or male gametophyte
	S32J	0.19	4.35	Infertile or male gametophyte
	S33J	0.05	2.16	Cystocarp**
	S34J	0.19	4.44	Infertile or male gametophyte

*Reproductive structure observed during isolation. **Reproductive structure observed after crossing.

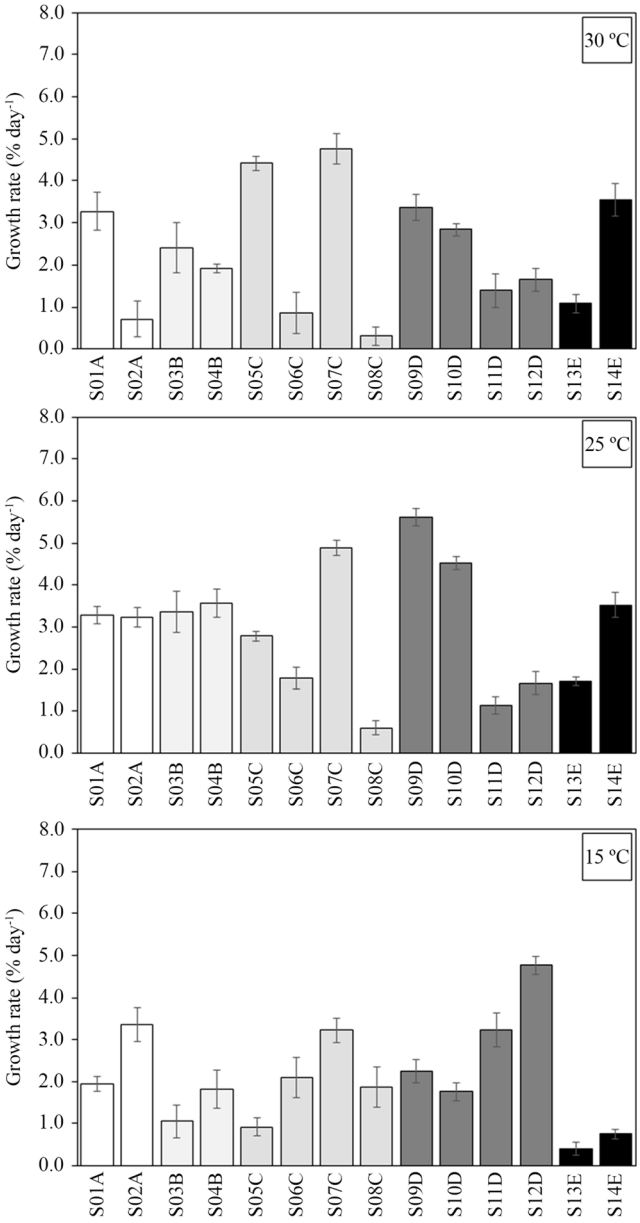


Fig. 4 Growth rates (GR % day^{-1}) of strains obtained from carpospore progeny of *Chondracanthus teedei* exposed to three temperatures (15, 25, and 30 °C). Bars represent mean ± SD for $n = 3$

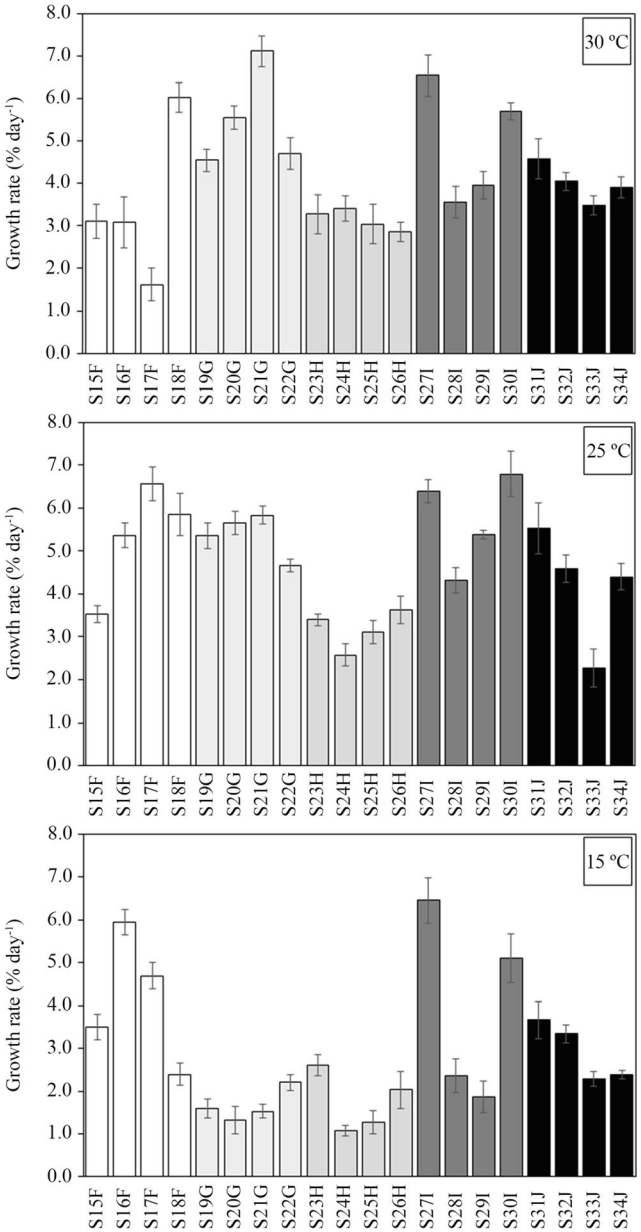


Fig. 5 Growth rates (GR % day^{-1}) of strains obtained from tetraspore progeny of *Chondracanthus teedei* exposed to three temperatures (15, 25, and 30 °C). Bars represent mean ± SD for $n = 3$

and 6.53 ± 0.49% day^{-1}, respectively) at 30 °C, followed by S30I, S17F, and S27I (6.79 ± 0.53% day^{-1}, 6.56 ± 0.39% day^{-1} and 6.39 ± 0.27% day^{-1}, respectively) at 25 °C and S27I (6.45 ± 0.53% day^{-1}) at 15 °C (Fig. 5). These results have no significant differences (two-way ANOVA, SNK; $p > 0.100$) between them (Tab S1 in the on-line Supplementary material).

Native carrageenan

Native carrageenan yield present in *C. teedei* strains was significantly influenced by temperature (gametophytic progenitor: two-way ANOVA: $F(26) = 7.68$, $p < 0.001$; tetrasporophytic progenitor: two-way ANOVA: $F(38) = 8.79$, $p < 0.001$), demonstrating heterogeneous responses at different temperatures between strains, including those belonging to the same progenitor (Figs. 6 and 7). In comparing the native carrageenan yield, we observed no significant differences among temperatures used in 12 strains, 4 strains from gametophytic progenitors (two-way ANOVA, SNK; $p > 0.100$) and 8 strains from tetrasporophytic progenitor (two-way ANOVA, SNK; $p > 0.200$) (Figs. 6, 7, and Tab S1 in the on-line Supplementary material). On the other hand, 4 strains, 1 strain from gametophytic progenitors and 3 strains from tetrasporophytic progenitors, showed higher levels of

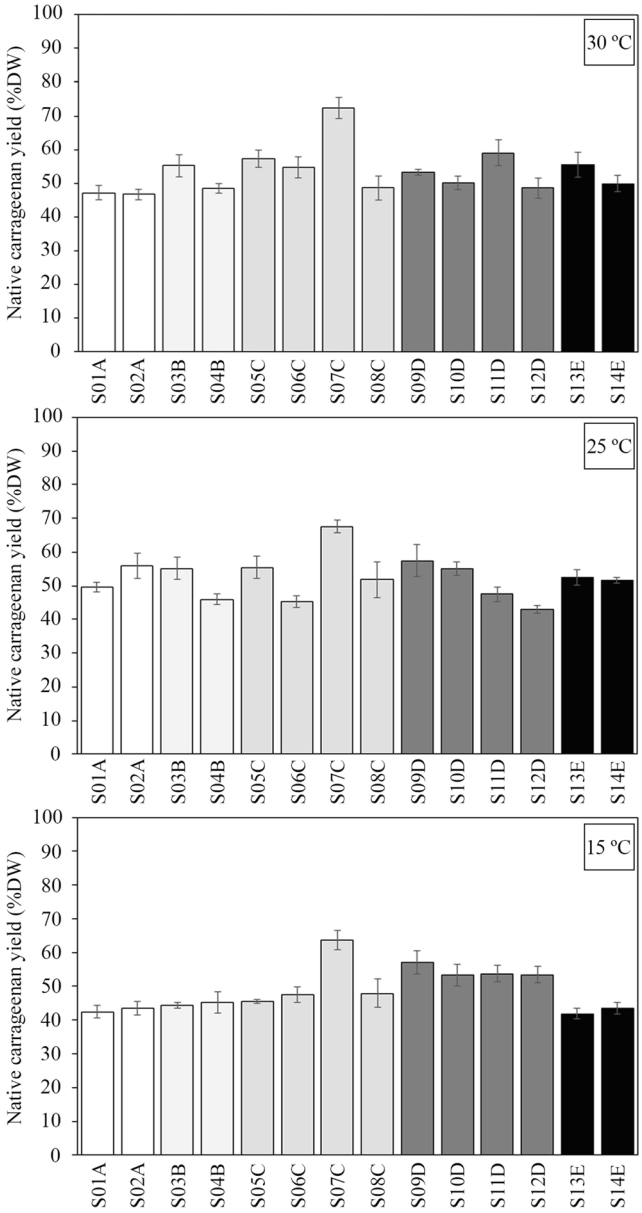


Fig. 6 Native carrageenan yield (% DW) of strains obtained from carpospore progeny of *Chondracanthus teedei* exposed to three temperatures (15, 25, and 30 °C). Bars represent mean ± SD for $n = 3$

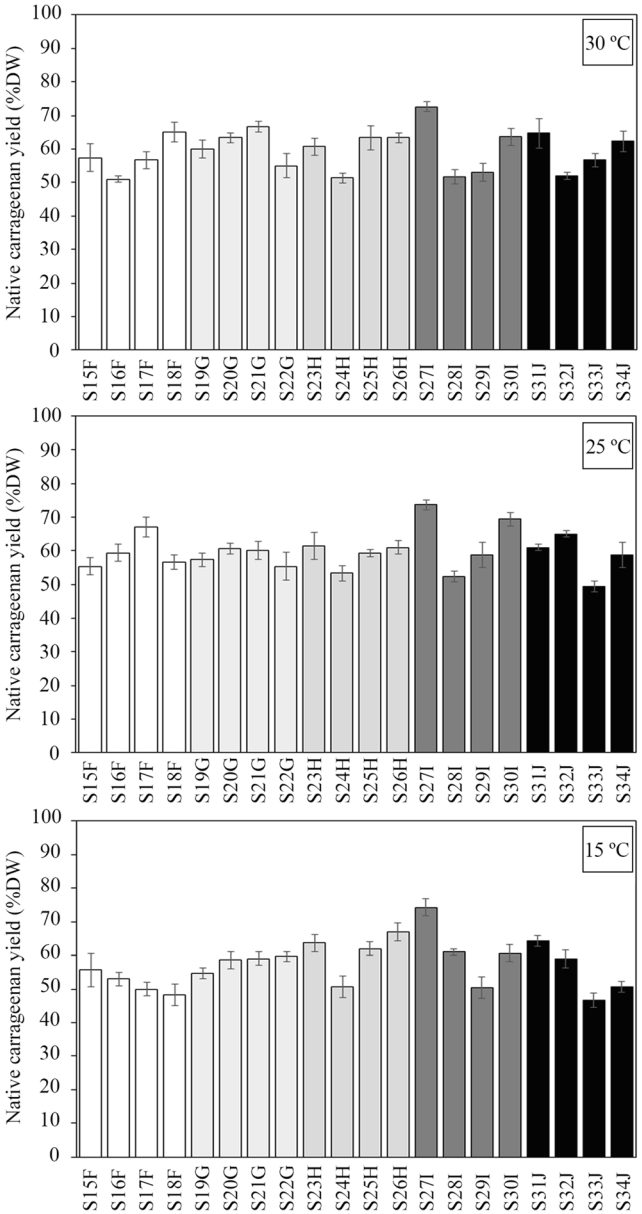


Fig. 7 Native carrageenan yield (% DW) of strains obtained from tetraspore progeny of *Chondracanthus teedei* exposed to three temperatures (15, 25, and 30 °C). Bars represent mean ± SD for $n = 3$

native carrageenan when kept at 15 °C, and 5 strains, 2 strains from gametophytic progenitors and 3 strains from tetrasporophytic progenitors, showed their highest values when kept at 30 °C. Among the strains from gametophytic progenitors, the highest carrageenan yield was observed in S07C at 30 °C (72.43 ± 3.11% DW), followed by 25 °C (67.60 ± 2.00% DW) and 15 °C (63.60 ± 2.78% DW) (Fig. 6). For these carrageenan values, only S07C at 30 °C is significantly different (two-way ANOVA, SNK; $p < 0.031$) (Fig. 6 and Tab S1 in the on-line Supplementary material). Meanwhile, from the strains of tetrasporophytic progenitors, the highest carrageenan yield was observed in S27I at 15 °C (74.3 ± 2.5% DW), 25 °C (73.7 ± 1.5% DW), and 30 °C (72.7 ± 1.5% DW) (Fig. 7). However, among these results, no significant differences (two-way ANOVA, SNK; $p > 0.600$) were noted (Fig. 7 and Tab S1 in the on-line Supplementary material).

Discussion

Our results showed the feasibility of selecting strains through the release of spores from wild gametophytic and tetrasporophytic fronds of *C. teedei* in southern Brazil. Differences were observed among the various strains obtained, even when they originated from the same progenitor. We noted heterogeneity in morphology, color, size, biomass, growth

rates, and yield of native carrageenan along development and different temperatures tested. These phenotypic differences may be the result of intraspecific genotypic differences and thus, eventually, express variations existing in the population of origin of the progenitors. Thus, we believe that populations of *C. teedei* may have intrinsic genetic diversity at the genotypic origin or heterosis. Accordingly, populations of *C. teedei* could serve as a source of individuals for strain selection from spores or matrices for crossbreeding aimed at a greater biomass production and/or carrageenan yield.

In general, strains obtained in this study visually showed morphological and color variability, with gametophytic strains more branched in relation to the tetrasporophytic strains, although differences were noted between strains at the same stage of the life cycle and those from the same progenitor (on-line Supplementary material). Although studies indicate that *C. teedei* is a promising species for carrageenan production (Pereira and van de Velde 2011), our results show that the morphologies found here meet the requirements for the Asian food market as well (see Figs. 2 and 3; Fig. S2 to S11 in the on-line Supplementary material) (Macchiavello et al. 2018). It is unlikely that this market could be sustained only by harvesting from natural populations (Contador et al. 2020). Therefore, it would be necessary to bolster production with preselected strains with the specific characteristics desired and maintained throughout development, as we observed for *C. teedei* in this study. Another reason for promoting strain selection is phycogastronomy directed toward the European food market. This species has already attracted a certain level of interest based on its use in small Italian villages and it has also recently gained interest from purveyors of haute cuisine (Palmieri and Forleo 2020).

Another important characteristic observed in this study was deviation in the sequence of the life cycle. Among the strains obtained from gametophytic progenitor C, cystocarp formation and release of viable carpospores in S07C were observed after 45 days of isolation, indicating that this is a monoic gametophytic strain, originating from carpospores (Fig. 2). The formation of a gametophyte from spores originating from cystocarps is commonly reported as apomictic and found in only a few macroalgae genera (West et al. 2001; Fierst et al. 2010), but so far, not reported to *Chondracanthus.* Although well documented for terrestrial plants (Majeský et al. 2017), little is known about the formation of apomictic individuals and the ecological implications in macroalgae. For *Mastocarpus papillatus* (C. Agardh) Kützing, for example, it is known that they are essential for the maintenance of some populations, mainly in their distribution limit (Fierst et al. 2010). This could be a basis for our finding once the *C. teedei* population that provided progenitor fronds is close to the southern limit of its distribution (Coll and Oliveira 1999). According to West et al. (1992), an apomictic system in red algae, similar to that in angiosperms, likely leads to the evolution of a greater genetic diversity. This has been understudied and explored but could reduce time and effort in future strain selection and maintenance.

We also observed the occurrence of self-fertilization in a strain obtained from tetrasporophytic progenitor H. Strain S24H showed cystocarps after 30 days of isolation and release of viable carpospores after 45 days. Self-fertilization in gametophytes is well documented for red algae (Rueness and Fredriksen 1998; Sano et al. 2020), as well as for some species of *Chondracanthus* spp. (Contador et al. 2020), although for *C. teedei* it is considered rare (Guiry et al. 1987; Braga 1990). Monoic individuals are considered a problem in breeding programs since self-fertilization hinders the hybridization process. On the other hand, they can help to maintain phenotypes obtained over generations (Meer 1990).

During this study, which included strain selection and temperature effects, the growth rates (GR) obtained in some strains of *C. teedei* showed values consistent with, or even higher than, those found for the species (Table 1) (see Bermejo et al. 2019) or for other economically important red algae (Bulboa et al. 2008; Ventura et al. 2020). When exposed to the temperature gradient, most strains (24 of 34) showed optimal GR values at the intermediate temperature used (25 °C) or without significant differences between 25 °C and one of the other temperatures used. These results corroborate the findings of Zinoun et al. (1993a) who identified an optimum temperature for growth between 20 and 25 °C for this species (formerly *Gigartina teedii* (Roth) Lamouroux). Thus, the breadth of growth performance would be related to the local temperature regime, as a reflection of adapting to these conditions (Eggert 2012). On the other hand, we obtained strains with responses different from the expected. Four strains showed optimal GR values only at the extreme temperatures used (S12D and S16F for 15 °C; S05C and S21G for 30 °C), and, interestingly, two gametophytic strains maintained their GR values without significant differences, irrespective of temperatures used, with a higher mean value for S27I ($6.46 \pm 0.07\%$ day^{-1}). These results demonstrate a possible strategy for maintaining the species in environments with wide thermal amplitude or a reflection of adaptation to the extensive geographic distribution (Pereira and Silva 2021). Optimum growth rates for temperatures different from the environmental average were also recorded for *C. chamissoi* (Bulboa and Macchiavello 2001) and other economically important red algae (Yokoya and Oliveira 1992).

The native carrageenan yield varied among strains and temperatures (see Figs. 6 and 7). Among the 34 selected strains, 12 showed higher yield of native carrageenan at the intermediate temperature used (25 °C) or without significant differences between 25 °C and one of the other temperatures used, with a maximum value of 69% DW for S30I. However, 4 strains showed higher yield when kept at 15 °C (maximum value 67% DW for S26H) and 5 when kept at 30 °C (maximum value 72% DW for S07C). On the other hand, 11 strains showed no

temperature effect on the yield of native carrageenan, presenting a maximum value for S27I (74% DW for 15 °C and 25 °C; 73% DW for 30 °C). These values corroborate those found by Saito and Oliveira (1990) (76% DW) and Zinoun et al. (1993a, 1993b) (70% DW) for this species. Although our data corroborate the findings of Zinoun et al. (1993b) demonstrating a strong temperature influence on native carrageenan yield in *C. teedei*, it must be noted that these responses do not represent a clear pattern for this strains in relation to the temperatures used. As we demonstrate here, both intraspecific and intra-progeny variability was noted and should be explored to obtain strains with higher native carrageenan yield, but less effect of temperature on GR. Additionally, a more in-depth analysis becomes necessary since carrageenan can be influenced by temperature, both in its yield and in its composition and physical properties (Pereira and Silva 2021).

We concluded that this study demonstrates the efficiency of strain selection in *C. teedei* using tetraspore and carpospore progeny once a variety of strains demonstrated satisfactory values of GR and yield of native carrageenan under different temperature conditions. In practice, this could help in biomass production in places where the amplitude of annual temperatures becomes a problem in cultivation, especially for tropical species such as *K. alvarezii*. It could even help in the selection of strains resistant to the effects of climate change or short climate events like heatwaves. Thus, our results demonstrate that intraspecific and intra-progeny differences do exist in *C. teedei*. This not only is of academic interest, helping to explain the distribution of species that occupy a variety of environments with characteristics of tropical and subtropical climates (Pereira and Silva 2021), but may also have implications for the practice of selecting strains of this or other genera of red algae of economic interest. Accordingly, we see that strains S12D and S16F could be grown in the winter months and strains S21G and S05C during the summer in southern Brazil. Strains S17F and S30I obtained good results at 25 °C, and S27I maintained its GR and native carrageenan yield high at all temperatures. Thus, we believe that a combination of strains in different seasons could provide greater stability in biomass production and native carrageenan to, in turn, provide economically viable production.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-021-02457-5.

Acknowledgements This research was supported by Projeto de apoio à Consolidação da Rede Brasileira de Pesquisas sobre Mudanças Climáticas (Rede CLIMA) (550990/2011-9). This study is part of the MS degree presented to the first author by the Graduate Program in Biology of Fungi, Algae and Plants (PPGFAP), Federal University of Santa Catarina, Santa Catarina, Brazil. The authors acknowledge financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 306917/ 2009-2 and 426215/2016-8 to P.A. Horta); CAPES/PNPD02828/09-0 and CAPES/PNADB2338000071/2010-61 to P.A. Horta; ProspecMar-*Islands-Sustainable prospecting in Ocean Islands*: *Biodiversity*, *Chemistry*, *Ecology and Biotechnology*; Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC); Rede Algas, ReBentos and INCT Clima. Eurico Cabral de Oliveira Filho for all the incentive and critical review of the manuscript. Bastos thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Postdoctoral fellowship (process no. 151637/2020-2).

References

Bermejo R, Macías M, Cara CL, Sánchez-García J, Hernández I (2019) Culture of *Chondracanthus teedei* and *Gracilariopsis longissima* in a traditional salina from southern Spain. J Appl Phycol 31:561–573

Braga MRA (1985) Taxonomia e biologia de *Gigartina teedii* (Roth) Lamouroux (Rhodophyta, Gigartinales) no literal do Estado de São Paulo. Masters Thesis, Universidade de São Paulo, São Paulo, p. 161

Braga MRA (1990) Reproductive characteristics of *Gigartina teedii* (Roth) Lamouroux (Rhodophyta, Gigartinales), a turf-forming species: field and laboratory culture studies. Bot Mar 33:401–409

Bulboa CR, Macchiavello JE (2001) The effects of light and temperature on different phases of the life cycle in the carrageenan producing alga *Chondracanthus chamissoi* (Rhodophyta, Gigartinales). Bot Mar 44:371–374

Bulboa CR, Paula EJ, Chow F (2007) Laboratory germination and sea out-planting of tetraspore progeny from *Kappaphycus striatum* (Rhodophyta) in subtropical waters of Brazil. J Appl Phycol 19: 357–363

Bulboa C, Macchiavello J, Oliveira E, Véliz K (2008) Growth rate differences between four Chilean populations of edible seaweed *Chondracanthus chamissoi* (Rhodophyta, Gigartinales). Aquac Res 39:1550–1555

Carneiro MA, do A, Resende JF de J, Oliveira SR, Fernandes F de O, Borburema HD dos S, Barbosa-Silva MS, Ferreira ABG, Marinho-Soriano E, (2021) Performance of the agarophyte *Gracilariopsis tenuifrons* in a multi-trophic aquaculture system with *Litopenaeus vannamei* using water recirculation. J Appl Phycol 33:481–490

Charrier B, Rolland E, Gupta V, Reddy CRK (2015) Production of genetically and developmentally modified seaweeds: exploiting the potential of artificial selection techniques. Front Plant Sci 6:127

Coleman MA, Minne AJP, Vranken S, Wernberg T (2020) Genetic tropicalisation following a marine heatwave. Sci Rep 10:12726

Coll J, Oliveira EC (1999) The benthic marine algae of Uruguay. Bot Mar 42:129–135

Contador CB, Massad IP, Contreras-Porcia L, Zapata J, Castañeda F, Ramírez ME, Gil-Kodaka P (2020) Concise review of genus *Chondracanthus* (Rhodophyta: Gigartinales). J Appl Phycol 32:773–785

Coutinho R (1982) Taxonomia, distribuição, crescimento sazonal, reprodução e biomassa das algas bentônicas no estuário da lagoa dos Patos (RS). Masters Thesis, Universidade do Rio Grande, Rio Grande, p. 25

de Oliveira EC (1997) Algas marinhas: um recurso ainda pouco explotado pelo Brasil. Panorama da Aqüicultura 7:24–26

Eggert A (2012) Seaweed responses to temperature. In: Wiencke C, Bischof K (eds) Seaweed biology. Springer, Berlin, pp 47–66

FAO (2020a). The State of World Fisheries and Aquaculture. FAO Fisheries Department, Rome, p. 208

FAO (2020b) FishStat. Global aquaculture production. http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en; searched on 10 September 2020.

Fierst JL, Kübler JE, Dudgeon SR (2010) Spatial distribution and reproductive phenology of sexual and asexual *Mastocarpus papillatus* (Rhodophyta). Phycologia 49:274–282

Garcia-Jimenez P, Marian F, Rodrigo M, Robaina R (1999) Sporulation and sterilization method for axenic culture of *Gelidium canariensis*. J Biotechnol 35:227–229

Guiry MD, Tripodi G, Lüning K (1987) Biosystematics, genetics and upper temperature tolerance of *Gigartina teedii* (Rhodophyta) from the Atlantic and Mediterranean. Helgol Meeresunters 41:283–295

Hayashi L, Paula EJ, Chow F (2007) Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical waters of São Paulo State, Brazil. J Appl Phycol 19:393–399

Hayashi L, Bulboa C, Kradolfer P, Soriano G, Robledo D (2014) Cultivation of red seaweeds: A Latin American perspective. J Appl Phycol 26:719–727

Hurtado AQ, Critchley AT, Neish IC (eds.) (2017) Tropical seaweed farming trends, problems and opportunities: focus on *Kappaphycus* and *Eucheuma* of commerce. Springer, Dordrecht, p. 216

Pinheiro-Joventino F, Dantas NP, Maraschin CDH (1998) Distribuição de algas marinhas no litoral de Fortaleza, Ceará, Brasil. Arq Ciências Mar 31:29–40

Levy I, Beer S, Friedlander M (1990) Strain selection in *Gracilaria* spp. 2. Selection for high and low temperature resistance in *G. verrucosa* sporelings. J Appl Phycol 2:163–171

Li D, Zhou Z, Liu H, Wu C (1999) A new method of *Laminaria japonica* strain selection and sporeling raising by the use of gametophyte clones. Hydrobiologia 398:473–476

Liu X, Bogaert K, Engelen AH, Lelialert F, Roleda MY, De Clerck O (2017) Seaweed reproductive biology: environmental and genetic controls. Bot Mar 60:89–108

Macchiavello J, Sepúlveda C, Basaure H, Sáez F, Yañez D, Marín C, Vega L (2018) Suspended culture of *Chondracanthus chamissoi* (Rhodophyta: Gigartinales) in Caleta Hornos (northern Chile) via vegetative propagation with secondary attachment discs. J Appl Phycol 30:1149–1155

Maggs CA, Callow ME (2006) Algal Spores. Encyclopedia of Life Sciences. https://doi.org/10.1038/npg.els.0000311

Majeský Ľ, Krahulec F, Vašut R (2017) How apomictic taxa are treated in current taxonomy: A review. Taxon 66(5):1017–1040

Mollion J, Braud JP (1993) A *Eucheuma* (Solieriaceae, Rhodophyta) cultivation test on the south-west coast of Madagascar. Hydrobiologia 260, 373-378

Oliveira EC, Paula EJ, Plastino EM, Petti R (1995) Metodologias para Cultivo no Axenico de Macroalgas Marinas in Vitro. In: Alveal K, Ferrario M, Olivelre EC, Sar E (eds) Material e Metodos Ficologicos. Universidad de Concepsion, Concepcion, p. 429–447

Palmieri N, Forleo MB (2020) The potential of edible seaweed within the western diet. A segmentation of Italian consumers. Int J Gastron Food Sci 20:100202

Park CS, Hwang EK (2014) Isolation and evaluation of a strain of *Pyropia yezoensis* (Bangiales, Rhodophyta) resistant to red rot disease. J Appl Phycol 26:811–817

Paula EJ, Pereira RTL, Ohno M (1999) Strain selection in *Kappaphycus alvarezii var. alvarezii* (Solieriaceae, Rhodophyta) using tetraspore progeny. J Appl Phycol 11:111–121

Pereira L, Mesquita JF (2003) Carrageenophytes of occidental Portuguese coast: 1 – spectroscopic analysis in eight carrageenophytes from Buarcos Bay. Biomol Eng 20:217–222

Pereira L, Mesquita JF (2004) Population studies and carrageenan properties of *Chondracanthus teedei var. lusitanicus* (Gigartinaceae, Rhodophyta). J Appl Phycol 16:369–383

Pereira L, Silva PA (2021) A concise review of the red macroalgae *Chondracanthus teedei* (Mertens ex Roth) Kützing and *Chondracanthus teedei var. lusitanicus* (J.E. De Mesquita Rodrigues) Bárbara & Cremades. J Appl Phycol 33:111–131

Pereira L, van de Velde F (2011) Portuguese carrageenophytes: carrageenan composition and geographic distribution of eight species (Gigartinales, Rhodophyta). Carbohydr Polym 84:614–623

Pereira L, Amado AM, Critchley AT, van de Velde F, Ribeiro-Claro PJA (2009) Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). Food Hydrocoll 23:1903–1909

Reddy CRK, Gupta MK, Mantri VA, Jha B (2008) Seaweed protoplasts: status, biotechnological perspectives and needs. J Appl Phycol 20: 619–632

Reis RP, Caldeira AQ, dos Miranda AP, S, Barros-Barreto MB, (2006) Potencial para maricultura da carragenófita *Hypnea musciformis* (Wulfen) J.V. Lamour. (Gigartinales - Rhodophyta) na Ilha da Marambaia, Baía de Sepetiba, RJ. Brasil. Acta Bot Bras 20:763–769

Robinson N, Winberg P, Kirkendale L (2013) Genetic improvement of macroalgae: status to date and needs for the future. J Appl Phycol 25:703–716

Rueness J, Fredriksen S (1998) Intraspecific reproductive variation in *Gelidium pusillum* (Stackh.) Le Jol. (Gelidiales, Rhodophyta) from Europe. J Appl Phycol 10:253–260

Saito RM, de Oliveira EC (1990) Chemical screening of Brazilian marine algae producing carrageenans. Hydrobiologia 204:585–588

Sano F, Murata K, Niwa K (2020) Identification, growth, and pigment content of a spontaneous green mutant of *Pyropia kinositae* (Bangiales, Rhodophyta). J Appl Phycol 32:1983–1994

Shannon E, Abu-Ghannam N (2019) Seaweeds as nutraceuticals for health and nutrition. Phycologia 58:563–577

Simioni C, Hayashi L, Oliveira MC (2019) Seaweed resources of Brazil: What has changed in 20 years? Bot Mar 62:433-441

van der Meer JP (1990) Isolation and genetic characterization of self-sterile mutants in a monoecious red alga *Gelidium vagum* (Gelidiales, Rhodophyta). In: Lindstrom S.C., Gabrielson P.W. (eds) Thirteenth International Seaweed Symposium. Developments in Hydrobiology, vol 58. Springer, Dordrecht pp 389-395

Ventura TFB, Bruzinga CP, dos Santos AA, Simioni C, Hayashi L (2020) Addition of carbon dioxide, followed by irradiance increase, as optimization strategy for the cultivation of the red seaweed *Kappaphycus alvarezii*. J Appl Phycol 32:4113–4126

West JA, Zuccarello G, Calumpong HP (1992) Bostrychia bispora sp. nov. (Rhodomelaceae, Rhodophyta), an apomictic species from Darwin, Australia: reproduction and development in culture. Phycologia 31:37–52

West JA, Zuccarello GC, Kamiya M (2001) Reproductive patterns of *Caloglossa* species (Delesseriaceae, Rhodophyta) from Australia and New Zealand: multiple origins of asexuality in *C. leprieurii*. Literature review on apomixis, mixed-phase, bisexuality and sexual compatibility. Phycol Res 49:183–200

Yan XH, Lv F, Liu CJ, Zheng YF (2010) Selection and characterization of a high-temperature tolerant strain of *Porphyra haitanensis* Chang et Zheng (Bangiales, Rhodophyta). J Appl Phycol 22:511–516

Yokoya NS, Oliveira EC (1992) Temperature responses of economically important red algae and their potential for mariculture in Brazilian waters. J Appl Phycol 4:339–345

Yokoya NS, Oliveira EC (1993) Effects of temperature and salinity on spore germination and sporeling development in South American agarophytes (Rhodophyta). Jap J Phycol 41:283–293

Zinoun M, Cosson J, Deslandes E (1993a) Physicochemical characterization of carrageenan from *Gigartina teedii* (Rooth) Lamouroux (Gigartinales, Rhodophyta). J Appl Phycol 5:23–28

Zinoun M, Cosson J, Deslandes E (1993b) Influence of culture conditions on growth and physicochemical properties of carrageenans in *Gigartina teedii* (Rhodophyceae — Gigartinales). Bot Mar 36:131–136