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Differential ecological responses to environmental stress in the life history phases of the isomorphic red alga *Gracilaria chilensis* (Rhodophyta)

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Abstract In order to better understand the alternation of generations that characterizes haploid–diploid life cycles, we assessed the existence of ecological differences between the two phases (haploid gametophyte and diploid tetrasporophyte) in *Gracilaria chilensis*, a rhodophyte with a typical *Polysiphonia*-type life cycle. We investigated the effect of light intensity and salinity on viability and growth of both phases at different ontogenetic stages: juveniles and adults. In our study, the survival of juvenile gametophytes (n) was higher than the survival of juvenile tetrasporophytes (2n) despite culture conditions; however, low salinity had greater effect on carpospores (2n) than on tetraspores (n). On the other hand, a complex interaction between salinity and light intensity within each life history phase generated observed differences between juvenile growth rates. Low light was shown to trigger early onset of alteration of the holdfast growing pattern. In addition, adult tetrasporophytes showed, despite the conditions, a faster vegetative growth than female and male gametophytes. These differences between phases could have led to the complete dominance of tetrasporophyte fragments of fronds observed in *G. chilensis* farms. We hypothesize that Chilean fishers could have unknowingly selected for tetrasporophyte thalli during domestication of the species, thus enhancing the natural trend of tetrasporophytes dominance already present in estuarine natural populations of free-floating plants.

Keywords Life cycle evolution · Gametophyte · Sporophyte · Ecological differences · Asexual reproduction · Light · Salinity

Introduction

A wide variety of haploid–diploid life cycles can be found in different groups of seaweeds, and one of the challenges of biology is to understand how haploid–diploid life cycles have evolved. Several theoretical arguments have been proposed to explain the evolutionary stability of different life cycle strategies (see reviews by Mabble and Otto 1998; Coelho et al. 2007). Hughes and Otto (1999) argued that the stability of haploid–diploid life cycles could be promoted by slight, but ecologically significant, differences between haploid and diploid phases. This hypothesis is well supported in heteromorphic haploid–diploid species where it has been demonstrated that the two morphologically different phases alternate in response to ecological factors (Thornber 2006). For example, grazing pressure has been demonstrated to play a substantial role in controlling the seasonal abundance of crustose/upright stages in five

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common heteromorphic algae species (Lubchenco and Cubit 1980). Although differences between isomorphic phases might be less conspicuous than the ones described between heteromorphic phases, it is clear that ecological differences exist between them at both microscopic and macroscopic stages, as is seen in red algae such as the Gigartinales and Gracilariales (Hannach and Santelices 1985; Destombe et al. 1993; Thornber 2006; Thornber et al. 2006), but not in the brown alga *Dictyota cililata* (Cronin and Hay 1996).

Gracilaria chilensis Bird, McLachlan & Oliveira, 1986 is a rhodophyte (Gracilariales) that presents a typical *Polysiphonia*-type life cycle with two free-living isomorphic generations—the tetrasporophytes (diploid) and the gametophytes (haploid) (Bird et al. 1986). Meiosis takes place in the tetrasporophytic plants, giving rise to haploid tetraspores. Tetraspores develop into gametophytes (males or females), which produce gametes by mitosis. Fertilization (syngamy) occurs in the female gametophyte, and the fertilized female gamete develops into a carposporophyte. Cystocarps are macroscopic hemispherical swellings observed on the surface of female branches, within which the carposporophyte produces thousands of diploid carpospores by repeated mitosis. Finally, completing the cycle, each carpospore can develop into a tetrasporophyte. Moreover, gametophyte and sporophyte individuals are able to reproduce vegetatively by fragmentation of erect fronds. The free-floating fronds detached from the holdfast grow indefinitely and propagate naturally (Santelices et al. 1984). The ability to alternate between sexual reproduction and vegetative propagation allows this species to colonize different environments, from intertidal rocky pools to muddy or sandy bottom of bays and estuaries. *G. chilensis* is able to cope with marked diurnal and seasonal fluctuations in temperature, water turbidity, light intensity and salinity caused by the tidal regime characteristic of the shallow waters used as habitat by the species (Gómez et al. 2005). In a study including 11 natural populations and 15 farms of *G. chilensis* along the extensive Chilean coast, Guillemin et al. (2008) showed that the haploid/diploid ratio was highly variable in natural populations reproducing sexually, while tetrasporophytes (2n) clearly dominated the farmed free-floating populations, which reproduced asexually. In order to explain the pattern of diploid dominance in farms, it has been hypothesized that tetrasporophytes (2n) are better at fragmenting than gametophytes (n) or that tetrasporophytes have been preferentially propagated by farmers due to an advantage in growth over gametophytes (Guillemin et al. 2008). However, to unravel the causes underlying such fluctuations in life history phase ratios among populations, experimental tests of adaptive differences between isomorphic generations are required (Fierst et al. 2005).

In this study we tested the occurrence of ecological differences between gametophyte and tetrasporophyte individuals. We hypothesized that (1) subtle adaptive ecological–physiological differences exist between life history phases; (2) the early developmental stages, especially spores and juveniles, are more susceptible to environmental stress than adults; and (3) tetrasporophytes (2n), which may benefit from genetic buffering conferring better cellular regulation, are more vigorous and tolerant to environmental stress than gametophytes (n). We investigated the effect of light intensity and salinity on viability and growth of both gametophytes and tetrasporophytes at different ontogenetic stages: spores, juveniles and adults.

Materials and methods

Gametophytes and tetrasporophytes of *G. chilensis* were sampled in the Maullin estuary (41°37′S, 73°35′W, Puerto Montt, Southern Chile). The population is characteristic of the estuarine and mudflat systems of southern Chile where most of the large natural beds and commercial farms are encountered (Buschmann et al. 2001). At this location, the mean annual water temperature is about 13°C, with an annual range from 7.5 to 20°C (Westermeier et al. 1991) with salinity varying between 17 and 31 ‰ (Santelices et al. 1993). The lowest salinity values were observed during low spring tides throughout the austral winter months (i.e. June to August), when heavy rains occur in this area (Santelices et al. 1993). *G. chilensis* is a shade-adapted species characterized by low light requirements (light saturating point between 60 and 170 μmol photons m^{-2} s^{-1}, Gómez et al. 2005). The beds of the mouth of the Maullin River extend between 1 and 3 m in depth.

Reproductive individuals, attached to pebbles by their holdfast, were collected during low tide in the natural population of Maullin (41°37′S, 73°35′W) in February 2010. Fronds were brushed and washed repeatedly in sterile seawater to remove epiphytes. Individuals were separated according to their reproductive structures using a binocular microscope following the protocol of Guillemin et al. (2008). Twenty diploid tetrasporophytic individuals, 20 haploid females and 20 haploid males were selected for the experiments.

Light and salinity treatments

Two contrasted conditions of salinity (15 and 35 ‰) and light intensity (20 and 60 μmol photons m^{-2} s^{-1}) were used in this study to compare the survival of carpospores and tetraspores and the survival and growth rates of the gametophytes and tetrasporophytes (juveniles and adults). Low salinity (15 ‰) conditions were obtained by adding distilled

water to filtered seawater before enriching (Modified SFC culture medium, Correa and McLachlan 1991); both 15 ‰ and 35 ‰ salinity cultures were controlled by a refractometer (Westover TM, model RHS-10ATC, USA). The two contrasting light intensities were obtained by adjusting the distance between the culture plates and the light source. Light was provided by cool white fluorescent tubes (18 W). The photon flux density was measured with a LI-COR LI-189 meter.

Sources of tetraspores (n) and carpospores (2n)

Fifteen centimeters of linear reproductive thallus were excised from 20 tetrasporophytes and placed jointly in filtered seawater in a 50 mL Falcon tube (Becton Dickinson, USA) for 24 h. In addition, 40 cystocarps were excised from 20 female gametophytes. These 800 cystocarps (i.e. 20 individuals × 40 cystocarps) were placed together in filtered seawater in a 50 mL Falcon tube for 24 h. Spore release was triggered by gentle dehydration and immersion in 4°C filtered seawater (modified from Edding et al. 1987). The spore density was estimated after 24 h by counting spores in ten replicates of 50 μL seawater aliquots using a microscope. The density of both tetraspores and carpospores was adjusted to 2500 spores mL^{-1}.

Spores were inoculated in six-well cell culture plates (BD Biosciences, USA). In each well, 400 μL (≈ 1,000 spores) of spore suspension aliquot was inoculated into 20 mL culture medium (Modified SFC culture medium, Correa and McLachlan 1991) adjusted to 15 and 35 ‰ salinity and subsequently placed under two different light intensities (20 and 60 μmol photons m^{-2} s^{-1}). A total of eight plates were used during this experiment, each corresponding to one life history phase grown under a specific light intensity level and salinity level.

After 24 h, non-attached spores were discarded by gentle rinsing, and the culture medium was replaced (i.e. day 0 of the experiment, T_0). Culture plates were distributed randomly in a culture chamber at 14 ± 1°C and 12:12 h LD. Culture medium was changed weekly, and juvenile individuals were cleaned using a soft brush.

Source of tetrasporophyte (2n), female and male gametophyte (n) adult fronds

For the three types of individuals (see above), 50 vegetative apical fragments (tips of 5 mm in length) were excised from each individual. Three pools of apical fragments, made of 1000 apices of each individual type (tetrasporophyte, female and male), were created. Fifteen apical fronds of the same individual type were randomly chosen and placed into 50 mL culture flasks. Twenty culture flasks were generated per individual type and distributed randomly into four culture conditions (five flasks for each combination treatment) in a culture chamber at 14 ± 1°C and 12:12 h LD.

Measured parameters

The diameter of 25 carpospores and 25 tetraspores (both unattached) was recorded just after spores release.

Using an inverted light microscope, the number of pigmented spores attached to the bottom was counted 24 h after inoculation in culture plates (T_0) in five areas of 4 mm^2 selected arbitrarily within each well. The total area of each culture well was 960 mm^2 (i.e. well diameter=3.5 cm). The survival rate of juveniles was measured by counting the number of individuals after 30 days of culturing (T_{30}) in the five selected areas of 4 mm^2. The survival rate of juveniles (T_0–T_{30}) was estimated as the number of living individuals observed at day 30 relative to the number of attached spores at T_0.

The growth rate of juveniles was estimated by measuring the diameter of the holdfasts, the length of the frond and the number of secondary frond ramifications when possible, at T_0, T_3, T_9, T_{15}, T_{30}, T_{45} and T_{60}. A minimum of five juvenile individuals were measured for each time interval and well. Only three wells for each culture plate were considered. Relative growth rates (RGR, percent day^{-1}, Hunt 1982) were estimated as follows: $RGR = 100x(\ln N_{30} - \ln N_0)/t$, where N_{30} is the holdfast diameter after 30 days and N_0 is the size at the beginning of the experiment (T_0).

The growth rate of adult apical fronds was estimated by measuring the length of the main axis and the number of secondary ramifications at T_0, T_3, T_9, T_{15} and T_{30}. Five replicates per treatment and for each type of individuals were considered, with a total of 15 apices measured per replicate. RGR (percent day^{-1}) were estimated with the formula above, considering N_{30} as the size (length of the principal axis) after 30 days and N_0 as the size at the beginning of the experiment (T_0).

Data analyses

The sizes of carpospores and tetraspores were compared using the Student *t*-test (Quinn and Keough 2002).

General lineal models (GLMs) were used to test for differences in spore settlement, juvenile survival and holdfast growth rate between "light intensity" (20 and 60 μmol photons m^{-2} s^{-1} treatments), "salinity" (15 ‰ and 35 ‰ treatments) and "life history phases" (carpospores and tetraspores treatments in the case of spore settlement variable, and haploid gametophyte and diploid tetrasporophyte treatments in the case of juvenile survival and holdfast growth variables). All these sources of variation were treated as fixed factors. The "culture well" was used as experimental unit in these analyses and considered as a random factor

nested in the interaction salinity × light intensity (Quinn and Keough 2002).

In the adult experiment, we used a full factorial statistical design (three-way ANOVA) using "type of individual" (diploid tetrasporophyte, haploid female and haploid male treatments), "light intensity" (20 and 60 μmol photons m^{-2} s^{-1} treatments) and "salinity" (15 and 35 ‰ treatments), all treated as fixed factors. The "culture flask" was considered as experimental unit in this analysis. To compare a posteriori the variation between types of individual, we used HSD-Tukey's test (Quinn and Keough 2002).

To meet homoscedasticity and normality assumptions, we used Cochran's and Kolmogorov–Smirnov's tests, respectively. Transformations preceded the analyses when needed (y=Arcsine $\sqrt{(x/100)}$ for the juvenile survival rate; $y=1/(x+1)$ for the number of secondary ramifications on the main axis in the adult apical fronds experiment).

Results

Spores size and settlement

The size of the carpospores (2n) was larger than the size of the tetraspores (n) with 32.6±2.6 μm and 28.1± 2.2 μm mean diameter, respectively (Student's t-test, $t_{(48)}=6.628$, $P<0.001$).

The attachment rates ranged from 50 to 95 % and correspond to a density of about 0.5–1.0 spore per square millimetre (Fig. 1). No difference in settlement was detected between carpospores and tetraspores (Table 1). Similarly, light conditions did not trigger a significant effect on spore settlement (Table 1). However, this feature varied significantly according to salinity (Table 1), showing a higher spore settlement at 35 ‰ salinity (Fig. 1).

Table 1 GLM statistical results on *Gracilaria chilensis* spore settlement from carpospores (2n) and tetraspores (n) 24 h after inoculation

	Spore settlement			
Source of variation	df	MS	F value	P value
Phase	1	1.559	2.245	0.150
Salinity	1	14.465	24.976	< 0.001
Light intensity	1	0.814	1.405	0.250
Phase × salinity	1	2.021	2.910	0.103
Phase × light	1	1.251	1.802	0.195
Salinity × light	1	1.317	2.274	0.147
Wells (salinity × light)	20	0.579	0.834	0.656
Phase × salinity × light	1	0.470	0.677	0.420
Residual	20	0.694		

Survival rate of juveniles

There was a significant interaction of life history phase, light intensity and salinity with the survival rate of juvenile individuals (Table 2). Tetrasporophytes displayed lower survival than gametophytes in all combinations of light intensity and salinity (Fig. 2). Tetrasporophytes juvenile individuals were more affected by salinity than were gametophytes at 15 ‰. Regardless of the light condition, tetrasporophyte survival was lower at 15 ‰ salinity than at 35 ‰ (i.e. less than 3 % had survived after 30 days of culture, Fig. 2). The effect of low salinity on tetrasporophytes was rapid, and only 5–8 % of the juveniles were still alive after 3 days of culture (data not shown). In fact, after 60 days of culture, all juvenile tetrasporophytes cultured at low salinity and low light conditions had died. At low salinity, after 30 days of culture, the survival rate of juvenile gametophytes was greater under low light than under high light conditions (Fig. 2). The lowest gametophytes

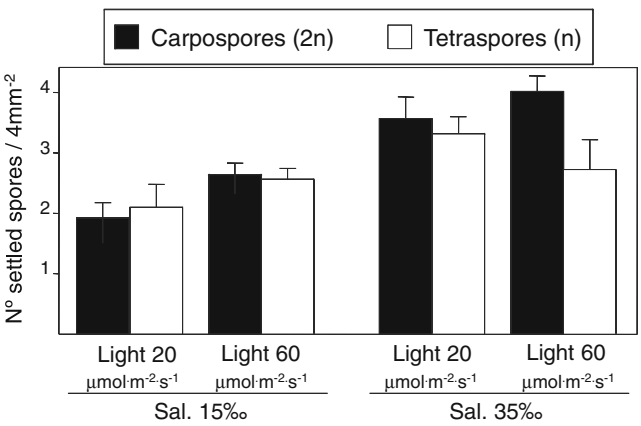

Fig. 1 Comparison of the settlement of tetraspores (n) and carpospores (2n), 24 h after inoculation, under four combinations of light intensity and salinity. Error bars = 1 SE (n=6)

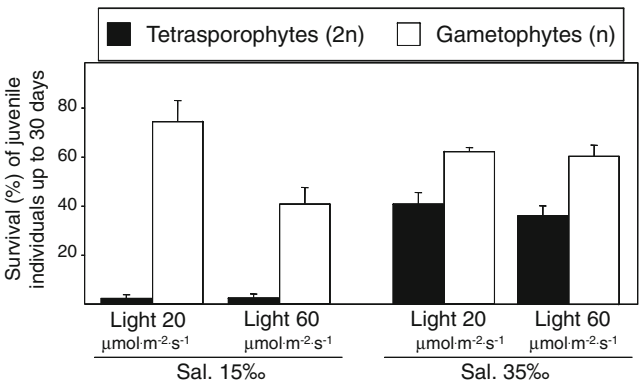

Fig. 2 Comparison of the survival rate of gametophytic (n) and tetrasporophytic (2n) juveniles after 30 days of culture under four combinations of light intensity and salinity. Error bars = 1 SE (n=6)

Table 2 GLM statistical results on *Gracilaria chilensis* survival and holdfast growth rate of juveniles from gametophyte (n) and tetrasporophyte (2n) after 30 days of culture

	Juvenile survival				Holdfast growth rate			
Source of variation	df	MS	*F* value	*P* value	df	MS	*F* value	*P* value
Phase	1	3.201	115.744	< 0.001	1	1.899	18.111	0.003
Salinity	1	0.999	53.338	< 0.001	1	0.567	4.197	0.075
Light intensity	1	0.158	8.447	0.009	1	0.409	3.024	0.120
Phase × salinity	1	0.941	34.025	< 0.001	1	1.298	12.379	0.008
Phase × light	1	0.115	4.148	0.055	1	3.288	31.354	0.001
Salinity × light	1	0.070	3.721	0.068	1	0.458	3.386	0.103
Wells (salinity × light)	20	0.019	0.677	0.805	8	0.135	1.289	0.364
Phase × salinity × light	1	0.157	5.692	0.027	1	1.135	10.827	0.011
Residual	20	0.028			8	0.105		

survival (41.1±14.7 %) was observed at low salinity and high light (Fig. 2).

Growth rate of juveniles

Variation of holdfast growth rate of juveniles after 30 days of culture cannot be explained by a single factor since this variable depends mainly on the interaction of life history phases with salinity and light intensity (Table 2). Under low light conditions, both phases showed a reduction in the holdfast diameter after 45 days (Fig. 3). This process began earlier (after 30 days) in tetrasporophytes, eventually leading to the death of all tetrasporophytes under the low salinity and low light condition, with erect fronds never developing (Fig. 4). In all other situations, erect fronds developed from the holdfasts after 30 days of culture (Fig. 4). Once again, under low light conditions, frond growth was stopped at day 45 with a noted decrease in size of the erect fronds (i.e. necrosis of the main fronds) for gametophytes under low salinity (Fig. 4). Despite salinity, tetrasporophyte holdfast growth rate under high light was greater than that of gametophyte after 30 days of culture (Fig. 5). Very few new secondary ramifications were observed on the main fronds of juveniles after 60 days of culture. The mean number of secondary ramifications per main frond of gametophytes cultured under high light with 15 and 35 ‰ of salinity reached 0.058±0.050 and 0.059±0.004, respectively, whereas in the tetrasporophytes cultured under high light and high salinity, the mean number of ramifications reached 0.016 ±0.027. In the remaining conditions, erect fronds lacked secondary ramifications at T_{60}.

Growth rate of adult apical fronds

Relative growth rates and the number of secondary ramifications of apices from adult plants were significantly affected by the type of thalli they were sampled from (Table 3). Diploid tetrasporophytes grew faster and produced more lateral branches than gametophytic individuals. In particular, males consistently displayed lower growth rates when compared to females or tetrasporophytes (Fig. 6a). The number of secondary ramifications of adult males was lower and represented only one third to one sixth of the number of lateral branches produced by females or tetrasporophytes (Fig. 6b). Moreover, the effect of salinity

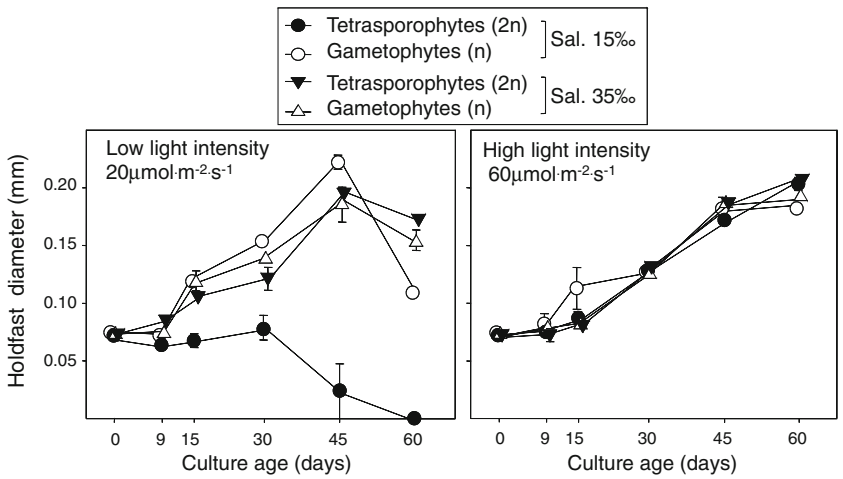

Fig. 3 Holdfast diameters of gametophytic (n) and tetrasporophytic (2n) juveniles according to time of cultivation under four combinations of salinity and light intensity. Holdfast diameter was recorded at five different times of culture (up to 60 days). Points were slightly separated for visual clarity, error bars = 1 SE (n=3)

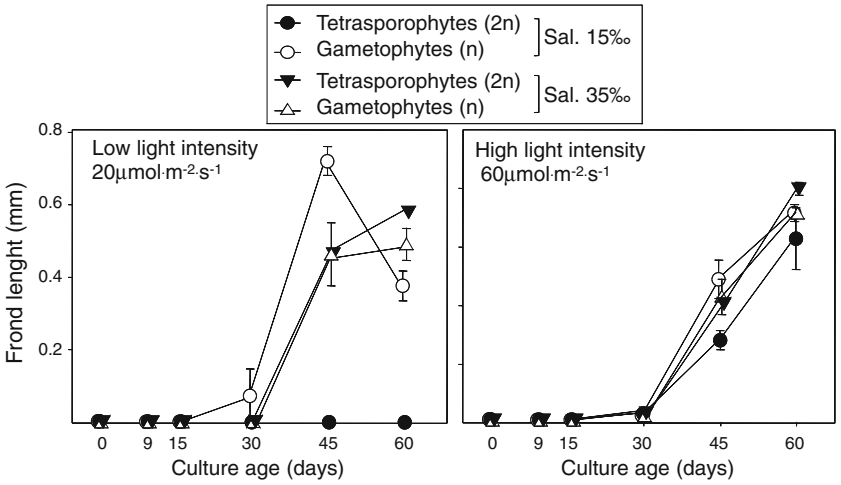

Fig. 4 Frond sizes of gametophytic (n) and tetrasporophytic (2n) juveniles according to time of cultivation under four combinations of salinity and light intensity. Frond size was recorded at five different times of culture (up to 60 days). Points were slightly separated for visual clarity, error bars = 1 SE (n=3)

was found to be significant (Table 3), with lower relative growth rate of the main axis recorded at 15 ‰ salinity (Fig. 6a). The relative growth rates of males and females represented 75 and 85 %, respectively, of the relative growth rate recorded in tetrasporophytic thalli, regardless of salinity conditions (HDS-Tukey's test, $P<0.05$ for all comparisons between individual types). Finally, light had no significant effect on thallus growth or branching (Table 3).

Discussion

Our results provide experimental support for the existence of some ecological differences among isomorphic gametophytic and tetrasporophytic individuals in *G. chilensis*. Differences were detected in juveniles and adults exposed to various conditions of light and salinity. In this general context, juvenile gametophytes seem to exhibit a higher tolerance to stress (low salinity) than young tetrasporophytes, whereas adult tetrasporophytes grew faster than female and male gametophytes.

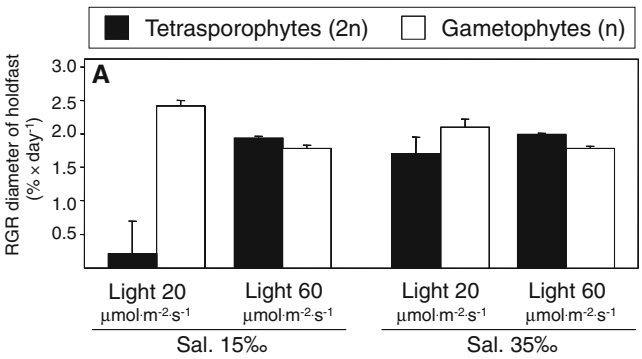

Fig. 5 Comparison of the holdfast relative growth rate of gametophytic (n) and tetrasporophytic (2n) juveniles after 30 days of culture under four combinations of light intensity and salinity. Error bars = 1 SE (n=6)

The relative frequency of tetrasporophytic versus gametophytic individuals in field populations has received considerable attention in the Rhodophyta (see review by Thornber 2006). In *Gracilaria*, depending on the population under study, the phases were either approximately similar or tetrasporophytes predominated. Kain and Destombe (1995) suggested that the predominance of tetrasporophytes indicates that the diploid phase is fitter at least in some environmental conditions. In a broader aspect, the variability in gametophyte/sporophyte ratios in the field can be partly explained by the differences in dispersal and survival rates between life history phases at the microscopic stage. For instance, haploid tetraspores and haploid germlings of *G. gracilis* had higher mortality rates than diploids, but tetraspores had the potential for longer dispersal distances (Destombe et al. 1989, 1992). In contrast, *G. pacifica* tetraspores showed higher survival rates during winter under detrimental conditions (Garza-Sánchez et al. 2000). Our results are consistent with this last observation, as gametophytic germlings displayed higher survival than tetrasporophytic individuals in all tested conditions, and juvenile tetrasporophytes appeared to be the most affected by low salinity.

The strong effect of low salinity on growth rate has been observed in various species of *Gracilaria* (17 species and strains from the Atlantic and eastern Pacific oceans, Bird and McLachlan 1986; *G. verrucosa* and *G. chorda*, Choi et al. 2006; seven species of temperate and tropical species from Asia, Raikar et al. 2001). In some cases, low salinity triggered early bleaching and tissue necrosis leading to death in *Gracilaria* (*G. arcuata* and *G. incurvata* from Japan, Raikar et al. 2001). In our study, the effect of salinity is low, and the mechanism that explains the higher sensitivity of juvenile tetrasporophytes to stress due to hyposalinity is unknown. Conversely, the high growth rates of adult apical segments observed at 15 ‰ salinity in our study are consistent with an earlier report (Bird and McLachlan 1986)

Table 3 Factorial ANOVA on *Gracilaria chilensis* adult frond growth from diploid tetrasporophytes, and haploid males and females after 30 days of culture

		Relative growth rate of main frond axis			Number of secondary ramifications		
Source of variation	df	MS	*F* value	*P* value	MS	*F* value	*P* value
Type of individuals	2	4.273	95.150	< 0.001	0.292	17.309	< 0.001
Salinity	1	1.355	30.163	< 0.001	0.025	1.499	0.227
Light intensity	1	0.003	0.078	0.781	0.010	0.570	0.454
Type × salinity	2	0.069	1.547	0.223	0.006	0.335	0.717
Type × light	2	0.058	1.283	0.287	0.003	0.195	0.824
Salinity × light	1	0.004	0.100	0.753	0.002	0.100	0.753
Type × salinity × light	2	0.005	0.103	0.902	0.002	0.095	0.910
Residual	48	0.045			0.017		

showing that a strain of *G. chilensis* from Maullin had a high tolerance to hyposalinity, thus suggesting that *G. chilensis* has genetically adapted to its estuarine habitat (Bird and McLachlan 1986).

The detrimental effect of high light and low salinity on juvenile gametophytes survival suggests that a synergism exists between the two factors. Seasonal bleaching and decrease in production of *G. chilensis* thalli have been associated to periods where cultivated stands were exposed to intense light and high temperature (Pizarro and Santelices 1993). As previously observed in kelps (Fredersdorf et al. 2009), the detrimental effect of stress factors on *G. chilensis* individuals was much more subtle in older developmental stages (i.e. apices of adult thalli as compared to spores and juveniles).

Higher vegetative growth and better vegetative propagation of tetrasporophytes have been demonstrated in other *Gracilaria* species (as *G. tikvahiae*, Patwary and van der Meer 1984), as well as in other haploid–diploid isomorphic species (as *Gelidium amansii*, Akatsuka 1986; *G. sesquipedale*, Juanes and Puente 1993; Carmona and Santos 2006; *Caloglossa leprieurii*, Zuccarello et al. 2001). In our study, higher growth rates and branching of diploids fragments of thalli strongly suggest that diploid sporophytes possess a greater capacity for vegetative propagation than haploid

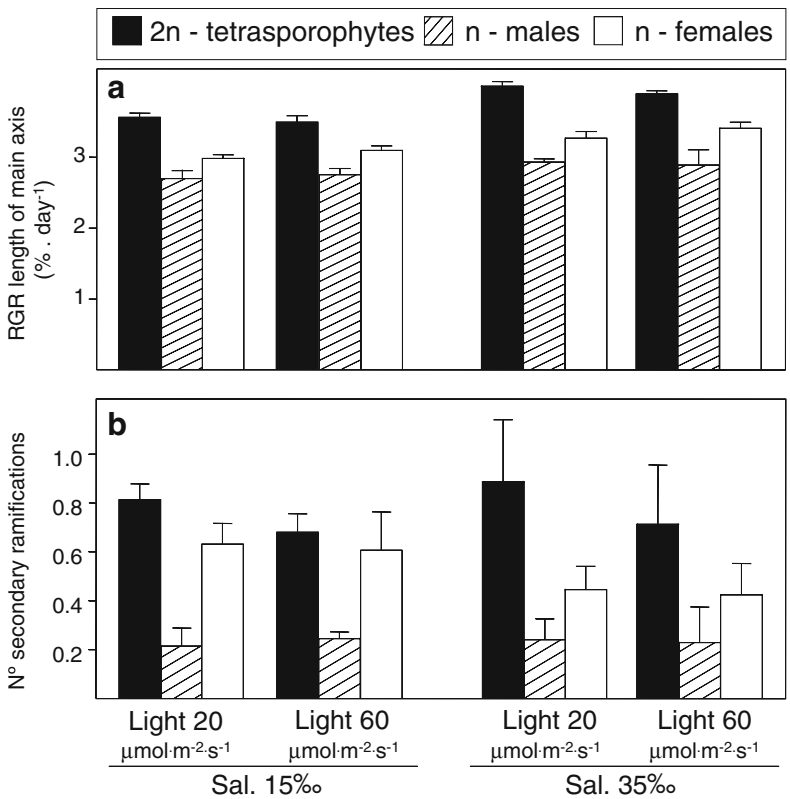

Fig. 6 Comparison of the adult tetrasporophyte, male and female growth rates after 30 days of culture under four combinations of light intensity and salinity: **a** apical frond relative growth rate and **b** number of secondary ramifications. Error bars = 1 SE (n=5)

gametophytes. These results are in agreement with the "genetic buffering hypothesis", suggesting that diploids should be more vigorous than haploids due to a better cellular regulation and metabolism (Raper and Flexer 1970; Maynard-Smith 1978). The clear sporophyte advantage for vegetative growth recorded in our study differs from previous results obtained by Santelices and Varela (1995) demonstrating that females of *G. chilensis* grow faster than sporophytes. However, contrary to our study based on vegetative apices, the study by Santelices and Varela (1995) only used reproductive tetrasporophytic thalli, suggesting that reproduction might decrease growth rate (Santelices and Valera 1995; Guimaraes et al. 1999).

In addition, our study demonstrated that non-cystocarpic female tips grew faster than immature males, regardless of culture conditions. This observation differs from previous reports on *G. parvispora* (as *G. bursapastoris*) and *G. coronopifolia* demonstrating that the relative growth rate of females was lower than that observed in males and sporophytes (Hoyle 1978). On the other hand, (Zhang and van der Meer 1987) showed that females grew much faster than males in *Gracilariopsis lemaneiformis* (as *G. sjoestedtii*). This difference in growth between sexes was attributed to the fact that reproduction was followed by senescence of male thalli (Kain and Destombe 1995). Interestingly, dominance of female gametophytes was observed in one farm of *G. chilensis* (Lenga, in the Concepcion region), while there did not exist a single male dominated farm (Guillemin et al. 2008).

With the exception of the farm in Lenga, diploid *G. chilensis* dominated in all 14 studied soft bottom estuaries and/or farms, where individuals reproduced mainly by vegetative propagation (Guillemin et al. 2008). In *G. chilensis*, most of the cultivated clones were heterozygous diploids, and it has been hypothesized by Guillemin and collaborators (2008) that the sporophyte advantage could have a genetic base (heterosis or over-dominance). One hypothesis that explains the advantage of diploids over haploids is that the substratum is important in determining the type of reproduction, without taking into account the level of heterozygosity. In fact, when plants are growing on soft bottom, development from spores is unlikely and vegetative propagation could favor a single phase (Kain and Destombe 1995). In *G. chilensis*, faster growth displayed by tetrasporophytes should be advantageous for this ploidy phase. For instance, in intensively planted and exploited farms in Chile, only diploid tetrasporophytic fronds of *G. chilensis*, mostly immature, were recorded (Guillemin et al. 2008). This observation suggests that Chilean fishermen could have unknowingly selected diploid fronds, having likely accelerated the natural trend of tetrasporophytes dominance already present in natural estuarine populations of floating fronds (Prieto et al. 1991). Similarly, Martín et al. (2010) reported in *G. gracilis* predominance of tetrasporophytes in a southern Argentina population where individuals naturally propagated mainly by fragmentation. Interestingly, in the red algae *C. leprieurii* and *Bostrychia moritziana*, it has been reported that most strains that were randomly selected to be maintained in culture were sporophytes (West and Zuccarello 1999; Zuccarello et al. 2001 and references herein).

The Gracilariaceae include potentially invasive seaweed species (Williams and Smith 2007), and the formation of extensive stands that reproduce mostly through fragmentation of vegetative thalli in muddy bays seems to be characteristic of the newly established populations after range expansion (i.e. *G. vermiculophylla*: Weinberger et al. 2008; Thomsen et al. 2009). Considering that tetrasporophytes are characterized by better propagation in Gracilariales, one could expect that diploids play a key role in the colonization of new habitats, as it was demonstrated for a Bonnemaisoniales (*Asparagopsis armata*), where the tetrasporophytic thalli had spread all along the Irish coast, likely due to propagation after introduction (Kraan and Barrington 2005).

In conclusion, our study demonstrates the existence of differences between the two life history phases (i.e. gametophytes and tetrasporophytes) in *G. chilensis* for spores, juveniles and adults. Slight adaptive differences between isomorphic generations have been proposed to promote the maintenance of complex life cycles (Hughes and Otto 1999) and may explain why morphologically indistinguishable phases co-occur in space and time in natural populations of *G. chilensis*. However, no inferences about the relationship between changes in salinity and light intensity on phase's recruitment or fertility in the field can be yet drawn with certainty. On the other hand, the faster vegetative growth that favored tetrasporic thalli over both females and males gametophytes under all conditions tested could easily explain the sporophytes dominance in floating populations of *G. chilensis* that are mainly maintained by fragmentation of vegetative thalli (Guillemin et al. 2008).

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