J Appl Phycol (2008) 20:897–906
DOI 10.1007/s10811-007-9276-2

UVB effects on early developmental stages of commercially important macroalgae in southern Chile

N. P. Navarro · A. Mansilla · M. Palacios

Received: 21 April 2007 / Revised and Accepted: 9 October 2007 / Published online: 28 November 2007

Abstract High levels of ultraviolet-B radiation (UVB) could represent a danger to seaweeds by affecting their physiological processes and development. The aim of this work was to study the effects of UVB radiation on early developmental stages of commercially and ecologically important marine algal species in southern Chile, considering spores survival and embryos growth. Spores of *Mazzaella laminarioides*, *Gigartina skottsbergii*, *Sarcothalia crispata* and embryos of *G. skottsbergii* and *Macrocystis pyrifera* were submitted to treatments of a) photosynthetically active radiation (PAR: Control), b) PAR+UVA (PA) and c) PAR+UVA+UVB (PAB). UV radiation did not affect spore survival of *M. laminarioides S. crispata* and *G. skottsbergii* (P=0.55, P=0.6 & P=0.25 respectively), but did provoke differences in the growth rate of *G. skottsbergii* embryos (P=0.00). Differences in survival and growth of *M. pyrifera* embryos were also observed (P=0.001 & P=0.007, respectively). Differences in growth of *M. pyrifera* embryos were observed only in the first five days, whereas changes in survival persisted until the end of the experiment. Additionally, UVB provoked morphological alteration in *M. pyrifera* embryos, as evidenced by progressive curling. These results suggest that the initial stages of the subtidal algae species *G. skottsbergii* and *M. pyrifera* cultivated in laboratory conditions were sensitive to UVA and UVB radiation, and their recruitment and development could be affected as well in natural conditions found in southern South America, where the ozone layer has thinned more than in other parts of the planet.

N. P. Navarro (✉) · A. Mansilla · M. Palacios
Facultad de Ciencias, Universidad de Magallanes,
Casilla 113-D, Avenida Bulnes,
01855 Punta Arenas, Chile
e-mail: nelso.navarro@umag.cl

Keywords Commercial seaweeds · Spores · UVB impact

Introduction

One of the most recognised atmospheric changes during the last few decades has been the thinning of the stratospheric ozone layer (Kirchhoff et al. 1997). This phenomenon has resulted in increasing levels of ultraviolet radiation B (UVBR: 280–320 nm) (Seckmeyer and Mckenzie 1992), reaching not only the Antarctic region, but also the southernmost part of South America (Chile and Argentina) (Kirchhoff et al. 1997; Bianciotto et al. 2003). During spring, when the area of the ozone hole increases and the stratospheric vortex elongates, the southern tip of South America is often under the influence of the ozone hole (Diaz et al. 2006).

Since ultraviolet radiation also penetrates the water column (Smith et al. 1992; Figueroa 2002), marine organisms are exposed to its harmful effects as well. Benthic macroalgae, in contrast to phytoplankton, are fixed and restricted to their growth sites, thus lacking the ability to avoid solar radiation, especially during low tide (Franklin and Forster 1997).

Sensitivity and tolerance of certain species to UVB radiation depends on their capacity to prevent and repair damage (van De Poll et al. 2001). When the levels of ultraviolet radiation increase, as they have in recent years, algal defense mechanisms may be insufficient, causing irreversible cellular damage. This sensitivity to UVB radiation might also vary according to developmental stages of species (Dring et al. 1996).

Ultraviolet radiation has been found to affect marine macroalgae in several ways, including effects on photosynthesis, DNA lesions, nitrogen metabolism and growth, as shown in numerous publications (reviewed by Franklin and Forster 1997; Xue et al. 2005). However, most of these

studies have been focused primarily on the adult stages in the life history of macroalgae (Cordi et al. 2001).

Research evaluating the effects of UVB radiation on juvenile stages and spores is not frequent in literature, but early growth forms are of great importance since reproductive structures and the first stages of development are essential for recruitment, especially for those species of great ecological and economic importance, as their recovery over time depends almost entirely on reproductive ability. Considering the smaller size and greater structural simplicity of microscopic life stages, any kind of stress that affects the biology of a species may exert more evident effects on their early developmental stages, as has been shown for zoospores and germlings of various brown algae (Dring et al. 1996; Wiencke et al. 2000, 2006; Altamirano et al. 2002), unicells of Ulvales (Cordi et al. 2001) and for early life stages of Gigartinales (Roleda et al. 2004a).

Knowledge of the sensitivity of these ontogenetic stages is crucial, since recruitment of species depends on the survival of these stages, and conclusions obtained with macroscopic stages should not be extrapolated to microscopic ones (Altamirano et al. 2002). However, information regarding macroalgae, especially commercially important species, is still limited. The aim of this work was to evaluate the effects of UVB radiation on early developmental stages of commercially and ecologically important species in southern Chile, considering spore survival of *Mazzaella laminarioides*, *Gigartina skottsbergii*, *Sarcothalia crispata* and embryo growth of *G. skottsbergii* and *Macrocystis pyrifera*.

In Chile, *Sarcothalia crispata* (Bory) Leister, *Gigartina skottsbergii* Setchell & Gardner, and *Mazzaella laminarioides* (Bory) Fredericq are considered important carragenan-producing red alga. For this reason, all are harvested as a single group to obtain this important phycocolloid, employed in the food and pharmaceutical industry (Marín et al. 2002). *Macrocystis pyrifera* (Linnaeus) C. Agardh is an economically and ecologically important brown algae used as food in abalone farming and as an alginate resource. In the Magellan Region is also an ecosystem engineer that serves as a refuge and reproductive site for a great diversity of organisms such as sea urchins, king crabs, snow crabs and scallops.

Material and methods

Reproductive fronds of *Mazzaella* and *Sarcothalia* were collected by hand from intertidal zones, while *Gigartina* and *Macrocystis* were collected by SCUBA diving in the subtidal zone of Punta Santa Ana (53 37′ S; 70 59′ W), Strait of Magellan (Chile) in autumn 2005 and transported to the Center for Aquiculture and Subantarctic Marine Resources Laboratory at the Department of Natural Sciences and Resources, University of Magallanes, where unialgal cultures were established as described by Oliveira et al. (1995). All cultures were maintained with Provasoli enriched seawater (20 mL L^{-1}; 31 psu salinity), in a temperature-controlled room at 9±1°C with artificial irradiation of 55 μmol photons m^2 s^{-1} PAR (provided Philips TLT 20W/54 daylight fluorescent tubes) on a 12–12 h light-dark cycle.

Segments (2 cm^2) from different reproductive fronds were cut and put in Petri dishes with sterile filtered seawater. These were kept in a culture chamber until the release of spores. Three hundred spores from differents species were cultivated in triplicate in plastic Petri dishes. In order to obtain embryos of *Gigartina*, tetraspores were cultivated. To obtain young sporophytes (embryos) of *Macrocystis*, gametophytes were obtained from zoospores in the laboratory. Afterwards, gametophytes were cultivated for 2 weeks to reach 3 mm long young sporophytes. Afterwards, the sporophytes were unfastened and collected by Pasteur pipettes and put in plastic Petri dishes immediately.

Experimental light conditions The UV radiation was supplemented with UV-A (provided by UV-A-340 fluorescent tubes, Q-Panel Lab Products, Cleveland, USA) and UV-B (TL20W/12RS, Philips, Netherlands). Petri dishes were covered with different radiation filters in order to set three light/UV conditions: PAR only (λ≥400 nm, achieved by Ultraphan URUV, Digefra, Munich, Germany), PAR+UV-A (λ≥320 nm, Folanorm SF-AS, Folex, Cologne, Germany) and PAR+UV-A+UV-B (λ≥295 nm, Ultraphan URT, Digefra, Munich, Germany). Radiation conditions in the experimental treatments are summarised in Table 1.

PAR was kept low and constant during the entire experimental period and the algae were exposed to a constant UVB and UVA irradiance for only 3 h a day (from 11.00 to 14.00 h). Under these conditions, the spores of red macroalgae were exposed for 4 subsequent days to calculate survival, while *Macrocystis* and *Gigartina* embryos were exposed for 15 days.

Survival and growth measurements From each Petri dish that contained red macroalgae spores, survival was calculated by percentage alive after two and four days. In each sample about 300 spores were counted and the percentage of live spores was determined using a light microscope. The live cells were easily distinguishable (pigmented and dividing cells) from the dead cells (no pigment and sometimes not settled).

In order to calculate the survival of *Macrocystis* and *Gigartina* embryos, survival after 15 days was determined. Thirty 0.1 μm diameter and 1 week old gametophytes were

Table 1 Irradiance treatments applied to test survival and growth rates of spores and embryos of commercially important macroalgae in the laboratory

	PAR (μmol photons $m^{-2}.s^{-1}$)	UVA ($W.m^{-2}$)	UVB ($W.m^{-2}$) unweighted	UVB ($W.m^{-2}$) weighted
P (control)	55			
PA	55	0.9		
PAB	55	0.9	0.2	0.003

For weighted irradiance, the action spectra for DNA damage (Setlow 1974) was applied
P = PAR photosynthetic active radiation, PA=PAR+UVA, PAB=PAR+UVA+UVB

obtained from tetraspores of *G. skottsbergii*, which were cultivated in Petri dishes in triplicate for growth rate and survival determination. Diameter was recorded for a period of 15 days. Relative growth rates (RGR) were estimated from the following equation:

$$\mathrm{RGR}(\%\ \mathrm{per\ day}) = \left((\ln \mathrm{D_f} - \ln \mathrm{D_i}) * \mathrm{t}^{-1}\right) * 100$$

(Lignel and Pedersén 1989), where D_i is the initial and D_f is the final diameter of the young tetrasporophytes after t days of culture under the different treatments. The diameter of discoid embryos was measured in triplicate, using photographs, and analyzed by optical microscopy software Image Pro Plus 4.0.

Recovery After the experiment with UV radiation (PA and PAB), embryos of *Gigartina*, which had been cultivated under this radiation, were transferred to the control treatment (P) for 14 days, to observe if there was recovery of the growth rate after radiation.

Data analysis Arcsine transformation was applied to percentage data of spores and embryo survival. Data were compared by one-way (UV irradiance treatment) analysis of

Table 2 One-way (UV irradiance treatment) analysis of variance (ANOVA) and *Newman-Keuls* test for spores survival of *Mazzaella laminarioides*, *Sarcothalia crispata* and *Gigartina skottsbergii*, and growth rate of *Macrocystis pyrifera* and *G. skottsbergii* embryos exposed to different conditions (P = PAR photosynthetic active radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB)

		P-values				
	df	*F*		P × PA	P × PAB	PA × PAB
M. laminarioides carpospore survival at 2 days	2	1.80	0.243 ns			
M. laminarioides carpospores survival at 4 days	2	1.56	0.284 ns			
M. laminarioides tetraspores survival at 2 days	2	0.64	0.557 ns			
M. laminarioides tetraspores survival at 4 days	2	0.63	0.561 ns			
S. crispata tetraspores survival at 2 days	2	4.99	0.052 ?	0.079 ns	0.048 *	0.363 ns
S. crispata tetraspores survival at 4 days	2	0.55	0.602 ns			
G. skottsbergii tetraspores survival at 3 days	2	0.93	0.445 ns			
G. skottsbergii tetraspores survival at 6 days	2	1.74	0.253 ns			
G. skottsbergii embryos growth after 15 days	2	315.9	0.000 *	0.000 *	0.000 *	0.000 *
G. skottsbergii embryos growth between the beginning and the 3th day	2	5.28	0.048 *	0.056 ns	0.039 *	0.739 ns
G. skottsbergii embryos growth between the 3th and the 7th day	2	9.39	0.014 *	0.012 *	0.137 ns	0.042 *
G. skottsbergii embryos growth between the 7th and the 9th day	2	11.80	0.008 *	0.032 *	0.007 *	0.086 ns
G. skottsbergii embryos growth between the 9th and the 15th day	2	65.82	0.000 *	0.002 *	0.000*	0.001*
G. skottsbergii embryos recovery after14 days	2	3.41	0.102 ns			
G. skottsbergii embryos recovery between the beginning and the 7th day	2	0.181	0.839 ns			
G. skottsbergii embryos recovery between the 7th and the 14th day	2	5.37	0.046 *	0.22 ns	0.11 ns	0.039 *
M. pyrifera embryos growth after 9 days	2	8.80	0.008 *	0.748 ns	0.007 *	0.011 *
M. pyrifera embryos growth between the beginning and the 5th day	2	56.14	0.000 *	0.276 ns	0.000 *	0.000 *
M. pyrifera embryos growth between the 5th and the 9th day	2	0.66	0.542 ns			
M. pyrifera embryos survival after 16 days	2	13.11	0.006 *	0.082 ns	0.006 *	0.024 *
M. pyrifera embryos survival between the beginning and the 5th day	2	17.27	0.003 *	0.077 ns	0.003 *	0.011 *
M. pyrifera embryos survival between the 5th and the 9th day	2	84.10	0.001 *		0.001 *	
M. pyrifera embryos survival between the 9th and the 16th day	1	60.62	0.000 *		0.000 *	

* = significant ($p<0.05$); ns = not significant.

variance (ANOVA). *Newman-Keuls* test was used to establish statistical differences. In all cases the RGR mean value of each Petri dish was considered as a replicate, thus for each treatment the number of replicate was three. All statistical tests were performed in accordance with Zar (1999).

Results

Spore survival In general, spore survival of red macroalgae studied was not affect by UV radiation after the first 4 days of UV exposure (Table 2). A higher percentage of survival (>80%) was observed in tetraspores and carpospores of *Mazzaella* exposed to all treatments tested (Figs. 1 and 2). ANOVA of carpospores and tetraspores percentage survival of *Mazzaella* showed no significant effects among all tested treatments (P=0.561 & P=0.284, respectively).

For *Sarcothalia* a higher percentage of survival was observed in tetraspores exposed to the control conditions (92±4%), when compared with PA (73±6%) and PAB (64±17%) treatments at two days (Fig. 3). However, there were no differences among percentage survival of tetraspores exposed to all treatments (P=0.052). Nonetheless, this p-value is at the limit of accepting hypothesis of the existence of significant differences in the survival percentages observed in the used treatments. In fact, the Newman-Keuls test showed differences between control and PAB treatment (P=0.048).

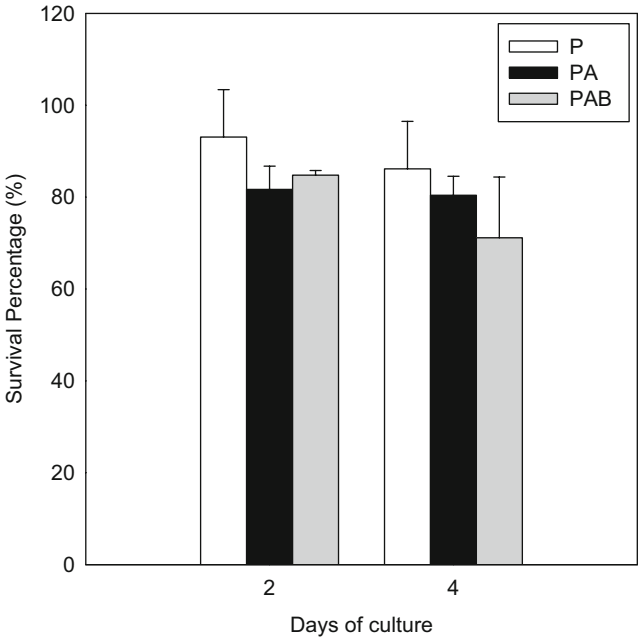

Fig. 1 Mean survival percentage of tetraspores of *Mazzaella laminarioides* exposed to different conditions (P=PAR photosynthetic active radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 2 and 4 days. Data are expressed as mean values ± S.D. (n=3). Letters on the graph show the result of the *Newman-Keuls* test (P<0.05); different letters refer to significant differences between mean values

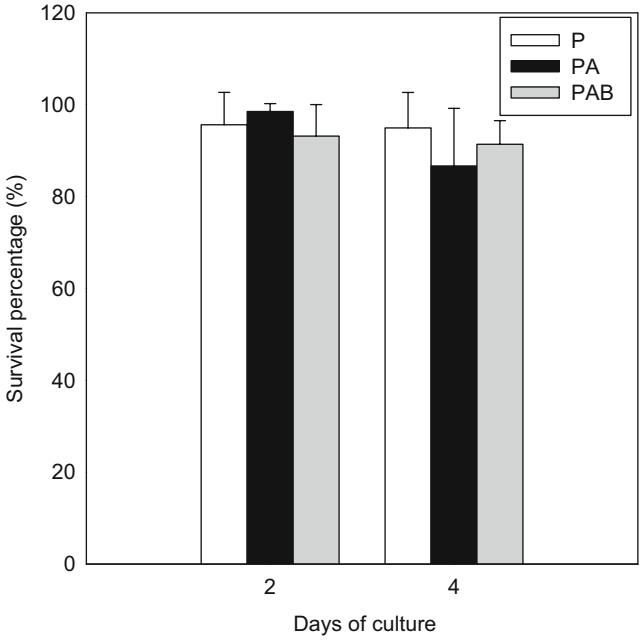

Fig. 2 Mean survival percentage (± S.D.) of carpospores of *Mazzaella laminarioides* exposed to different conditions (P=PAR photosynthetic active radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 2 and 4 days. ANOVA results are as in Fig. 1

In the case of *Gigartina*, UV radiation (UVA and UVB) did not lead to differences in tetraspore survival (P=0.445 at three days and P=0.253 at six days). However, spore survival was lower in all treatments (Fig. 4).

Growth of embryos

Gigartina

Higher RGRs were observed in *Gigartina* embryos exposed to the control conditions when compared with those cultivated at PA and PAB (P=0.000: Fig. 5). Detailed analysis of the relative growth rates at all data collection dates (days 3 to 15) showed that there was daily variation (Table 2: Fig. 6). Higher RGRs were observed in embryos cultivated in control conditions, when compared with those exposed to UVR treatments at 3, 9 and 15 days, except at day 7, where the higher RGRs were similar between control and PAB treatment, but different to PA treatment.

Those embryos of *Gigartina* which were transferred from PA and PAB to P treatment showed a similar growth rate to those of the control treatment after 14 days (Fig. 7), indicating that the effect of ultraviolet radiation on the growth rates of *Gigartina* embryos could be reversible.

Macrocystis

The analysis of the growth rate calculated from the length of the *Macrocystis* embryos over the total period of the

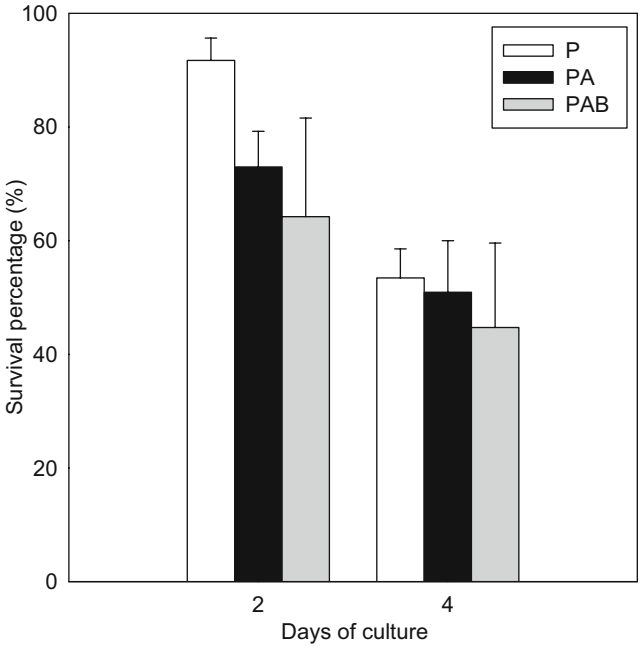

Fig. 3 Mean survival percentage (± S.D.) of tetraspores of *Sarcothalia crispata* exposed to different conditions (P=PAR photosynthetic active radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 2 and 4 days. ANOVA results are as in Fig. 1

experiment (9 days) showed differences between the treatments (P=0.007). The *post-hoc* test indicated that the differences were between the P treatment (control) and the PAB, with lower growth being observed under this last

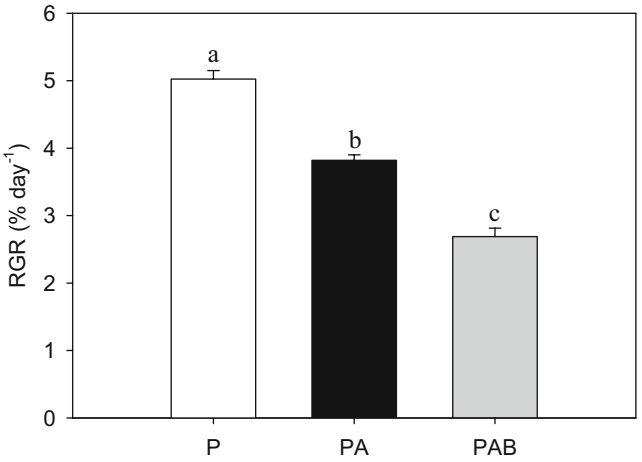

Fig. 5 Mean relative growth rate (RGR; % day-1) of *Gigartina skottsbergii* embryos exposed to different conditions (P=PAR (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 15 days. Data are expressed as mean values ± S.D. (n=4); different letters refer to significant differences between mean values with *Newman-Keuls* post-hoc test (P<0.05)

treatment (Fig. 8). A more detailed analysis showed that the major differences in the growth rates were observed in the first 5 days of cultivation (P=0.000), whereas in the period between the 5th and the 9th day there were no significant differences (P=0.542) (Fig. 9).

Regarding survival, it was found that embryos cultivated under the PAB treatment showed a survival rate lower than

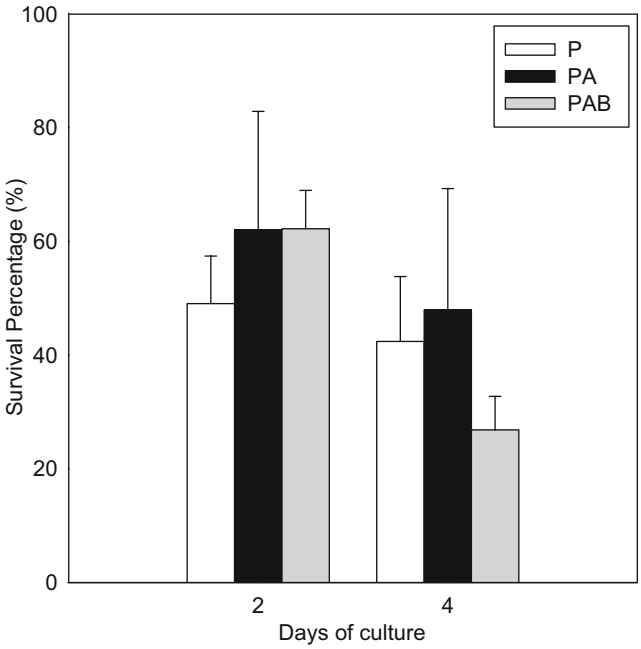

Fig. 4 Survival percentage of tetraspores of *Gigartina skottsbergii* exposed to different conditions (P=PAR Photosynthetic Active Radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 3 and 6 days. Data are expressed as mean values ± S.D. (n=3). Letters on the graph show the result of the Newman-Keuls test (P<0.05); different letters refer to significant differences between mean values

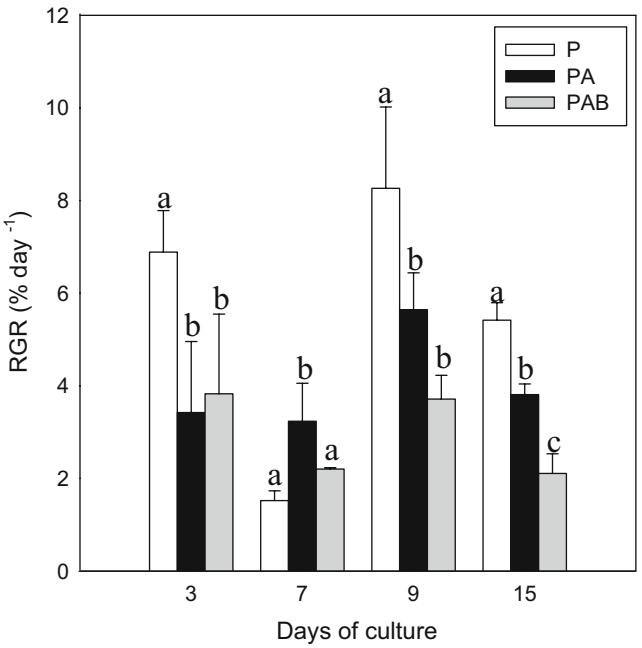

Fig. 6 Mean relative growth rate (RGR; % day-1) of *Gigartina skottsbergii* embryos exposed to different conditions (P=PAR (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) of all data collection dates (days 3 to 15). Data are expressed as mean values ± S.D. (n=4); different letters refer to significant differences between mean values with *Newman-Keuls* post-hoc test (P<0.05)

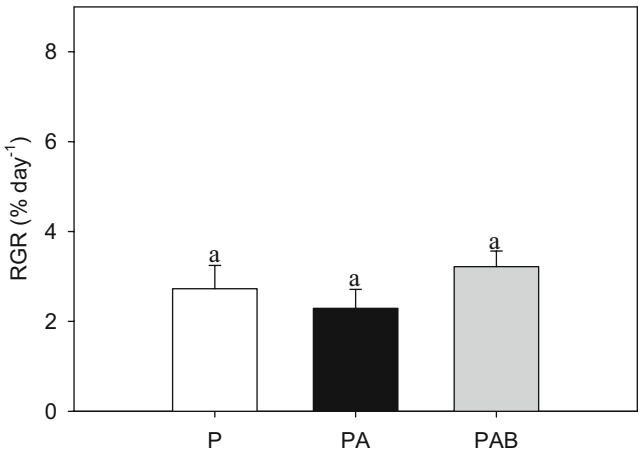

Fig. 7 Relative growth rate (RGR; % day-1) of *Gigartina skottsbergii* embryos after 14 days of recovery period under only PAR. Insert shows daily growth rate. Data are expressed as mean values ± S.D. (n=4); different letters refer to significant differences between mean values with *Newman-Keuls* post-hoc test (P<0.05)

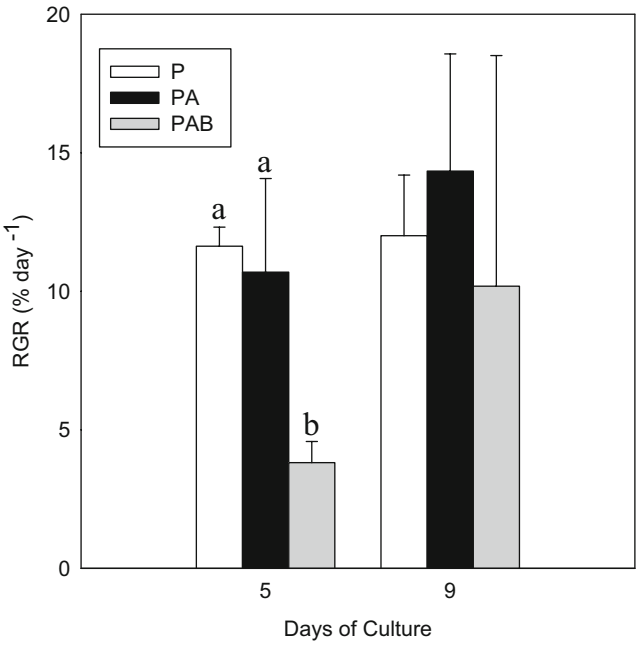

Fig. 9 Detailed analysis of the mean relative growth rates (RGR; % day^{-1}) of *Macrocystis pyrifera* embryos of all data collection dates (days 5 to 9) under different UV conditions (P=PAR Photosynthetic Active Radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB). Data are expressed as mean values ± S.D. (n=4); different letters refer to significant differences between mean values with *Newman-Keuls* post-hoc test (P<0.05)

20%, whereas the survival of those cultivated under the control condition (P) was higher than 70% (Fig. 10).

After the evaluation of the growth and survival rates, some embryos (sporophytes) of *Macrocystis* continued to be cultivated with the purpose of determining the effect of UVB radiation on their morphology. The observations performed showed that UVB damages the morphological development of the sporophytes (Fig. 11), with samples maintained in the treatment with ultraviolet B (PAB), from the 9th day of cultivation, showing changes in their morphology. These changes appeared as sporophyte curling, alterations at the edges of the thalli and/or tissue necrosis (Fig. 11); alterations that caused a decrease in the

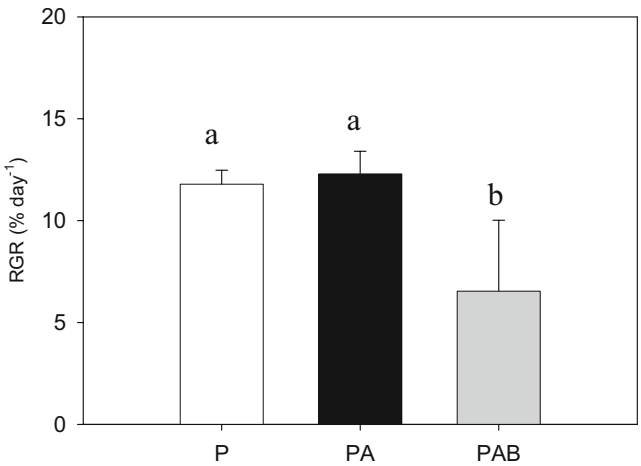

Fig. 8 Mean relative growth rate (RGR; % day-1) of *Macrocystis pyrifera* embryos exposed to different conditions (P=PAR Photosynthetic Active Radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 9 days. Data are expressed as mean values ± S.D. (n=4); different letters refer to significant differences between mean values with *Newman-Keuls* post-hoc test (P<0.05)

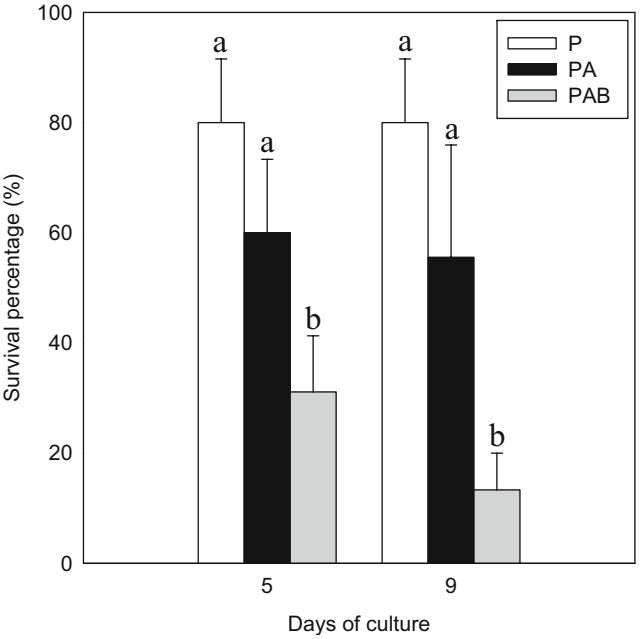

Fig. 10 Mean survival percentage (± S.D.) of *Macrocystis pyrifera* embryos exposed to different conditions (P=PAR Photosynthetic Active Radiation (control), PA=PAR+UVA, PAB=PAR+UVA+UVB) after 5 and 9 days. Data are expressed as mean values ± S.D. (n=4)

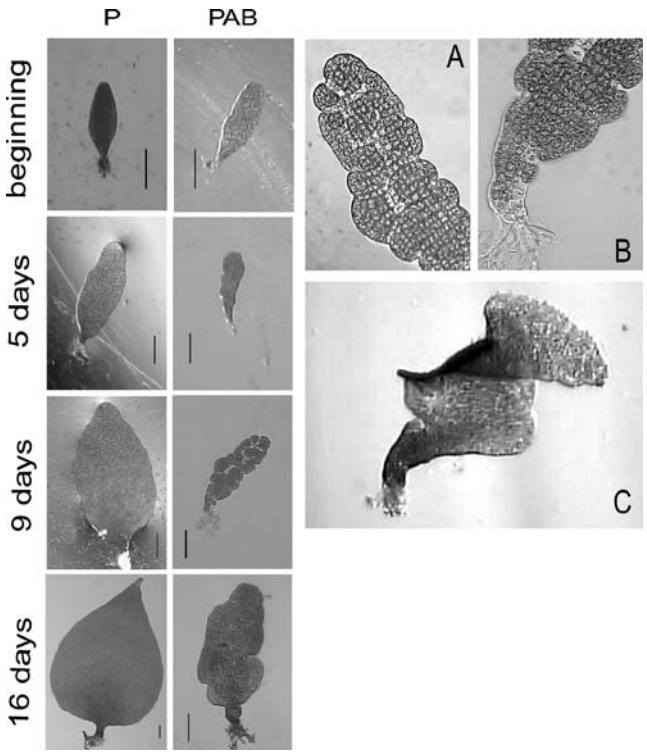

Fig. 11 Mean morphological responses (± S.D.) of *Macrocystis pyrifrea* embryos after 16 days under different condition (P=PAR photosynthetic active radiation (control) and PAB=PAR+UVA+UVB). Letters A, B and C show details of *M. pyrifera* embryos exposed to UVB radiation at 9 days

growth (P=0.000) (Fig. 12). This morphological alteration was observed mainly in embryos cultivated under the PAB treatment.

Discussion

In natural ecosystems, seaweeds are influenced not only by PAR, but also by ultraviolet radiation (UVA and UVB) (Makarov and Voskoboinikov 2001). These radiations may or may not be harmful for the development of particular species. It has been shown previously that there was decreased sensitivity to UV irradiation with increasing age (Dring et al. 1996) and with high visible light stress (Lüning 1980; Hanelt et al. 1997). In this regard, our results showed that, in general, there were no negative effects on the survival of spores, but negative effects were indeed observed in embryo growth of *Gigartina* and the sporophytic embryos of *Macrocystis*.

These data seem contrary to the evidence presented by the above-mentioned authors; however, our data should be analyzed separately, considering the bathymetric distribution of these species and their evolutionary history. Of the species studied, *Mazzaella* is an abundant species within the upper eulittoral to the upper sublittoral zone; *Sarcothalia* inhabits the lower eulittoral and upper sublittoral, whereas *Gigartina* and *Macrocystis* are found in sublittoral environments. In their natural environment, eulittoral (intertidal) species are more exposed to solar radiation and therefore are more likely to be adapted to UV exposure. On the other hand, sublittoral macroalgae are less exposed and may not be adapted to enhanced levels of this radiation. In fact, van De Poll et al. (2001) also reported that littoral species had a greater tolerance to UVB radiation than subtidal taxa.

In our experiments, spores (tetraspores and carpospores) of *Mazzaella* showed a high survival rate in the treatments used, which would indicate the acclimation of spores through the existence of defense mechanisms against UV, or that levels of UVB used are not harmful for the survival of the spores. In the case of the survival of *Sarcothalia* tetraspores, differences were observed among the treatments with a higher survival in the control treatment and a lower level in the treatment with UVB. However, these differences, besides being small, did not remain over time, since on the sixth day survival was similar among the treatments. Similar results have been reported in the other two Gigartinales species that co-inhabit the rocky littoral zone of North Atlantic coasts. Carpospores of *Mastocarpus stellatus* were found to be more tolerant of the deleterious effect of UVR compared with *Chondrus crispus* carpospores (Roleda et al. 2004a). Therefore, differences in the sensitivity between *Mastocarpus* and *Chondrus* carpospores to high light and UVB radiation reflect the differences in their zonation.

While *Mastocarpus* recruits at higher shore levels, *Chondrus* would limit recruitment at lower shore levels (Roleda et al. 2004a). In this way, it is possible that differences in the sensitivity between *Mazzaella* and *Sarcothalia* spore survival to UVB radiation could play a

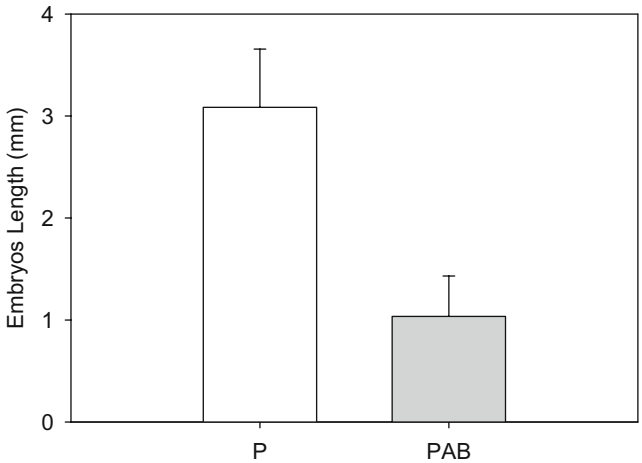

Fig. 12 Mean size measurements (± S.D.) of *Macrocystis pyrifera* embryos exposed to different conditions (P=PAR (control) and PAB=PAR+UVA+UVB) after 16 days. ANOVA values are as Fig. 1

role in the establishment of the zoning pattern at southern South American shorelines, at least for *Mazzaella* and *Sarcothalia*.

Sarcothalia, like *Mazzaella*, seems to have defense mechanisms against UV. However these mechanisms would not act immediately, but in a gradual acclimation process, since the *Sarcothalia* is not directly exposed to UV, with the exception of those days of spring tide. One of the most important physiochemical acclimation mechanisms against biologically harmful UV radiation involves the biosynthesis and accumulation of UV-screening substances. The most common photoprotective sunscreens in Antarctic macroalgae are the mycosporine-like amino acids (MAAs), which have been reported for species of *Mazzaella* and *Sarcothalia* in Chile (Huovinen et al. 2004).

Because *Gigartina* inhabits the sublittoral zone and often is protected from UV by the shadowing of big brown algae, mainly *Macrocystis*, we expected that its spores would be sensitive to UV radiation, however UVA and UVB radiation did not lead to differences in tetraspore survival when compared with the control treatment. On the other hand, the existence of a low survival of tetraspores in the treatments used, could be due to the use of PAR, whose intensity in the experiment was higher than that suggested by Romo et al. (2001) for the cultivation of this species in its first stages of development. However, the possibility of the existence of mycosporine-like amino acid compounds can not be dismissed. Research into this phenomenon could provide evidence of the synthesis of mycosporine-like amino acids in spores.

Embryo growth The growth of *Gigartina embryos* was negatively affected by UV, being significantly more inhibited by the UVB. These results are in agreement with those reported Mansilla et al. (2006), which showed that embryos of *Gigartina* cultivated under the different intensities of UVB had lower growth rates in comparison with those cultivated without UVB, referring to subtidal algae, and therefore more sensitive to UVB. In this study, the embryos were also treated with UVA, unlike Mansilla et al. (2006). UVA also inhibited the growth of embryos of *Gigartina*, even though high levels of UVA and PAR have been reported to increase the induction of mechanisms for repairing damage and protection against UVB and to increase tolerance to UVB (Takayanagi et al. 1994; van De Poll et al. 2001). From our results, it is difficult to assume this. However, it can be concluded that the effects caused by UVA and UVB radiation do not remain over time, since on the exclusion of UVA and UVB during cultivation allowed the recovery of the growth rate. This would indicate that the embryos of *Gigartina* have mechanisms to avoid harm, changing the energy initially destined for growth, towards a process of protection.

In the case of *Macrocystis*, inhibiting effects of UVB were also detected in the growth of sporophytic embryos. In addition, morphological changes were observed. The interaction of these effects would have significant effects on the survival of those embryos treated with UVB. On the other hand, those surviving embryos have the capacity to acclimate to UVB, since the differences in the growth rate among the treatments were only maintained for the first 5 days and disappeared on the ninth day of cultivation.

The mechanisms that could be involved in the acclimation of embryos to UVB could be a) the establishment of physical barriers that protect the tissues (or part of them) as morphological changes, or b) the induction or synthesis of phlorotannin compounds, which have a photoprotective function in brown algae (Schoenwaelder 2002; Henry and Van Alstyne 2004). An induction of phlorotannins due to UVB and UVA radiation has been described for *Macrocystis integrifolia* (Swanson and Druehl 2002) and in zoospores of five species of Laminariales (Wiencke et al. 2004).

Phlorotannins occur in cells in vesicles known as physodes and are also deposited in cell walls (Schoenwaelder and Clayton 1998, 2000). These compounds play a role in the determination of depth zonation of brown algae (Bischof et al. 2006). Recently it was demonstrated that zoospores of Arctic *Alaria esculenta* and *Saccorhiza dermatodea* are less sensitive to UVR or have a better capacity to recover from UVR-induced stress than zoospores of species from deeper water, *Laminaria digitata*, *L. saccharina* and *L. solidungula* (Wiencke et al. 2004).

Although the existence of phlorotannins has not been shown in adults or the early development stages of *Macrocystis pyrifera*, evidence does exist that to believe that it does occur in them, or there is at least some other mechanism that protects them from UV radiation. First, although *Macrocystis* is a sublittoral alga, its apical meristem sections reach the water's surface, thus being exposted to solar radiation (PAR and UV). Consequently, it is essential to guarantee protection of the delicate meristematic cells to UV radiation by means of photoprotective compounds. Second, zoospores and embyos of *Macrocystis* preferentially grow a few centimeters from parental plants, as demonstrated by the zoospores of *Sargassum spinuligerum* (Kendrick and Walker 1991). For this reason, zoospores and embryos of *Macrocystis* would be more sensitive to UV radiation than the adults, which could be attenuating the UVB radiation that arrives in the sublittoral zone. However, in our experiments some embryos had the capacity to acclimate to UVB. This suggests the existence of a protection and acclimation system. Also, kelp phlorotannin exudates from macroalgal sources have been proposed to reduce the impact of UVB radiation by forming UV refugia for kelp zoospores within the water column (Swanson and Druehl 2002). Additionally, it has been

suggested that kelp spores and embryos are pre-adapted to the UV conditions of the parent plant (Swanson and Druehl 2000). Genetic adaptations to PAR light have been previously demonstrated within the Laminariales (Gerard 1988), and may also exist for potent environmental stresses such as UV (Swanson and Druehl 2000).

On the other hand, embryos of *Macrocystis* show morphological changes, seen as deformation in the thallus and the loss of cells, possibly from necrosis. These changes have been reported for *Laminaria ochroleuca* (Roleda et al. 2004b) and *L. solidungula* (Michler et al. 2002), and the red algae *Iridaea cordata* (Navarro 2004). In this last case, the deformation was manifest as curving of the thallus, diminishing in the area of exposure to UVB, and thus being a protective factor against radiation.

Implications Our results provide firm evidence of UVB radiation effects on the development of *Macrocystis*, *Gigartina*, *Sarcothalia* and *Mazzaella*, all of which are of great commercial and interest in southern Chile. Such effects have a potential to interfere with natural populations of these macroalgal species, as well.

In nature, the early developmental stages of macroalgae species could grow with the protection of nearby adults. Adult individuals could be attenuating the UVB radiation to the young stages, reducing radiation levels which could cause irreparable harm. The occurrence of microhabitats could favour the survival of spores and juveniles stages (Brawley and Johnson 1991). This protection has been suggested for other species (Ang 1991; Brawley and Johnson 1991; Johnson and Brawley 1998) and could attenuate the UVB effect, preventing the irreparable harm caused by high levels. Nonetheless, if the adults are extracted because of their economic importance, the result would be an increase of UVB levels reaching the intertidal and subtidal zone. In this scenario, the effects on growth and morphology may not be reversible, and the defense mechanisms might be insufficient, causing irreparable cellular damage and unbalances in early developmental stages.

The importance of kelp forests of *Macrocystis* is being increasingly recognised in southern Chile for both its role as a keystone species and ecosystem engineer. In addition *Macrocystis* has high commercial value and is under increasing pressure for industrial exploitation. Within kelp forests we also find many other species of algae, including *Gigartina* (Zamorano and Westermeier 1996), which would be affected by the future extraction of *Macrocystis*. Consequently, for both ecological and economic reasons, there is a great necessity to understand the role of UVB on natural populations of algae and their long-term recruitment systems. In addition, complementary studies regarding the effects of UVB on the carrageen and algine acid content of these species will allow researchers to better understand the real effects that this kind of radiation has on commercial species of macroalgae in Chile. A final aspect that should be included in future work is the need to incorporate an outlook of long-term recuperation of stratospheric ozone levels, which are currently projected to not reach historic levels until 2050 (WMO/UNEP 2002; Valkama et al. 2003).

Acknowledgement We gratefully acknowledge Susana Ovando, Clare Brown and Dr. Christopher Anderson for valuable suggestions and improvement of this manuscript. This work was financed in part by Project Mineduc Acuicultura PR 334 (Chilean Ministry of Education), and by Project 26600 (University of Magallanes).

References

Altamirano M, Flores-Moya A, Figueroa F (2002) Effects of the radiation and temperature on growth of germling of three species of *Fucus* (Phaeophyta). Aquat Bot 1606:1–12

Ang PO Jr (1991) Natural dynamics of a *Fucus distichus* (Phaeophyta, Fucales) population: reproduction and recruitment. Mar Ecol Prog Ser 78:71–85

Bianciotto OA, Pinedo LB, San Roman NA, Blessio AY, Collantes MB (2003) The effects of natural UV-B radiation on a perennial *Salicornia* salt-marsh in Bahía San Sebastián, Tierra del Fuego, Argentina: a 3-year field study. J Photochem Photobiol B Biol 70:177–185

Bischof K, Gómez I, Molis M, Hanelt D, Karsten U, Lüder U, Roleda MY, Zacher K, Wiencke C (2006) Ultraviolet radiation shapes seaweed communities. Rev Environmental Sci Biotechnol 5:141–166

Brawley SH, Johnson LE (1991) Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. J Phycol 27:179–186

Cordi B, Donkin ME, Peloquin J, Price DN, Depledge MH (2001) The influence of UV–B radiation on the reproductive cells of the intertidal macroalga, *Enteromorpha intestinalis*. Aquat Toxicol 56:1–11

Diaz S, Camilio C, Deferrari G, Fuenzalida H, Armstrong R, Booth C, Paladini A, Cabrera S, Casiccia C, Lovengreen C, Pedroni J, Rosales A, Zagarese H, Vernet M (2006) Symposium-in-Print: UV effects on aquatic and coastal ecosystems ozone and UV radiation over southern south America: climatology and anomalies. Photochem Photobiol 82:834–843

Dring M, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183–191

Figueroa FL (2002) Bio-optical characteristics of Gerlache and Bransfield Strait waters during an Antarctic summer cruise. Deep Sea Res II 49:675–691

Franklin L, Forster R (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32:207–237

Gerard VA (1988) Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. Mar Biol 97:25–36

Hanelt D, Wincke C, Karsten U, Nultsch W (1997) Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). J Phycol 33:387–395

Henry EB, Van Alstyne KL (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. J Phycol 40:527–533

Huovinen P, Gómez I, Figueroa F, Ulloa N, Morales V, Lovengreen C (2004) Ultraviolet-absorbing mycosporine-like amino acids in red macroalgae from Chile. Bot Mar 47:21–29

Johnson LE, Brawley SH (1998) Dispersal and recruitment of canopy-forming intertidal alga: the relative roles of propagule availability in post-settlement processes. Oecologia 117:517–526

Kendrick GA, Walker D (1991) Dispersal distances for propagules of *Sargassum spinuligerum* (Sargassaceae, Phaeophyta) measured directly by vital staining and venturi suction sampling. Mar Ecol Prog Ser 79:133–138

Kirchhoff VWJH, Zamorano F, Casiccia C (1997) UVB enhancements at Punta Arenas, Chile. J Photochem Photobiol B Biol 38:174–177

Lignel A, Pedersén M (1989) Agar composition as a function of morphology and growth rate. Studies on some morphological strains of *Gracilaria secundata* and *Gracilaria verrucosa* (Rhodophyta). Bot Mar 32:219–227

Lüning K (1980) Critical levels of light and temperature regulating the gametogenesis of three *Laminaria* species (Phaeophyceae). J Phycol 16:1–15

Makarov MV, Voskoboinikov GM (2001) The influence of ultraviolet-B radiation on spores release and growrh of kelp *Laminaria saccharina*. Bot Mar 44:89–94

Mansilla A, Werlinger C, Palacios M, Navarro NP, Cuadra P (2006) Effects of UVB radiation on the initial stages of growth of *Gigartina skottsbergii*, *Sarcothalia crispata* and *Mazzaella laminarioides* (Gigartinales, Rhodophyta). J Appl Phycol 18:451–459

Marín SL, Westermeier R, Melipillan J (2002) Simulation of alternative management strategies for red algae, luga roja, (*Gigartina skottsbergii* Setchell and Gardner) in southern Chile. Ecol Mod 154:121–133

Michler T, Aguilera J, Hanelt D, Bischof K, Wiencke C (2002) Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperature macroalgae. Mar Biol 140:1117–1127

Navarro N (2004) Efeitos da radiação UVB em *Iridaea cordata* (Gigartinales, Rhodophyta). Dissertação Universidade de São Paulo

Oliveira EC, De Paula EJ, Plastino EM, Petti R (1995) Metodologías para el cultivo no axenico de macroalgas marinas *in vitro*. In: Alveal K, Ferrario M, Oliveira E, Sar E (eds) Manual de métodos ficológicos. Universidad de Concepción, Chile, pp 429–447

Roleda M, van de Poll W, Hanelt D, Wiencke C (2004a) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. Mar Ecol Prog Ser 281:37–50

Roleda M, Hanelt D, Krabs G, Wiencke C (2004b) Morphology, growth, photosynthesis and pigment in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. Phycologia 43(5):603–613

Romo H, Avila M, Nuñez M (2001) Manual de técnicas de cultivo y repoblación de "Luga Roja" (*Gigartina skottsbergii*) Proyecto FONDEF D97I1064. IFOP- Universidad de Concepción, Chile

Schoenwaelder M (2002) The occurrence and cellular significance of physodes in brown algae. Phycologia 41:125–139

Schoenwaelder M, Clayton M (1998) Secretion of phenolic substances into the zygote wall and cell plate in embryos of *Hormosira* and *Acrocarpia* (Fucales, Phaeophyceae). J Phycol 34:969–980

Schoenwaelder M, Clayton M (2000) Physode formation in embryos of *Phyllospora comosa* and *Hormosira banksii* (Phaeophyceae). Phycologia 39:1–9

Seckmeyer G, Mckenzie RL (1992) Elevated ultraviolet radiation in New Zealand (45 S) contrasted with Germany (45 N). Nature 359:135–137

Setlow RB (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Nat Acad Sci USA 71:3363–3366

Smith RC, Prézelin BB, Baker KS, Bidigare R, Boucher N, Coley T, Karentz D, Macintyre S, Matlick H, Menzies D, Ondrusek M, Wan Z, Waters J (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959

Swanson A, Druehl L (2000) Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient. Mar Biol 136:657–664

Swanson A, Druehl L (2002) Induction, exudation and the UV protective role of kelp phlorotannins. Aquat Bot 73:241–253

Takayanagi S, Trunk J, Sutherland JC, Sutherland BM (1994) Alfalfa grown outdoor are more resistant to UV-induced DNA damage than plants grown in a UV-free environmental chamber. Photochem Photobiol 60:363–367

Valkama E, Kivimaenpaa M, Hartikainen H, Wulff A (2003) The combined effects of enhanced UV-B radiation and selenium on growth, chlorophyll fluorescence and ultrastructure in strawberry (*Fragaria* x *ananassa*) and barley (*Hordeum vulgare*) treated in the field. Agric For Meteorol 120:267–278

van De Poll W, Eggert A, Buma A, Breeman A (2001) Effects of UV-induced DNA damage and photoinhibition on growth of temperature marine red macrophytes: habitat - related differences in UV-B tolerance. J Phycol 37:30–37

Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa FL (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Mar Ecol Prog Ser 197:217–229

Wiencke C, Clayton M, Schoenwaelder M (2004) Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. Mar Biol 145:31–39

Wiencke C, Roleda M, Gruber A, Clayton MN, Bischof K (2006) Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments. J Ecol 94:455–463

Xue L, Zhang Y, Zhang T, An L, Wang X (2005) Effects of enhanced ultraviolet-B radiation on algae and cianobacteria. Crit Rev Microbiol 31:79–89

WMO/UNEP (2002) Scientific assessment of ozone depletion: 2002. Available at http://www.wmo.ch/web/arep/reports/o3_assess_rep_2002_front_page.html

Zamorano J, Westermeier R (1996) Phenology of *Gigartina skottsbergii* (Rhodophyta, Gigartinales) in Ancud Bay, southern Chile. Hydrobiologia 326/327:253–259

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Upper Saddle River, NJ