Effect of ultraviolet-B radiation on growth, photosynthetic pigments, and cell biology of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) macroalgae brown strain

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Abstract

Kappaphycus alvarezii is a seaweed of great economic importance for the extraction of *kappa* carrageenan from its cell walls. The most common strains are dark red, brown, yellow, and different gradations of green. It is known that ultraviolet radiation (UVR) affects macroalgae in many important ways, including reduced growth rate, reduction of primary productivity, and changes in cell biology and ultrastructure. Therefore, we examined the brown strain of *K. alvarezii* exposed to ultraviolet-B radiaton (UVBR) for 3 h per day during 28 days of cultivation. The control plants showed growth rates of 7.27% d^{-1}, while plants exposed to UVBR grew only 4.0% d^{-1}. Significant differences in growth rates and in phycobiliproteins between control and exposed plants were also found. Compared with control plants, phycobiliprotein contents were observed to decrease after UV-B exposure. Furthermore, the chlorophyll *a* (Chl *a*) contents decreased and showed significant differences. UVBR also caused changes in the ultrastructure of cortical and subcortical cells, which included increased thickness of the cell wall and number of plastoglobuli, reduced intracellular spaces, changes in the cell contour, and destruction of chloroplast internal organization. Reaction with Toluidine Blue showed an increase in the thickness of the cell wall, and Periodic Acid-Schiff stain showed a decrease in the number of starch grains. By the significant changes in growth rates, photosynthetic contents and ultrastructual changes observed, it is clear that UVBR negatively affects intertidal macroalgae and, by extension, their economic viability.

Additional keywords: brown strain of *Kappaphycus alvarezii*; chloroplast; growth rates; photosynthetic pigments; ultrastructure; ultraviolet-B radiation.

Introduction

The stratospheric ozone layer provides natural protection against UVR exposure for all biological organisms (Madronich 1992). It has been nearly three decades since the first reports about man-made changes in the stratospheric ozone layer, which result from atmospheric pollutants, such as chlorofluorocarbons (CFC), halocarbons, carbon dioxide (CO_2) and methyl chloroform (MCF) (Kerr and McElroy 1993). Increasingly, UVBR

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Abbreviations: APC – allophycocyanin; *ANOVA* – analysis of variance; CBB – Coomassie Brilliant Blue; CFC – chlorofluorocarbons; Chl *a* – chlorophyll *a*; CO_2 – carbon dioxide; FM – fresh mass; GRs – growth rates; LM – light microscopy; MCF – methyl chloroform; MAAs – mycosporine-like amino acids; PAR – photosynthetically active radiation; PAS – Periodic Acid-Schiff; PC – phycocyanin; PE – phycoerythrin; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; TB-O – Toluidine Blue; TEM – transmission electron microscope; UVA – ultraviolet-A radiation; UVBR – ultraviolet-B radiation; UVC – ultraviolet-C radiation; UVR – ultraviolet radiation.
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(280–320 nm) reaches the earth's surface as a result of this ozone layer depletion (Mitchell *et al*. 1992, Lubin and Jensen 1995). Consequently, UV energy induces photodamage in proteins, nucleic acids and other compounds in biological tissues (Mitchell *et al.* 1992). According to Pakker *et al*. (2000), the principal target of UVBR in the cell is nuclear DNA. When UVBR is absorbed by DNA, the formation of pyrimidine dimers can occur. This process leads to a distortion of the DNA and, consequently, the arrest of replication and transcription.

Similar to other places in middle and high latitudes in the northern and southern hemispheres (Santee *et al*. 1995, Kirchhoff *et al*. 1996, Rousseaux *et al*. 1999), southern Brazil has been exposed to a gradual increase in the levels of UVR. According to the Brazilian Institute for Space Research (INPE), this region receives natural solar radiation from 2.2–3.5 W m^{-2}, based on a daily UV index that varies from 9 to 14 during a typical summer.

Therefore, the effects of ultraviolet radiation (UVA and UVB) on biological matter have become an increasingly important issue (Holzinger and Lütz 2006). Ultraviolet radiation affects all biological organisms, especially those in the aquatic ecosystem, in many important ways. Several studies have shown a decreased macroalgae growth rate (Wood 1987), reduced primary productivity (Worrest 1983), DNA damage (Karentz *et al*. 1991), and many alterations in biochemical metabolism of marine microalgae (Premkumar *et al*. 1993). The photosynthetic process is also potentially affected by inhibiting the activity of 1,5-bisphosphate carboxylase/oxygenase (Rubisco), or D1 protein, of the photosystem II reaction center (Lesser and Shick 1994) and by altering the thylakoid membrane composition of chloroplasts (Grossman *et al*. 1993).

One of the strategies used by macroalgae to survive exposure to high levels of UVR is the synthesis and accumulation of photoprotective compounds, such as mycosporine-like amino acids (MAAs) and carotenoids, which directly or indirectly absorb UVR energy (Karsten and Wiencke 1999, Karsten *et al*. 1999, Sommaruga, 2001, Sonntag *et al*. 2007). In spite of these defense mechanisms, some papers have reported changes in the ultrastructure and cell biology of macroalgae exposed to UVBR (Poppe *et al*. 2002, 2003; Garbary *et al*. 2004, Holzinger *et al.* 2004, 2006; Holzinger and Lütz 2006, Steinhoff *et al*. 2008). These changes mainly occur in the chloroplasts, modifying the quantity, size, organization, as well as the number of thylakoids (Talarico and Maranzana 2000). *K. alvarezii* (Doty) Doty ex P. Silva is a red macroalgae that presents several colors: red, brown, yellowish and different gradations of green (Areces 1995). It exists in reef environments of the Indo-Pacific, China, Japan, the islands of Southeast Asia, and the East Africa region to Guam (Doty 1987). This seaweed is of great economic importance for the extraction from its cell walls of *kappa* carrageenan, a phycocolloid composed of polymers of sulphated galactans. This is one of the phycocolloids most used by the food, pharmaceutical, textile, and cosmetic industries because of its properties as a thickener, emulsifier, stabilizer, and gelling agent (Paula and Pereira 1998).

The native species *Hypnea musciformis* (Wulfen) Lamouroux has great potential to produce carrageenan. However, its production is dependent on natural stocks, and its seasonal cultivation is complicated, making it unstable and reducing its productivity (Oliveira 1997, Reis *et al*. 2006). Therefore, in 1995, *K. alvarezii* was introduced in Brazil to meet the increasing demand for *kappa* carrageenan. However, because of the potentially damaging effects of UVBR on the cellular structure, growth, and photosynthetic pigments of the *K. alvarezii* brown strain, we were motivated to undertake this investigation.

Materials and methods

The brown strain of *K. alvarezii* was taken from a culture collection at *LAMAR-UFSC* (Macroalgae Laboratory, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil).

Culture conditions: The apical thalli portions were selected (±50 mg) from each specimen and cultivated in the culture room of *LAMAR* for 28 days in 250 ml beakers with 200 ml natural sterilized seawater, ±34 practical salinity units (p.s.u.), with salinity enriched by 0.8 ml von Stosch medium (Edwards 1970).

Culture room conditions were 24°C temperature, continuous aeration, illumination from above with fluorescent lights (*Philips C-5 Super 84 16W/840*, São Paulo, Brazil), photosynthetically active radiation (PAR) at 80 μmol(photon) m^{-2} s^{-1} (*Li-cor* light meter *250*, Lincoln, USA), and 12 h photocycle (starting at 8 h). PAR was carried out according to Paula *et al*. (1999). UVBR was provided through a *Vilber Lourmat* lamp (*VL-6LM*, Marne La Vallée, France) with peak output at 312 nm. The intensity of UVBR was 1.6 W m^{-2} (*Radiometer-model IL 1400A*, *International Light*, Newburyport, MA, USA). To avoid exposure of UVC radiation, a cellulose diacetate foil with thickness of 0.075 mm was utilized. The apices were subjected to 3 h of UVBR exposure per day in a culture room, from 12 to 15 h. During these 3 h, the flow of air was increased in order to expose apices to UVBR irradiation all along the thalli.

A random rotation of the beaker positions was carried out so that all apices would receive the same irradiation during the experiment. The experimental space inside the culture room was isolated with thick plastic protecting the ambient environment and people from UVR exposure. Apical thalli controls were evaluated using PAR alone,

while exposed apical thalli were cultivated under PAR + UVBR. Samples for transmission electron microscopy were fixed directly on day 28, the last day of experimentation, and samples of photosynthetic pigments were frozen by immersion in liquid nitrogen on day 28, after the final exposure to UVBR at 15:00 h.

Medium was replaced weekly. Four replicates were made for each experimental group.

Growth rates (GRs): Growth rates for treatment groups and control were calculated using the following equation: GR [% day^{-1}] = $[(W_t/W_i) - 1] * 100/t$, where W_i is initial wet mass, W_t is wet mass after t days, and t is internal time (t = 28 d) (Penimann *et al*. 1986).

Pigments analysis: The content of photosynthetic pigments (Chl *a* and phycobiliproteins) of the *K. alvarezii* brown strain was analyzed between treatment group and control. Samples (fresh mass) were frozen by immersion in liquid nitrogen and kept at –40ºC until the analyses. The photosynthetic pigments were extracted according to Aguirre-von Wobeser (2001), using 0.100 g of each sample. All pigments were extracted in quadruplicate samples.

Chl *a* was extracted from approximately 0.100 g of tissue in 1 ml of dimethylsulfoxide (*DMSO, Merck*, Darmstadt, FRG) at 65ºC, during 30 min, using a glass tissue homogenizer (Hiscox and Israelstam, 1979). Pigments were quantified spectrophotometrically according to Wellburn (1994).

Phycobiliproteins: About 0.100 g of plant material was ground to a powder with liquid nitrogen and extracted at 4ºC in darkness in 0.1 M phosphate buffer, pH 6.4. The homogenates were centrifuged at 2,000 × *g* for 20 min. Phycobiliprotein levels [allophycocyanin (APC), phycocyanin (PC), and phycoerythrin (PE)] were determined by *UV-Vis* spectrophotometry, and the equations of Kursar *et al*. (1983) were used for calculations.

Light microscope (LM): Samples approximately 5 mm in length were fixed in 2.5% paraformaldehyde in 0.2 M (pH 7.2) phosphate buffer overnight. Subsequently, the samples were dehydrated in increasing series of ethanol aqueous solutions. After dehydration, the samples were infiltrated with *Historesin* (*Leica Historesin*, Heidelberg, Germany). Sections 5 µm in width were stained with different histochemical techniques and were investigated with an epifluorescent microscope (*Olympus BX 41*, *Olympus Corporation*, Tokyo, Japan) equipped with an image *Q Capture Pro 5.1 Software* (*Qimaging Corporation*, Austin, TX, USA).

Histochemical staining: LM sections were stained as follows: Periodic Acid-Schiff (PAS) was used to identify neutral polysaccharides (Gahan 1984); Toluidine Blue (TB-O) 0.5% pH 3.0 (*Merck* Darmstadt, Germany) for acid polysaccharides through a metachromatic reaction (Gordon and McCandless 1973); Coomassie Brilliant Blue (CBB) 0.02% in Clarke's solution (*Serva*, Heidelberg, Germany) for proteins (Gahan 1984). Controls consisted of applying solutions to sections without the staining component (*e.g*., omission of periodic acid application in the PAS reaction).

Transmission electron microscope (TEM): For observation under the transmission electron microscope (TEM), samples approximately 5 mm in length were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) plus 0.2 M sucrose overnight. The material was post-fixed with 1% osmium tetroxide for 4 h, dehydrated in a graded acetone series and embedded in Spurr's resin. Thin sections were stained with aqueous uranyl acetate followed by lead citrate, according to Reynolds (1963). Four replicates were made for each experimental group; two samples per replication were then examined under TEM JEM 1011 (*JEOL Ltd.* Tokyo, Japan, at 80 kV). Similarities based on the comparison of individual treatments with replicates suggested that the ultrastructural analyses were reliable.

Data analysis: Data were analyzed by unifactorial Analysis of Variance (*ANOVA*) and the *Tukey a posteriori* test. All statistical analyses were performed using the *Statistica* software package (release *6.0*), considering $p \leq 0.05$. Analyses were performed in order to evaluate the effect on the growth rates and concentration of photosynthetic pigments between treatment group and control.

Results

Growth rates: After 28 d in culture, the brown strain of *K. alvarezii* showed statistical differences ($p \leq 0.05$) in GRs between thalli cultured under PAR (control condition) and thalli cultured under a combination of PAR + UVBR (Fig. 1). UV-exposure caused a significant reduction in GRs.

Pigments: Changes in the contents of photosynthetic pigments in the brown strain of *K. alvarezii* plants exposed to UVBR are shown in Table 1. The exposed plants of *K. alvarezii* showed a decrease of Chl *a* content compared with control plants. Samples of the control plants showed significant difference ($p < 0.05$). After UVBR exposure, phycobiliprotein concentrations [allophycocyanin (APC), phycocyanin (PC) and phycoerythrin (PE)] were reduced. Phycobiliprotein contents were statistically different between control and exposed plants.

Observations under LM and histochemistry: The cortical region of the brown strain, when observed in transversal section, showed two to three layers of cells. In the outermost layer, the cells were elongated, forming a cup-shape, whereas the second and third cell layers became spherical (Fig. 2*A*).

Toluidine Blue staining showed a metachromatic reaction in the cell wall, indicating the presence of acidic polysaccharides. Orthochromatic cytoplasm was also observed (Fig. 2*A*). Samples stained with Periodic Acid-Schiff exhibited a strong reaction, suggesting the presence of cellulosic compounds in the cell wall. This reaction also occurred in the cytoplasm with neutral polysaccharides, especially with many floridean starch grains, the main substance of red algae reserve (Fig. 2 *B*). Both the cortical and subcortical cells had abundant

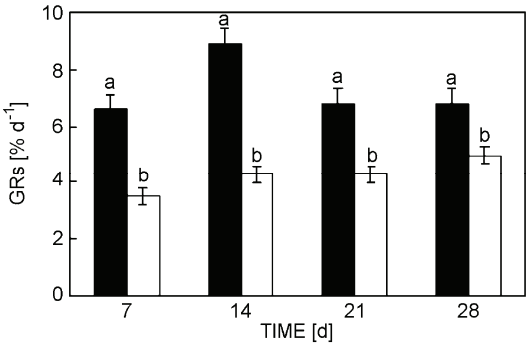

Fig. 1. Growth rates (GRs) of the brown strain of *K. alvarezii* under UVBR. Vertical bars represent ±SD for means (n = 4). Letters indicate significant differences according to the *Tukey* test ($p \leq 0.05$). ■ – control plants; □ – exposed plants.

Table 1. Changes in photosynthetic pigments [μg g^{-1}(FM)] of brown strain of *K. alvarezii* under UVBR. Means ±SD, n = 4. Different letters indicate significant differences according to the *Tukey* test ($p \leq 0.05$).

Treatment	Chl *a*	APC	PC	PE
Control plants	156±0.28[a]	270±0.50[a]	50±0.25[a]	153±0.28[a]
Exposed plants	140±0.47[b]	150±0.25[b]	30±0.25[b]	97±0.25[b]

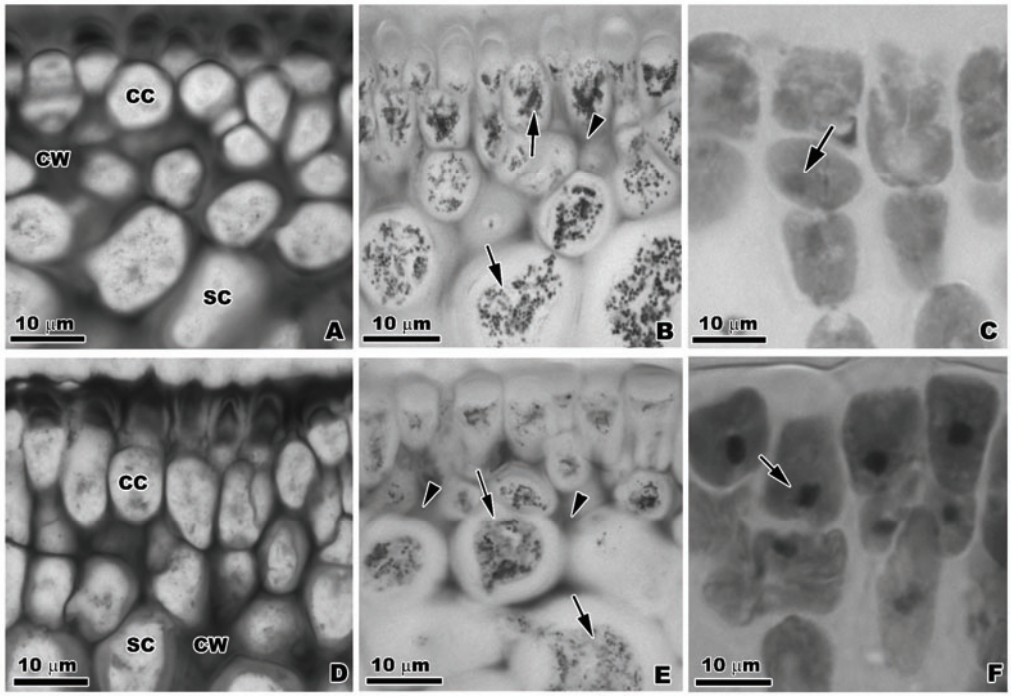

Fig. 2. Light microscopy of the transversal sections of thallus control (*A–C*) and UVBR-exposed (*D–F*) brown strain of *K. alvarezii*. *A*: Section stained with TB-O. The cell wall of cortical and subcortical cells shows metachromatic reaction. The cytoplasm shows orthochromatic reaction. *B*: Section stained with PAS. PAS-positive floridean starch grains (*arrows*) in the cortical and subcortical cells. The arrowhead indicates the positive reaction with cell wall. *C*: Section stained with CBB. Positive reaction with the nuclei (*arrow*) and with all cellular proteins. *D*: Section stained with TB-O. Metachromatic reactions in the cell wall of cortical and subcortical cells. *E*: Section stained with PAS. PAS-positive floridean starch grains (*arrows*) in the cortical and subcortical cells. The arrowheads show the PAS-positive reaction in the cell wall. *F*: Section stained with CBB. The cytoplasm shows the densest cellular reaction. The nuclei have become more evident (*arrow*). CC – cortical cell; CW – cell wall; SC – subcortical cell.

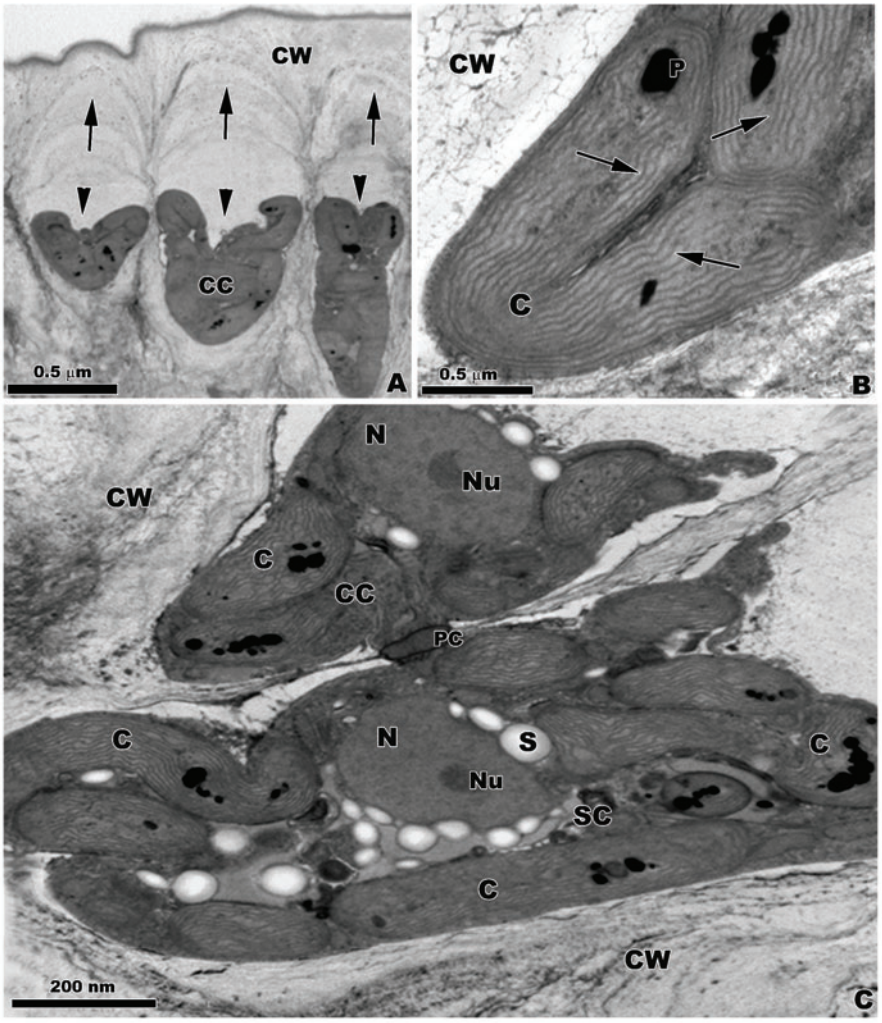

Fig. 3. Transmission electron microscopy (TEM) micrographic images of control group of brown strain of *K. alvarezii*. *A*: Detail of cortical cells showing the cup-shape (*arrowheads*) thick cell wall with concentric microfibrils embedded in an amorphous matrix (*arrows*). *B*: Detail of the chloroplast of a cortical cell. Observe the thylakoid (*arrows*) and plastoglobuli. *C*: Detail of connection of the cortical and subcortical cells between the pit connections. Observe the elongated chloroplast in subcortical cell and perinuclear starch grains. C – chloroplast; CC – cortical cell; CW – cell wall; N – nucleus; Nu – nucleolus; PC – pit connection; S – starch grain; SC – subcortical cell.

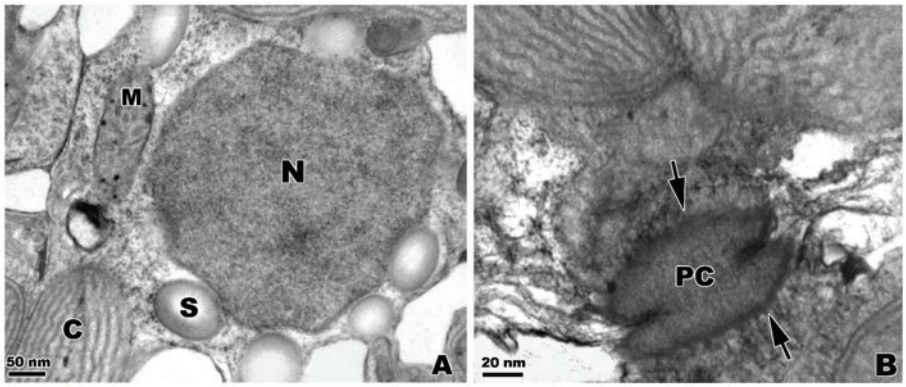

Fig. 4. TEM control group of brown strain of *K. alvarezii*. *A*: Detail of nucleus of the cortical cell, with large nucleolus. Perinuclear starch grains, chloroplast, mitochondria near the nucleus. *B*: Detail of the pit connection between cortical and subcortical cell. Note the striated pit plug (*arrows*). C – chloroplast; M – mitochondria; N – nucleus; PC – pit connection; S – starch grains.

starch grains which increased in number toward the subcortical cells (Fig. 2*B*).

Finally, when stained with Coomassie Brilliant Blue, the most external layer of the cortical region reacted more intensely than other cells. This reaction showed numerous organelles in these cells (Fig. 2*C*).

UVBR-exposed *K. alvarezii* as observed under LM: When stained with TB-O, the UVBR-exposed brown strain of *K. alvarezii* showed a reaction in the cytoplasm similar to that observed in control plant. The cytoplasm of the cortical and subcortical cells was denser as compared to the control (Fig. 2*D*).

By PAS reaction, it was also possible to detect a reduction in the density of starch grains in the cortical and subcortical cells when compared to control. However, the PAS-positive reaction was more intense in the cell wall of plants treated with UVBR (Fig. 2*E*).

Finally, the cytoplasm of cortical and subcortical cells was uniformly marked with the CBB, indicating a homogeneous distribution of organelles or structures rich in protein. However, the colour was more intense than that observed in the cytoplasm of the control cells. With this reaction, the nuclei became more evident (Fig. 2*F*).

Observations under TEM: When observed by transmission electron microscopy (TEM), the cells of the brown strain of the cortical region were surrounded by a thick cell wall. This wall was formed by concentric microfibrils embedded in an amorphous matrix which consisted of sulfated polysaccharides, such as carrageenans (Fig. 3*A*).

The chloroplasts were large, elongated and able to adjust to the cell morphology (Fig. 3*B*). The chloroplasts assumed an internal organization typical of the red algae, with unstacked, evenly-spaced thylakoids. The chloroplasts apparently consisted of an individual and flat thylakoid surrounded by a single peripheral thylakoid (Fig. 3*B*,*C*). Plastoglobuli were observed between the thylakoids (Fig. 3*B*).

Small mitochondria were present in association with the chloroplasts and nuclei (Fig. 4*A*). They were spherical to elongated, with tubuli-type structure.

In addition, cortical cells were connected with the subcortical cells through pit connections. The pit plug filled the pit connection with slightly granular and electron-dense material. This plug was covered by two membranes (Fig. 3*C*, 4*B*) and was composed of protein filling the channel between the daughter cells, resulting in partial cytokinesis.

UVBR-exposed brown strain of *K. alvarezii* as observed under TEM*:* After exposure to UVBR for 3 h per day during a 28-d period, the brown strain of *K. alvarezii* was observed to undergo some ultrastructural changes, including an increase in the thickness of the cell wall of the first cortical cells, with a concomitant increase in the number of concentric microfibrils (Fig. 5*A*). The first cortical cells lost their cup-shape and showed an irregular outline (Fig. 5*A*).

Chloroplasts of the cortical and subcortical cells also showed visible changes in ultrastructural organization with irregular morphology. The thylakoids were disrupted (Fig. 5*B*,*C*,*D*). The number of plastoglobuli increased in the chloroplasts (Fig. 5*B*,*D*). However, the mitochondria and nuclei showed no ultrastructural changes after exposure to UVBR.

Discussion

Effects of UV on GRs: The first days of cultivating the brown strain of *K. alvarezii* provided a period of acclimation for the plants exposed to UVBR. During that time, they showed lower GRs when compared to control plants. Altamiro *et al*. (2000) also reported that *Ulva rigida* C. Agardh showed a decrease in GRs during the first days of exposure to UVBR, but with no significant differences after 20 days.

According to Altamiro *et al*. (2000), intertidal macroalgae are the most likely to suffer from the effects of enhanced UVBR. The reduction in the GRs of the brown strain of *K. alvarezii* observed in this study in the first days of UVBR exposure may therefore indicate that UVR is the key factor limiting growth. According to van de Poll *et al*. (2001), growth reduction results from the combined effects of damage to several cellular components, such as proteins from PSII reaction centers and DNA. These processes are directly affected by UVR, and the ability to repair or prevent damage eventually determines the UV tolerance of species. Thus, to assess their responses to these new light conditions, it is necessary to understand the ability of macroalgae to acclimate to UVR stress.

Specifically, with increasing UVBR, as previously described, the failure of protective mechanisms can cause changes and cellular imbalances (Bowler *et al*. 1992). These imbalances lead to conformational changes in DNA molecules and a resultant breakdown in replication, transcription and translation (Lão and Glazer 1996, Buma *et al*. 2000). Such interference with these processes ultimately leads to decreased macroalgae growth rates (Wood 1987) and, finally, increased mortality (Franklin and Forster 1997). UV-mediated growth inhibition and the simultaneous occurrence of DNA damage indicate that growth is halted at the higher doses because DNA damage is not effectively repaired (Buma *et al*. 2000). The DNA molecules absorb 50% of the incident UVBR and are the primary targets of photodamage. This reduction in growth observed in the green and red strains studied in this report may also be related to the delay in the process of cell division, resulting from the formation of pyrimidine dimers (Buma *et al*. 1995, van de Poll *et al*. 2001).

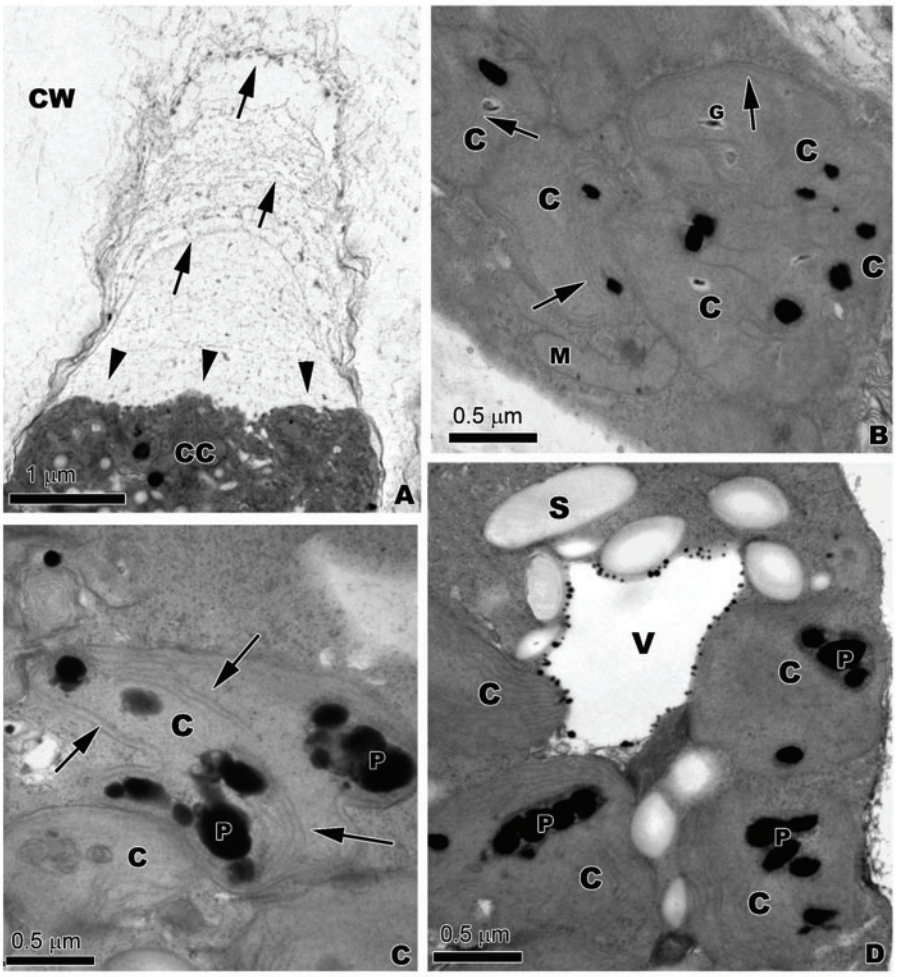

Fig. 5. TEM micrographic images of UVBR-exposed brown strain of *K. alvarezii*. *A*: Detail of cortical cells showing the thickening of cell wall (*arrows*) and cortical cell with an irregular outline (*arrowheads*). *B*: Details of chloroplasts in a cortical cell without thylakoid. Detail of disrupted chloroplast with some intact thylakoids (*arrow*) and intact genophore. Mitochondria close to the chloroplast. *C*: Detail of cortical cell showing large plastoglobuli and some preserved thylakoids (*arrows*). *D*: Detail of chloroplast of the subcortical cell with some preserved thylakoids and large plastoglobuli. Observe some starch grains near the vacuole. C – chloroplast; CC – cortical cell; CW – cell wall; G – genophore; P – plastoglobulus; S – starch grain; V – vacuole.

This, in turn, causes a decline in GRs when these strains are exposed to UVBR. The decrease in GRs observed in the brown strain of *K. alvarezii* studied here may therefore be related to the use of energy for activation of mechanisms of adaptation and repair of damage induced by UVBR. For example, Chl and other pigments in plants and photosynthetic organisms may significantly contribute to shielding of the DNA from UVR (Lao and Glazer 1996). Although algae have developed such defense mechanisms, it is important to note that the increase of UVR levels over the last several years may have rendered the process insufficient in reversing cellular damage.

UV radiation generates a decrease of photosynthetic activity that is result of various biomolecules such as: nuclei acids, lipids, and proteins being damaged due to absorption of the high energetic quanta of the waveband (Aguilera *et al*. 1999). The changes of these biomolecules may be related to the decrease in the growth rates that occurred in brown strain of *K. alvarezii* exposed to UVBR.

Our results are corroborated by findings based on studies carried out on other species of algae, which were also exposed to UVBR for three hours daily. These include *Delesseria sanguinea* (Hudson) JV Lamouroux (Pang *et al*. 2001), *Phyllophora pseudoceranoides* (S.G. Gmelin) Newroth & A.R.A, *Rhodymenia pseudopalmata* (J.V. Lamouroux) P.C. Silva, *Phycodrys rubens* (Linnaeus) Batters, and *Polyneura hilliae* (Greville) Kylin (van de Poll *et al*. 2001).

Effects of UVR on photosynthetic pigments: In the present study, Chl *a* contents decrease upon exposure to UVBR. Many investigations with red macroalgae have shown a decrease in Chl *a* concentration after UVBR exposure, including, for example, *Eucheuma strictum*

F. Schmitz cultivated *in vitro* during 16 days of exposure (Wood 1989); *K. alvarezii* cultured in long line and incubated with UVBR during 30, 60, 90, 120, 150, and 180 min (Eswaran *et al*. 2001); *Chondrus crispus* Stackhouse cultivated *in vitro* and subjected to 20 h of UVBR exposure (Yakovleva and Titlyanov 2001), *Palmaria palmata* (Linnaeus) Kuntze, and *Phycodrys rubens* (Linnaeus) Battersea (Bischof *et al*. 2000).

The study of Bischof *et al*. (2000) with five macroalgae (*Monostroma arcticum*, *Laminaria solidungula*, *Alaria esculenta*, *P. palmata*, and *Ph. rubens*) during a 72-h exposure to UVR showed that a decrease in Rubisco activity seems to play an important role in the UV-induced inhibition of photosynthesis. In addition to the change in Rubisco activity induced by UV radiation, loss of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) activity was demonstrated in the same study. However, compared with Rubisco, G3PDH was less affected.

The levels of phycobiliproteins decreased in the brown strain of *K. alvarezii* exposed to UVBR. The phycobiliproteins are located in the phycobilisomes outside of the thylakoid of chloroplasts. In red algae, phycoerythrin (PE) is used during the acclimation process; therefore, it is located more externally in the phycobilisomes (Talarico 1996). Our results demonstrated that phycobiliprotein levels, including allophycocyanin (APC), phycocyanin (PC), and phycoerythrin (PE), in the brown strain of *K. alvarezii* decreased after exposure to UVBR. These molecules absorb solar energy, transferring it to the reaction center of photosystem II (Gantt 1981).

Studies performed by Aguirre-von Wobeser *et al*. (2001) with green and red morphotype "strains" of *K. alvarezii* from the Philippines exposed to UVA+ UVB+PAR showed that decrease in both morphotypes the effective quantum yield. These authors demonstrated a greater photoinhibition of the green morphotype when compared with the red morphotype.

Our results showed a decrease in the phycobiliprotein contents similar to the findings of Eswaran *et al*. (2001) with *K. alvarezii* cultivated in long line and incubated with UVBR during 30, 60, 90, 120, 150, and 180 min. According to the same authors, PE, which is responsible for the major light harvesting function (>90%), was seriously affected by UVBR. This indicates that UVBR strongly inhibited the accumulation of phycobiliproteins. Eswaran *et al*. (2002) reported a drastic decline in the absorbance of phycobilisomes with increasing UVBR exposure time.

PE is the first pigment affected by UVR, followed by PC, APC, the carotenoids and, finally, Chl *a*, which is the most resistant (Gerber and Hader 1993, Sinha *et al*. 1995). This pattern of destruction of pigments in chloroplasts was observed in the red macroalgae *Ahnfeltiopsis concinna* (J. Agardh) PC Silva & De Cew (Beach *et al*. 2000). Similar results were observed with the cyanobacterium *Anabaena* and *Nostoc* Vaucher ex Bornete & Flahault, where the concentration of PE decreased upon exposure to UVR (Sinha *et al*. 1995).

Effects of UVR on ultrastructure as observed by light microscopy and transmission electron microscopy: The cell wall of the control and UVBR-exposed brown strain of *K. alvarezii* reacted positively to TB-O. This occurred because the cell wall contains sulfated polysaccharides in the form of carrageenan. The cell wall of cortical and subcortical cells of brown control strain of *K. alvarezii* showed a slightly positive PAS reaction. However, the reaction was more intense in the cell walls of plants exposed to UVBR, indicating greater deposition of neutral polysaccharides, possibly cellulose.

When analyzed under TEM, the control strain of *K. alvarezii* showed a microfibrillar texture with microfibrils structured in concentric layers with different degrees of compression. The increase in the thickness of the cell wall of the brown UVBR-exposed strain of *K. alvarezii* can be interpreted as a defense mechanism against exposure to ultraviolet radiation. According to Hóllosy (2002), an increase in leaf thickness has been interpreted as a protective mechanism against damage caused by UVR. Staxén and Bornman (1994) reported the alteration of the deposition of cell wall microfibrils. Microtubules, which have been implicated in the deposition of cell wall microfibrils, depolymerized when irradiated by UVBR. This caused the disruption of the cortical microtubule network of epidermal cells, leading to the alteration of cell shape and, consequently, alteration of leaf morphology.

Proteins are the main constituents of cellular organelles, and in the cytoplasm, they are present as molecules involved in various metabolic pathways (Bouzon 2006). When stained with CBB for the detection of proteins, most of the subcortical and cortical cells of the control and UVBR-exposed brown strain of *K. alvarezii* exhibited a strong nuclear reaction. The cytoplasm of cells exposed to UVBR reacted with a stronger intensity, showing a higher concentration of cytoplasmic organelles.

Repair mechanisms for UVBR-induced damage of membranes or electron transport components demand increased enzymatic activity with higher nitrogen requirements (Poppe *et al.* 2003). For example, important photosynthetic proteins, such as Rubisco or D1 protein, show an increased turnover under UVBR exposure, leading to a decrease in photosynthetic activity (Aro *et al.* 1993, Bornmann and Teramura 1993, Strid *et al.* 1994).

The nuclei of the *K. alvarezii* brown strain exposed to UVBR showed no evidence of ultrastructual changes. The same result was found in *P. palmata* (L.) O. Kuntze and *P. decipiens* (Reinsch) Ricker exposed to UVA+ UVB. In both plants, the nucleus remained intact (Poppe, *et al.* 2002, Poppe *et al.* 2003). Other studies report ultrastructural changes in mitochondria of *P. palmata* and *P. decipiens*. Changes in these species were manifested by an apparent swelling and by changes in the inner

mitochondrial membrane from a tubuli- to sacculi-type structure when exposed to UVA+UVB radiation (Poppe *et al.* 2003). The green macroalgae *Prasiola crispa* (Lightfoot) Kützing exposed to 24 h UVA+UVB radiation also showed changes in mitochondria (Holzinger *et al.* 2006). However, in the mitochondria of the *K. alvarezii* brown strain exposed to UVBR, no ultrastructural changes were observed.

In red algae, the thylakoids that are not associated with each other are free in chloroplasts. In chloroplasts, the stroma is filled by plastidial ribosomes. The chloroplasts of the brown control strain of *K. alvarezii* showed a structure very similar to that of red algae, having one peripheral thylakoid surrounded by parallel thylakoids. The number of parallel thylakoids is variable and this number mainly depends on the spatial location of the cell in the algae.

In contrast, when compared to controls, the chloroplasts of specimens exposed to UVBR show significant structural changes, including modification in the quantity, size, and organization of thylakoids. Studies on red macroalgae exposed to UVA+UVB radiation, including *P. palmata*, *P. decipiens*, *Phycodrys austrogeorgica*, and *Bangia atropurpurea* (Roth) C. Agardh, also showed ultrastructural changes in chloroplasts which manifest as dilation and disorganization of thylakoids and the formation of translucent vesicles between thylakoids (Poppe *et al*. 2003). In *Ph. austrogeorgica*, after 12 h of exposure to UVA+UVB radiation, phycobilisomes became detached from the thylakoid membranes (Poppe *et al*. 2003).

Additional changes in chloroplasts can occur after a longer period of exposure, causing changes in pigment composition. In the long term, these could be considered as acclimation strategies (Talarico and Maranzana 2000). Meanwhile, in green algae, such as *P. crispa* exposed to 24-h UVA+UVB radiation, the chloroplasts showed mild changes in thylakoids, a decrease in plastoglobuli and the appearance of numerous electron-dense cells (Holzinger *et al*. 2006). The green microalgae *Micrasterias denticulada* C. Agardh also showed disintegration of the grana and a loss of the net-like arrangement of the thylakoids under UVR (Meindl and Lutz 1996, Lütz *et al*. 1997).

Photosystem II (PSII) is a major target of UVBR in plants and macroalgae (Post *et al*., 1992; Neale *et al*. 1993, Vass 1997). According to Holzinger *et al.* (2004), PSII photochemical efficiency in *P. palmata* and *Odonthalia dentata* (Linnaeus) Lyngbye strongly decreased to about one third the initial value under UV. Since UVBR radiation increases the difficulty of establishing a proton gradient across the thylakoid membrane, photosynthetic reactions will be impaired (Poppe *et al*. 2003). Other reports of UVBR-induced changes include the depolarization of membrane potential, net leakage of Cl^-, Na^+ and K^+, and different ATPase activities in cell membranes (Doughty and Hope 1973, Murphy 1983, Gallo *et al*. 1989) and in thylakoid membranes (Iwanzik *et al*. 1983, Chow *et al.* 1992, Hideg and Vass 1996).

The electron-dense lipid droplets described in the chloroplast of *K. alvarezii* are plastoglobuli and are interpreted as lipid material with a reserve role. The number of plastoglobuli increased when exposed to UVBR in the brown strain of *K. alvarezii*. Similar results were reported with formation of plastoglobuli in *P. palmata* and *O. dentata* (Holzinger *et al*. 2004) and with the zoospores of the brown macroalgae *Laminaria hyperborea* (Gunnerus) Foslie (Steinhoff *et al*. 2008) after exposure to UVR.

In summary, the present study demonstrates that UVBR negatively affects the intertidal macroalgae. According to Han *et al*. (2004), in recent years, there has been considerable interest in the physiological and ecological consequences of UVBR in various organisms, partially because UVBR at the earth's surface is increasing as a consequence of a global decrease in stratospheric ozone concentrations. In the case of the present study, this became obvious after 3 h of daily exposure to UVBR over a 28-d experimental period, resulting in ultrastructural damage changes observed primarily in the internal organization of chloroplasts and cell wall of the *K. alvarezii* brown strain. Moreover, this exposure might have caused photodamage and photoinhibition of photosynthetic pigments, leading to a decrease in photosynthetic efficiency and a decrease in growth rates. The relevance of these findings is highlighted both by economic consequences and the current index of stratospheric ozone depletion, especially in the southern hemisphere.

References

Aguilera, J., Karsten, U., Lippert, H., Vögele, B., Philipp, E., Hanelt, D., Wiencke, C.: Effects of solar radiation on growth, photosynthesis and respiration of macroalgae from the Arctic. – Mar. Ecol. Progress Ser. **191**: 109-119, 1999.

Aguirre-von-Wobeser, E., Figueroa, F.L., Cabello-Pasini, A.: Photosynthesis and growth of red and green morphotypes of *Kappaphycus alvarezii* (Rhodophyta) from the Philippines. – Mar. Biol. **138**: 679-686, 2001.

Altamirano, M., Flores-Moya, A., Figueroa, F.L., Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated *in situ*. – Bot. Mar. **43**: 119-126, 2000.

Areces, A.J.: [Commercial cultivation of carrageenophytes genera *Kappaphycus* Doty.] – In: Alveal, K., Ferrario, M.E., Oliveira, E.C., Sar, E. (ed.): Phycologycal Methods Manual. Pp. 529-549. University of Concepción, Concepción 1995. [In Spanish]

Aro, E.-M., Virgen, I., Andersson, B.: Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. –

Biochim. Biophys. Acta **1143**: 113-134, 1993.
Beach, K.S., Smith, C.M., Okano, R.: Experimental analysis of rhodophyte photoacclimation to PAR and UV-radiation using *in vivo* absorbance spectroscopy. – Bot. Mar. **43**: 525-536, 2000.
Bischof, K., Hanelt, D., Wiencke, C.: Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. – Planta **211**: 555-562, 2000.
Bornmann, J. F., Teramura, A. H.: Effects of ultraviolet-B radiation on terrestrial plants. – In: Young, A.R. , Björn, L.O., Moan, J., Nultsch, W.(ed.). Environmental UV Photobiology . Pp. 427-471. Plenum Press, New York – London 1993.
Bouzon, Z.L.: Histochemistry and ultrastructure of the ontogenesis of the tetrasporangia of Hypnea musciformis (Wulfen) J. V. Lamour. (Gigartinales, Rhodophyta). – Rev. Bra. Bot. **29**: 229-238, 2006. [In Portuguese]
Bowler, C., van Montagu, M., Inzé, D.: Supeoxide dismutase and stress tolerance. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **43**: 83-116, 1992.
Buma, A.G.J., van Hannen, E.J., Roza, L., Veldhuis, M.J.W., Gieske, W.W.C.: Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. – J. Phycol. **31**: 314-321, 1995.
Buma, A.G.J., van Oijen, T., van de Poll, W., Veldhuis, M.J.W., Gieskes, W.W.C.: The sensitivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. – J. Phycol. **36**: 296-303, 2000.
Chow, W.S., Strid, A., Anderson, J.M.: Short-term treatment of pea plants with supplementary Ultraviolet-B radiation: Recovery time-courses of some photosynthetic functions and components. – In: Murata, N, (ed.).: Research in Photosynthesis. Pp. 361-364. Kluwer Academic Publishing, Dordrecht 1992.
Doty, M.S., Caddy, J.F., Santelices, B.: The production and use of *Eucheuma*. – In: Doty, M.S., Caddy, J.F., Santelices, B. (ed.): Case Studies of Seven Commercial Seaweed Resources. Pp. 123-164. FAO United Nations Fisheries Technical Paper, Rome 1987.
Doughty, C.J., Hope, A.B.: Effects of ultraviolet radiation on the membranes of *Chara corallina*. – Membr. Biol. **13**: 185-198, 1973.
Edwards, P.: Illustrated guide to the seaweeds and sea grasses in the vicinity of Porto Arkansas, Texas. – Contr. Mar. Sci. **15**: 1-228, 1970.
Eswaran, K., Subba Rao, P.V., Mairh, O.P.: Impact of ultraviolet-B radiation on Kappaphycus alvarezii (Solieraceae, Rhodophyta). – Indian J. Mar. Sci. **30**: 105-107, 2001.
Eswaran, K., Mairh, O.P., Subba Rao, P.V.: Inhibition of pigments and phycocolloid in a marine red algae Gracilaria edulis by ultraviolet-B radiation. – Biol. Plant. **45**: 157-159, 2002.
Franklin, L.A., Forster, R.M.: The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. – Eur. J. Phycol. **32**: 207-232, 1997.
Gantt, E.: Phycobilisomes. – Annu. Rev. Plant Physiol. **32**: 327-347, 1981.
Gahan, P.B.: Plant Histochemistry and Cytochemistry: An Introduction. – Academic Press, London 1984.
Gallo, R.L., Kochevar, I.E., Granstein, R.D.: Ultraviolet radiation induces a change in cell membrane potential *in vitro*: a possible signal for ultraviolet radiation induced alteration in cell activity. – J. Photochem. Photobiol. **49**: 655-662, 1989.
Garbary, D.J., Kim, K.Y., Hoffman, J.: Cytological damage to the red alga *Griffithsia pacifica* from ultraviolet radiation. – Hydrobiologia **512**: 165-170, 2004.
Gerber, S., Häder D.P.: Effect of solar irradiation on motility and pigmentation of three species of phytoplankton. – Environ. Exp. Bot. **33**: 515-521, 1993.
Gordon, E.M., McCandless, E.L.: Ultrastructure and histochemistry of *Chondrus crispus* Stackhouse. – In: Harvey, M.J., McLachlan, J. (ed.): *Chondrus Crispus*. Pp. 111-133. Nova Scotian Institute Science, Halifax 1973.
Grossman, A. R., Schaefer, M.R., Chiang, G.G., Collier, J.L.: The phycobilisome a light harvesting complex responsive to environmental conditions. – Microbiol. Rev. **57**: 725-749, 1993.
Han, T., Kong, J.A., Han, Y. S., Kang, S.H., Häder, D.P.: UV-A/blue light-induced reactivation of spore germination in UV-B irradiated *Ulva pertusa* (Chlorophyta). – J. Phycol. **40**: 315-322, 2004.
Hideg, É., Vass, I.: UV-B induced free radical production in plant leaves and isolated thylakoid membranes. – Plant Sci. **115**: 251-260. 1996.
Hóllosy, F.: Effects of ultraviolet radiation on plant cells. – Micron **33**: 179-197, 2002.
Holzinger, A., Lütz, C., Karsten, U., Wiencke, C.: The effect of ultraviolet radiation on ultrasctruture and photosynthesis in the red macroalgae *Palmaria palmata* and *Odonthalia dentata* from Artic waters. – Plant Biol. **6**: 568-577, 2004.
Holzinger, A., Karsten, U., Lütz, C., Wiencke, C.: Ultrastructure and photosynthesis in the supralittoral green macroalga *Prasiola crispa* from Spitsbergen (Norway) under UV exposure. – Phycologia **45**: 168-177, 2006.
Holzinger, A., Lütz, C.: Algae and UV irradiation: Effects on ultrastructure and related metabolic functions. – Micron **37**: 190-207, 2006.
Iwanzik, W., Tevini, M., Dohnt, G., Voss, M.; Weiss, W.; Gräber, P.; Renger, G.: Action of UV-B radiation on photosynthetic primary reactions in spinach chloroplasts. – Physiol. Plant. **58**: 401-407, 1983.
Karentz, D., Cleaver, J.E., Mitchell, D.L.: DNA damage in the Antarctic. – Nature **350**: 28, 1991.
Karsten, U., Wiencke, C.: Factors controlling the formation of UV-absorbing mycosporine-like amino acids in the marine red alga *Palmaria palmata* from Spitsbergen (Norway). – J. Plant Physiol. **155**: 407-415, 1999.
Karsten, U., Bischof, K., Hanelt, D., Tüg, H., Wiencke, C.: The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). – Physiol. Plant **105**: 58-66, 1999.
Kerr, J.B., Mc Elroy, C. T.: Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. – Science **262**: 1032-1034, 1993.
Kirchhoff, V.W.J.H., Schuch, N.J., Pinheiro, D.K., Harris, J.M.: Evidence for ozone hole perturbation at 30º South. – Atmospheric Environ. **30**: 1481-1484, 1996.
Kursar, T.A., van Der Meer, J., Alberte, R.S.: Light-harvesting system of the red alga *Gracilaria tikvahiae*. I. Biochemical analyses of pigment mutations. – Plant Physiol. **73**: 353-360, 1983.
Lão, K., Glaser, A.N.: Ultraviolet-B photodestruction of light-harvesting complex. – Proc. Nat. Acad. Sci. USA **93**: 5258-5263, 1996.
Lesser, M.P., Shick, J.M.: Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia*

pallida: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. – Mar. Biol. **102**: 243-255, 1994.

Lubin, D., Jensen, E. H.: Effects of clouds and stratospheric ozone depletion on ultraviolet-radiation trends. – Nature **377**: 710-713, 1995.

Lütz, C., Seidlitz, H.K., Meindl, U.: Physiological and structural changes in the chloroplast of the green alga *Micrasterias denticulata* induced by UV-B simulation. – Plant Ecol. **128**: 55-64, 1997.

Madronich, S.: Implications of recent total atmospheric ozone measurements for biological active ultraviolet radiation reaching the Earths surface. – Geophys. Res. Lett. **19**: 37-40, 1992.

Meindl, U., Lütz, C.:Effects of UV irradiation on cell development and ultrastructure of green alga *Micrasterias*. – J. Photochem. Photobiol. B-Biol. **36**: 285-292, 1996.

Mitchell, D.L., Jen, J., Cleaver, J.E.: Sequence specificity of cyclobutane pyrimidine dimers in DNA treated with solar (ultraviolet B) radiation. – Nucleic Acids Res. **20**: 225-229, 1992.

Murphy, T. M.: Membranes as targets of ultraviolet radiation. – Physiol. Plant **58**: 381-388. 1983.

Neale, P. J., Cullen, J. C., Lesser, M. P., Melis, A.: Physiological bases for detecting and predicting Photoinhibition of aquatic photosynthesis by PAR and UV radiation. – In: Yamamoto, H.Y., Smith, C.M. (ed.) Photosynthetic Responses to the Environment. Pp. 61-77. Amer. Soc. Plant Physiol. Rockville 1993.

Oliveira, E.C.: Seaweed: A feature still little exploited by Brazil. – Panorama da Aqüicul. **7**: 24-26, 1997. [In Portuguese]

Pakker, H., Martins, R.S.T., Boelen, P., Buma, A.G.J., Nikaido, O., Breeman, A.M.: Effects of temperature on the photoreactivation of ultraviolet-b-induced DNA damage in *Palmaria palmata* (Rhodophyta). – J. Phycol. **36**: 334-341, 2000.

Pang, S., Gomez, I., Lüning, K.: The red macroalga *Delesseria sanguinea* as a UVB-sensitive model organism: selective growth reduction by UVB in outdoor experiments and rapid recording of growth rate during and after UV pulses. – Eur. J. Phycol. **36**: 207-216, 2001.

Paula, E.J., Pereira, R.T. L.: The "marinomia" mariculture of exotic seaweed *Kappaphycus alvarezii* to carrageenans produce in Brazil. – Panorama da Aqüicul. **8**: 10-15, 1998. [In Portuguese]

Penniman, C.A., Mathieson, A.C, Penniman C.E.: Reproductive phenology and growth of *Gracilaria tikvahiae* McLachlan (Gigartinales, Rhodophyta) in the Great Bay Estuary, New Hampshire. – Bot Mar. **29**: 147-154, 1986.

Poppe, F., Hanelt, D. Wiencke, C.: Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic Red Alga *Palmaria decipiens* during acclimation to UV radiation. – Bot. Mar. **45**: 253-261, 2002.

Poppe, F., Schmidt, R.A.M., Hanelt, D., Wiencke, C.: Effects of UV radiation on the ultrastructure of several red algae. – Phycol. Res. **51**: 11-19, 2003.

Post, A., Gentle, S., Larkum, A.W.S.: Algal photosynthesis: inhibition by UV-B radiation, recovery and UV-absorbing pigments. – In: Murata, N. (ed.): Research in Photosynthesis. Pp. 847-850. Kluwer Academic Publ., Dordrecht – Boston – London 1992.

Premkumar, A., Eswaran, K., Kulandaivelu, G.: Growth, biochemical and photosynthetic responses of greengram seedlings under water stress to ultraviolet-B radiation. – Plant Physiol. Biochem. **20**: 31-35, 1993.

Reis, R.P.: Caldeira, A.Q.: Miranda, A.P.S., Barros-Barreto, M.B.: Potencial of carrageenophyte mariculture *Hypnea musciformis* (Wulfen) J.V. Lamour. (Gigartinales - Rhodophyta) in Marambaia Island, Baía de Sepetiba, RJ, Brazil. – Acta Bot. Bras. **20**: 763-769, 2006. [In Portuguese]

Reynolds, E.S.: The use of lead citrate at light pH as an electron opaque stain in electron microscopy. – J. Cell. Biol. **17**: 208-212, 1963.

Rousseaux, M.C., Ballaré, C.L., Giordano, C.V., Scopel, A.L., Zima, A.M., Swarcberg-Bracchitta, M., Searles, P.S., Caldwell, M.M., Díaz, S.B.: Ozone depletion and UVB radiation: impact on plant DNA damage in southern South America. – Proc. Natl. Acad. Sci. USA **96**: 15310-15315, 1999.

Santee, M.L., Read, W.G., Waters, J.W., Froidevaux, L., Manney, G.L., Flower, D.A, Jarnot, R.F., Harwood, R.S., Peckham, G.E.: Interhemispheric differences in polar stratospheric HNO_3, H_2O, ClO, and O_3. – Science **267**: 849-852, 1995.

Sinha, R.P., Lebert, M., Kumar, A., Kumar, H.D., Hader, D.P.: Spectroscopic and biochemical analyses of UV effects on phycobiliproteins of *Anabaena* sp and *Nostoc carmium*. – Bot. Acta **108**: 87-92, 1995.

Sommaruga, R.: The role of solar UV radiation in the ecology of alpine lakes. – J. Photochem. Photobiol. B-Biol. **62**: 35-42, 2001.

Sonntag, B., Summerer, M., Sommaruga, R.: Sources of mycosporine-like amino acids in planktonic *Chlorella*-bearing ciliates (Ciliophora). – Freshwater Biol. **52**: 1476-1485, 2007.

Staxén, I., Bornman, J.F.: A morphological and cytological study of *Petunia hybrida* exposed to UV-B radiation. – Physiol. Plant **91**: 735-740, 1994.

Steinhoff, F.S., Wiencke, C., Müller, R., Bischof, K.: Effects of ultraviolet radiation and temperature on the ultrastructure of zoospores of the brown macroalga *Laminaria hyperborea.* – Plant Biol. **10**: 388-397, 2008.

Strid, A., Chow, W.S., Anderson, J. M.: UV-B damage and protection at the molecular level in plants. – Photosynth. Res. **39**: 475-489, 1994.

Talarico, L.: Phycobiliproteins and phycobilisomes in red algae: Adaptive responses to light. – Sci. Mar. **60**: 205-222, 1996.

Talarico, L., Maranzana, G.: Light and adaptative responses in red macroalgae: an overview. – J. Photochem. Photobiol. B-Biol. **56**: 1-11, 2000.

van de Poll, W.H., Eggert, A., Buma, G.J., Breeman, A.M.: Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. – J. Appl. Phycol. **37**: 30-37, 2001.

Vass, I.: Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus. – In: Pessarakli, M. (ed.). Handbook of photosynthesis. Pp. 931-949. Marcel Dekker, New York – Basel – Hong Kong 1997.

Wellburn, A.R.: The spectral determination of chlorophyll *a* and chlorophyll *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. – J. Plant Physiol. **144**: 307-313, 1994.

Wood, W.F.: Effect of solar ultraviolet radiation on the kelp *Ecklonia radiata*. – Mar. Biol. **96**: 143-150, 1987.

Wood, W.F.: Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet

radiation. – Aquat. Bot. **33**: 41-5, 1989.
Worrest, R.C.: Impact of solar ultraviolet-B radiation (290-320 nm) upon marine microalgae. – Physiol. Plant **58**: 428-434, 1983.
Yakovleva, I.M., Titlyanov, E. A.: Effect of high visible and UV irradiance on subtidal *Chondrus crispus*: stress, photo-inhibition and protective mechanism. – Aquat. Bot. **71**: 47-61, 2001.