

# Effects of solar UV radiation on germination of conchospores and morphogenesis of sporelings in *Porphyra haitanensis* (Rhodophyta)

Hongxia Jiang · Kunshan Gao · E. Walter Helbling

Received: 1 August 2006 / Accepted: 4 January 2007 / Published online: 15 February 2007  
© Springer-Verlag 2007

**Abstract** The effects of ultraviolet radiation (UVR 280–400 nm) on the germination of *Porphyra haitanensis* conchospores and on the growth and morphogenesis of the subsequent sporelings were investigated by culturing the released conchospores under natural sunlight from 29 September to 6 October 2005. Germination increased with time and was faster when UV-B was excluded using cut-off filters. There were significant negative effects of UV-B radiation on growth and cell division of sporelings, with decreases up to 18% for thallus length, between 6 and 18% for thallus width, up to 29% for thallus area, and between 6 and 14% for cell size as compared to PAR-controls. UV-A had a significant positive effect on morphogenesis, enhancing the formation of sporelings with cells dividing transversely; on the other hand, UV-B delayed the formation of such sporelings. We also tested the effects of solar UVR on the growth of *P. haitanensis* juveniles

and found no significant effects. Our results indicate that UV-A has an important role in the germination and morphogenesis of the species, but on the other hand, sporelings of *P. haitanensis* are more sensitive to UV-B radiation than juveniles.

## Introduction

Solar UV-B radiation (280–315 nm) reaching the earth's surface has been increasing due to the depletion of the stratospheric ozone layer (Lubin and Jensen 1995). The primary production of algae in aquatic media might be affected, as biologically effective doses of UV-B can penetrate deep into the water column (Franklin and Forster 1997).

Previous studies showed that ultraviolet radiation (UVR 280–400 nm) can affect macroalgae, and various targets might be implicated such as photosynthesis (Franklin and Forster 1997), DNA (Pakker et al. 2000a, b), pigments (Aguilera et al. 1999a) and enzymes related to nutrient uptake, such as nitrate reductase and carbonic anhydrase (Flores-Moya et al. 1998). UVR also influences the content of UV-absorbing compounds (Korbee Peinado et al. 2004; Han and Han 2005), production of reactive oxygen species (Aguilera et al. 2002) and spore movement of macroalgae (Flores-Moya et al. 2002). However, short-term responses to UVR do not usually provide enough information to relate to the long-term effects. For example, *Laminaria ochroleuca* showed partial acclimation to chronic UVR exposure in photosynthesis, but not in growth (Roleda et al. 2004). Growth is an important parameter that integrates stress effects in several biochemical processes within the cell

---

Communicated by K. Yin.

---

H. Jiang · K. Gao (✉)  
Marine Biology Institute, Shantou University,  
Shantou, Guangdong, 515063, China  
e-mail: ksgao@stu.edu.cn

K. Gao  
State Key Laboratory of Freshwater Ecology  
and Biotechnology, Institute of Hydrobiology,  
Chinese Academy of Sciences, Wuhan,  
Hubei, 430072, China

E. W. Helbling  
Estación de Fotobiología Playa Unión and  
Consejo Nacional de Investigaciones Científicas y  
Técnicas (CONICET), Casilla de Correos No 15 - (9103),  
Rawson, Chubut, Argentina

(Altamirano et al. 2000) and indispensable to estimate the impact of future ozone depletion and resulting enhancement of UV-B radiation on productivity. Previous studies on the impact of UV-B on spore germination (Han et al. 2004) and growth of macroalgae (Dring et al. 1996; Makarov 1999; Altamirano et al. 2000; Pang et al. 2001; Roleda et al. 2006) concluded that macroalgae had different degrees of growth inhibition due to UV-B. The growth rate of the eulittoral green alga, *Ulva rigida*, was enhanced (50%) in the absence of UV-B after 1 week of exposure, but no differences were found after 20 days (Altamirano et al. 2000). Furthermore, UV-B has no effects on eulittoral species, but significantly inhibited the growth of sublittoral red macrophytes (van de Poll et al. 2001).

Different life stages of macroalgae showed different sensitivity to light stress (Dring et al. 1996; Hanelt et al. 1997). Most studies conducted to evaluate the impact of UV-B on seaweeds used macrothallus stages; however, early developmental life stages of intertidal algae seemed to be more sensitive to UVR than adult stages (Major and Davison 1998; Coelho et al. 2000; Hoffman et al. 2003). Studies to establish the sensitivity of early developmental stages are critical since the survival and growth of these stages will determine the recruitment of a species and thus productivity.

*Porphyra haitanensis* is endemic in the southern part of China and is commercially cultivated. In its natural habitat, it grows in the eulittoral zone and is frequently exposed to air and thus to full solar radiation during low tides, which may threaten the survival of the species. So far, most of the growth-related studies have been done in the laboratory (Dring et al. 1996; van de Poll et al. 2001; Roleda et al. 2006), but results from natural sunlight exposure are essential in order to try to extrapolate to the field. The aim of this study was to determine the sensitivity of *P. haitanensis* conchospores, the subsequent sporelings and juveniles to solar UVR, by investigating germination of the conchospores, growth and morphogenesis of the sporelings, as well as the growth of juveniles.

## Materials and methods

### Plant materials and maintenance

Conchospores of *P. haitanensis*, Chang and Zheng, were collected at 11:00 am after being released from the mature conchocelis, in shells cultivated indoor at Nori cultivation base of Yingke Seaweed Cultivation Ltd. during September 2005. The conchospores were transported to a nearby laboratory (20 min) in a flask

containing seawater and covered with a black bag. Both the cultivation base and the laboratory were located in Nan'ao Island (23.47°N, 117.09°E), China. In the laboratory, the spore suspension was filtered through gauze, thereby allowing debris to be removed. The spores were allowed to settle on rectangle coverslips (26 × 21 mm) with 6–8 spores in one field of view under 100× magnification in a microscope (Olympus BX50). The coverslips with the settled spores were transferred to three UV-transparent quartz containers (length 29 cm, width 17 cm, height 5 cm), each container with 10 coverslips and 1,500 ml filtered (Whatman GF/F) and sterilized, enriched seawater (F medium, Guillard and Ryther 1962). The cultures were maintained in the lab at 23–25°C under dim window light before being used in experiments the following day.

Juvenile *P. haitanensis* (3–6 cm long, 1.0–1.5 cm wide) with no visible epiphytes were collected from the farmed areas around Nan'ao Island during November 2004 and transported to the laboratory, and maintained in filtered aerated seawater at room temperature (15–20°C) under window light ( $\leq 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) before being used for the experiments the day after collection.

### Radiation measurements and treatments

Outdoor incident solar radiation was monitored with an ELDONET radiometer (Real Time Computers, Erlangen, Germany) permanently installed on the roof at Shantou University (23.42°N, 116.59°E). This instrument is equipped with sensors for three wavelength bands: 400–700 nm (PAR), 315–400 nm (UV-A) and 280–315 nm (UV-B), and an Ulbricht integrating sphere to collect direct and indirect radiation, recording at 1-min intervals.

Three radiation treatments were implemented under sunlight using UV-cutting off filters: (1) samples exposed to PAR alone (P), quartz containers or tubes covered with an Ultraphan 395 filter (UV Opak, Dige-fra, Munich, Germany); (2) samples exposed to PAR + UV-A (PA), quartz containers covered with a Folex 320 filter (Folex, Dreieich, Germany) and (3) samples exposed to PAR + UV-A + UV-B (PAB), uncovered quartz containers. The spectra of these materials are published in Figueroa et al. (1997). PAR levels inside the containers were determined using a spherical micro sensor (Li-Cor, US-SQS/L) that was intercalibrated with the ELDONET radiometer. The filters that were placed above the open containers to allow airflow through reflect 8% PAR, so that the samples in the uncovered quartz containers received about

8% higher PAR. One option to account for this difference is to also cover the quartz tubes with a film filter that cuts at 295 nm, as have been done in other studies (Figuroa et al. 1997). We decided against the use of such a filter, as this would cut short wavelengths in the UV-B that are important at our tropical location. Since our study focused on the effects of UVR, we preferred to have this small difference in the PAR and not in the UV-B part of the spectra. The quartz containers holding the coverslips were maintained in a flow-through water bath for temperature control ( $27 \pm 2^\circ\text{C}$ ) and exposed to solar radiation for 8 days without aeration. Half of the volume of the enriched seawater in each treatment was renewed every day.

In the case of the juvenile experiments, 50 individuals were randomly placed into nine 800-ml quartz tubes (inner diameter 5.4 cm, length 35 cm) with 500 ml enriched seawater and aerated at a flow rate of about  $0.4\text{--}0.5\text{ l min}^{-1}$ . The cultures were maintained in a flow-through water bath for temperature control within a range of  $25 \pm 2^\circ\text{C}$  from 20 to 25 November 2004. Half of the volume of the enriched seawater in each treatment was renewed every day.

#### Observation of germination, growth and morphogenesis

Two coverslips with the settled spores from each radiation treatment were taken at 8:00 am every other day for observation. Germination percentage was determined by counting the number of spores that germinated producing at least a rhizoid cell and a blade cell and then divided by the total number of spores in ten fields of view that were chosen randomly under  $100\times$  magnification.

The length of the germinated spores, from the base of the rhizoid to the distal end of the sporelings (not including the rhizoid), and the width, across the widest portion of the sporelings, were measured with the eyepiece micrometer that was demarcated by a stage micrometer. Both the observed sporelings and the stage micrometer were photographed using the same magnification with a digital camera (Canon S50), which was placed tightly above the eyepiece. The photographs were analyzed using the software Adobe Photoshop (version 7.0) to get the pixels of each thallus and a square ( $10,000\ \mu\text{m}^2$ ) drawn in the photograph of a stage micrometer. And then the pigmented thallus area was determined with the pixel ratio of the thallus to the square and the area of the square. Cell size was expressed as the area (in the plane of the thallus) per cell; this was determined by dividing the thallus area by the number of cells in the thallus. At the same time, the

sporeling size in terms of cell number per thallus was recorded and then the percentage of sporelings of different sizes in all observed individuals was counted to get a frequency distribution based on size.

The relative inhibition (%) due to UV-B was calculated as  $\text{Inh}_{\text{UV-B}} (\%) = (P_{\text{PA}} - P_{\text{PAB}}) / (P_{\text{P}}) \times 100$ , where  $P_{\text{P}}$ ,  $P_{\text{PA}}$  and  $P_{\text{PAB}}$  represented the values for samples exposed to PAR, PAR + UV-A and PAR + UV-A + UV-B, respectively. The relative growth rates (RGR) of sporelings and juveniles were estimated from changes in surface area, via the equation  $\text{RGR} (\% \text{ d}^{-1}) = 100 \times (\ln W_f - \ln W_0) \times t^{-1}$ , where  $W_0$  was the initial surface area, and  $W_f$  the surface area after 6 days ( $t$ ) of incubation under different treatments. The surface area of juveniles was estimated by taking photographs of the thalli and one known square ( $4\text{ cm}^2$ ) and applying the pixel-area ratio to obtain the area of each thallus.

In order to get information about the morphogenesis of the sporelings, the percentage of individuals containing cells dividing transversely was recorded in all observed ones.

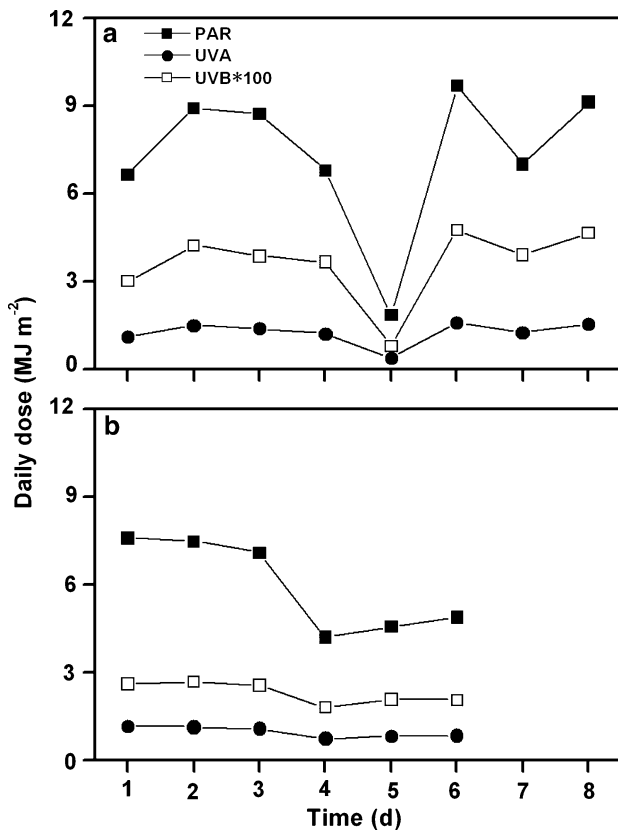
#### Statistics

The data were expressed by the mean values  $\pm$  standard deviation ( $n \geq 3$ ). Statistical significance among different treatments was tested with an ANOVA test at a significant level of  $P < 0.05$ .

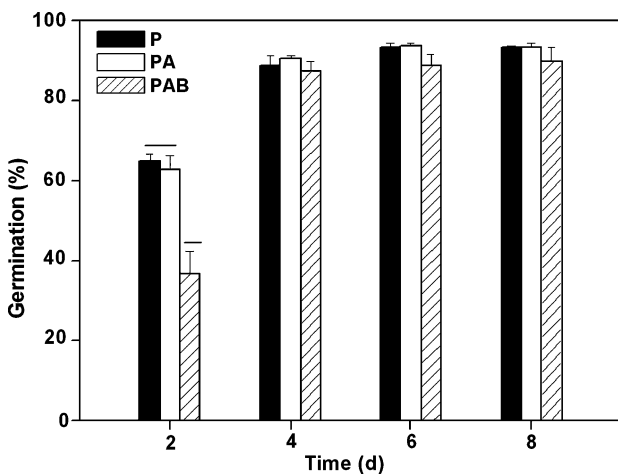
## Results

Solar radiation data during the experimental periods are shown in Fig. 1. Daily doses of solar radiation during the experiments of *P. haitanensis* conchospores and sporelings (29 September–6 October 2005) varied from 1.8 to  $9.7\text{ MJ m}^{-2}$  for PAR, from 0.3 to  $1.6\text{ MJ m}^{-2}$  for UV-A and from 8 to  $47\text{ kJ m}^{-2}$  for UV-B (Fig. 1a); and those during the experiments of *P. haitanensis* juveniles (20–25 November 2004) varied from 4.2 to  $7.6\text{ MJ m}^{-2}$  for PAR, from 0.7 to  $1.1\text{ MJ m}^{-2}$  for UV-A and from 18 to  $27\text{ kJ m}^{-2}$  for UV-B (Fig. 1b). The observed variability between daily doses was due to cloud cover.

The percentage germination of *P. haitanensis* conchospores increased with time in all radiation treatments, but there was a significant inhibition due to UV-B during the first 2 days of exposure (Fig. 2). The percentages of germination on day 2 were 37, 63 and 65% for samples exposed in the PAB, PA and P treatments, respectively. As the experiment progressed, germination in the PAB treatment had no differences with the PA and P treatments, reaching all 90% after day 4.

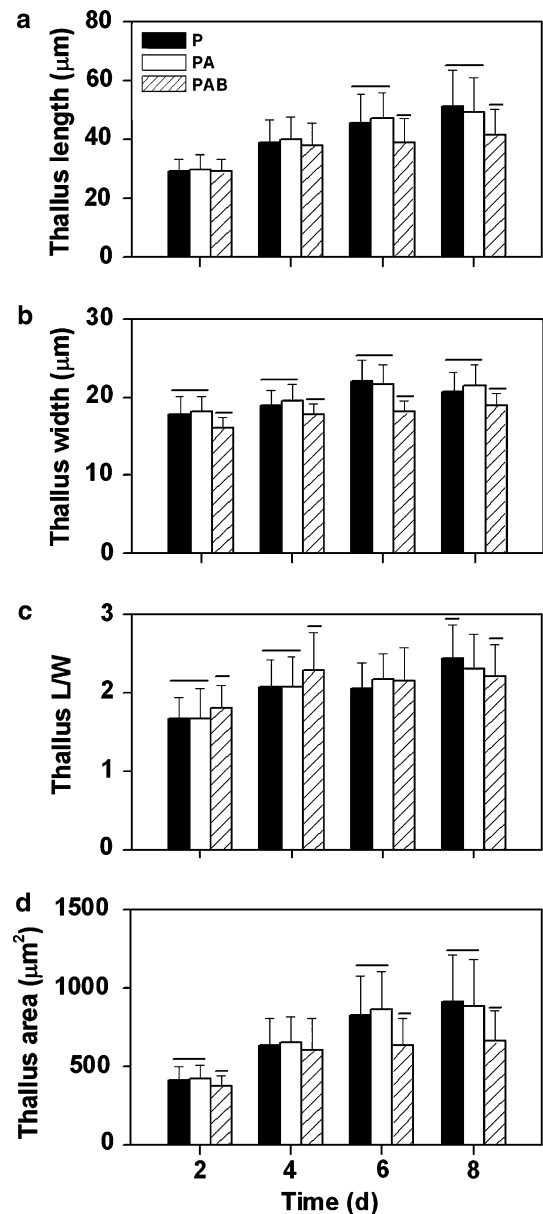


**Fig. 1** Daily doses of solar PAR, UV-A and UV-B\*100 ( $\text{MJ m}^{-2}$ ) from 29 September to 6 October 2005 for *Porphyra haitanensis* conchospores (a) and from 20 to 25 November 2004 for the juveniles (b)



**Fig. 2** Percent germination of *P. haitanensis* conchospores, cultured under solar radiation and exposed to PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB) from 29 September to 6 October 2005. Data were the means  $\pm$  SD ( $n = 50\text{--}80$ ). The horizontal lines over the bars indicate significant differences between treatments

There were significant differences in the thallus dimensions and area along the experiment and between radiation treatments (Fig. 3). The thallus



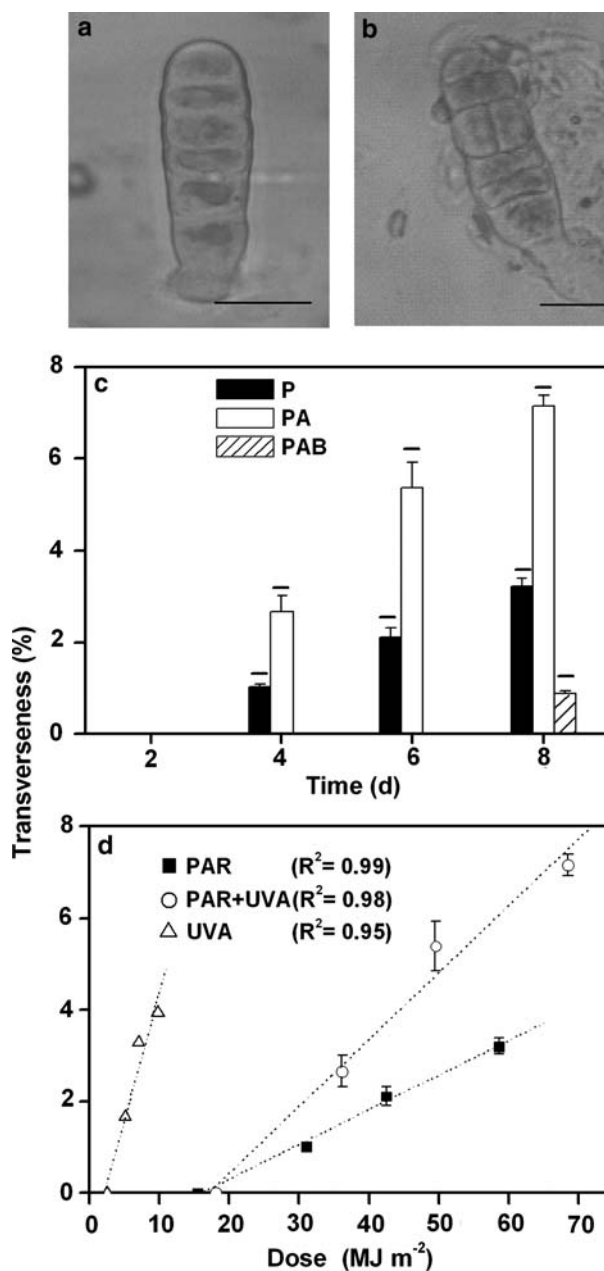
**Fig. 3** The mean length (a), width (b), ratio of length to width (c) and area (d) of all observed *P. haitanensis* sporelings with two cells or more, from the germinated conchospores exposed to different solar radiation treatments (as in Fig. 2). Data were the means  $\pm$  SD ( $n = 50\text{--}80$ ). The horizontal lines over the bars indicate significant differences between treatments

length of *P. haitanensis* sporelings increased from a mean value of  $29.5 \mu\text{m}$  on day 2 to  $51.1 \mu\text{m}$ , and  $49.3 \mu\text{m}$  on day 8 in P and PA treatments, respectively, but only to  $41.7 \mu\text{m}$  in PAB treatment (Fig. 3a). No negative influence of UV radiation on thallus length could be detected during the first 4 days, but during the latter 4 days the thallus length was significantly lower in PAB treatment than in the P and PA treatments. The thallus width had a slight increase with time and was significantly affected by UVB throughout the

experiments (Fig. 3b), with narrower width in the thalli under PAB treatment as compared to the ones in the P and PA treatments. The ratio of thallus length to width also showed a slight increase with time in all radiation treatments, but was significantly higher in the PAB treatment than in both P and PA treatments on days 2 and 4; however, the ratio was lower in the PAB treatment than in the P and PA treatments on day 8 (Fig. 3c). The mean thallus area of *P. haitanensis* increased with time from 408.4 and 417.0 to 910.6 and 887.5  $\mu\text{m}^2$  in the P and PA treatments, respectively. The area also increased with time in the PAB treatment from 374.9  $\mu\text{m}^2$  (day 2) to 658.0  $\mu\text{m}^2$  (day 8), but these values were significantly lower than in the P and PA treatments (Fig. 3d).

Most of the sporelings had no cells in the transversal sense (Fig. 4a); however, some of them contained cells that were dividing transversely (indicated by an arrow in Fig. 4b), and this was the main reason for the sporelings becoming wider and wider. These latter type of sporelings accounted for a very small portion of the total observed sporelings during our experimental period, with a maximum of 7% in PA treatment on day 8 (Fig. 4c). There was a significant difference in the amount of sporelings with divided transverse cells (Fig. 4b) between the three radiation treatments throughout the experiments (Fig. 4c). The formation of “transverse cells” was faster (appeared early in the experiments) and higher (higher percentage of sporelings) in the P and PA than in the PAB treatment; the cells in the sporelings in this latter treatment did not divide transversely until day 8. The percentage of transverse cells increased with increasing dose; in addition, samples receiving UV-A had a faster increase in transverseness (i.e., slopes in Fig. 4d) than those exposed only to PAR.

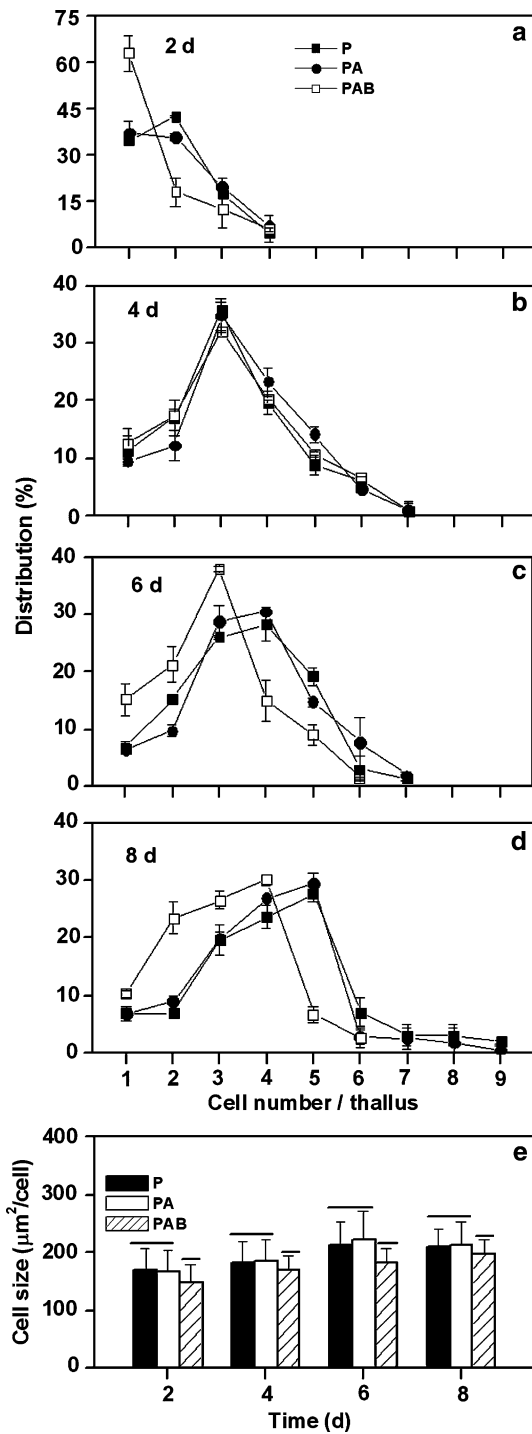
The frequency distribution of *P. haitanensis* sporelings with different size, in terms of cell number per thallus, was established as the percentage of sporelings with a certain size in the total observed individuals during the experimental period (Fig. 5a–d). The number of cells per thallus increased with time in all treatments, but was significantly less in PAB treatment than in the P and PA treatments (Fig. 5a, c, d), with the exception of day 4 (Fig. 5b). On day 2, both P and PA treatments had the largest percentage of sporelings with two cells, while in the PAB treatment the spore was without germination (Fig. 5a). There were no significant differences between the P and PA treatments, and most sporelings contained four cells on day 6, and five cells on day 8. The PAB treatment, however, had significantly lower percentage of sporelings that contained four cells and five cells, on day 6 and 8, respec-



**Fig. 4** Percentage of sporelings in *P. haitanensis*. Most sporelings were arranged in one column (a), while some contained cells dividing transversely (b). Percentage of transverseness in the various radiation treatments (c) and correlation between transverseness and doses of PAR, PAR + UV-A and UV-A (d). Data were the means  $\pm$  SD ( $n = 50$ –80). The horizontal lines over the bars indicate significant differences between treatments. (scale: 20  $\mu\text{m}$ )

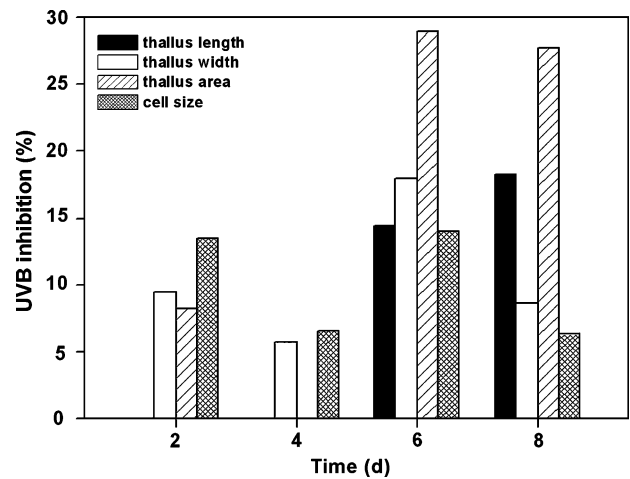
tively, and most sporelings contained three cells on day 6 and four cells on day 8 (Fig. 5c, d). The cell size in the sporelings increased with time in all treatments, but again was significantly smaller in PAB treatment than in the other two treatments throughout the experiments (Fig. 5e).

All our data presented so far indicate that UV-B had a significant impact in all parameters measured in



**Fig. 5** Frequency distribution of *P. haitanensis* sporelings with different size, in terms of cell number per thallus. Expressed as the percentage of sporelings with certain size in total observed individuals, including the spores without germination on day 2 (a), day 4 (b), day 6 (c) and day 8 (d). e The mean cell size in the sporelings with two cells or more. Data were the means  $\pm$  SD ( $n = 50\text{--}80$ ). The horizontal lines over the bars indicate significant differences between treatments

*P. haitanensis*. In Fig. 6, the relative inhibition of thallus length, thallus width, thallus area and cell size due to solar UV-B are shown over the experimental period.



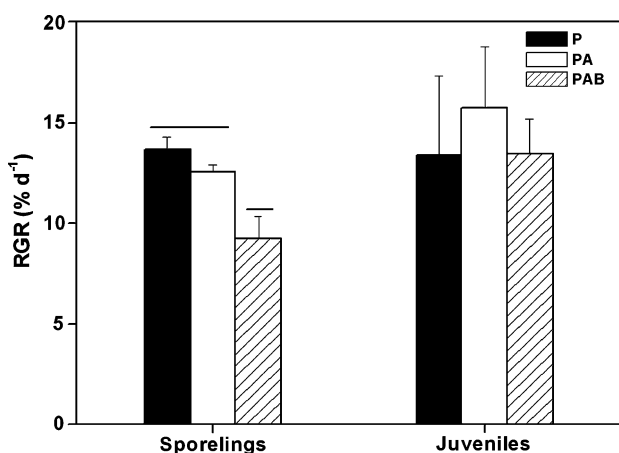
**Fig. 6** Relative inhibition of thallus length, thallus width, thallus area and cell size due to UV-B in the sporelings germinated from *P. haitanensis* conchospores, which were cultured under solar radiation

The relative inhibition due to UV-B was up to 18% for thallus length, from 6 to 18% for thallus width, up to 29% for thallus area and from 6 to 14% for cell size.

The growth rate of *P. haitanensis* sporelings was significantly lower under full solar radiation than when UVR was screened out, with an inhibition of 32% as compared to the P treatment (Fig. 7). The growth of juveniles, however, was not significantly different among radiation treatments. The growth rates of sporelings and juveniles were similar in the P treatment ( $\sim 14\% \text{ d}^{-1}$ ); however, they were significantly lower in sporelings receiving either UV-A or UV-A + UV-B than in comparable treatments of the juveniles.

## Discussion

Exposure to UVR had been reported to affect macroalgal ecophysiology and productivity (Franklin and Forster 1997). In nature, the thalli of *P. haitanensis* grow in the eulittoral zone, appearing after mid-autumn when the decreasing temperature and day length stimulate the release of conchospores, which germinate into sporelings and flourish in winter. Our studies were carried out at the same time with the germination of *P. haitanensis* conchospores and the growth of juveniles in cultivation receiving exactly the same solar irradiances as recorded in our experiments. Thus, the experimentally measured effects of solar UVR could be extrapolated to that in nature. Germination of *P. haitanensis* conchospores was not affected by solar UV-A, but slowed by UV-B during the first 2 days of exposure;



**Fig. 7** Relative growth rate (RGR) of *P. haitanensis* sporelings and juveniles in terms of thallus area. Juveniles were cultured under solar radiation from 20 to 25 November 2004. Data were the means  $\pm$  SD ( $n = 3$ ). The horizontal lines over the bars indicate significant differences between treatments

however, no differences between radiation treatments were observed after 4 days (Fig. 2). Thus under ambient UV-B, *P. haitanensis* thalli could become established in the intertidal zone, although with a slight delay. Similar delay of germination was found in the green alga *Ulva pertusa* spores by solar UV-A (Han et al. 2004); however, this species seems to be more sensitive as the germination delay was seen even with 2 h exposure to sunlight in December, whereas germination was completely inhibited in samples exposed to PAR + UV-A + UV-B. Germination of zoospores of *Laminaria* plants from southern Spain was prevented by UV-B at water depths down to 7 m (Wiencke et al. 2000). As mentioned above, the PAR levels inside PAR + UV-A + UV-B treatment were about 8% higher than in the other two treatments, and so a small part of the observed results might be due to this slight difference in PAR.

The subsequent sporelings of *P. haitanensis* exhibited no effects of solar UV-A, but strong inhibiting effects of UV-B on growth (Fig. 3, 5), as also found in brown, green and red macroalgae (Grobe and Murphy 1994, 1998; Makarov 1999; Pang et al. 2001). UV-B-specific harmful delay or cessation of cell division in the red algae *Rhodymenia pseudopalmata* and *Palmaria palmata* was mainly due to the formation of thymine dimers and 6–4 photoproducts (Pakker et al. 2000a, b), which inhibited transcription and replication of DNA, consequently disrupted cell metabolism and division and finally inhibited growth of the plant. The low germination rate of *P. haitanensis* conchospores, together with the small sporeling size of most of the observed individuals, as well as the lower percentage of transverse division in sporelings, clearly were due to

UV-B inhibition of cell division, which might be directly related to DNA damage. In contrast, other studies (Grobe and Murphy 1994) did not find any solar UV-B effect on the cell size of *Ulva expansa* thalli, while in our study the inhibiting effect on cell size of *P. haitanensis* sporelings was seen throughout the experiments and the relative inhibition due to UV-B accounted for 14%. Both the inhibiting effects on cell division and cell size would then lead to the inhibition of thallus length and width, and subsequent thallus area of *P. haitanensis* sporelings. During the first 4 days, solar UV-B exhibited inhibition in thallus width, but not in thallus length. Therefore, the ratio thallus L/W was significantly higher in the treatment that received UV-B; this coincided with the delayed formation of transverse cells. Such reduced efficiency of thallus expansion by UV-B would result in a lower capacity to harvest light and nutrients for photosynthesis when self-shielding occurs within the population. *P. haitanensis* thallus consists of one layer of cells, and therefore changes in the thallus area also reflects in changes of biomass and growth. After 8 days, the relative inhibition of thallus area in *P. haitanensis* sporelings due to solar UV-B accounted for 29%, which was similar to the growth reduction of 31% in the subtidal brown alga *Dictyota dichotoma* over 3 weeks (Kuhlenkamp et al. 2001), but lower than 46–70% in the sublittoral red alga *Chondrus crispus* over 2 weeks (Franklin et al. 1999). Such inhibition suggests that any future increase of UV-B radiation, due to the diminishing ozone levels, would further limit the productivity of *P. haitanensis* and might affect their vertical distribution. Other studies (Altamirano et al. 2003a), working with germlings of three species of *Fucus*, found that UV-B-induced growth reduction appeared to be related to the vertical distribution of the species in the intertidal zone.

Many studies found that UV-A exhibited no obvious effects, while others found that UV-A inhibited macroalgal growth (Franklin and Forster 1997; Aguilera et al. 1999b; Michler et al. 2002; Hoffman et al. 2003); however, in some species UV-A mediated DNA damage repair and increased growth (Pakker et al. 2000a, b). Solar UV-A also enhanced the growth of *Fucus gardneri* embryos, both in terms of length and surface area (Henry and Van Alstyne 2004). In our study, solar UV-A showed no negative effects on both germination and growth in *P. haitanensis* during the experimental period, but showed significantly positive effect on the morphogenesis of sporelings. Cells of sporelings in PA treatment divided transversely earlier and the percentage of such sporelings in the total observed sporelings was the highest; while in PAB

treatment transverse division appeared 4 days later than in the other two treatments and with the lowest percentage. Transverse division was obviously enhanced in P and PA treatments as compared with the PAB treatment, and such a response allowed the thalli to expand in all potential directions and thus grow faster. This was reflected in higher values of thallus width and thallus area in the treatments without UV-B (Fig. 3). Such an enhanced expansion of the thallus would allow the alga to assimilate more efficiently under reduced levels of light or nutrients. This is especially significant for the few-celled sporelings that need to survive, competing with the phytoplankton in natural seawater, while being grazed by shrimp and fish. Transverse cells occurred at higher percentages under PA than under P treatment; however, the thallus width and area in PA treatment did not show significant differences than those in P treatment. This might be due to the relatively low percentage, with a maximum of 7%, and the relatively small cell size, just after transverse division, being difficult to significantly enhance the mean width and area of all observed thalli during our experimental period. The mechanism to enhance this transverse division is unknown, but should not be related to the repair of DNA damage, as the sporelings in PA treatment did not receive UV-B radiation throughout the experimental period. It might, however, be related to the activation of some cellular factor controlling the division direction, as light was suggested to induce morphological changes as photomorphogenetic responses to wavelength (Dring 1988). Besides, our morphogenetic response (i.e. formation of transverse cells) was dose dependent, with a good correlation between the percentage of transverseness and the doses over the experimental period. Such enhancement by UV-A was ecologically important for sporelings, partly counteracting the negative effects of UV-B in natural sunlight.

Our data showed no significant effects of solar UVR on the growth of juvenile *P. haitanensis* and suggested that juveniles exhibited higher tolerance to UV-B than the earlier stage sporelings. Similar results were found in the intertidal alga *F. gardneri*, as solar UV-B showed no effects on the growth of juveniles, but inhibited significantly the growth of embryos (Henry and Van Alstyne 2004). Low growth sensitivity to UVR had also been found in mature sporophytes than in young sporophytes of three species of *Laminaria* (Dring et al. 1996). The stage of 5–10 h after fertilization was the most UV-sensitive during the first 24 h of the development of *Fucus serratus* zygotes, because those germ-lings that developed from zygotes exposed to UVR at such a stage showed the strongest inhibition of growth

(Altamirano et al. 2003b). The levels of solar radiation received by *P. haitanensis* sporelings during September to October was relatively higher than that received by the juveniles during November, and this might account for the differences of inhibitory effects due to UV-B. However, tolerance to many environmental stresses was found to increase with age, as individuals became more differentiated and developed improved repair and defense mechanisms (Davison and Pearson 1996; Dring et al. 1996; Franklin and Forster 1997; Hanelt et al. 1997). Inhibition in our sporelings increased from day 2 to day 8, but later on the juveniles were not inhibited at all, suggesting a morphological development and photoacclimation during the development. Despite both consisting of one layer of cells, the thalli of sporelings are much thinner than those of juveniles due to the smaller size and greater structural simplicity, which would allow UVR to easily penetrate into the UV-sensitive cellular molecules. Besides, the conchocelis of *P. haitanensis*, which released conchospores, had been grown for long periods in UVR-low conditions, whereas the juveniles collected from the field had been receiving solar UVR for months.

In conclusion, germination of *P. haitanensis* conchospores was inhibited and slowed by solar UV-B, while UV-A enhanced the transverse division of cells in sporelings; UV-B only exhibited strong inhibition on the growth of sporelings and not on juveniles. Thus, our results suggest that the establishment of *P. haitanensis* in the intertidal zone is not strongly affected by the levels of ambient solar UVR during wintertime, and solar UV-A can partly counteract the negative effect of UV-B on the growth of sporelings, which develops a tolerance to UV-B during maturation.

**Acknowledgments** This work was supported by the Chinese “863” project (No. 2006AA10A413) and National Natural Science Foundation of China (Project No. 90411018). We acknowledge Yingke Seaweed Cultivation Ltd. in Nan’ao Island for providing *P. haitanensis* conchospores. We thank Gang Li for the experimental assistance and Weizhou Chen for his kind support. The experiments performed comply with the current laws of China.

## References

- Aguilera J, Jiménez C, Figueroa FL, Lebert M, Häder D-P (1999a) Effect of ultraviolet radiation on thallus absorption and photosynthetic pigments in the red alga *Porphyra umbilicalis*. J Photochem Photobiol B Biol 48:75–82
- Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999b) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. Mar Ecol Prog Ser 191:109–119
- Aguilera J, Dummermuth A, Karsten U, Schriek R, Wiencke C (2002) Enzymatic defenses against photooxidative stress in-



- duced by ultraviolet radiation in Arctic marine macroalgae. *Polar Biol* 25:432–441
- Altamirano M, Flores-Moya A, Figueroa FL (2000) 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
- Altamirano M, Flores-Moya A, Figueroa FL (2003a) Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophyceae). *Aquat Bot* 75:9–20
- Altamirano M, Flores-Moya A, Kuhlenkamp R, Figueroa FL (2003b) Stage-dependent sensitivity to ultraviolet radiation in zygotes of the brown alga *Fucus serratus*. *Zygote* 11:101–106
- Coeelho SM, Rijstenbil JW, Brown MT (2000) Impacts of anthropogenic stresses on the early development stages of seaweeds. *J Aquat Ecosyst Stress Recovery* 7:317–333
- Davison IR, Pearson GA (1996) Stress tolerance in intertidal seaweeds. *J Phycol* 32:197–211
- Dring MJ (1988) Photocontrol of development in algae. *Annu Rev Plant Physiol Plant Mol Biol* 39:157–174
- Dring MJ, 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, Salles S, Aguilera J, Jiménez C, Mercado J, Viñegla B, Flores-Moya A, Altamirano M (1997) Effects of solar radiation on photoinhibition and pigmentation in the red alga *Porphyra leucosticta*. *Mar Ecol Prog Ser* 151:81–90
- Flores-Moya A, Gómez I, Viñegla B, Altamirano M, Pérez-Rodríguez E, Maestre C, Caballero RM, Figueroa FL (1998) Effects of solar radiation on the endemic Mediterranean red alga *Rissoella verruculosa*: photosynthetic performance, pigment content and the activities of enzymes related to nutrient uptake. *New Phytol* 139:673–683
- Flores-Moya A, Posudin YI, Fernandez JA, Figueroa FL, Kawai H (2002) Photomovement of the swimmers of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia*: effect of photon irradiance, spectral composition and UV dose. *J Photochem Photobiol B Biol* 66:134–140
- Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur J Phycol* 32:207–232
- Franklin LA, Yakovleva I, Karsten U, Lüning K (1999) Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. *J Phycol* 35:682–693
- Grobe CW, Murphy TM (1994) Inhibition of growth of *Ulva expansa* (Chlorophyta) by ultraviolet-B radiation. *J Phycol* 30:783–790
- Grobe CW, Murphy TM (1998) Solar ultraviolet-B radiation effects on growth and pigment composition of the intertidal alga *Ulva expansa* (Setch.) S. and G. (Chlorophyta). *J Exp Mar Biol Ecol* 225:39–51
- Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can J Microbiol* 8:229–239
- Han Y-S, Han T (2005) UV-B induction of UV-B protection in *Ulva pertusa* (Chlorophyta). *J Phycol* 41:523–530
- Han T, Kong J-A, Han Y-S, Kang S-H, Häder D-P (2004) UV-A/blue light-induced reactivation of spore germination in UV-B irradiated *Ulva pertusa* (Chlorophyta). *J Phycol* 40:315–322
- Hanelt D, Wiencke 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 BE, Van Alstyne KL (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J Phycol* 40:527–533
- Hoffman JR, Hansen LJ, Klinger T (2003) Interactions between UV radiation and temperature limit inferences from single-factor experiments. *J Phycol* 39:268–272
- Korbee Peinado N, Abdala Díaz RT, Figueroa FL, Helbling EW (2004) Ammonium and UVR stimulate the accumulation of mycosporine-like amino acids (MAAs) in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. *J Phycol* 40:248–259
- Kuhlenkamp R, Franklin LA, Lüning K (2001) Effect of solar UV radiation on growth in the marine macroalga *Dictyota dichotoma* (Phaeophyceae) at Helgoland and its ecological consequences. *Helgoland Mar Res* 55:77–86
- Lubin D, Jensen EH (1995) Effects of clouds and stratospheric ozone depletion on ultraviolet radiation trends. *Nature* 377:710–713
- Major KM, Davison IR (1998) Influence of temperature and light in growth and photosynthetic physiology of *Fucus evanescens* (Phaeophyta) embryos. *Eur J Phycol* 33:129–138
- Makarov M (1999) Influence of ultraviolet radiation on the growth of the dominant macroalgae of the Barents Sea. *Chemosphere Glob Change Sci* 1:461–467
- 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-temperate macroalgae. *Mar Biol* 140:1117–1127
- Pakker H, Beekman CAC, Breeman AM (2000a) Efficient photoreactivation of UVBR-induced DNA damage in the subtidal macroalga *Rhodomenia pseudopalmata* (Rhodophyta). *Eur J Phycol* 35:109–114
- Pakker H, Martins R, Boelen P, Buma AGJ, Nikaido O, Breeman AM (2000b) Effects of temperature on the photoreactivation of ultraviolet-B induced DNA damage in *Palmaria palmata* (Rhodophyta). *J Phycol* 36:334–341
- Pang S, Gómez I, Lüning K (2001) 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
- van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. *J Phycol* 37:30–37
- Roleda MY, Hanelt D, Kräbs G, Wiencke C (2004) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. *Phycologia* 43:603–613
- Roleda MY, Hanelt D, Wiencke C (2006) Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea). *Mar Biol* 148:1201–1211
- 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