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Article

Effects of Artificial UV-B and Solar Radiation on Four Species of Antarctic Rhodophytes

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Abstract : During austral summer 1998 we examined the impacts of artificial UV-B and solar radiation on chlorophyll *a* content and fresh weight of four species of Antarctic red algae namely, *Georgiella confluens*, *Iridaea cordata*, *Pantoneura plocamioides* and *Porphyra endiviifolium*. These subject species were taken in consideration of clear demarcations of their vertical distribution and classified as shallow water group (*Iridaea* and *Porphyra*) and deep water group (*Georgiella* and *Pantoneura*). When irradiated with artificial UV-B at the irradiance of 2.0 W m^{-2} the shallow water inhabitants were much more resistant than the algae from deep water the fresh weight of which was reduced by 40-50 % relative to control apart from loss of pigmentation. Direct solar radiation was lethal to the deep water group with a sign of complete bleaching whereas the shallow water group did not show any change in the physiological parameters. We were unable to discriminate difference in the algal sensitivity between UV-filtered and UV-transparent treatments since samples tested were either all unaffected or dead. Spectrophotometric measurements of methanolic extracts revealed a strong absorption peak in the UV range in the shallow water group of algae, *Iridaea* and *Porphyra*, but not in the deep water counterparts. Species difference in sensitivity to artificial UV-B and solar radiation is discussed in relation to biochemical and morphological characteristics and the role of the radiation in the algal vertical distribution is suggested from ecological perspective.

Key words : *Georgiella*, *Iridaea*, *Pantoneura*, *Porphyra*, UV-B, solar radiation.

1. Introduction

There is a spectrum of responses with individual species being more or less able to tolerate high light stress. Some algae can tolerate increasing photon irradiances up to full sunlight with no apparent inhibition of photosynthesis or growth whereas the same physiological parameters of others are significantly reduced by high light treatment. Drew (1974) found that photosynthesis of *Laminaria hyperborea* was rapidly reduced to zero in bright surface sunlight but *L. digitata* was completely unaffected and suggested that the sensitivity to high levels of light corresponded with their ecological habitats, i.e. sublittoral for the former species and upper sublittoral/intertidal for the latter. Therefore, the extent to which adaptation to high light stress seems to be genetically determined.

Photoinhibition also occurs when organisms grown in low light environments are suddenly exposed to higher irradiances. When young sporophytes of *Laminaria digitata* and *L. hyperborea* were acclimated to different irradiances and then exposed to direct sunlight survival of both species acclimated to higher irradaince was remarkably higher than those adapted to lower irradiance (Han and Kain 1996). In the fields at shallow water depths, experimental removal of kelp canopy in summer was found to damage the subcanopy plants (Wood 1987).

It is well known that the UV part of solar spectrum can cause damage to living systems since UV radiation is

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absorbed by biologically important molecules such as DNA, proteins and lipids (Kramer 1991; Mitchell and Karentz 1993; Yu and Björn 1997). In recent years, considerable attention has been paid to the effects of UV-B radiation on marine algae since UV-B flux reaching at the Earths surface is increasing due to global depletion of the ozone layer. Researches related to UV-B effects on macroalgae have shown that the radiation induces phytotoxic effects such as growth retardation, destruction of photosynthetic pigments and decline of photosynthesis (Wood 1987; Larkum and Wood 1993; Grobe and Murphy 1994; Dring *et al*. 1996; Han 1996). Despite remarkable progress in our understanding of the role of UV relatively little information is available on UV-related traits of macroalgae occurring in Antarctica where the rising levels of UV-B in connection to seasonal reduction of ozone concentrations have been first noticed. In addition, Antarctic macroalgae are adapted to the low light conditions due to ice cover in winter and low solar angle (Kirst and Wiencke 1995). Flexibility in response to changes in irradiance may thus determine the tolerance of Antarctic algae to high irradiance.

In this study we compared the effects of artificial UV-B and solar radiation on four species of Antarctic rhodophytes collected from different depths based on the idea that the implication of photoinhibition may bear a strong relation to the distribution of algae. The hypotheses tested were as follows:

1) Are the species from deep water more susceptible to UV-B and high solar radiation than shallow water counterparts, thus unable to have a chance to grow in shallow water?

2) Is difference in sensitivity to artificial UV-B and solar radiation, if any, related to species-specific biochemical and morphological characteristics?

In this context, the subject algal species were taken in consideration of clear demarcations of their vertical distribution. *Georgiella confluens* (Reinsch) Kylin and *Pantoneura plocamioides* Kylin rarely growing above the upper sublittoral. The rich aggregations of *Iridaea cordata* (Turner) Bory de Saint-Vincent occur in the tidal zone as well as in the sublittoral down to 10 m. *Porphyra endiviifolium* (A. & E.S. Gepp) Chamberlain is mainly found in the upper littoral zone.

2. Materials and methods

During austral summer 1998 four species of Antarctic rhodophytes were collected each from different depths by scuba diving near Marian Cove, King George Island. After collection in a black vinyl back, the samples were transported to King Sejong Marine Research Centre and maintained for 2-3 days in tanks with ambient seawater inflow at 1-2 ^{o}C. Sunlight incidence was partly reduced using a nylon net shade. Algal discs (12 mm, diam.) or tips (trimmed to a constant fresh weight) were taken out of healthy thalli depending on thallus types, i.e. parenchymatous or filamentous type.

In laboratory, samples were placed in unlidded Petri-dishes (100×10 mm) filled with 40 m*l* of filtered fresh seawater (0.45 μm, filter size, Whatman) for UV treatments. Two artificial UV-B lamps (BLE-1T158, Spectronics) were employed to produce UV-B radiation of 2.0 W m^{-2}. Immediately after UV exposures samples were transferred to newly prepared Petri-dishes containing filtered seawater and cultured at 15-20 μmol m^{-2} s^{-1} of continuous white fluorescent light with temperature kept at 1-2 ^{o}C. The seawater medium was renewed everyday and the culture period was for 5 days.

In outdoor culture experiments, samples were put out in tanks with and without cellulose filters which cut off 80 % of UV-B and less than 10 % of UV-A waveband from solar radiation. Samples were incubated for 5 days in the tanks with continuous inflow of ambient seawater before harvesting for analysis of physiological parameters.

The effects of high solar radiation with/without UV-B portion and artificial UV-B radiation were assayed by measuring chlorophyll *a* content and fresh weight. Chlorophyll *a* concentrations were determined spectrophotometrically after 100 % methanol extraction for 24 h in the dark at 5 ^{o}C (Hipkins and Baker 1986). Samples were blotted dry with tissue paper and their wet weight was then measured.

Methanolic extraction was also made for determination of the UV-absorbing pigments within thalli of the four rhodophytes and the relative amount was quantified by normalizing the peak values to the respective fresh weight of thalli.

3. Results and discussion

When the four Antarctic rhodophytes were exposed to artificial UV-B radiation and then cultured at 15-20 μmol m^{-2} s^{-1} of white light in laboratory the chlorophyll *a* content of the shallow water dwelling species (*Iridaea* and *Porphyra*) remained unaffected by the ambient ground levels of UV-B radiation (2.0 W m^{-2}), but the deeper water species (*Georgiella* and *Pantoneura*) were bleached (Fig. 1). Chlorophyll content has often been used as a sensitive indicator of effect of UV irradiation (Caldwell 1983).

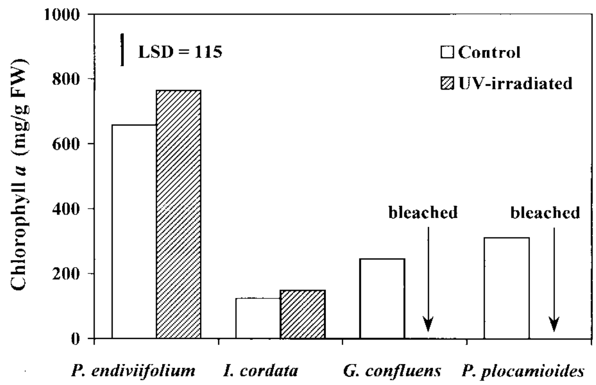

Fig. 1. Effect of artificial UV-B radiation on chlorophyll *a* content of four species of Antarctic rhodophytes. Samples were exposed to the UV irradiance of 2.0 W m^{-2} for 3 h. Mean and least significance difference (LSD).

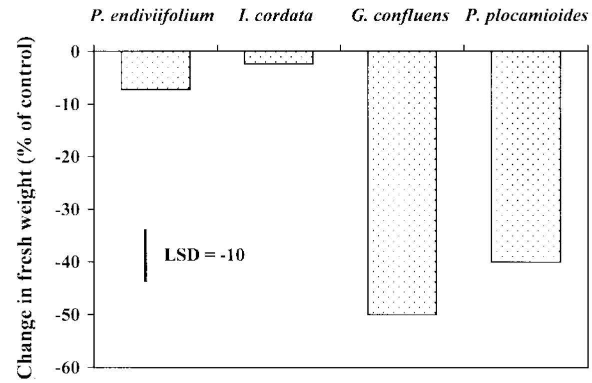

Fig. 2. Effect of artificial UV-B radiation on fresh weight of four species of Antarctic rhodophytes. Samples were exposed to the UV irradiance of 2.0 W m^{-2} for 3 h. Mean and least significance difference (LSD).

High levels of UV-B irradiance produced significant reductions in chlorophyll concentrations in various macroalgae. The total chlorophyll content of the green alga, *Ulva pertusa*, declined with increasing durations of UV-B radiation with the result that plants lost more than 50 % of the total chlorophyll in 3 days after exposure to only 2 h UV at the irradaince (2.0 W m^{-2}) that simulated ambient levels (Han 1996). Under unfiltered natural solar radiation chlorophyll *a* concentrations of the red alga, *Eucheuma striatum*, were significantly reduced compared with those cultured in the absence of UV (Wood 1989). Post and Larkum (1993) observed that there was reduction in chlorophyll content in Antarctic green alga, *Prasiola crispa*, when grown in UV enhanced conditions. In relation to photosynthetic pigments there have been reports of a variety of negative responses to UV including photo-oxidation of newly synthesized pigments, down-regulation of the expression of genes for chlorophyll-binding proteins and disruption of light harvesting pigment complexes (Clendennen *et al.* 1996; Mackerness *et al.* 1996). Concerning the main cause of chlorophyll degradation by UV irradiation, however, confirmation is still required as to whether it involves indirect way of decreased pigment synthesis through physical disturbances in chlorophyll biosynthesis pathways or rather direct way of increased degradations of chlorophylls or their precursors due to absorption of high energy quanta.

There were also differential responses in fresh weight to UV-B radiation between the shallow and deep water group. Fresh weight was reduced only by less than 10 % for the algae occuring in shallow water while the degree of reduction was 40 and 50 % for *Pantoneura* and *Georgiella* respectively (Fig. 2). These observations are similar to the results from studies on other species exposed to artificial UV-B radiation (Friedlander and Ben-Amotz 1991; Grobe and Murphy 1994). Algal biomass may be a good indicator of UV radiation effects on growth since it represents an integration of many inherent variables. Large decrease in fresh weight might have been ascribed to UV-B induced reduction in photosynthetic rates. Although there are species difference in sensitivity UV-B radiation generally results in reductions in net photosynthesis, which may be reflected in decrease in biomass.

Exposure to natural solar radiation resulted in different physiological responses among the four Antarctic rhodophytes. During the 5 day culture period mean midday solar irradiance was 1,500 μmol m^{-2} s^{-1} and the water temperature was maintained at 1-2 °C. In the two algal species growing mainly in shallow water there was no difference in fresh weight and chlorophyll *a* content between plants cultivated in UV transparent and UV-filtered condition (Figs. 3, 4). In contrast, the deep water dwelling species were found to succumb to complete photobleaching in the same conditions. Unrelated biophysical and biochemical processes may result in lowered photosynthesis at high irradiance levels and this adverse effects of intense illumination on physiological processes have well been studied in algae (Neale 1987). The absorption of excessive energy by the photosystems produces photosensitizer, and subsequent reactions leads to photo-oxidative breakdown of chlorophyll molecules.

It was impossible to discriminate the effect of UV-B portion on the physiological parameters from that of full spectrum of solar radiation since the red algal species tested were either unaffected by solar radiation both with and without UV-B portion or all dead (Figs. 3, 4). However, the response pattern observed was not dissimilar to that found in laboratory experiments which revealed differential effects of UV-B depending species groups of different habitats. Wood (1987) reported that solar UV-B radiation was deleterious to the survival of the kelp *Ecklonia radiata*, thus preventing the selttled sporophytes from recruitments at shallow depths. Removing solar UV-B significantly reduced photoinhibition, and the quantum yield of plants exposed to UV-filtered solar radiation was much higher than those irradiated with full solar radiation (Hanelt *et al.* 1997). It is well documented in higher plants that low light adapted plants respond with significant photoinhibition to excessive radiation that does not affect high light adapted plants as much as low light adapted counterparts (Björkman 1973). Antarctic algae are supposed to be dim light adapted plants, thereby with extremely high sensitivity to high light, but two of the investigated algae seem to cope well with UV-B and even surface solar radiation over relatively long term periods. Hanelt *et al.* (1997) pointed out that some polar algae are able to optimize photosynthesis under strong light by undergoing dynamic photoinhibition which serves photoprotective mechanism to make excessive absorbed energy harmless in the form of thermal dissipation. Recently, it has been reported that polar macroalgae from different depths show different degree of responses to light stress (Hanelt *et al.* 1997). Photosynthetic parameters of algae growing in the intertidal were not apparently stressed by high light, but there was significant reduction in those of sublittoral algae albeit subsequent recovery followed in dim light. Photosynthetic activity of lower sublittoral species was severely declined, unable to recover due perhaps to photodamage. The ability of the Antarctic intertidal species (*Iridaea* and *Porphyra*) to withstand ambient levels of UV-B and high solar radiation found in the present study may stem from operation of photoprotective mechanism which could be lacking in *Georgiella* and *Pantoneura*.

Methanolic thalli extracts of the two littoral species showed a strong absorbance peak in the UV range while there was no significant peak detected in the other two species (Fig. 5). When the relative amounts of UV absorbing pigments as expressed by mean of absorbance peak values were compared, *Porphyra* was shown to have the highest amount (approximately 15) followed by *Iridaea* with 10 (Fig. 6). *Georgiella* seems to contain less than half the amount recorded in the former species. No UV peak was observed in methanol extracts of *Pantoneura*.

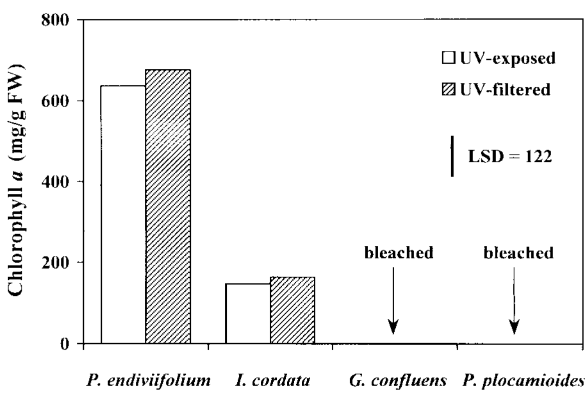

Fig. 3. Effect of direct and UV-B filtered solar radiation on chlorophyll *a* content of four species of Antarctic rhodophytes. Mean and least significance difference (LSD). Mean total midday solar and UV-B irradiance were 1500 μmol m^{-2} s^{-1} and 2.0 W m^{-2} respectively.

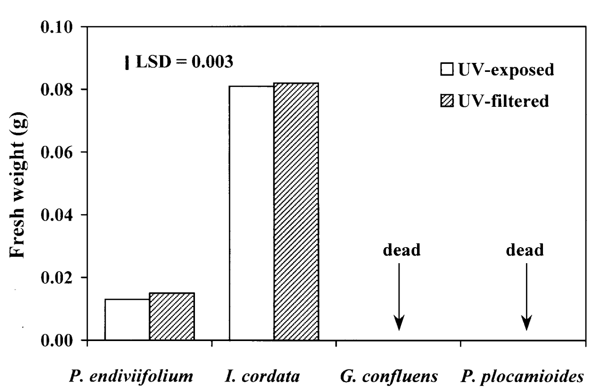

Fig. 4. Effect of direct and UV-B filtered solar radiation on fresh weight of four species of Antarctic rhodophytes. Mean and least significance difference (LSD). Mean total midday solar and UV-B irradiance were 1500 μmol m^{-2} s^{-1} and 2.0 W m^{-2} respectively.

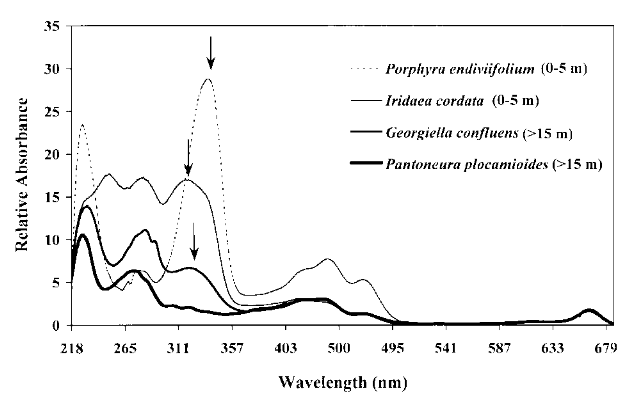

Fig. 5. Spectrophotometric absorbance characteristics of 100 % methanol extracts of the four Antarctic rhodophytes. Absorbance values were all normalized to fresh weight.

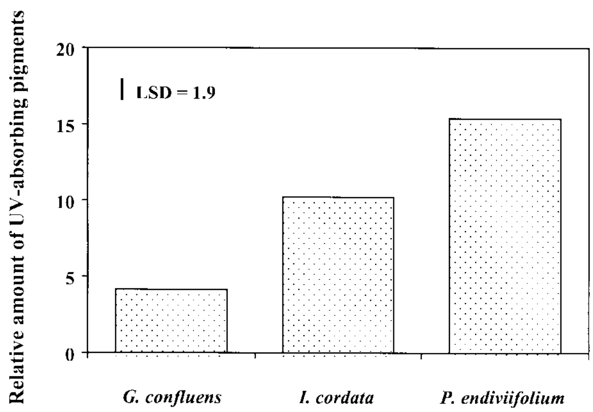

Fig. 6. Relative amounts of UV absorbing pigments in four species of Antarctic rhodophytes. Mean and least significance difference (LSD).

A recent survey made on UV-absorbing pigments has shown that the physicochemical characteristics of these substances are mycosporine-like amino acids (MAAs), which are water soluble and strongly absorb in the UV wavebands ranging from 310 to 360 nm (Karentz *et al.* 1991; Dunlap and Shick 1998). The UV-absorbing pigments are generally known to act as a natural sunscreen, protecting biologically important organelles from damaging UV radiation. For instance, the photosynthetic capacity of Antarctic *Palmaria* has been observed to increase under intense solar radiation in austral summer, which corresponds with increase in the levels of UV-absorbing pigments (Post and Larkum 1993). In the tropical red alga, *Eucheuma striatum*, UV-absorbing pigments were significantly higher in plants subjected to UV than in those grown in the absence of UV (Wood 1989). The sunscreen role of MAAs can also be envisaged from an action spectrum showing that UV radiation is most efficient in eliciting increases in MAAs (Garcia-Pichel *et al.* 1993). Differences in sensitivity to artificial UV-B and solar radiation between Antarctic rhodophytes studied may therefore be partly related to the presence and amount of UV-absorbing pigments. On the other hand, the mere presence of UV absorbing pigments does not ensure protective function against damaging UV radiation. Methanol extracts of Korean red alga *Pachymeniopsis* sp. showed a broad peak covering the range 300-350 nm whereas brown alga *Kjellmaniella crassifolia* has UV-absorbing pigments with the absorption peak at 340 nm (Han unpublished). Despite the existence of UV absorbing pigments in both species difference in their sensitivity to UV-B radiation was substantial. The chlorophyll *a* content of the red alga remained unchanged relative to control, but that of the brown was reduced by as much as 90 % after exposure to the same dose of artificial UV-B radiation. The results appear to suggest the importance of spectral correctness between the UV absorbing pigments and the incoming UV radiation for efficient protective function.

Thallus thickness may be another characteristics that determine the sensitivity of the algal species to high levels of irradiances. Thicker tissue could help mitigate damaging effects of high energy by greater absorption of harmful quanta reaching biologically important organelles. Halldal (1964) reported that monostromatic/filamentous algae are more susceptible to high irradiances than multicellular algae with thicker thalli. It has also been observed in laminarian plants that early stages of plants with optically thin thalli are more vulnerable to high PAR and UV radiation than mature thalli (Dring *et al.* 1996). If this holds true, the differential sensitivity recognized between the shallow and deep group of Antarctic red algae may not be unexpected since the shallow water group has multicellular thalli as thick as 200-375 μm in contrast to deep water species with filamentous thalli (data not shown). While macrophytes of different morphology exhibit different tolerances to high irradiances the sensitivity is not always in parallel with thallus thickness. When a green alga, *Ulva pertusa*, with thin thalli of only two cell layers and a brown, *Kjellmaniella crassifolia*, possessing thick thalli of several cell layers were compared for their sensitivity to UV-B radiation, chlorophyll *a* content and fresh weight were much less reduced in *Ulva* than in *Kjellmaniella* after exposure to the same dose of artificial UV-B radiation (Han unpublished).

In the four species of Antarctic rhodophytes *Iridaea* and *Porphyra* from shallow waters appear to adapt effectively to solar PAR+UV and artificial UV-B radiation compared to *Georgiella* and *Pantoneura* from deep waters. The data presented here support the idea that this difference in sensitivity to high solar and UV-B radiation may be correlated to a combination of biochemical and anatomical differences including UV-absorbing pigments and thallus thickness. The present study also suggests that UV-B portion of solar radiation may be a potent factor in determination of the upper limit of Antarctic rhodophytes.

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