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ORIGINAL ARTICLE

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Effect of solar ultraviolet radiation on growth in the marine macroalga *Dictyota dichotoma* (Phaeophyceae) at Helgoland and its ecological consequences

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Abstract At Helgoland, in the North Sea, growth of the high sublittoral brown macroalga *Dictyota dichotoma* (Hudson) Lamoroux was examined in October (the time of tetraspore release) in an outdoor tank by exposing 2-day-old germlings to four solar radiation treatments achieved with different filter materials or an additional artificial light source: photosynthetically active radiation (PAR; 395–700 nm), PAR plus ultraviolet (UV)-A (320–700 nm), full solar spectrum, or solar spectrum plus artificial UV radiation (UVR). Based on length measurements over a period of 3 weeks, the growth rate in germlings strongly decreased in conditions with UVR compared to PAR: by 14% under PAR+UV-A, by 31% under the full solar spectrum and by 65% with additional UVR. Although growth rates of germlings under UVR were reduced mainly in the first week, the plants did not regain the size of the untreated plants even after 9 weeks. Regardless of the exposure, no defects in morphology or anatomy including the exposed apical meristem were detected, except for a reduction in cell division rates perhaps due to additional cost for photoprotective or repair mechanisms. Depending on the actual position of *D. dichotoma* plants in the natural habitat, individuals in high positions receive substantial amounts of the more harmful UV-B while those lower down might only receive UV-A during part of the day, thus the effect of UV-B on the growth of *D. dichotoma* will depend on its position in the field. The effects of tidal variation of the light climate and the implications of our results for the zonation of *D. dichotoma* are discussed.

Keywords *Dictyota* · Growth rate · Solar radiation · Ultraviolet radiation · Sublittoral zonation

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Introduction

Ozone-depletion at temperate and polar latitudes and the consequent increase in levels of ultraviolet (UV)-B radiation (280–315 nm; Commission Internationale d'Eclairage definition) have spurred recent interest in the effects of UV radiation (UVR, 295–400 nm; UV-A, 320–400 nm) on marine macroalgae (Wängberg et al. 1996; Franklin and Forster 1997; Häder and Figueroa 1997). It has been suggested that damage caused by UV-B may determine current and future vertical zonation patterns of some species (Wood 1987; Hanelt et al. 1997b; Bischof et al. 1998a; Yakovleva et al. 1998; Aguilera et al. 1999; Makarov 1999; Wiencke et al. 2000); this has also been substantiated by in situ studies (Häder et al. 1996; Häder et al. 1998). Potential targets of UV-B damage are broad, as UV-B is absorbed by numerous molecules, including several amino acids, lipids, and nucleic acids.

Short-term studies of UVR effects, focused especially on sublittoral species, indicate the potential for substantial inhibition of photosynthesis by UV-B (Dring et al. 1996a; Hanelt et al. 1997a; Bischof et al. 1998a, 1998b). Despite their value in determining targets of UVR stress, short-term studies are often of limited use in evaluating the ecological significance of UVR, since UV-B doses may not be reciprocal (cf. Caldwell and Flint 1994). Long-term studies demonstrate that growth can be even more susceptible to UV-B (Wood 1987, 1989; Friedlander and Ben-Amotz 1991; Grobe and Murphy 1994; Franklin et al. 1999), since growth integrates the multiple biological effects of UVR, e.g. direct damage to DNA or indirect effects on general metabolism. Furthermore, growth and survival of an individual, and subsequently of the

population, depend on the combined effects of the received UVR, and on the possible multiplication of concurrent stress effects like photoinhibition, desiccation and nutrient limitation (Franklin and Forster 1997). Specific analyses of UV-B effects on growth are few, as many long-term growth experiments with natural radiation have either examined the effects of photosynthetically active radiation (PAR; 400–700 nm) alone, or not specifically isolated the effects of UV-B and UV-A from those of the total solar spectrum.

In order to predict the effects of any environmental stress on macroalgal populations and subsequently on community structure, we need to know the relative sensitivity of early ontogenetic stages, since recruitment of a species depends primarily on the survival of those stages. For example, Wiencke et al. (2000) have shown that the UV-B susceptibility of spores of several brown algae was correlated with the vertical distribution of the parent plants. The germination of spores, as well as photosynthesis and growth of gametophytes and young sporophytes of all three *Laminaria* species occurring at Helgoland were more sensitive to UV-B than the photosynthesis and growth of adult sporophytes (Dring et al. 1996b), while juvenile *L. saccharina* sporophytes were more susceptible to stress from high levels of PAR than were older sporophytes (Hanelt et al. 1997c). The basis for these differences might lie in the different metabolic rates, cell division rates, or acclimation history of the particular stage.

The marine macroalga *Dictyota dichotoma* (Hudson) Lamoroux (Dictyotales, Phaeophyceae) is a common brown alga occurring in cold temperate to tropical regions of the Atlantic and Mediterranean, growing in the intertidal or sublittorally in the sunlit upper photic zone (Hörnig et al. 1992a, 1992b). Beginning with the observations during the nineteenth century by Kuckuck, *D. dichotoma* was abundant on the intertidal sandstone terraces at the north side of the island of Helgoland (German Bight, North Sea) (Nienburg 1930), but gradually disappeared around 1960 (Kornmann and Sahling 1977). Since the implementation of diving surveys at Helgoland in 1965 (Lüning 1970), *D. dichotoma* was rarely encountered in the sublittoral, until small, but persistent, populations of sporophytes and gametophytes were found again in 1989 (Kornmann and Sahling 1994). These populations appeared to be restricted to two sublittoral habitats of predominantly artificial substrata at the south harbour. There is no obvious reason why *D. dichotoma* disappeared completely from the intertidal location, since it previously reappeared after eradication of the entire intertidal community during the severe winter of 1922, when the soft sandstone substratum was frozen, fractured, and destroyed (Nienburg 1930). Despite the fact that *D. dichotoma* occurs also in the intertidal zone in the Mediterranean, the photosynthetic performance of mature specimens from Helgoland is reduced by high levels of PAR (Nultsch et al. 1987; Hanelt et al. 1995). Thus, light may play a role in limiting the upper distribution of *D. dichotoma* in this region. Even mature thalli of field material from southern Spain have lower photosynthetic performance in the presence of UVR than when UVR is removed (Flores-Moya et al. 1999). Following the suggestion by Dring et al. (1996b) that only experiments with natural radiation would further elucidate the role of UV-B on macroalgal community structure, we studied at Helgoland the long-term growth of *D. dichotoma* as affected by present levels of solar UVR and by enhanced UV-B. Experiments started with very young germlings at a time when the natural population was releasing tetraspores which enabled us to follow the impact of UVR during early ontogenesis. As a model organism for the study of UVR effects on macroalgal growth including the early stages, *Dictyota* has several advantages: it has a mainly two-dimensional thallus morphology with surface area as a reliable factor for calculating growth rate, and its relatively large tetraspores are easily obtained and germinate immediately, with a germination rate of nearly 100% (R. Kuhlenkamp et al., personal observation). The present study was designed to test the effects of natural and enhanced UVR on germling growth under semi-field conditions with the invaluable advantage of natural ratios of PAR and UVR (see Fiscus and Booker 1995), and naturally high PAR levels which are usually difficult to reach in laboratory systems. This is particularly important for the later interpretation of physiological responses (Dring et al. 1996a), as it has long been known from higher plant studies that UV-B effects are less pronounced when plants are grown under the high PAR and UV-A levels of solar radiation normally seen in outdoor experiments (Teramura 1980; Caldwell et al. 1994).

Materials and methods

Materials

Attached specimens of *D. dichotoma* were collected by SCUBA diving at the island of Helgoland, south-eastern North Sea, Germany (54°11'N, 7°53'E) on 25 September 1997. Plants of up to 7 cm in length grew in the sublittoral zone (0.5–2 m below MLWS) in a sheltered location without any overgrowing canopy.

The material was kept in seawater and darkened containers during the immediate transport to the laboratory. Twelve fertile tetrasporophytes were selected and cleaned of any debris and epiphytes. Thalli were precultured for 3 days in sterile, enriched seawater [Provasoli 0.5 ES; Starr and Zeikus (1993)] at 16°C and a light:dark regime of 16:8 h with about 60 µmol m^{-2} s^{-1} of irradiance from daylight fluorescent tubes (L 58 W; Osram, Germany). Then, segments with mature sporangia were excised from ten healthy specimens and placed in seawater in sterile glass dishes. Release of sufficient numbers of tetraspores occurred overnight and about 40 to 50 spores were seeded as evenly as possible on the bottom of each polystyrene Petri dish (50 mm in diameter) used in the experiment. Prior to exposure in the outdoor experimental setup, all dishes were left for 3 days under the same culture regime as used for the parent plants to facilitate secure attachment of the germlings. Germlings were placed in their respective test condition simultaneously in the morning after the first measurement had been taken (initial value). Five dishes were used in each treatment, totalling about 200–250 germlings per light condition. Before each measurement plants and dishes were cleaned carefully with a soft nylon brush to remove the inevitably occurring diatoms and other small algae. The procedure was sufficient to keep the epiphyte

Table 1 Characteristics of light treatments in the outdoor tank system (measurements with LI-1800 UW spectroradiometer). *PAR* Photosynthetically active radiation, *UV* ultraviolet, *UVR* UV radiation

Treatment		Spectral range (nm)	Filter	Company
1	PAR	395–700	UVR-blocking acrylic sheet 5 mm, GS233 Plexiglas	Röhm, Darmstadt, Germany
2	PAR+UV-A	320–700	UV-B-blocking foil 140 µm, Folex	Folex Dr. Schleusner, Dreireich, Germany
3	Solar spectrum (PAR+UV-A+UV-B)	300–700	UVR-transparent acrylic sheet 5 mm, Altuglas 123	Altumax, Bonn, Germany
4	Solar spectrum plus additional UVR	300–700	UVR-transparent acrylic sheet 5 mm, Altuglas 123 plus one or two UVA-340 fluorescent lamps	Altumax, Bonn, Germany; Q-Panel, Cleveland

cover very low for about 1 week. Due to the strict haplo-genotypic differentiation into male and female tetraspores, the plant population used in the experiment consisted of equal numbers of male and female gametophytes. Since both sexes are morphologically identical with the same growth characteristics, it was assumed that UVR would have the same effect on the growth of both stages.

Experimental tank system

Open dishes with attached *D. dichotoma* germlings were placed in an open tank situated in the outside experimental area of the Biologische Anstalt Helgoland (ground level) with running seawater and exposed to natural solar radiation. Seawater was pumped in from the surrounding sea, filtered through a large scale sedimentation filter and flowed at 40–70 l h^{-1} through a shallow tank (1 m×1.5 m surface area) containing 450 l water. Dishes were attached to black polyvinylchloride (PVC) plates (30 cm×90 cm× 5 mm) and submerged horizontally to about 6 cm below the water surface (9 cm below the upper edge of the tank). The dishes were placed between silicone strings wound around the PVC plates. This assured a secure, horizontal fastening and provided a black background, important for establishing the photomorphogenetic polarity of rhizoids and upright phylloids. The tank was orientated such that all dishes of one treatment were exposed to the same light dose. Light treatments consisted of four combinations of solar PAR, UV-A and UV-B obtained by using filter materials with different cut-off characteristics, or by the addition of artificial UVR (see Table 1 for filter materials, lamps, and sources). Filters were placed above the dishes on pins in the PVC plate, and were kept always submerged to reduce surface reflection. A slightly oblique attachment with the southfacing edge being about 5 cm lower prevented accumulation of gas bubbles. Two UVA-340 Q-Panel lamps were situated 17 cm above the germlings of treatment 4 on the northward side of the tank, avoiding shading by the tubes as much as possible. At the position of the test organisms, the supplemental UVR doses were 10.358 Wm^{-2} UV-A (316–400 nm), 0.502 Wm^{-2} UV-B (300–315 nm), and 0.098 Wm^{-2} as weighted UV-B [300–313 nm, generalized plant action spectrum; Caldwell (1971); Björn and Murphy (1985)] During the first week of the treatment, both UVA-340 lamps were switched on for 6 h between 1000 and 1600 hours. Since incident irradiance <340 nm declined during the second and third weeks, the supplementary dose was reduced to 5.179 Wm^{-2} UV-A (316–400 nm), 0.251 Wm^{-2} UV-B (300–315 nm), and 0.049 Wm^{-2} as weighted UV-B (300–313 nm) by using only one lamp during the same daily period. UVR-blocking acrylic sheets were placed between treatments as UVR barriers.

Spectral characteristics of the light fields under each treatment were measured using a LI-1800UW spectroradiometer (Li-Cor, Lincoln, Neb.) submerged in seawater with the sensor in the same position as the *D. dichotoma* germlings. Sample spectra were measured during clear skies between 1320 hours and 1340 hours on 3 September 1997 and compared to the incident solar irradiance just above the water surface (variable weather conditions during the actual experiment did not permit repeated measurements). Solar irradiance (PAR: 400–700 nm; 380, 340, 320 and 305 nm) at Helgoland was monitored continuously during the experiment with a PUV-500 radiometer (Biospherical Instruments, San Diego, Calif.) mounted permanently on the roof of the research laboratory. Water temperature in the tank was monitored using an electronic temperature logger (0.1°C accuracy, ama-digit ad 20 th with Pt 100 sensor, Germany).

Growth measurements

The morphology of *D. dichotoma* with its thin, flat phylloid is already distinct in 5-day-old germlings and facilitates measuring length or surface area as a valid parameter for growth rate. At the beginning of the experiment (day 0=initial) and then every week between 2 and 23 October 1997, 50 germlings in each treatment were measured always during early morning. Petri dishes were taken out of the tank and black and white photographic images were made of germlings in the centre of the dish, avoiding areas possibly shaded by the rim. Although most of the water had to be removed from the dish for the germlings to lie flat, this rapid procedure had no obvious impact on viability. Ten individuals in each dish were randomly selected from the initial photographs and their length and width were obtained for each sample date. Tests with an automated image analysis system failed to reproduce accurate measurements of area or length because the germlings were too small, and the system could not distinguish between the actual phylloid area and rhizoids.

In order to calculate the area of *D. dichotoma* germlings in millimetres squared, a factor was applied to the value of length multiplied by width. The factor was established from five randomly selected plants of each sample date and treatment by measuring the actual area in millimetres squared and comparing the result to the value obtained by the multiplication of length by width (which is larger than the actual size). Since the factor was relatively consistent for each sample date, without much variation between dates, the average factor of 0.705 (±0.05) was applied in all area calculations.

The relative growth rate (RGR) per day was calculated on the basis of the means of the length measurements as $RGR=(\ln x_1-\ln x_0)t^{-1}$ where t=time in days, x_0=mean length at the start of the growth interval and x_1=mean length at the end of the growth interval. The RGR of *D. dichotoma* was computed for each treatment for the whole experimental period and for each weekly interval.

At the end of the experiment several plants out of each treatment were observed under the light microscope for any visible damage or deformation of cell structures. About 50 plants in each treatment were further cultivated under the experimental conditions for another 6 weeks in order to observe the continued development of plants and possible aberrations in growth morphology due to long-term damage of the apical meristem.

Statistical analysis

Mean values and SDs were calculated for the 50 replicates of each of the four treatments and sample dates (initials, 7, 14 and 21 days). Normality tests showed a near-normal distribution for all samples (n=50 per sample). Significance was tested by one-way ANOVA (Statistica version 5.5; Statsoft) using the Cochran test of homogeneity of variances and Tukey honest significant difference test.

Results

Environmental parameters

The spectral characteristics of the four light treatments are shown in Fig. 1. Although the UVR-blocking acrylic sheet transmitted some of the UV-A radiation (about 34% of the full UV-A spectrum from 360 to 400 nm), the total UV-A in treatment 1 (PAR) was strongly reduced and the shorter wavelengths of 300–360 nm were completely removed. The Folex-foil in treatment 2 was slightly reflective underwater, and reduced the incoming light by an average of 3.6% per wavelength calculated for the PAR range. The emission spectrum of the UVA-340 lamps was similar to the solar spectrum at wavelengths <345 nm and contained no radiation <295 nm.

The natural light doses received by the plants in treatments 3 and 4 under the full solar spectrum are listed in Table 2, calculated for the wavelengths measured by the Biospherical radiometer and for each week of the 3-week experimental period. The weekly integrals of PAR and 380-nm UVR (UV-A) were relatively constant during the 3 weeks of the experiment, showing a slight drop by about 6% and 10%, respectively, in the third week. In contrast, the short UVR range decreased continuously during this period. The strongest and most prominent drop of about 60% from the first week integral occurred in the shortest wavelength of 305 nm. This is due to the stronger seasonal decline of UV-B compared to the other wavelengths as shown by the low ratio UVR:PAR for UV-B (305 and 320 nm) during autumn, while ratios for UV-A (340 and 380 nm) stay constant throughout the year (Dring et al. 2001).

Temperature in the experimental seawater tank dropped continuously over the test period from about 17°C at the beginning to about 12.5°C at the end of the experiment, following the decrease in water temperatures of the surrounding sea. Since the tank was exposed to ter-

Table 2 Incident solar irradiance for each wave band measured by the Biospherical PUV-500 radiometer at Helgoland. Values are integrals for each weekly interval and for the total period of the experiment (21 days)

Spectral band Units	PAR mol m^{-2}	380 nm kJ m^{-2}	340 nm kJ m^{-2}	320 nm kJ m^{-2}	305 nm kJ m^{-2}
Week 1	81.7	34.6	23.5	11.1	0.5
Week 2	83.8	34.9	22.9	10.0	0.3
Week 3	78.1	31.2	20.0	8.6	0.2
Total period	243.6	100.7	66.4	29.7	1.0

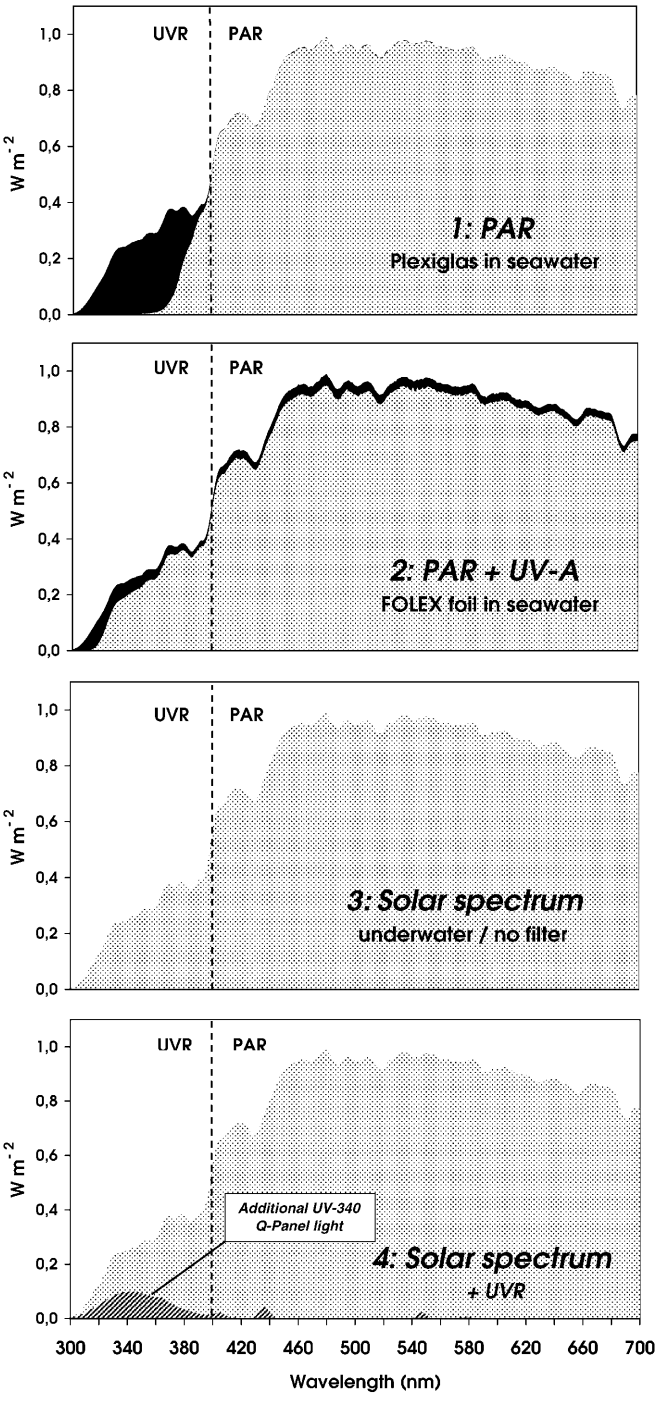

Fig. 1 Spectral characteristics of the four light treatments photosynthetically active radiation (*PAR*), PAR plus ultraviolet-A (*UV-A*), solar spectrum and solar spectrum plus UV radiation (*UVR*) in the range 300–700 nm. The spectra (*shaded areas*) were measured underwater with the light sensor in the position of the test-organisms in the tank. The *black area* (unfiltered spectrum of solar irradiance at water surface) indicates the part of the solar spectrum which is blocked by each filter. The spectral distribution of the additional UVR source used in treatment 4 (*hatched area*) shows the similarity in spectral distribution in the range of 300–340 nm. All spectra were measured on 3 September 1997 with a Licor LI-1800UW spectroradiometer

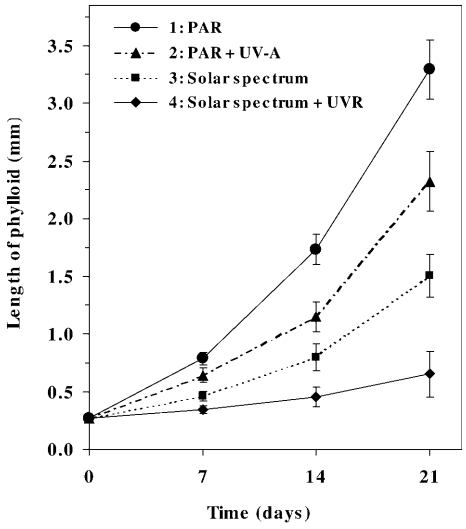

Fig. 2 Increase in length of phylloids of *Dictyota dichotoma* germlings under the four treatments PAR (●), PAR+UV-A (■), solar spectrum (◆) and solar spectrum plus UVR (▲). Measurements were taken at day 0 (initials), after 7, 14 and 21 days, and the means and SDs for 50 replicates each are shown. For abbreviations, see Fig. 1

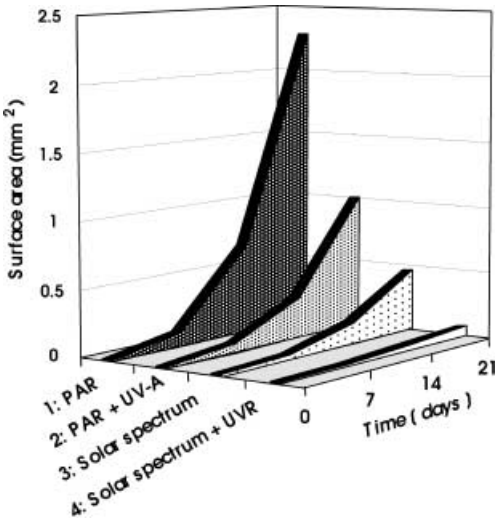

Fig. 3 Increase in surface area of phylloids of *D. dichotoma* germlings under the four light treatments. Values are shown as means calculated from the length and width measurements of 50 replicates each. For abbreviations, see Fig. 1

restrial temperatures and insolation, values in the tank were about 1°C higher during the first period (until 1 October) and 1°C lower during the second period than the actual seawater temperatures monitored by the Helgoland Reede time series (Biologische Anstalt Helgoland – AWI; Scharek, Mangelsdorf, personal communication).

Growth experiment

All *D. dichotoma* specimens taken from the field were healthy plants of typical morphology and were fertile, corresponding to the reproductive season between August and November (R. Kuhlenkamp et al, personal observation). Germination of tetraspores occurred immediately after release and was nearly 100% with germlings attaching to the Petri dishes within 2–3 days, rhizoids at the shaded side. When the dishes were outplanted into the outdoor tank, the average length of the germlings was 0.2 mm. During the 21 days of the main experiment, *D. dichotoma* plants exhibited nearly exponential growth under all conditions while strong differences in length among treatments were recorded based on measurements of 50 germlings each (Fig. 2). Within each of the four treatments, differences in length among the sampling days were all highly significant with P=0.004. When comparing values within the same sample date (7, 14 and 21 days), differences among treatments were highly significant with P=0.00001. Reduction in length was clearly related to the spectral characteristics of the applied solar and artificial radiation. Plants under PAR (treatment 1, UVR-blocking acrylic sheet) were always larger than those under any of the other treatments, and every additional amount of UVR significantly decreased growth. Germlings growing under

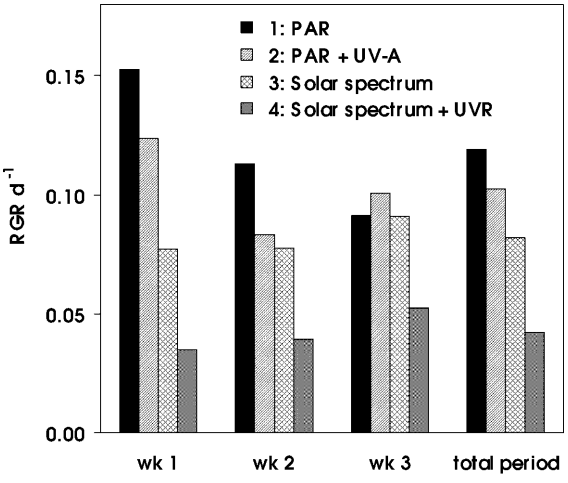

Fig. 4 Relative growth rates (*RGR*) per day of *D. dichotoma* germlings calculated from the means of the length measurements shown in Fig. 2. The growth rates are indicated for the total experimental period of 21 days (*d*) (total period) and for 3 weekly intervals. *wk 1* first week, *wk 2* second week, *wk 3* third week; for other abbreviations, see Fig. 1

the full solar spectrum supplemented by artificial UVR from the Q-Panel UVA-340 lamps (treatment 4) were the smallest plants at all sampling dates. Since juvenile *D. dichotoma* plants grow mainly in a two-dimensional way with a factor of about 0.7 between length and width, using phylloid area as the indicator for growth results in a more realistic representation of the exponential growth characteristics and size differences among plants from different treatments (Fig. 3).

The RGRs per day calculated from the means of the length measurements are based on the total period of 21 days and on weekly intervals (Fig. 4). Variation in the

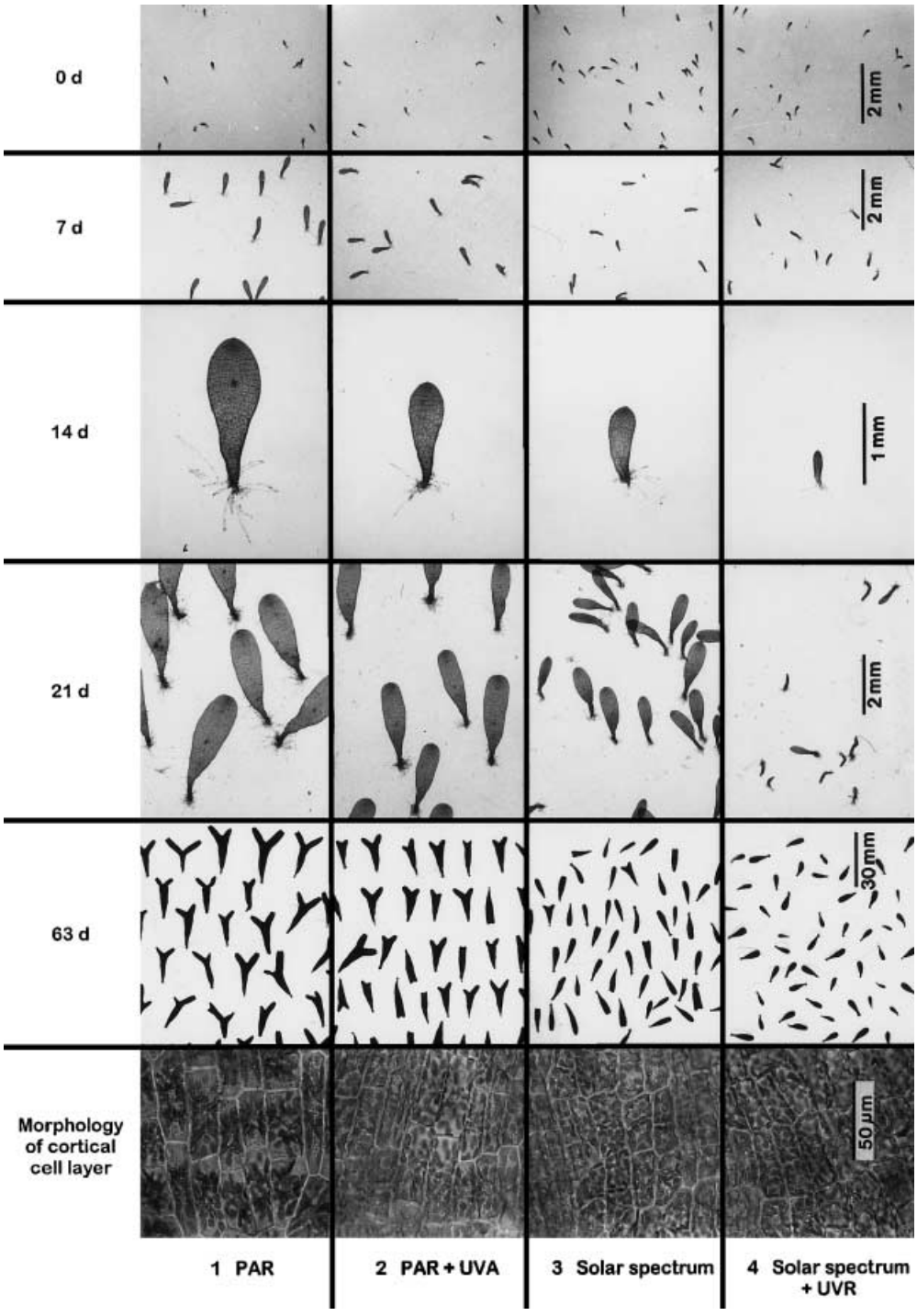

Fig. 5 Photo-micrographs of representative examples of *D. dichotoma* plants from the experimental groups. The *first four rows* show images of the successive stages attached to the Petri dishes during the exposure to the four light treatments taken at the same time as the growth measurements. The normal development of the juvenile plants after 9 weeks is shown in row *63 d*. Representative surface views of the cortical cell layer of germlings after the 21 days of exposure to the four treatments are presented in the *last row* showing the intact general anatomy of the cells (nuclei, chloroplasts, cytoplasm, etc.); cells and chloroplasts are all of equal size. *Scale bars* shown for one image apply for the respective row. For abbreviations, see Figs. 1 and 4

RGRs among the different intervals within one treatment were apparent. Whereas the length of plants increased steadily in all conditions, growth rates of plants under PAR and PAR+UV-A were higher in the first week than in the third, while in the treatments including UV-B (full solar spectrum) the growth rates increased slightly. The apparent acclimation of RGR in treatments 2 and 3 to the level in treatment 1 is a reflection of both reduced RGR in treatment 1 and increase in RGR in treatments 2 and 3. Calculated for the total period of 21 days, however, growth rates decreased significantly with increasing UVR. The shorter the wavelength of UVR, or the higher the UVR dose was, the greater was the negative effect on growth rate. While during the total period of 21 days, RGR under PAR+UV-A was reduced by 14% compared to the control condition PAR, the full solar spectrum reduced the RGR by about 31% and the full solar spectrum plus additional UVR by Q-Panel lamps resulted in nearly 65% reduction of the RGR.

Germlings and juveniles of *D. dichotoma* in treatments 1–3 did not exhibit any aberrations in their general

morphology, except for the very obvious differences in size depending on the amount of UVR received (Fig. 5). While germlings were all similar in size when outplanted, clear size differences were apparent after 1 week which became more pronounced and macroscopically visible after 2 and 3 weeks. Microscopic observations made at 14 and 21 days including the apical meristematic zone did not reveal any apparent differences in shape, cell structure and arrangement of cells and chloroplasts. Only in treatment 4 with the supplementary artificial UVR did many of the germlings show a slight deformation in their morphology during the first week with germlings attaining a crooked shape of the still narrow thallus. *D. dichotoma* plants kept in the tank for further observation after the main experiment continued to show the strong difference in size due to UVR. Those in treatments 1–3 developed a normal, flat thallus with its characteristic dichotomies (Fig. 5; 63 days), indicating that the apical, meristematic cell was not damaged despite the presence of solar UV-B in treatment 3. Plants in treatment 1 showed first signs of dichotomies during the fourth week and were up to 3 cm long after 9 weeks. Germlings in treatment 4 with the highest UVR were still the smallest (up to 1.5 cm in length) and continued to show an increasing variation in size, but most individuals developed a fairly normal morphology.

Discussion

The case for natural radiation in UV-B studies on marine macroalgae

Identification of specific targets of UV-B stress is often done using an artificial UV-B source, but translating these results into an ecological context is difficult, due to the unnatural spectral composition of most artificial sources. While they may mimic the natural UV-B spectrum very closely, they are usually deficient in UV-A and PAR. These wavelengths are critical for promoting the repair of UV-B-induced DNA dimerization (Sancar and Sancar 1988; Sancar 1994; Buma et al. 1995), for maintaining active photosynthesis, and in some cases, for inducing the synthesis of UV-absorbing protective pigments, the mycosporine-like amino acids (Riegger and Robinson 1997; Hannach and Sigleo 1998). In cases where the natural ratio of UV-B:UV-A:PAR has been maintained, sensitivity of plants to UV-B was shown to be less pronounced (Cen and Bornman 1990; Caldwell and Flint 1994). Therefore, one might expect a truer picture of the impacts of UV-B to come from designs using artificial radiation with near natural ratios of UV-B:UV-A:PAR, or those incorporating natural radiation. On the other hand, marine macroalgal habitats often have very high wave energy, presenting another set of difficulties for using filters, supplemental irradiance, and following the establishment of microscopic propagules in situ. Shore-based experiments then, are a compromise for following the growth and development of individuals under easily manipulated radiation conditions. It is particularly useful for very shallow subtidal organisms, where individuals are not periodically emersed. Our study shows that long-term outdoor experiments with large numbers of replicates can be performed with juvenile stages of macroalgae, as young as 2-day-old germlings. In species like *D. dichotoma* where propagules are easily obtained, germinate and adhere readily, the development of reproductive organs, spore release and the first cell divisions could also be tested under near natural conditions, thereby encompassing all ontogenetic stages. Such experiments would complement laboratory studies on early processes important for recruitment, such as those performed on *Padina boergensii*, a related species of *Dictyota*, which showed reduced tetraspore liberation under UVR (Ganesan et al. 1999).

UVR effects on growth of juvenile *D. dichotoma*

A number of studies on higher plants have demonstrated that enhanced levels of UV-B lead to changes in growth rate, leaf thickness, internode length, and tillering (reviewed in Jansen et al. 1998). While reductions in photosynthesis and growth have been observed for a number of macroalgae (Wood 1987; Grobe and Murphy 1998; Franklin et al. 1999), changes in thallus morphology as a result of UV-B exposure have not been reported. The first field and outdoor experiments studying growth of young stages of marine algae under natural UVR and high PAR levels, suggested that the kelp *Ecklonia radiata* was inhibited from growing at higher levels in the sublittoral by high UVR sensitivity in young stages (Wood 1987). However, exposure of the meristem of *E. radiata* to ambient PAR and UVR after removal of the shading canopy was lethal. Growth of *D. dichotoma* is based on divisions of a single apical cell (Katsaros and Galatis 1985) which is not shaded by any other cell layer. The apical cell is present in the earliest stage and the primary cell divisions are critical for the subsequent thallus differentiation. Because cell divisions occur continuously, any severe effect on cell division (e.g. permanent DNA damage) in the meristem should have a clear effect on morphology and plant viability. The experiments described here demonstrate that UV radiation has a strong impact on *D. dichotoma* growth, but no impact on morphology.

The effect on growth in *D. dichotoma* appears to be due to a reduction in cell division rate, as no cell or organelle size differences, or thallus deformation were detected, apart from those which occurred in the beginning under additional UVR from artificial lights. In a comparable long-term tank experiment, Grobe and Murphy (1998) reported a similar reduction in the growth of high subtidal *Ulva expansa*, with no change in pigmentation, nitrogen uptake, or cell size. The response of *D. dichotoma* to UVR suggests that there is an inverse linear relationship between wavelength or dose of UVR and growth rate, at least at the relatively low seasonal amounts of UVR encountered here. This relationship remains to be verified in reciprocity experiments using different UVR doses.

The reduction in growth rate may be due to additional metabolic costs for ongoing repair to cellular components such as the repair of UV-B-induced DNA dimerization, without which DNA replication cannot proceed. Formation of thymine dimers occurs in microalgae exposed to UV-B (Buma et al. 1997), and Pakker and Breeman (1997) have demonstrated that thymine dimers can form in macroalgae exposed to natural levels of UV-B. Growth reduction may be the result of lower photosynthetic rates. The photosynthetic performance under solar irradiance of *D. dichotoma* juveniles is lower than that of adults (Ramus and Rosenberg 1980), thus their ability to meet any increased metabolic cost may be reduced at the same time that UV-B exposure is maximal. Natural levels of UV-B have been shown to damage reaction centre II proteins in *Brassica* (Olsson et al. 2000) and the red alga *Chondrus crispus* (Franklin and Lüning 1998). In a number of macroalgae, activity of Rubisco is reduced when thalli are exposed to UV-B, though the amount of reduction depends on species and length of exposure (Bischof et al. in press). In *D. dichotoma*, the photosynthetically active region consists of the one-cell thick, cortical layer (Katsaros and Galatis 1985) which is, therefore, directly exposed to radiation. While chloroplasts in this layer change position with increasing irradiance, so as to limit absorbance and protect the chloroplast from photodamage (Hanelt and Nultsch 1991), UV-B is not effective in this process, and no additional protection might be expected.

Due to the late release of tetraspores, the study was conducted late in the season, when the level of solar irradiance is nearly 4 months past its yearly maximum. Not only are changes in daily irradiance levels high during that period, but UV-B decreases faster than PAR. The extreme drop (>50%) in UV-B over the 21 days of the experiment was likely responsible for the increasing growth rates of *D. dichotoma* germlings exposed to the full solar spectrum, since the available PAR was still sufficient for similar (week 3) or higher (week 2) growth rates as seen in plants in the PAR treatment. This could explain the convergence of growth rates for the first three treatments in the last week of the experiment, indicating no effect of UVR on growth rate. Plants, however, which had been exposed to UVR during early development (treatments 2 and 3 during week 1) and had very low growth rates during this time, were not able to regain the size of the control specimens even when UVR was reduced.

Effects relative to natural habitat

In contrast to populations of *D. dichotoma* in the clear waters of the Mediterranean, those at Helgoland experience turbid water with a wide tidal range (1–3 m). Therefore, the population is exposed to a wide range of UVR doses during the day. Plants near the MLSW level receive the full solar radiation for some time during the day, and those between 0–2 m depth are exposed to the more effective UV-B at least during part of the day. Based on the average yearly 1% depths for UV-A and UV-B at this location (Dring et al. 2001), plants growing at depths of 1–2 m below MLSW receive some UV-A throughout the day, while those below 2 m should receive nearly no UV-B. Despite the limitations of our experimental design to represent natural conditions, due to the constant depth of the samples etc., it is an appropriate system to measure effects of solar radiation on germination and juvenile stages. The long duration of the experiment, with gradual changes in radiation due to diurnal and meteorological patterns, brings results closer to natural conditions than might be expected for short-term measurements of physiological responses to short, sudden changes in radiation. At shallow depths, growth rates of *D. dichotoma* will be under the antagonistic effects of UVR and PAR because both increase towards the surface. High PAR may also be inhibitory to growth, as shown in *Ulva* (Henley and Ramus 1989). Therefore, depending on the actual level of PAR or UVR, plants could experience a negative, positive, or neutral effect on growth rate. Although action spectra for UVR-induced damage indicate that UV-A is far less effective than UV-B (Buma et al. 1997), and is beneficial to the repair of DNA damage by photoreactivation (Sancar 1994), the greater quantity of UV-A in the environment relative to UV-B leads to some UV-A-induced damage. During the first 2 weeks of the experiment, *D. dichotoma* growth rates were reduced by UV-A indicating a net stress on the germlings.

Flores-Moya et al. (1999) found that *D. dichotoma* from southern Spain recovered to a greater extent from a natural PAR+UV-A+UV-B treatment than from a PAR+UV-A exposure, and suggested that UV-B might play an essential role in recovery. A similar acclimation to UV-B was shown for a Mediterranean population of *Ulva rigida* (Altamirano et al. 2000). The contrast between the *D. dichotoma* distribution in southern regions and Helgoland may reflect ecotypic differences and warrant further investigation. If future studies on *D. dichotoma* at Helgoland demonstrate similar or higher susceptibility to UVR in summer to that in the present study, then UVR and particularly UV-B could be a strong factor limiting *D. dichotoma* to depths below those incurring a critical dose of UVR in this northern habitat.

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