Effect of visible and UV radiation on early sporophytes of species of the Laminariales

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By

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To my parents

'In the beginning, God created the heaven and the earth. And God said, Let there be light. And God saw the light, that it was good; and God divided the light from the darkness (Genesis 1: 1, 3)'

Contents

Abstract: Effect of visible and UV radiation on early sporophytes of species of the Laminariales

Light-related behaviour of early sporophytes of species of the Laminariales was investigated in laboratory culture.

The growth of four species (***Alaria esculenta, Laminaria digitata, Laminaria hyperborea*** and ***Laminaria saccharina***) was similarly light-saturated at about 30 μmol $m^{-2}s^{-1}$. The minimum irradiance for growth of ***L. hyperborea*** (the only species used) seemed to be less than 1-2 μmol $m^{-2}s^{-1}$. In all four species, there was a reduction in the growth rate with age.

The length/width ratios of thalli of ***A. esculenta*** and ***L. digitata*** were high, irrespective of irradiance, while that of ***L. hyperborea*** was lower. The thallus shape of ***L. saccharina*** seemed related to growth rate.

In 17:7 h light-dark cycle, the growth rate of early sporophytes increased with increased irradiance up to 57 μmol $m^{-2}s^{-1}$, beyond which there was no significant increase. In 7:17 h light-dark, however, ***A. esculenta*** showed a significant increase in the growth rate up to 127 μmol $m^{-2}s^{-1}$ (the highest irradiance used). In the short-day an increase above 10 mol $m^{-2}s^{-1}$ MDI (Mean Daily Irradiance) had no effect on the growth rate of ***L. hyperborea*** and ***L. saccharina,*** but in the long day there was a significant increase.

The ratio of maximum growth rate under continuous light and 12:12 h light-dark cycle was 1.5:1 for ***L. hyperborea,*** 1.2:1 for ***L. saccharina*** and 1.1:1 for both ***A. esculenta*** and ***L. digitata.***

After 24 days, ***L. digitata*** but not ***L. hyperborea*** was found to require higher irradiance for faster growth with time. Compared with ***L. hyperborea, L. digitata*** was short-survived in the dark and showed a slower growth in extremely reduced daylight conditions.

L. hyperborea showed a significantly lower growth rate in red than in blue or green light at low irradiances but the growth rate of ***A. esculenta*** seemed to depend more on light quantity than on light quality. ***L. saccharina*** appeared to be sensitive to the red waveband in response to changes in irradiance.

In ***L. hyperborea*** (the only species tested), the growth response did not seem to be correlated with the arrangement of phaeoplasts.

Of the four species, a limited population of ***L. hyperborea*** and ***L. saccharina*** showed a significant growth inhibition at 180 μmol $m^{-2}s^{-1}$. Furthermore, excessive blue light was found to be involved in the photoinhibition of growth of ***L. hyperborea.*** ***A. esculenta*** and ***L. digitata*** were more tolerant of high irradiance of sunlight than ***L. hyperborea.***

Early sporophytes of ***L. hyperborea*** acclimated to 13-19 μmol $m^{-2}s^{-1}$ for 14-20 days were growth-inhibited or photobleached with no sign of growth when they were transferred to 135-159 μmol $m^{-2}s^{-1}$. On the other hand, ***L. digitata*** showed a fast growth even when the plants were transferred from the low to the high irradiance. Neither species acclimated to 55-63 μmol $m^{-2}s^{-1}$ showed any inhibition in growth at 135-159 μmol $m^{-2}s^{-1}$. In addition, acclimation at 55 μmol $m^{-2}s^{-1}$ allowed ***L. digitata*** and ***L. hyperborea*** higher survival under direct sunlight of sublethal dose than did that at 8 μmol $m^{-2}s^{-1}$.

Exposure of UV-irradiated early sporophytes to visible light resulted in recovery from UV damage that would otherwise cause much higher mortality. For this photoreactivation, blue light was highly effective, whereas negligible reactivation was produced either in green or red light. The response in white light was proportional to the blue band it contained. The blue quantum requirement for 50% response was 1.2 mol m^{-2} for ***L. saccharina,*** 1.9 mol m^{-2} for ***A. esculenta*** and 2.5 mol m^{-2} for ***L. hyperborea.***

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General introduction

Growth is a complex phenomenon and subject to many input variables (Lobban et al. 1985). Many ecological studies have indicated that light, water temperature and nutrient availability are important factors affecting algal growth (see Darley 1982, Lüning 1990). On the other hand, the role of light in structuring patterns of benthic algal distributions has received a great deal of attention (see reviews by Hellebust 1970, Drew 1983, Lüning 1981a). However, the prediction of light demands of algae is not simple, because even within one species they differ between different stages (Fei and Neushul 1984, Fei 1985, Fei et al. 1989, Hales and Fletcher 1989, Gerard 1990). In order to understand the effect of light on the basic features of algal ecology, therefore, it is necessary to examine the growth response to light separately.

The establishment stage is clearly of great importance for the coming generation, affecting the performance of the adult population (Kain, 1964, 1965, 1969, Vadas 1972, Hruby and Norton 1979, Deyser and Norton 1982). Perhaps due to their supposedly extreme shade environment and relative ease of culturing, brown algal microscopic stages have been favourite subjects for studies on growth and reproduction in relation to light (Kain 1964, 1965, 1969, Norton and Burrows 1969, Lüning and Dring 1972, 1975, Vadas 1972, Dring and Lüning 1975, Lüning 1980, Fain and Murray 1982, Fei et al. 1989).

The Laminariales is one of the most important benthic orders in the sublittoral euphotic zone. On most parts of the rocky coasts of Britain, the sublittoral laminarian algae are observed to form certain features of distribution. The upper margin of the sublittoral region is dominated

by ***Laminaria digitata*** (Huds.) Lamour. and/or ***Alaria esculenta*** (L.) Grev. and at the lower limit below ELWS these are displaced by ***Laminaria hyperborea*** (Gunn.) Fosl. and/or ***Laminaria saccharina*** (L.) Lamour. (Kitching 1941, Kain, 1962, Norton et al. 1977). The life cycle typical of the Laminariales consists of an alternation of generations between a microscopic gametophyte stage and a more conspicuous sporophyte stage that forms the kelp canopy (Kain 1971a).

Recently, Gerard (1990) has reported a phase-specific adaptation between gametophyte and sporophyte stages in light-related traits of ***L. saccharina.*** This may highlight the importance of studying the growth response of early sporophytes to light as direct indicators of the later behaviour of the adult plants in preference to that of gametophytes. However, little attention has been given to the light-related behaviour of the very early sporophyte stage except that of Kain (1965, 1969).

In this study, a hypothesis that the major factor responsible for the distribution pattern may be light, whether it acts as a resource for competition between species or a direct stress to the plants, is tested with very early sporophytes of species of the Laminariales in a laboratory culture study. Thus, sensitivity of the four laminarian species to radiation (including UV) is assessed in the following ways. In chapter 1, the light requirement of early sporophytes is determined at various photon irradiances and at different ages. The effect of different photoperiods on growth is evaluated. For two species (***L. digitata*** and ***L. hyperborea***), investigations of their resistance to darkness and growth response to reduced daylight are made. Also, a study is extended to compare the morphological characteristics of each species in relation to light. Chapter 2 includes some growth experiments on the three species performed in different wavebands of

light and an observation of the phaeoplast arrangements of ***L. hyperborea***. Sensitivity of different species to high photon irradiances constitutes a main theme of chapter 3. This chapter consists of some investigations on the effect of high irradiance of white light, the growth response of ***L. hyperborea*** to high irradiance of coloured artificial light or the survival in colour-filtered sunlight and the resistance of different species to sunlight (***A. esculenta*** or ***L. digitata*** in comparison with ***L. hyperborea***). In the last chapter, the possibility of recovery from UV damage and the light requirement for the response are investigated.

General materials and methods

Culture of plants

Fertile sporophytes of members of the Laminariales were collected near Port Erin Bay, Isle of Man (54º 05' N, 4º 54' W) during the period from October,1988 to December, 1991. The plants were stored overnight in a polythene bag in a controlled temperature room maintained at 10 ºC before they were wiped with soft paper to remove diatoms and mucus. Suitable fertile blades of single plants were rinsed in two or three separate baths of filtered seawater and immersed with sterile seawater, being magnetically stirred to stimulate release of zoospores for 1-2 h, after which the spore solution was poured off into a cylinder and left to stand in darkness for an hour to allow diatoms or debris to settle. Magnetic stirring prevented clumping of spores which might have affected their later growth (Kain 1965). After microscope examination to confirm the presence of zoospores, liquid was drawn off the top for use. Five to ten percent of spore solution, by volume, was poured into an appropriate volume of medium. This mixture was distributed into petri-dishes (100 mm diam., 10 mm high) each containing 35 ml of medium, with 12 coverslips (18 x 18 mm, square form) or 27-30 coverslips (13 x 13 mm, round form) or into lidded flat-bottomed crystallizing dishes (80 mm diam., 43 mm high) each containing 50 ml of medium with 5-6 coverslips (22 x 22 mm, round or square form) lining the bottom. All the glassware and culture dishes were washed in 'Lypsol' cleansing liquid, thoroughly rinsed with tap water followed by distilled water, then dried in a dry oven (40 ºC for plastic dishes and 120 ºC for glassware) prior to use.

Growth medium

The culture medium was a modified BG (mBG) which contained fewer vitamins than BG used by Kain (personal communication) as was described by Kain (1964), using the vitamins listed by Kain and Fogg (1960). The effect of omission of 8 vitamins from BG on the fertility of ***L. hyperborea*** and on the time taken for the appearance of sporophytes in three laminarian species was tested (in preliminary experiment A). Composition of the medium used in this study is shown in Table 0.1.

Table 0.1. Composition of mBG (per litre filtered seawater).

KNO_3 (1000 mM)		1.0 ml	
K_2HPO_4 (20 mM)		5.0 ml	
$FeCl_3$ (10 mM)		0.5 ml	
3 Vitamins		5.0 ml	
3 V	Thiamine (1000 mM)	5.0 ml	
	Cobalamine (0.1 μM)	0.5 ml	made up to 100 ml
	Biotin (10 μM)	2.0 ml	distilled water

When medium was autoclaved (15 lb/in, 415 A), K_2HPO_4 was added after autoclaving.

Seawater pumped from Port Erin Bay was filtered through LP depth filter cartridges (0.22 μm filter size, Balston filter products). The medium was

replaced at least weekly and GeO_2 was included in the medium for the first week to prevent the cultures from being contaminated by any remaining diatoms (Lewin 1966). No obvious inhibitory effect has been found in ***Laminaria saccharina*** grown in 2 ml saturated GeO_2/liter-added medium (Lüning 1981c) whereas in the same species, Markham and Hagmier (1982) demonstrated a slight inhibition of growth at 0.22 mg GeO_2/ 1 l medium. Therefore, careful consideration was given to regulation of GeO_2 concentration. In this study, 0.5 ml saturated GeO_2/ 1 l medium was used following Holt (1984). Autoclaved seawater was used initially in the preparation of media because it has been successfully used in ***L. hyperborea*** (Kain 1964, 1965). Later, however, seawater was filtered and not autoclaved, because fertilization of gametophytes, and hence the appearance of sporophytes, of ***L. hyperborea*** seemed to be stimulated if this was done (refer to preliminary experiment A).

Irradiation

The main sources of illumination were 'Polylux 4000 (100 W)' and 'Northlight (125 W)' white fluorescent tubes (Thorn). Coloured fluorescent tubes were also used combined with Cinelux gelatin filters (Strand Lighting). The spectral distribution of quanta emitted from light tubes was converted from the manufacturers' data on spectral energy output between 400 and 700 nm and are shown in Fig. 0.1. Measurement of photon irradiance was made with a Li-Cor LI-1000 Datalogger. As no numerical conversion is involved, the unit $\mu mol\ m^{-2}s^{-1}$ was used in preference to $\mu E\ m^{-2}s^{-1}$ which appears on the quantum meter (Dring 1984). Photon irradiance was varied by different distance or thickness of black nylon net interposed between the dishes and

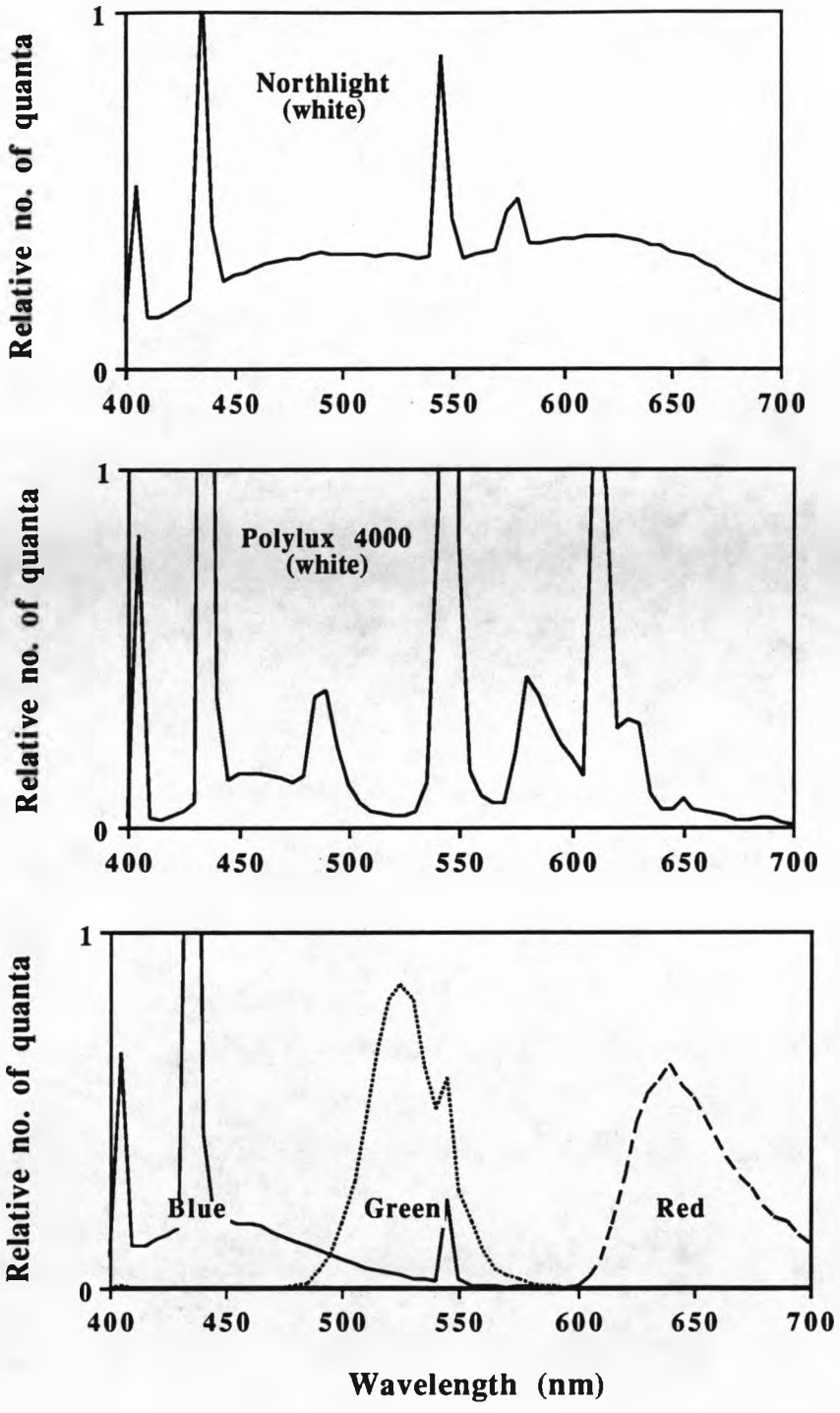

Fig. 0.1. Spectral distribution of relative number of quanta emitted from various light sources. The coloured light sources are combined forms of coloured fluorescent tubes (Thorn) with the corresponding colour of Cinelux filters (Blue: No. 444, Green: No. 424, Red: No. 406).

light sources. Because of considerable fluctuations of voltage and the resultant variation in irradiance, the measurements were taken at various time intervals in the same position. Temperature was maintained within the range of 10-15 °C, differences within which were not considered of any great importance (Kain 1965).

Determination of growth rate

Growth rates of early sporophytes at various irradiances were normally determined after pretreatment of the cultures in a saturating irradiance of continuous white light (Northlight), allowing uniform production of sporophytes. In members of the Laminariales 7-14 days has been reported to be taken for the appearance of sporophytes when saturated in continuous light (Kain 1969). For experimental treatments, Polylux 4000 white fluorescent tube was used in place of Northlight. The use of different light sources between pretreatment and treatment was made for two reasons. First, as the Northlight tube shows relatively even spectral output distribution of quanta compared with the Polylux 4000 tube (Fig. 0.1), any possibility of preferential effect of specific waveband of light on physiological state of plants before experimental treatments are given may have been excluded in the former. The second reason was of practical concern that the latter but not the former tube produced high irradiances easily. This consideration was necessary for some experiments in which the effect of high irradiance on the growth of sporophytes was investigated.

At the beginning of transfer some cultures were maintained in the dark, following the pretreatment period to obtain a mean control value for calculation of relative growth rate (RGR) under a specific treatment. The

purpose of placing the cultures in the dark was so that early sporophytes should metabolise any organic reserves they might have accumulated during the pretreatment period, confirming that all growth resulted from responses to the experimental treatments. Kain (1969) has found that after the transfer of laminarian young sporophytes from saturating to low irradiance a high proportion of the cells divided during the first 2 days, even if the low irradiance would normally allow only a small proportion of the cells to divide. The state of development of sporophytes in culture was determined by measuring the length and width after preparation of microscope slides from cover slips by use of corn syrup solution (30-40 % of tapwater).

As a method for estimating growth, counting the number of cells has adequately been used in filamentous forms of algae (Green 1973, Guillard 1973). In this study, however, it would be indispensable to replace the estimate of number of cell with length or width because of appearance of polystromatic stages. Growth in the polystromatic stages takes place in three dimensions, and three cell divisions would be necessary for the length to be doubled. For this reason there is possibility that distorted growth rate would be produced although Kain (1965) has shown that in the early sporophyte of *L. hyperborea,* increase in cell number is logarithmic up to 1000-cell stage, being proportional to the logarithm of length. Therefore, instead of counting the number of cells, measurement of length and width was made for largest normal sporophytes per cover slip (Kain 1965). The criteria for discriminating normal sporophytes from abnormal ones are that the latter have irregular patterns of cell walls, lack of rhizoids and, therefore, apparent polarity (Schreiber,1930, Svendsen and Kain 1971). As suggested by Kain (1965) random sampling was avoided and 2 largest plants on each coverslip were selected for measurement (refer to preliminary experiment B). The

length (and width) measured was converted to natural logarithm value, and a relative growth rate (RGR) was determined from the following relationship:

$$RGR = \frac{Log_e\, Lt_2 \text{ (or } Wt_2) - Log_e\, Lt_1 \text{ (or } Wt_1)}{t_2 - t_1}$$

Lt_1 (or Wt_1) and Lt_2 (or Wt_2) are the values of length (or width) at the beginning and the end, respectively, and t_1 and t_2 are corresponding times at which values are determined, in days (Brinkhuis 1985).

Statistical analysis

Main and combined effects were tested by the appropriate analyses of variance (ANOVA, Zar 1984). Differences between the levels of a factor (or factors) were further analysed by the Least Significance Difference (LSD, Sokal and Rohlf 1969) or Student-Newman-Keuls (S-N-K) test (Zar 1984). Percentage data with replicates were arcsine-transformed prior to analysis by one-way ANOVA and a significant F ratio permitted a posteriori testing using S-N-K procedure (Zar 1984). Confidence intervals were also used to denote statistical differences based on the table for percentages supplied by Rohlf and Sokal (1969).

<Preliminary experiments>

A. Effect of mBG on the appearance of early sporophytes

The basic medium used throughout this study was modified BG (mBG) which was the same as the medium (so-called BG; Kain, personal communication) described by (Kain 1964) but without some vitamins. As exclusion of the vitamins might affect the time lapse to be taken for sporophytes to appear, the relative effectiveness of the medium lacking 8 micronutrients compared with BG seemed worthy of testing. Also, it has been shown that autoclaving a medium delayed fertilization of ***Alaria esculenta*** gametophytes (Walton 1986) although gametophytes of species of ***Laminaria*** and ***Saccorhiza*** matured rapidly in autoclaved medium (Kain 1969). In this context, preliminary work was done to determine whether the vitamins omitted from BG were essential for the appearance of sporophytes of some members of the Laminariales and whether the autoclaving process together with omission of vitamins caused any effect on the advent of the sporophyte stage.

Firstly, four slightly different media were tested for effect on the fertility of ***L. hyperborea.*** Duplicate cultures were made under continuous white light (Northlight) of 30-40 μmol $m^{-2}s^{-1}$ and two coverslips were placed in each petri-dish (100 mm diam., 10 mm high) with 35 ml of medium renewed every 4 days. The plants were harvested after 11 days from inoculation in Expt. 1 and 9 days in Expt. 2 and counts were made of dehisced oogonia or sporophytes based on 1000 plants in groups of 50 observed with a microscope at a magnitude of x 250.

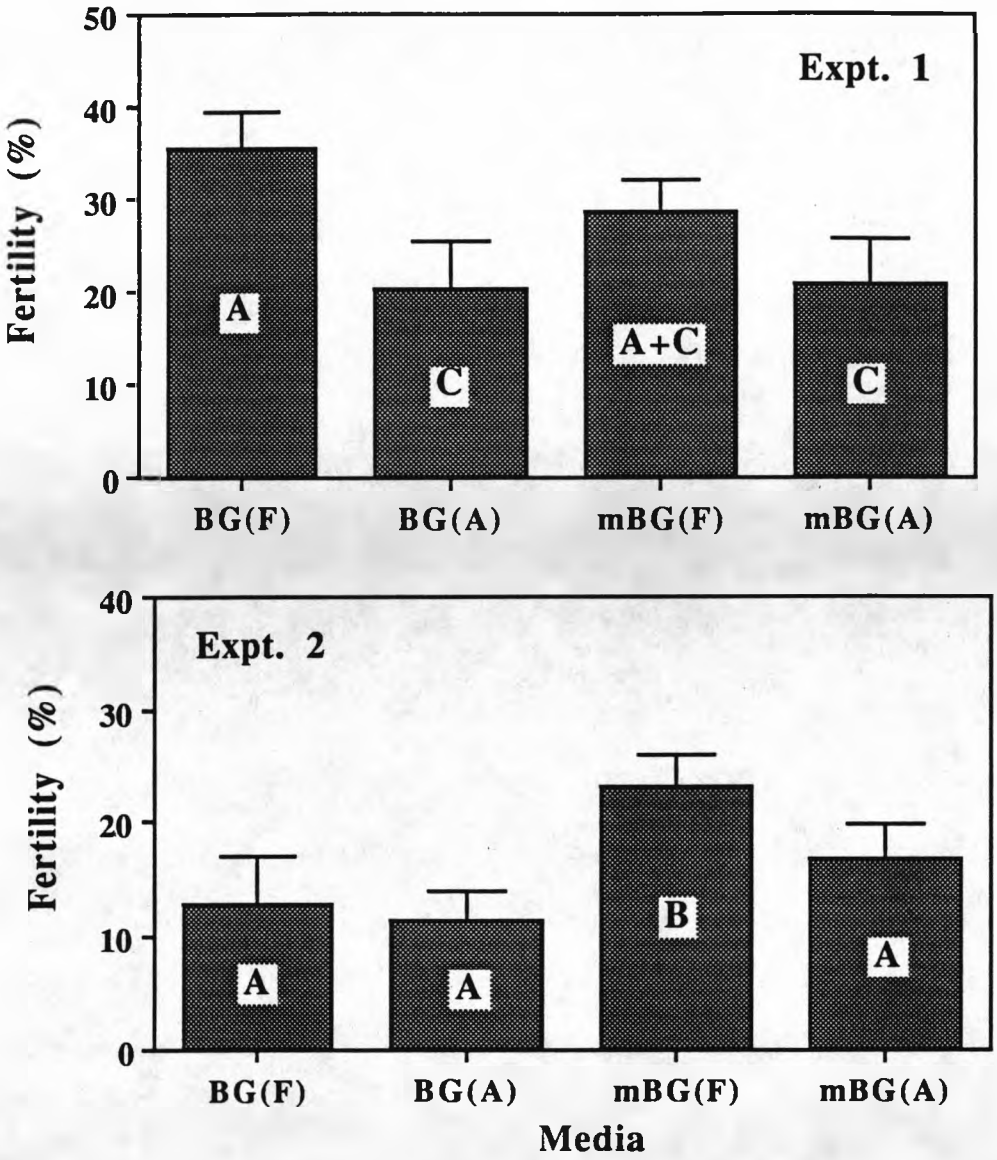

Fig. 0.2. Fertility of ***Laminaria hyperborea*** in different media (F: filtered seawater, A: autoclaved seawater). Vertical bars indicate 95 % confidence intervals. Values having different letters are significantly different at p= 0.05 (S-N-K test based on the ANOVA table in Appendix 1).

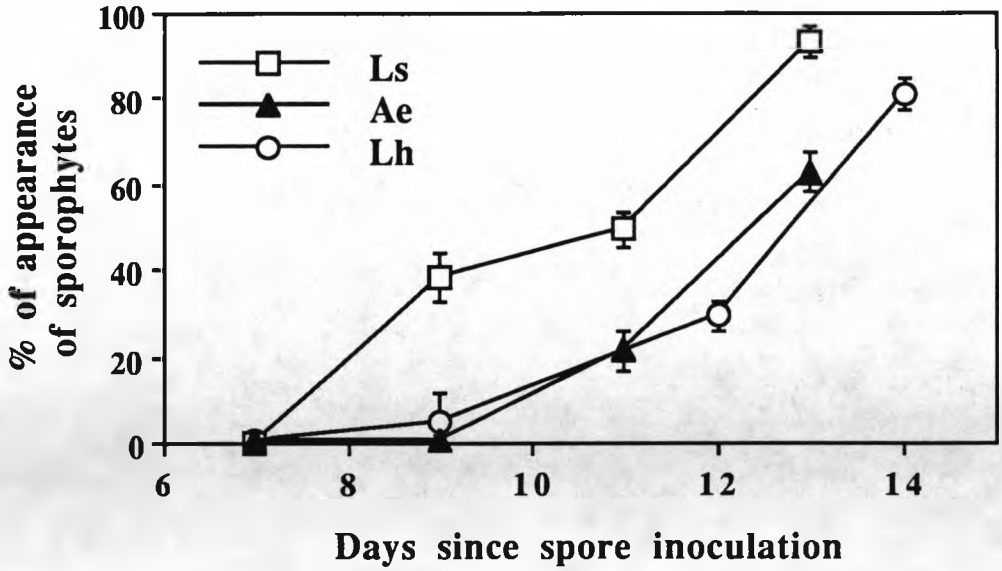

Fig. 0.3. Effect of varying periods of illumination on the appearance of sporophytes of three species of the Laminariales in mBG (filtered seawater). Vertical bars denote 95% confidence intervals (n=500). Ls: ***Laminaria saccharina,*** Ae: ***Alaria esculenta*** and Lh: ***Laminaria hyperborea.***

In experiment 1, fertility seemed to be higher in BG (filtered) than in BG or mBG ($p < 0.05$, Fig. 0.2). On the other hand, experiment 2 showed that the fertility in mBG (filtered) was significantly higher than that in all the other media.

When spores of three species of the Laminariales were grown in filtered seawater-based mBG at 25-35 μmol $m^{-2}s^{-1}$ of continuous white light (Northlight), the fastest sporophytes to appear were those of ***L. saccharina,*** taking 7-8 days, ***L. hyperborea*** was next, taking 9-10 days and ***A. esculenta*** slowest, taking 11-13 days (Fig. 0.3). These are very similar to the days taken for the very first dehisced oogonia or sporophytes of the same species as the former two to appear, when BG was used for the cultures (Kain 1969).

From the results of experiments here, it seems unlikely that all the vitamins used in the preparation of BG are essentially required by laminarian gametophytes for gametogenesis. Anderson and North (1969) have used a simplified medium similar to mBG for culturing ***Macrocystis*** early sporophytes.

There seemed some difference in the fertilization of ***L. hyperborea*** between in medium of filtered seawater and of autoclaved seawater (Fig. 0.2) although the latter had been successfully used in three ***Laminaria*** species (Kain 1969). As gametophytes of ***A. esculenta*** have also been reported to grow and become fertile better in a medium made of filtered and not autoclaved seawater (Walton 1986), the media of filtered seawater seemed to have a stimulatory effect to some extent on the fertility compared with the media of autoclaved seawater (Fig. 0.2). Therefore, together with some other advantage, i.e. rapidity and simplicity in its preparation, use of filtered seawater-based mBG seemed to be desirable for this study.

B. Sample size determination

Kain (1965) has suggested that random sampling should be avoided in measuring the length of laminarian early sporophytes for determination of growth rate since the sporophytes derived from eggs produced at different times are likely to be selected and therefore actual trends in growth rate may be masked. In the beginning of this study, considering the inappropriateness of random sampling, the relative growth rate (RGR) was determined by measuring the length of the six largest sporophytes of ***Laminaria hyperborea*** present on a standard area of each coverslip taken from replicate culture dishes. However, a considerable variability in length was found even between the selected plants. For this reason, it was required to determine the sample size, i.e. the number of plants to be measured in order to reduce the variation which might be ultimately manifested in RGR.

To establish the number of individuals to be measured, length measurements were made at intervals of all sporophytes on each coverslip, and then the mean values of the natural logarithms of the length data were plotted against time to obtain a regression coefficient. This coefficient was then considered as the mean growth rate of a population, which was 0.121 and 0.128 respectively in two separate experiments (Fig. 0.4). As the regression lines calculated for the different populations showed a good agreement ($t= 0.57$, $p> 0.5$), the mean slope was taken as the mean growth rate. If the selected sporophytes had been synchronously developed ones, the difference in length between the plants would have at least been less than that between the plants having 24 h (1 day) gap in their time of fertilisation. Therefore, the difference in the logarithm of lengths (DL) between the largest and smallest of selected plants was not supposed to exceed the

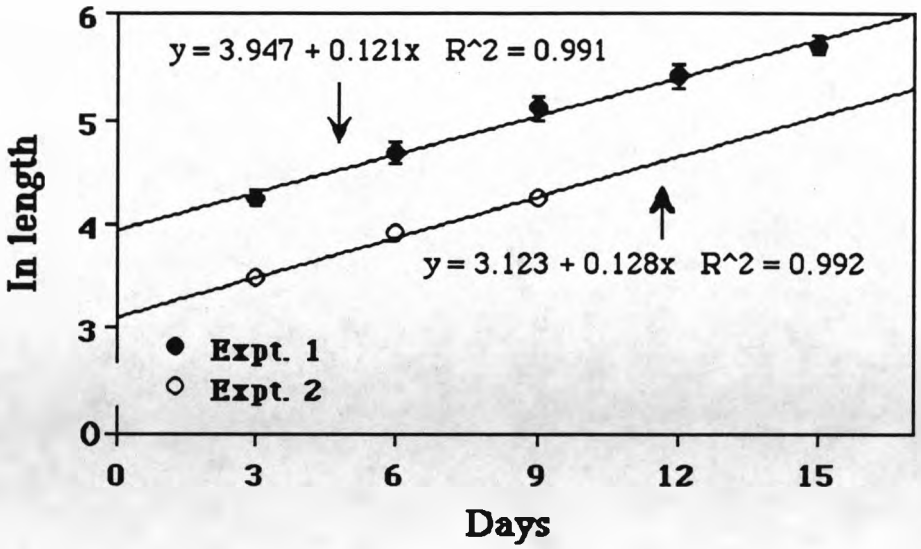

Fig. 0.4. Increase in the mean logarithms of length of sporophytes found on a whole coverslip with time in separate experiments. Vertical bars indicate 95% confidence intervals.

Table 0.2. Comparison of DL between the largest and the smallest of the selected plants with MDG (Total: 67 cases).

No. of selected plants	DL < MDG	DL ≥ MDG	Percentage of DL lower than MDG
2	53	14	79.1%
3	37	30	55.2%
4	32	25	47.8%
5	22	45	32.8%
6	14	53	20.9%

mean daily growth (MDG; mean growth rate x 1 day). For this reason, DL was calculated for selected largest plants (from 2 to 6) from 67 cultures grown at 50-65 μmol $m^{-2}s^{-1}$ of continuous white light (Northlight or Polylux 4000) and compared with MDG. As shown in Table 0.2, when 2 plants were sampled, the DL was less than MDG in 53 cases out of 67 (79.1%). On the other hand, selection of 6 plants turned out to have only 19 cases (20.9%) of DL less than MDG.

However, as this procedure was based on the supposition that variations in length of young sporophytes might have been only due to different times of fertilization, a large natural variation in length increase between individual plants, if found, could affect the procedure. Therefore, in order to see how much variation individual plants show in relation to length growth, the lengths of individual plants were traced for 12 days at 30-40 μmol $m^{-2}s^{-1}$ of continuous white light (Northlight). Initial length of the plants was 65.9 $\pm$ 3.8 μm and 71.8 $\pm$ 7.6 μm (mean $\pm$ SD, n=8) respectively in two separate experiments with different genetic materials and measurements were made at 3 day intervals. When the logarithm of lengths was plotted against time intervals as shown in Fig. 0.5, the resulting slopes appeared to be similar to one another ($p > 0.5$ in each population, Table 0.3). Also the mean regression line from the population A was comparable to that from the population B ($t = 0.556$, $p > 0.5$), suggesting that length growth of individual plants is relatively constant.

If it is assumed that growth rate values form a normal curve, these data can be viewed in another way. In population A (Table 0.3), where the mean RGR was 0.302 $\pm$ 0.00907, a sporophyte starting with a nominal length of ln 1 (0) would reach an ln length of 3.02 $\pm$ 0.0907 after 10 days growth (the usual period before sampling). The difference in ln length (DL) of a sporophyte arising one day before another would

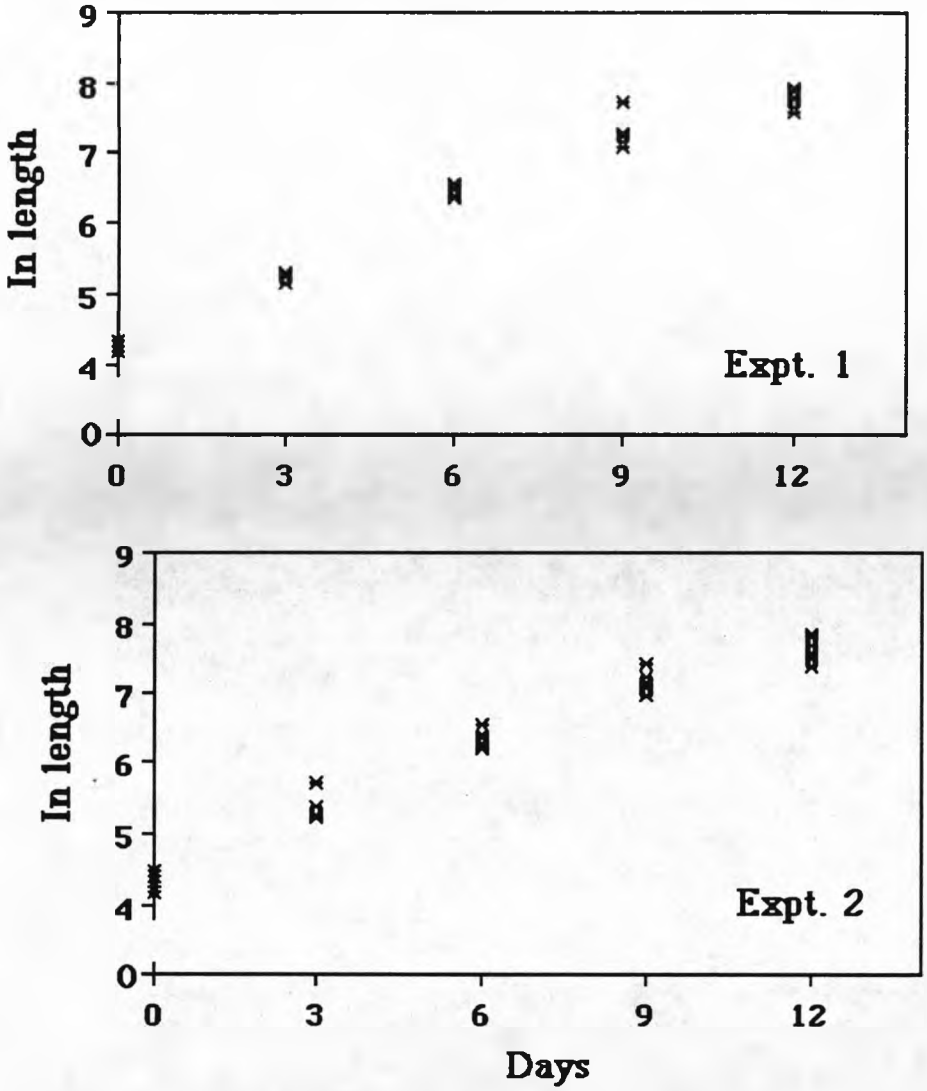

Fig. 0.5. Increase in the logarithm of length of individual plants with time. Different genetic materials were used for the different experiments.

Table 0.3. Comparison of the regression coefficients calculated from the logarithm of length of individual plants with time. A and B were from different genetic populations.

Plant	b	SS	F	P	Results of test for difference between slopes
A1	0.299	0.196	121.8	0.002	0.302 ± 0.00907 (mean$\pm$S.D.)
A2	0.293	0.236	98.3	0.002	
A3	0.292	0.185	124.5	0.002	$F=0.116 < F_{0.05(1),7,24} = 2.42$
A4	0.302	0.234	105.3	0.002	
A5	0.294	0.153	153.0	0.001	Therefore, accept Ho at $p > 0.25$
A6	0.305	0.205	122.1	0.002	
A7	0.315	0.183	146.5	0.001	
A8	0.314	0.181	147.5	0.001	
B1	0.264	0.161	116.9	0.002	0.287 ± 0.01580 (mean$\pm$S.D.)
B2	0.263	0.122	153.1	0.001	
B3	0.287	0.165	134.2	0.001	$F=0.456 < F_{0.05(1),7,24} = 2.42$
B4	0.288	0.201	111.6	0.002	
B5	0.306	0.215	117.2	0.002	Therefore, accept Ho at $p > 0.25$
B6	0.297	0.158	150.8	0.001	
B7	0.296	0.082	287.8	0.000	
B8	0.297	0.067	357.2	0.000	

be 0.302; half of this value, 0.151, is the difference from the mean. Dividing this by the 10-day standard deviation, 0.0907, gives the 1/2DL in standard deviation units. This can be used to find the appropriate area of half a normal curve from Table P of Rohlf and Sokal (1969). The value of 1.66 yields 0.4515 for half the area, 90% for the whole. From this and a similar calculation for population B it can be deduced that in populations A and B respectively, 10% and 36% of pairs of sampled sporophytes would differ by at least one day's growth. This is similar to the result of 20% in the previous experiment (Table 0.2).

To determine the number of plants to be measured, finally, variations between the logarithms of length of plants grown on different coverslips in a dish or between those of plants grown in different dishes (replicates) had to be taken into consideration. Mean and standard errors of the standard errors between coverslips or between replicates were calculated for logarithms of length of one and two selected plants from 7 different experiments. The mean and standard errors of standard error were 0.069 ± 0.017 between coverslips and 0.114 ± 0.024 between replicates in the case of selecting one plant per coverslip, whereas for 2 plants selection, the values were 0.053 ± 0.010 and 0.117 ± 0.022 respectively. Variation about the SE estimates seemed to drop when two plants were selected (Bros and Cowell 1987).

In conclusion, the estimated error of selecting the plants developed at different times for measurement would be of the magnitude of 20%, ensuring a small age range for the selected plants and, therefore, small variation and also variability in and between replicates could be reduced as seen in convergence of the SE estimates if the number of plants to be measured is restricted to 2. Selective sampling such as this must result in a selection of the fastest-growing individuals and thus

the growth rates be the highest attained in each culture. These are, however, likely to be the important rates in the sea (Kain 1969).

Chapter 1. Light requirements

1.1. Introduction

In a kelp community, the establishment stages such as gametophytes or early sporophytes normally start beneath the parental canopy, where they should encounter very different light conditions from those experienced by the later mature stages of the life cycle. Differences in growth responses to light between juvenile and mature stages within the same species have already been recognised in some brown algae (Fei and Neushul 1984, Fei et al. 1989, Hales and Fletcher 1989). For the maintenance of a population, therefore, the plants of establishment stages must have adequate physiology for the available light regime. Numerous studies have been made on the effect of photon irradiance on the growth of laminarian gametophytes, showing that the plants can be characterized as extreme shade plants with regard to their low light requirements either for photosynthesis (Kain 1964, Fain and Murray 1982) or growth (Vadas 1972, Lüning and Neushul 1978). Although distinct differences even between gametophytes and few-celled sporophytes have been reported in some laminarian species (Fei et al. 1989, Gerard 1990) only a few studies have been made to define the light requirements of laminarian early sporophytes such as those of Kain (1965, 1969).

Irradiance and daylength are variables demonstrated in laboratory studies to be important in regulating the growth of algae (Lüning 1981b). In their natural habitat, algae may experience light conditions that vary continuously due to rapid changes in incident irradiance and more gradual changes in daylength (Marra 1980). Therefore, in addition to its obvious significance as an energy source for photosynthesis, the tremendous fluctuation of light in both space

(depth and latitude) and time (day and season) suggests that light will often be a determining factor for algal growth. In this respect knowledge of growth behaviour of early sporophytes in relation to light in terms of both irradiance and photoperiod is essential for an understanding of the basic features of the ecology of an adult population.

On the other hand a number of studies have revealed various morphological responses of algae to different light levels (see reviews by Norton et al. 1981, 1982, Hay 1986). Burrows (1964) has observed in *Laminaria saccharina* sporelings that transferrence of plants from dim light to high irradiance led sometimes to abnormally widened laminae. Light may affect algal morphology resulting in a greater efficiency to capture light for growth (Hay 1986, Carpenter 1990).

In this context, the present study was designed to investigate the effect of photon irradiance and photoperiod on the growth and morphology of early sporophytes of four species of the Laminariales and to predict their growth behaviours in the sea.

1.2. Materials and Methods

1.2.1 Effect of photon irradiance on growth

Cultures were exposed to 30-40 μmol $m^{-2}s^{-1}$ of continuous white light provided by Northlight until fertilisation took place. Just after the appearance of sporophytes on the coverslips (13 mm diameter) contained in petri-dishes (100 mm diam., 10 mm high), 2 or 3 coverslips bearing sporophytes were transferred to 60 mm diam. plastic petri-dishes filled with 20 ml medium. Two to three replicate dishes were then subjected to 4-5 different irradiances (0.1-90 μmol $m^{-2}s^{-1}$) of continuous white light (Polylux 4000). After 9-10 days, the cultures were harvested and fixed for observation. Growth of early sporophytes was estimated from the lengths of the two largest plants on each coverslip, taken to the nearest 0.01 μm under a Dialux 20 EB (Leitz Wetzlar). Temperature was maintained between 12 °C and 14.5 °C during the culture period and medium was changed every 3-4 days.

In a second experiment for each species, the same cultures were grown for a further period (10-12 days) in the pretreatment condition and then distributed to three different irradiances (11, 33 and 59 μmol $m^{-2}s^{-1}$; maximum variation in irradiance $\pm$7%) in order to compare the growth responses with those of younger plants. Other conditions were the same as in the previous experiments. Length/width ratios were calculated for plants of the two ages grown at different irradiances.

1.2.2. Interactive effect of daylength and irradiance on growth

Early sporophytes of three species of the Laminariales (***Alaria esculenta, Laminaria hyperborea*** and ***Laminaria saccharina***) were grown under daylengths of 7 or 17 h of each 24 h cycle, in ventilated controlled chambers at temperatures of 12 to 14.5 °C. The cultures were in triplicate and kept at irradiances of 1, 13, 57, 128 μmol $m^{-2}s^{-1}$ (±4% variation) of white light (Polylux 4000) for each daylength. At the end of 10 days growth under each regime, length and width measurements were made for the 2 largest plants per replicate (one coverslip each) and the mean and standard errors of the RGRs calculated (n=3). Length/width ratios were calculated from measurements of 12-24 plants grown in different treatment conditions.

In a further experiment, ***L. hyperborea*** and ***L. digitata*** were grown at different irradiances (36 and 75 μmol $m^{-2}s^{-1}$, ±7% variation) under two different light periods (7 and 17 h out of 24 h) for 24 days. Triplicate cultures were set up and harvesting made on day 12 and day 24. The lengths of two sporophytes from the three replicates for each treatment were then pooled to produce the mean and 95 % confidence intervals (n=6).

1.2.3. Comparison of 12 hour photoperiod with continuous light

Two or three dishes were placed either in 24 h light or in 12 h light followed by 12 h darkness. Three irradiances (24, 55 and 109 μmol $m^{-2}s^{-1}$) were employed for each light period. After 8-10 days, RGRs of the plant were compared. For ***L. digitata,*** different genetic materials were used between 24 μmol $m^{-2}s^{-1}$ treatments and the higher irradiances

treatments, preventing direct comparisons between the conditions used.

1.2.4. Survival in the dark

Some cultures of *L. hyperborea* and *L. digitata* grown at 30-40 μmol $m^{-2}s^{-1}$ of continuous white light (Northlight) for 20-24 days were transferred to the dark and then 10 days later the dishes were retrieved to low illumination (2-3 μmol $m^{-2}s^{-1}$) for a brief period (1-2 min) in order to estimate the mortality of the young sporophytes under a microscope, after which observation was made at 5 days intervals. The number of dead individuals amongst 200 plants was counted.

1.2.5. Growth in shaded daylight

A culture experiment was run in a 650 mm long, 327 mm wide and 73 mm deep PVC tray in an open yard from 26th September to 16th October in 1990. Plants materials were *L. hyperborea* and *L. digitata* grown on 18 x 18 mm square coverslips for 12 and 17 days respectively in the routine laboratory condition since the spore inoculation. When the coverslips bearing sporophytes were transferred in crystallizing dishes containing 75 ml medium to the PVC tray, into which continuous cool water was siphoned, the mean length of sporophytes was initially 136.47 (±36.47: S.D.) μm for *L. hyperborea* and 161.44 (±6.09) μm for *L. digitata*. Duplicate cultures were made and the medium renewed every 8 days. Light levels employed in this experiment were 4, 15 and 35 % daylight. The varying light gradients

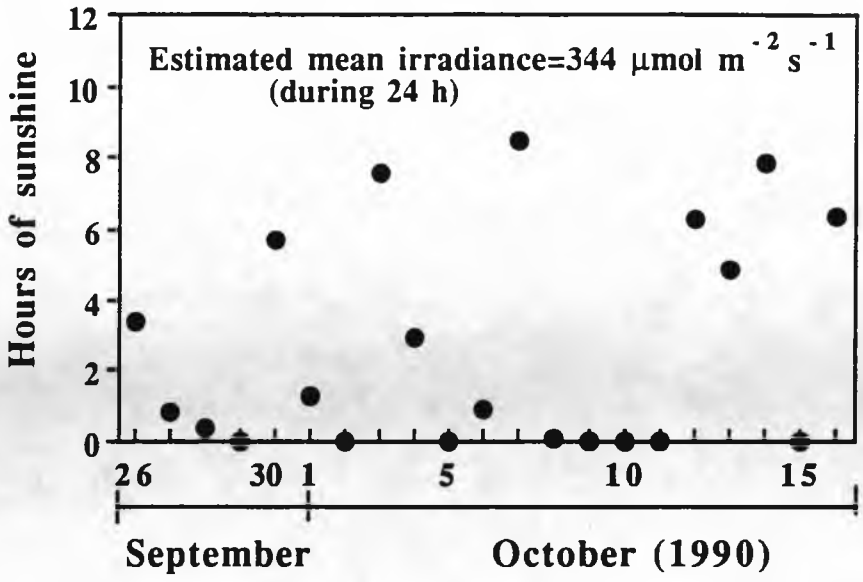

Fig. 1.1. Daily sunshine hours and mean photon irradiance during 26th September- 16th October, 1990. The data were provided from Ronaldsway Meteorological Office (Isle of Man).

were obtained by placing black nylon and a glass screen on the tray. Light levels were determined by measuring the highest irradiance at noon time on relatively fine sunny day and expressing the irradiances measured under a given experimental regime as percentages of the irradiance of full sunlight. The sunlight conditions prevailing during the experiment are shown in Fig. 1.1, which is based on climatological data provided by Ronaldsway Meteorological Office (Isle of Man). Mean photon irradiance was also calculated from the records of mean daily solar irradiation in mega joules on a square meter per sec at Eskdalemuir (55° 10' N) and Aughton (53° 35' N) which are near to the latitude from Isle of Man (54° 05' N). Temperature varied from 9.7 °C to 15.8 °C throughout this study.

1.3. Results

1.3.1. Effect of photon irradiance on growth

Growth rates of early sporophytes at various irradiances are shown in Fig. 1.2. With regard first to the minimum irradiance necessary for growth, even the lowest irradiance used (1-2 μmol $m^{-2}s^{-1}$) allowed some growth (0.03-0.057/day). A further experiment on ***L. hyperborea,*** however, showed that the minimum requirement seemed to be between 0.5 and 1.0 μmol $m^{-2}s^{-1}$ for increase in length under continuous white light (Fig. 1.3). In all the four species relative growth rates increased with increasing irradiance and became light-saturated at about 30 μmol $m^{-2}s^{-1}$ at 12-14.5° C (Fig. 1.2). ***L. saccharina*** had the highest mean RGR of length (0.259/day) at saturating irradiances but the difference between the two experiments was considerable. The mean daily RGR was 0.242 for ***A. esculenta,*** 0.196 for ***L. digitata,*** 0.216 for ***L. hyperborea.***

Mean RGRs of laminarian young sporophytes of different ages at three irradiances are shown in Table 1.1. Here 'age' does not mean the absolute age of early sporophytes counted from the very first appearance, but the time from the start of the first part of the experiment. In the case of younger plants, there was no substantial difference in growth pattern between length and width of ***L. hyperborea,*** whereas the other species showed faster growth in length than in width. When older sporophytes cultured in the pretreatment condition for a further period were grown at three different irradiances, RGR of the length was significantly lower ($p<0.001$, Table 1.2a,b) than that of younger ones although growth saturation seemed to occur at comparable irradiances (Table 1.1). In the ***Laminaria*** species, 30-50% growth reduction in length was found with age, whilst RGR of length in ***A.esculenta***

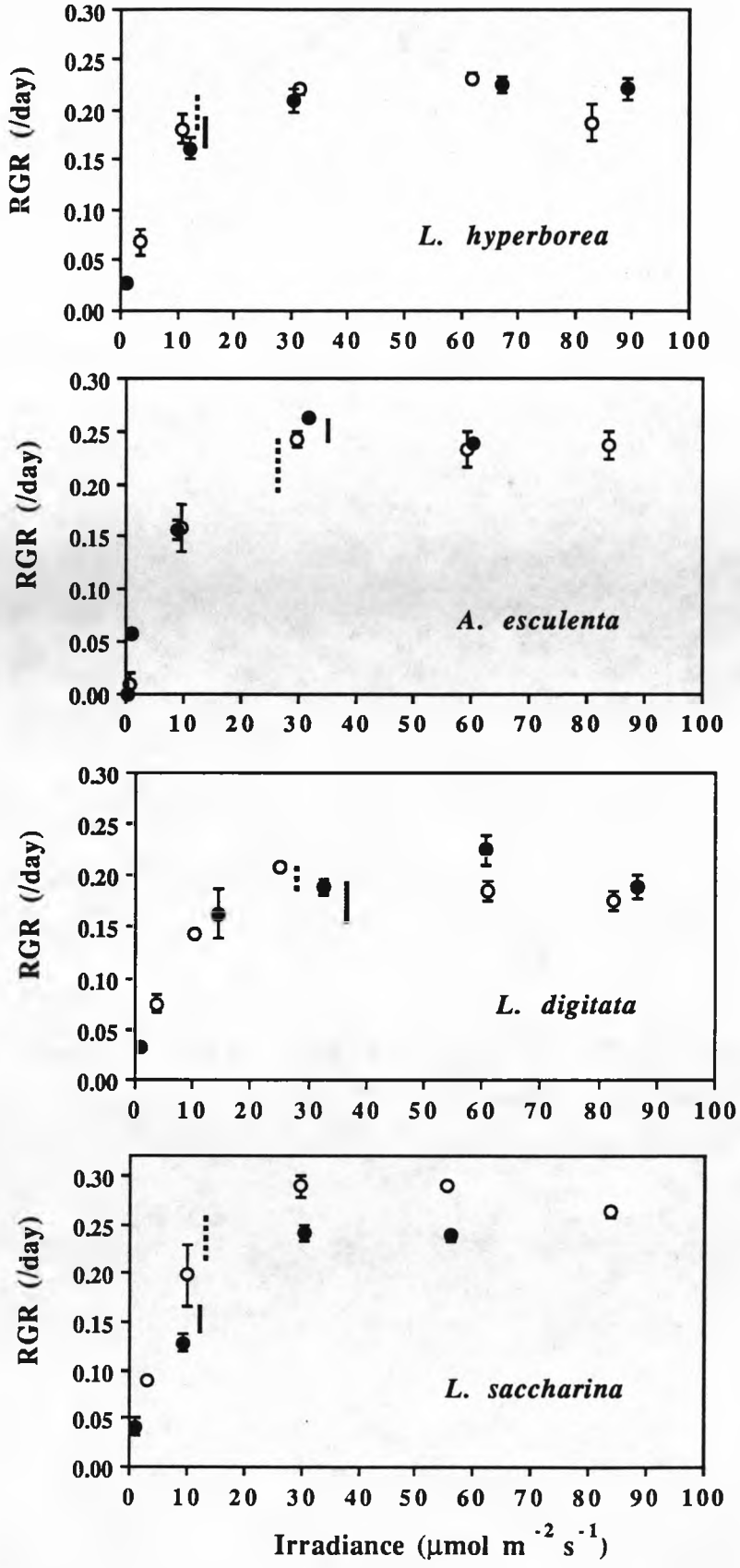

Fig. 1.2. Relative growth rate (RGR) of early sporophytes of four species of the Laminariales at various continuous photon irradiances. Different symbols are RGRs from different experiments for each species. Mean and standard error bars are shown (n=2-3). A broken bar indicates a least significant difference (LSD) at p=0.05 for open circles and solid bar for closed circles.

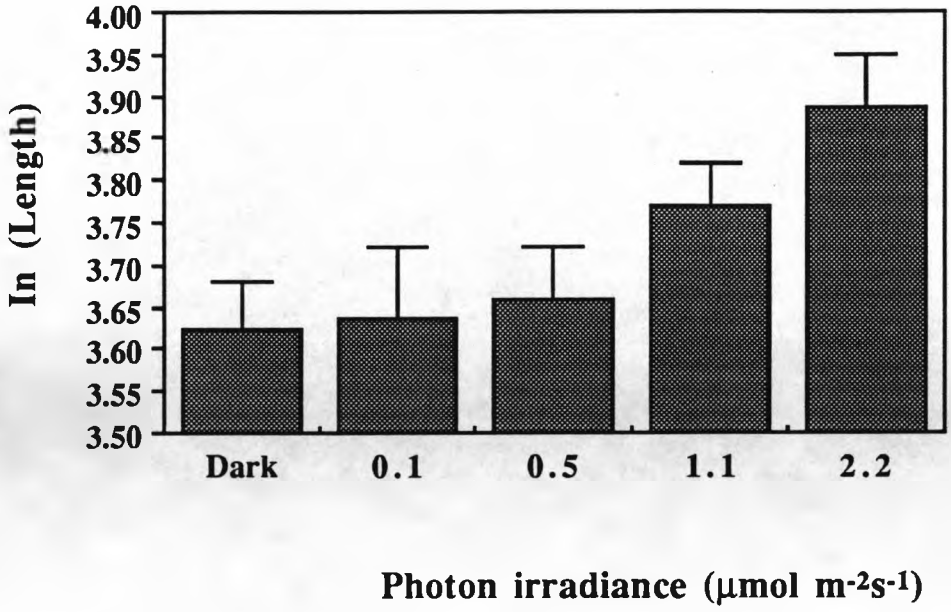

Fig. 1.3. Minimum light requirement of *L. hyperborea* for growth. Since the first appearance of sporophytes on coverslips, four dishes each containing two coverslips were distributed to different experimental conditions and then harvest was made after 9 days. Vertical bars indicate 95% confidence intervals (n=4).

Table 1.1. Relative growth rate (± S.E., n=3) of sporophytes transferred from 30-40 μmol $m^{-2}s^{-1}$ to different irradiances at different ages. Ae: ***A. esculenta***, Ld: ***L. digitata***, Lh: ***L. hyperborea***, Ls: ***L. saccharina***.

Species	Age (day)	Intial length / width (μm)	Relative growth rate (/day) Irradiance (μmol $m^{-2}s^{-1}$) 11	33	59
Lh	0	L: 49.6 ± 7.3	0.161±0.011	0.210±0.012	0.226±0.008
	10	L: 520.2±53.6	0.080±0.004	0.128±0.003	0.138±0.008
	0	W: 14.8 ± 2.6	0.149±0.009	0.196±0.014	0.205±0.012
	10	W: 110.3± 13.7	0.111±0.002	0.167±0.004	0.177±0.010
Ae	0	L: 87.5 ± 10.7	0.157±0.009	0.262±0.003	0.239±0.004
	12	L: 1382.3±179.2	0.027±0.004	0.073±0.015	0.062±0.008
	0	W: 23.1 ± 5.0	0.125±0.005	0.210±0.006	0.189±0.003
	12	W: 230.4± 53.4	0.026±0.006	0.083±0.013	0.068±0.011
Ld	0	L: 57.1 ± 5.8	0.148±0.001	0.200±0.003	0.185±0.002
	10	L: 399.8±63.1	0.102±0.010	0.166±0.009	0.153±0.011
	0	W: 15.6 ± 2.4	0.120±0.004	0.155±0.006	0.137±0.005
	10	W: 63.1±19.2	0.120±0.012	0.190±0.005	0.184±0.014
Ls	0	L: 46.0 ± 4.8	0.128±0.009	0.241±0.008	0.238±0.006
	11	L: 993.6±149.8	0.080±0.016	0.162±0.017	0.181±0.017
	0	W: 14.6 ± 2.7	0.075±0.006	0.177±0.011	0.169±0.010
	11	W: 225.0±56.3	0.081±0.009	0.199±0.012	0.210±0.011

<L. hyperborea>

(a) RGR-length

Source	DF	SS	MS	F
Age (A)	1	0.031	0.031	146.00***
Irradiance (I)	2	0.013	0.006	29.26***
A x I	2	4.744×10^{-4}	2.372×10^{-4}	0.11 n.s.
Error	12	0.003	2.146×10^{-4}	

(b) RGR-width

Source	DF	SS	MS	F
Age (A)	1	0.005	0.005	16.64**
Irradiance (I)	2	0.013	0.006	23.22***
A x I	2	1.013×10^{-4}	5.067×10^{-4}	0.18 n.s.
Error	12	0.003	2.769×10^{-4}	

<A. esculenta>

(a) RGR-length

Source	DF	SS	MS	F
Age (A)	1	0.114	0.114	506.48***
Irradiance (I)	2	0.018	0.009	39.34***
A x I	2	0.003	0.001	6.16*
Error	11	0.002	2.248×10^{-4}	

(b) RGR-width

Source	DF	SS	MS	F
Age (A)	1	0.056	0.056	279.73***
Irradiance (I)	2	0.015	0.008	38.44***
A x I	2	0.001	3.054×10^{-4}	1.54 n.s.
Error	11	0.002	1.987×10^{-4}	

Table 1.2. a. ANOVA table for RGR of laminarian sporophytes of different ages grown at three different irradiances. *$0.05 > p \geq 0.01$, ** $0.01 > p \geq 0.001$, *** $p < 0.001$, n.s. not significant at $p = 0.05$.

<L. digitata>

(a) RGR-length

Source	DF	SS	MS	F
Age (A)	1	0.004	0.004	34.13***
Irradiance (I)	2	0.009	0.005	36.81***
A x I	2	7.211×10^{-4}	3.605×10^{-4}	0.29 n.s.
Error	11	0.001	1.229×10^{-4}	

(b) RGR-width

Source	DF	SS	MS	F
Age (A)	1	0.002	0.002	13.48**
Irradiance (I)	2	0.008	0.004	30.62***
A x I	2	0.001	4.781×10^{-4}	3.56 n.s.
Error	11	0.001	1.344×10^{-4}	

<L. saccharina>

(a) RGR-length

Source	DF	SS	MS	F
Age (A)	1	0.014	0.014	42.70***
Irradiance (I)	2	0.034	0.017	52.73***
A x I	2	0.001	2.973×10^{-4}	0.93 n.s.
Error	9	0.003	3.183×10^{-4}	

(b) RGR-width

Source	DF	SS	MS	F
Age (A)	1	0.002	0.002	7.25*
Irradiance (I)	2	0.039	0.020	76.97***
A x I	2	0.001	3.444×10^{-4}	1.34 n.s.
Error	9	0.002	2.563×10^{-4}	

Table 1.2. b. ANOVA table for RGR of laminarian sporophytes of different ages grown at three different irradiances. *$0.05 > p \geq 0.01$, ** $0.01 > p \geq 0.001$, ***$0.001 > p$, n.s. not significant at $p = 0.05$.

was reduced by 80% (Fig.1.4). In contrast to the overall reduction of growth rate in length with age, a comparable reduction in width was found only in ***A. esculenta*** (Fig. 1.4). In ***L. hyperborea,*** the growth rate in width did not seem to slow down as much as in length. ***L. digitata*** and ***L. saccharina*** showed even higher width RGRs (23-34% and 12-24% respectively) in older plants than in younger ones at saturating irradiances (Table 1.1).

Length/width (L/W) relationships of the thallus of young sporophytes seem in general not to be influenced by photon irradiances (Table 1.3). However, in some cases, there was a significant change in L/W ratio (except for ***A. esculenta***) and the change was downwards. ***A. esculenta*** maintained ca. 6.0 times more length than width regardless of age and irradiance while ***L. hyperborea*** showed a L/W ratio of only 4.0 in all the irradiances, becoming even wider with age (Table 1.3). In ***L. digitata,*** as seen in Table 1.3, a significant but slight change was detected at 11 and 59 μmol $m^{-2}s^{-1}$, indicating an increase of L/W ratio at the lower irradiance and a decrease at the higher irradiance with age. The significant decrease in L/W ratios with age found in ***L. saccharina*** is consistent with correspondingly increased growth rate of width (Table 1.1, 1.2).

1.3.2. Interactive effect of daylength and irradiance on growth

Fig. 1.5 shows the growth rate of early sporophytes of three species of the Laminariales under different photon irradiances and photoperiods. The result of the analyses given in Table 1.4 suggests that both main factors, irradiance ($p< 0.001$) and photoperiod ($p<0.001$) significantly affected the growth rate and there was interaction between the two factors. In the case of plants grown under a 17 h light period, the growth in all the species increased with increased irradiance only to 57 μmol $m^{-2}s^{-1}$ beyond which there was no

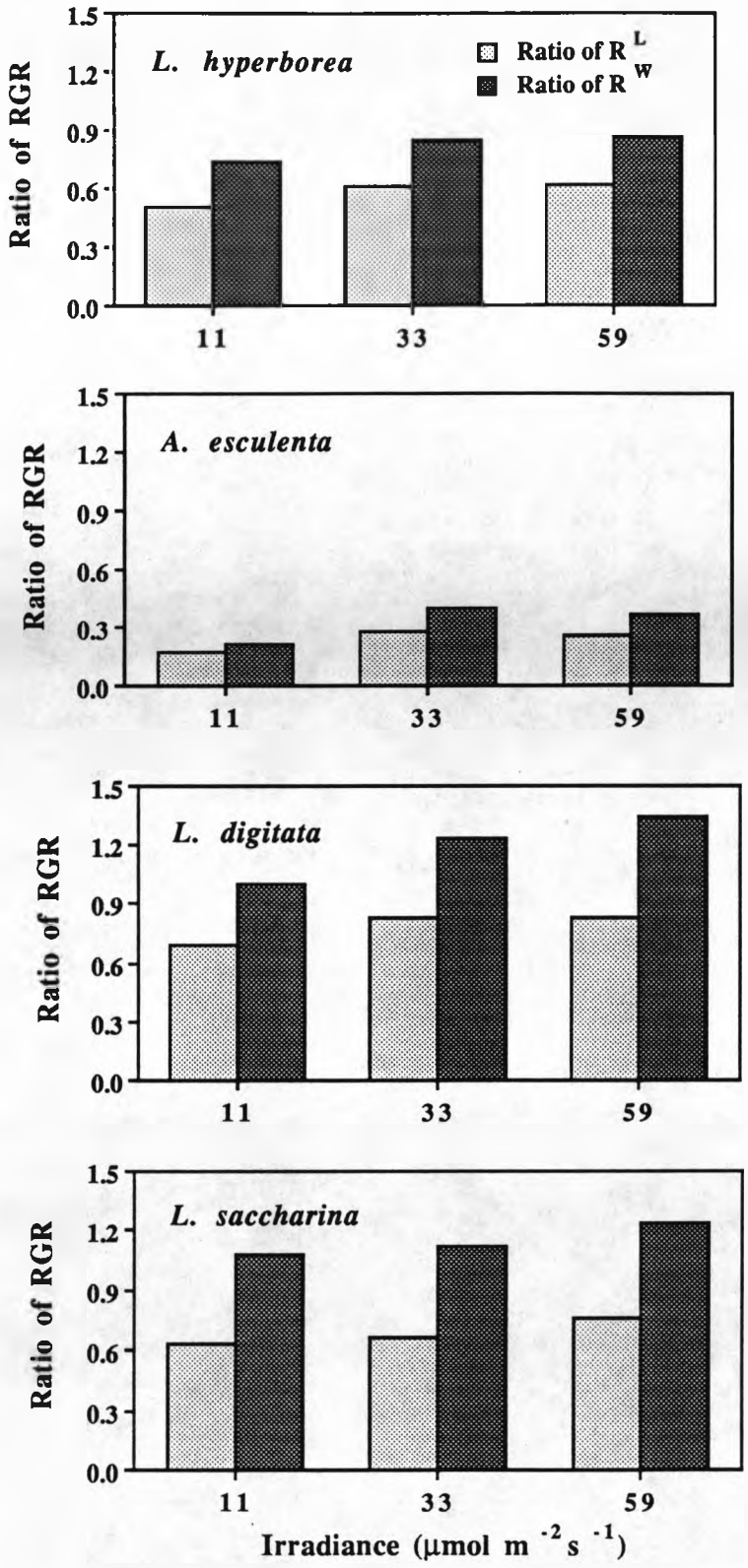

Fig. 1.4. Ratio of relative growth rate attained by sporophytes of different ages. The ratio was calculated by dividing RGR of older plants by that of younger plants at each irradiance for each growth estimate. R^L: RGR of length, R^W: RGR of width.

Table 1.3. Mean length/ width ratios (± 95% confidence intervals, n=12-24) of four species of laminarian sporophytes of different ages after growth at three different irradiances of continuous white light for 9-10 days.

Species	Age (day)	Irradiance (μmol $m^{-2}s^{-1}$) 11	33	59
L. hyperborea	0	3.82 ± 0.29	3.92 ± 0.27	4.29 ± 0.52
	10	3.51 ± 0.23	3.25 ± 0.22	3.26 ± 0.31
A. esculenta	0	5.38 ± 0.61	6.54 ± 0.85	6.46 ± 0.74
	12	6.22 ± 0.49	5.73 ± 0.52	5.91 ± 0.55
L. digitata	0	4.96 ± 0.49	5.92 ± 0.54	6.02 ± 0.58
	10	5.79 ± 0.46	5.17 ± 0.42	4.85 ± 0.47
L. saccharina	0	5.45 ± 0.40	6.17 ± 0.69	6.51 ± 0.83
	11	4.33 ± 0.82	3.06 ± 0.32	3.40 ± 0.67

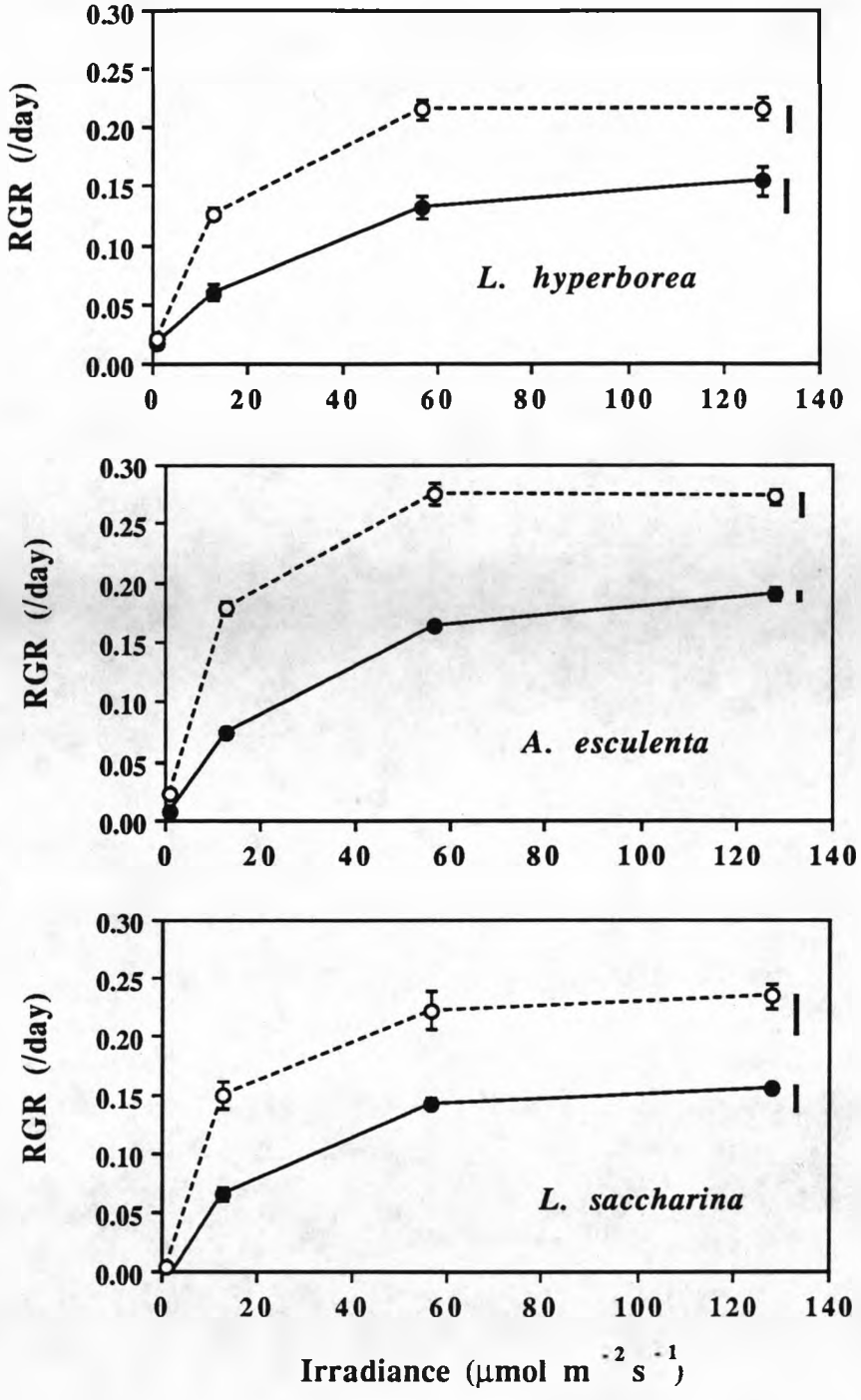

Fig. 1.5. Effect of different photoperiods and irradiances on the growth of three laminarian early sporophytes (open circle-17 h, closed circle-7 h light during a 24 h). Vertical bars show LSDs for mean RGR at different irradiances under each photoperiod (n=3).

Table 1.4. ANOVA table for RGR of three species of laminarian early sporophytes grown at three different irradiances under different photoperiods. (a) ***L. hyperborea*** (b) ***A. esculenta*** (c) ***L. saccharina***. **0.01 > p ≥ 0.001, ***0.001 >p.

(a)

Source	DF	SS	MS	F
Photoperiod (P)	1	0.017	0.017	91.88***
Irradiance (I)	3	0.108	0.036	192.57***
P x I	3	0.005	0.002	9.67**
Error	16	0.003	1.863×10^{-4}	

(b)

Source	DF	SS	MS	F
Photoperiod (P)	1	0.037	0.037	444.35***
Irradiance (I)	3	0.181	0.060	725.35***
P x I	3	0.009	0.003	35.64***
Error	16	0.001	8.304×10^{-4}	

(c)

Source	DF	SS	MS	F
Photoperiod (P)	1	0.025	0.025	110.17***
Irradiance (I)	3	0.148	0.049	221.58***
P x I	3	0.005	0.002	8.13**
Error	16	0.004	2.225×10^{-4}	

significant increase (Fig. 1.5). But, in a 7 h light period, ***A esculenta*** growth at 128 μmol $m^{-2}s^{-1}$ was significantly higher than at 57 μmol $m^{-2}s^{-1}$ whereas in the other species there was no significant growth rate increase over this range (Fig. 1.5).

There was a striking difference in the final length attained under the different photoperiods between the species (Table 1.5). At 13 and 57 μmol $m^{-2}s^{-1}$ the mean length at the final harvest of the long-day plants of ***A. esculenta*** was approximately 2.9 and 3.1 times those of the short day forms. In contrast, the same irradiances allowed the ratio of mean lengths of the long-day plants over those of the short-day ones to be 1.9 and 2.3 for ***L. hyperborea.*** In ***L. saccharina*** the ratios were similar albeit higher than those in ***L. hyperborea,*** showing 2.4 at the lower irradiance and 2.3 at the higher irradiance.

The data on irradiance and light period treatments were expressed in terms of total amount of quanta that plants received during each 24 h, i.e. MDI (Mean Daily Irradiance) used by Chapman and Burrows (1970) although irradiance was used instead of light intensity. The results (Fig 1.6) show that growth rate increased with total quanta/day up to a value of ca. 10 μmol m^{-2} s^{-1} MDI (17 h light at 13 μmol $m^{-2}s^{-1}$). In irradiation giving greater than 10 μmol $m^{-2}s^{-1}$ MDI the short-day was not effective in increasing growth rate of the ***Laminaria*** species ($p>0.20$; S-N-K test), but a significant increase in growth rate was found with the long-day treatment for all the species ($p<0.001$).

From a calculation of the L/W ratio of 12-24 plants grown either in the short-day or the long-day condition, no difference between L/W ratios of plants grown at different irradiances was detected in ***L. hyperborea*** and ***A. esculenta*** (Fig. 1.7) as was the case in the previous experiment conducted in continuous light. At 13 μmol $m^{-2}s^{-1}$, however, ***A. esculenta*** and ***L. saccharina*** showed a considerable difference in the L/W ratios under different photoperiods ($p<0.01$ for ***A. esculenta,*** $p< 0.002$ for ***L. saccharina***; t-test),

Table 1.5. Mean (± S.D., n=6) length (μm) of 10 day-old cultures of laminarian early sporophytes under different photoperiods (SD-7 h, LD-17 h light during a 24 h) of two different irradiances. Initial length was observed to be 49.1 μm for ***L. hyperborea,*** 95.8 μm for ***A. esculenta*** and 51.5 μm for ***L. saccharina*** respectively.

	13 μmol $m^{-2}s^{-1}$		57 μmol $m^{-2}s^{-1}$	
	SD	LD	SD	LD
L. hyperborea	88.8 ± 9.7	171.8 ± 13.3	182.8 ± 23.1	415.5 ± 54.0
A. esculenta	201.0 ± 9.1	573.9 ± 54.6	489.0 ± 32.2	1507.5±223.5
L. saccharina	99.2 ±11.2	234.4 ± 42.2	215.0 ± 18.8	485.0 ± 114.8

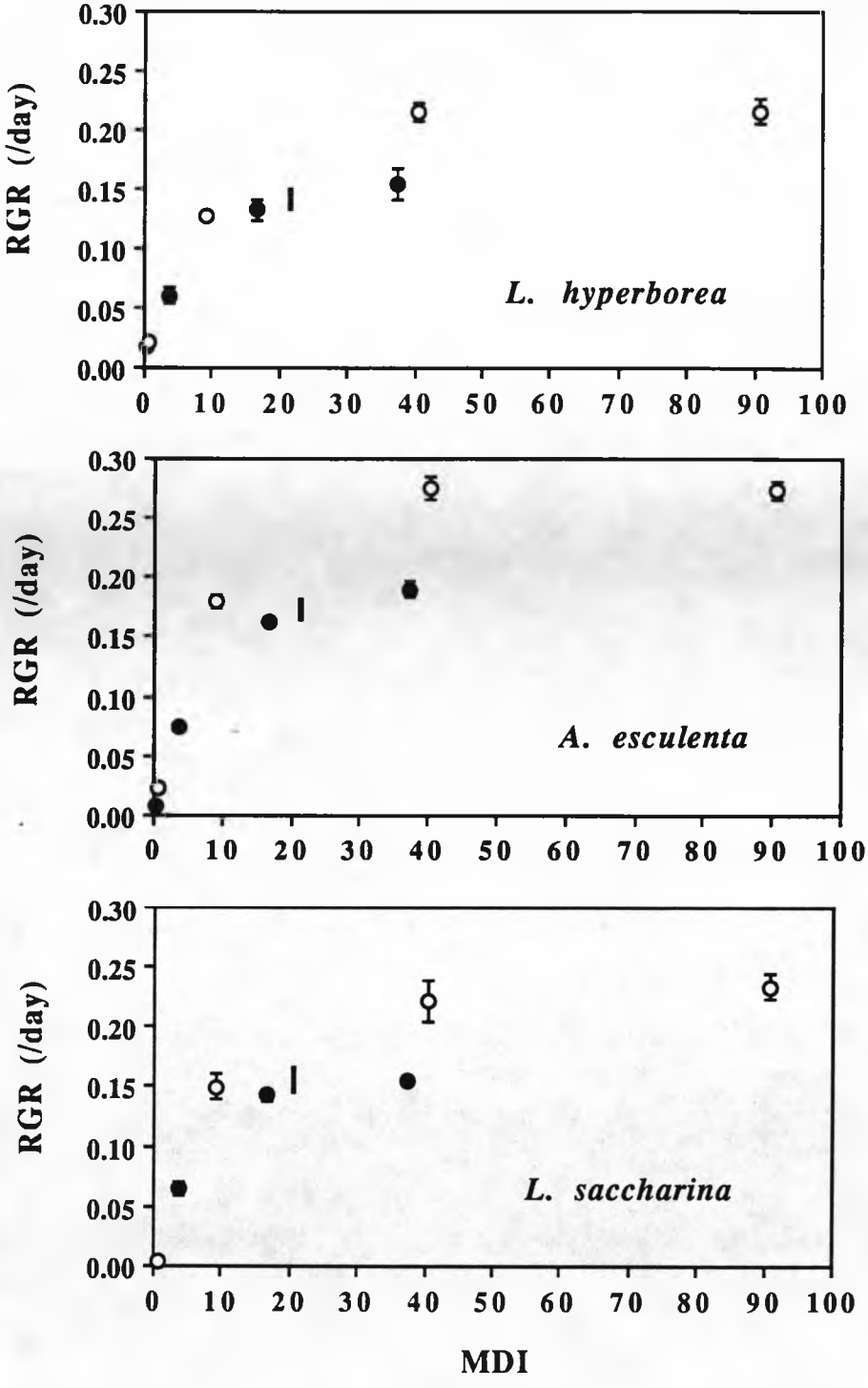

Fig. 1.6. Mean growth rate of early sporophytes under various µmol $m^{-2}s^{-1}$ MDI. Open circles are mean RGRs under 17 h x irradiance combinations and closed circles under 7 h x irradiance combinations. Vertical bars denote LSDs (at p=0.05, n=3).

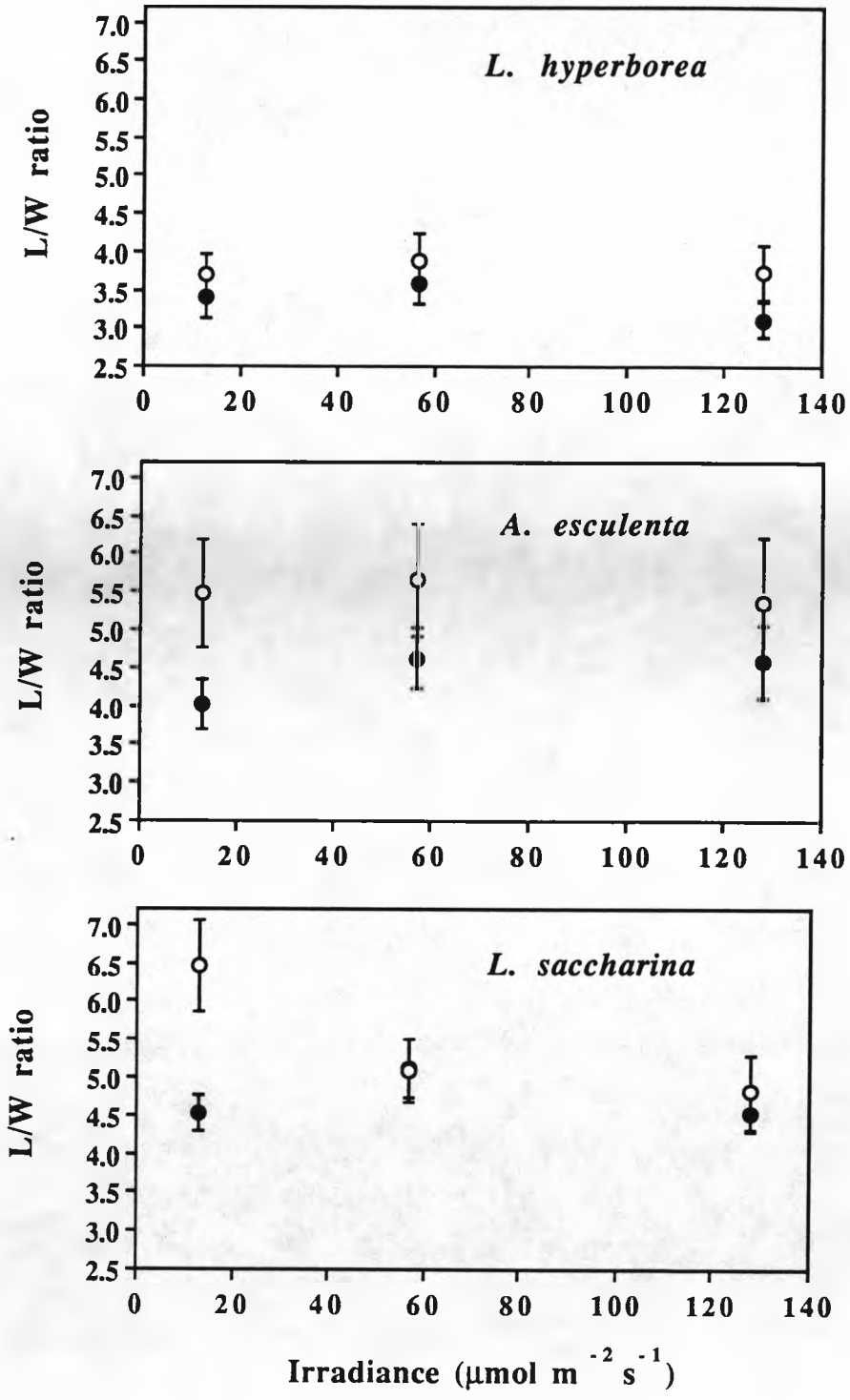

Fig. 1.7. Mean length/width ratios with 95% confidence intervals (n=12-24) of early sporophytes grown under different photoperiods and irradiances (open circle-17:7 h light: dark, closed circle-7:17 h light: dark).

while in *L. hyperborea* there was no suggestion of a difference in L/W ratio with photoperiods ($p > 0.50$, Fig. 1.7). In optimal light condition (17 h light of 57 μmol $m^{-2}s^{-1}$), the thallus of *A. esculenta* and *L. saccharina* showed 5.7 and 5.1 times more length than width, which were reduced to 4.0 and 4.5 in 7 h light of 13 μmol $m^{-2}s^{-1}$ (Fig. 1.7) The thallus shape of *L. hyperborea* consisted of only about 3.6 times more length than width irrespective of light conditions.

Early sporophytes of *L. hyperborea* and *L. digitata* showed higher growth in 17 h daylength than in 7 h daylength at photon irradiances of 36 and 75 μmol $m^{-2}s^{-1}$ during the first 12 days. Although plants were given the same dose of quanta during each 24 h plants of both species grown at 36 μmol m^{-2} s^{-1} under the long-day were longer than those at 75 μmol $m^{-2}s^{-1}$ under the short-day (Fig. 1.8). There was no significant difference in the mean length of plants at different irradiances when they were grown in the same photoperiod with the exception that after 24 days growth *L. digitata* sporophytes grown under 17 h daylength of 75 μmol $m^{-2}s^{-1}$ were significantly longer than those in the other conditions, whereas length of *L. hyperborea* grown at 75 μmol $m^{-2}s^{-1}$ was not different from that at 36 μmol $m^{-2}s^{-1}$ under either photoperiod (Fig 1.8). In addition, there was no significant difference in *L. digitata* between the plants grown in similar total quanta provided by either 17 h of 36 μmol $m^{-2}s^{-1}$ or 7 h of 75 μmol $m^{-2}s^{-1}$, whilst *L. hyperborea* exhibited significantly longer length in the lower irradiance of 17 h light period than in the higher irradiance of 7 h light period condition. At the final harvest *L. hyperborea* produced a maximum 3.12 ($\pm$0.53 S.D., n=6) mm of length in 17:7 light: dark of 36 μmol $m^{-2}s^{-1}$, whilst *L. digitata* had developed a mean maximum length of 5.04 ($\pm$0.43, n=4) mm in the same light-dark cycle but at 75 μmol $m^{-2}s^{-1}$.

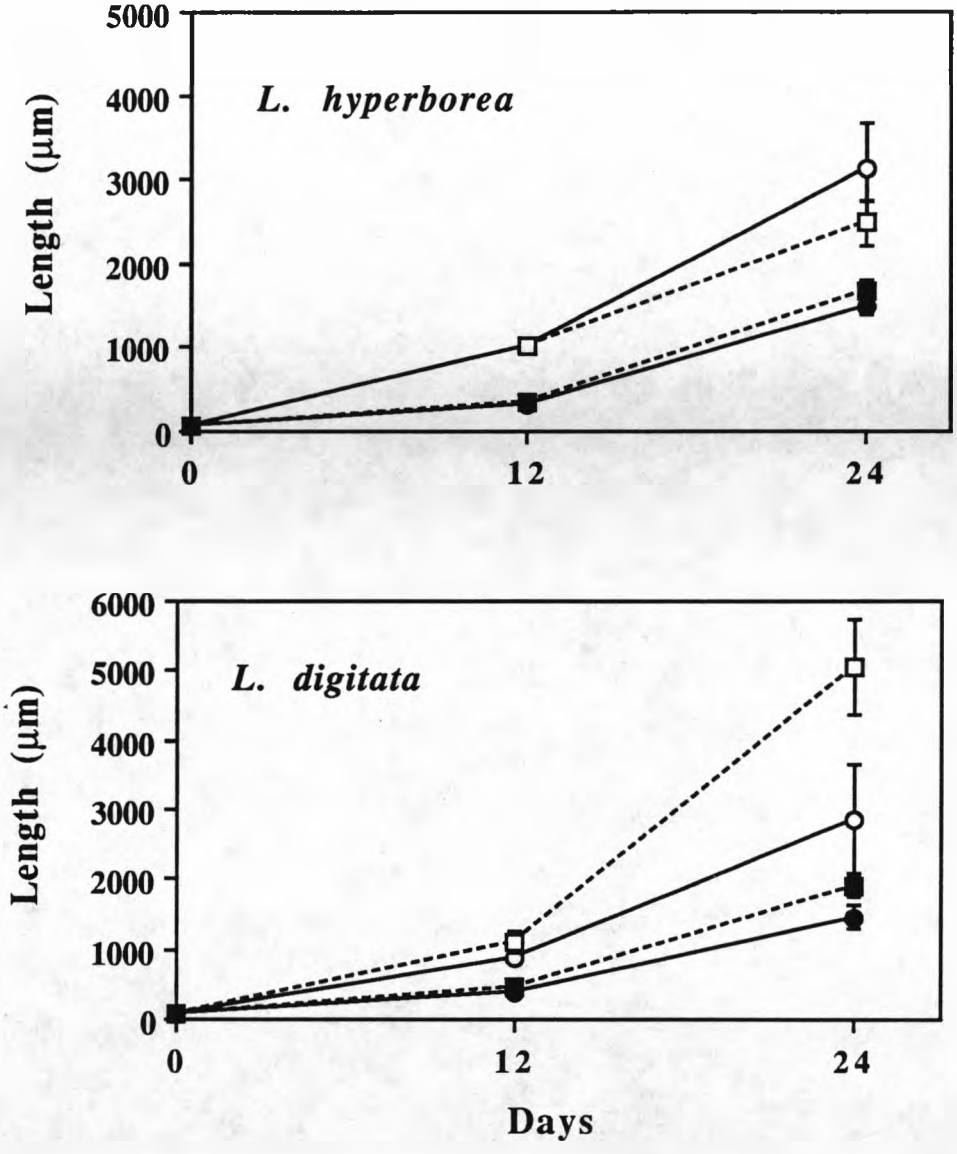

Fig. 1.8. Growth of *L. hyperborea* and *L. digitata* early sporophytes under different photoperiods and irradiances. Open symbols represent mean length under long days (17 h light), closed short days (7 h light); circles indicate 36 µmol $m^{-2}s^{-1}$, squares 75 µmol $m^{-2}s^{-1}$. Vertical bars indicate 95% confidence intervals (n=4-6).

1.3.3. Comparison of 12 hour photoperiod with continuous light

As can be seen from Fig. 1.9 perceptible effects of light period on the growth of early sporophytes of *L. hyperborea* were that light saturation of growth occurred at about the same irradiance in both 12:12 h light: dark cycle and in 24 h illumination, but growth rate was much lower in the former than in the latter. For the other *Laminaria* species, light saturation of growth did not seem to happen even up to 109 μmol $m^{-2}s^{-1}$ in the light-dark cycle, whereas the growth was light-saturated at 55 μmol $m^{-2}s^{-1}$ in continuous light. For *A. esculenta,* the maximum growth rate was not much lower in the cyclic illumination than in continuous light although the light saturation point for growth was found at a similar irradiance (Fig. 1.9).

1.3.4. Survival in the dark

Pronounced disparity in the ability to survive total darkness was found when mortality of *L. digitata* in the dark was compared with that in *L. hyperborea* (Fig. 1.10). The *L. digitata* kept in the dark for 10 days began to decay and 23% had died by the end of 20 days when 96% of *L. hyperborea* still survived.

1.3.5. Growth in shaded daylight

When some sporophytes were grown in shaded conditions under daylight, it was noticed that reduction of daylight from 35 to 4% caused a systematic decrease in the growth of early sporophytes of two *Laminaria* species (Fig.1.11). However, *L. digitata* showed lower growth rates than *L.*

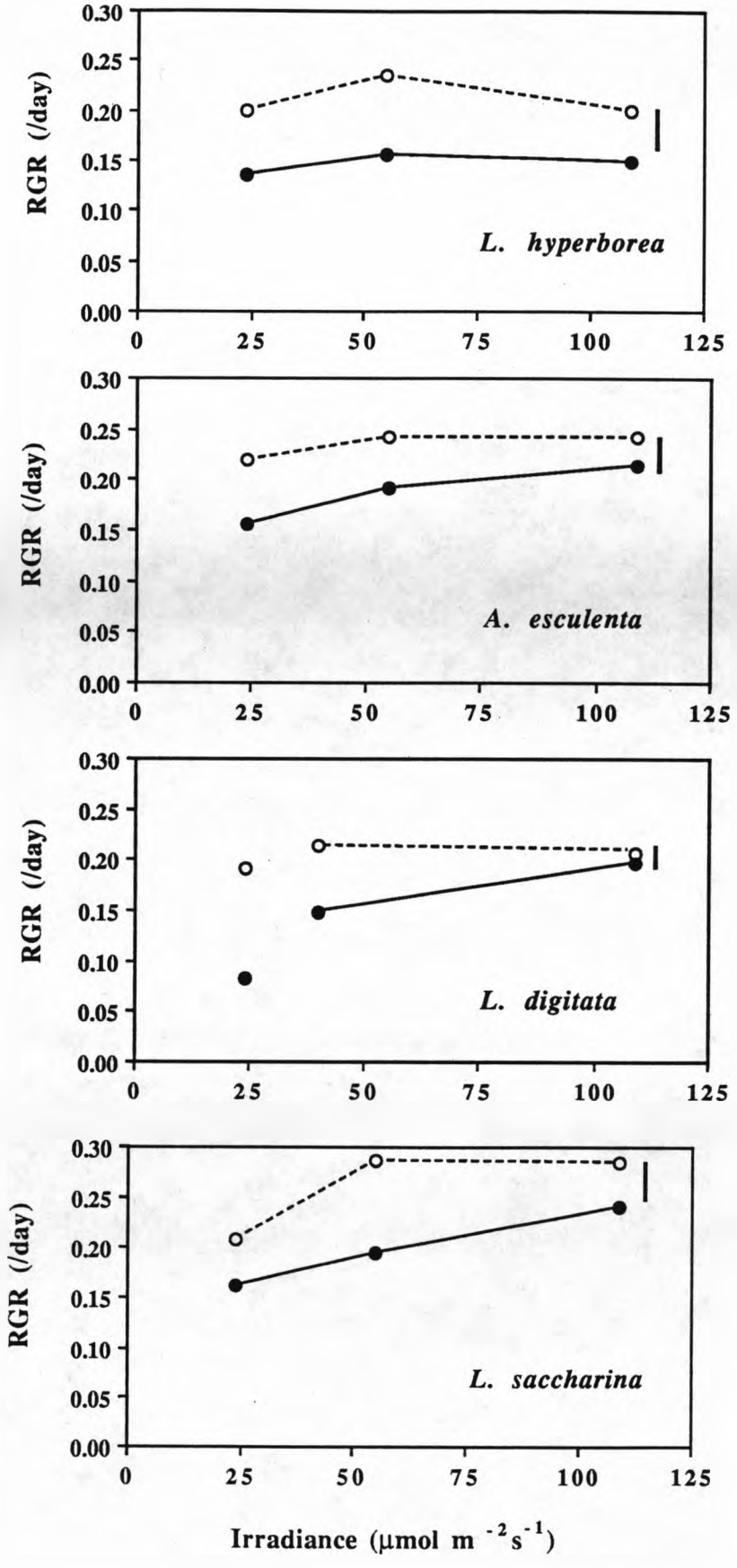

Fig. 1.9. Growth of four species of laminarian sporophytes under 12:12 h light: dark cycle (closed) and 24 h illumination (open) of three different irradiances (24 µmol $m^{-2}s^{-1}$, 55 µmol $m^{-2}s^{-1}$ and 109 µmol $m^{-2}s^{-1}$). Vertical bars show LSDs (at p=0.05, n=2-3).

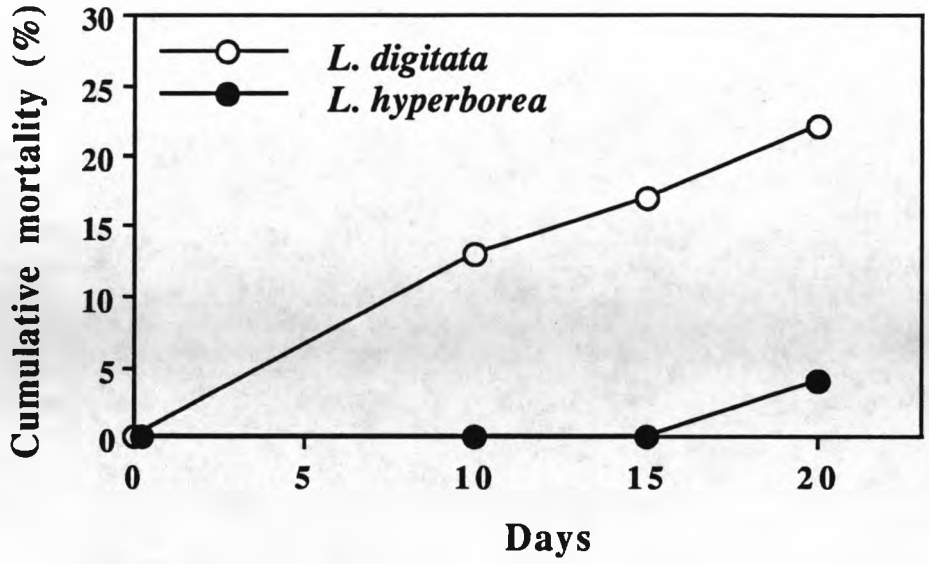

Fig. 1.10. Mortality in the dark of 20-24 day-old early sporophytes of *L. hyperborea* and *L. digitata*. Counts were made on 200 plants for each species.

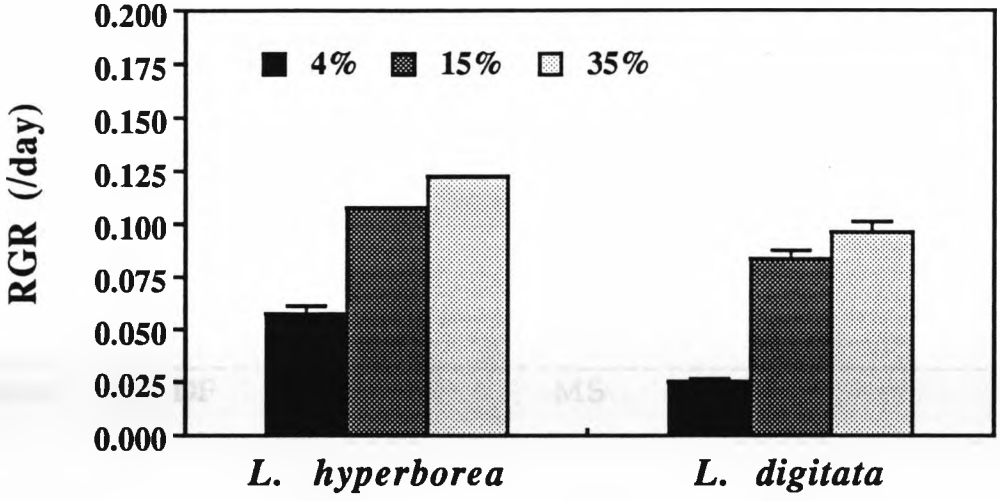

Fig. 1.11. Effect of reduced levels of daylight on growth of two species of ***Laminaria*** early sporophytes. Mean RGR and one standard error is shown (n=2).

hyperborea over all the light levels ($p<0.001$; S-N-K test based on Table 1.6) and there was an abrupt decrease of growth rate from 0.084 in 15% daylight to 0.025 in 4% daylight.

1.4. Discussion

Early sporophytes of four species of the Laminariales can be characterized as shade plants in that the growth was light-saturated (30 μmol $m^{-2}s^{-1}$) at even lower irradiances than some deep-growing red algae in which light saturation of photosynthesis occurs at about 50 μmol $m^{-2}s^{-1}$ (Mathieson and Norall 1975). This value of saturating irradiance is higher than that previously reported for early sporophytes of the same *Laminaria* species (10-20 μmol $m^{-2}s^{-1}$; Kain 1969). The minimum irradiance requirement for growth seems to be less than 1-2 μmol $m^{-2}s^{-1}$, which has been reported to be a typical compensating irradiance for photosynthesis of shade plants such as deep-water red algae and terrestrial plants growing on the floor of rain forests (Lüning 1990). In this study the compensation point for growth of ***L. hyperborea*** was found to be between 0.5 and 1.0 μmol $m^{-2}s^{-1}$. Although a detailed study on comparison of light compensation point between the species was not made, there is no evidence that in the other species compensating irradiance is substantially different from that of ***L. hyperborea*** (Kain 1969, Burrows 1971).

The very early stage sporophytes of the four species respond similarly to variations in irradiance up to 90 μmol $m^{-2}s^{-1}$, making it unlikely that light can be a determining factor in the outcome of plausible competition between the species. This may also explain the penetration of some of the species to a similar depth (Kain 1966).

Early sporophytes of all species investigated in this study seem to exert themselves for length growth during the first 9-10 days and then also for width growth as they grew older. A marked reduction of growth rate with age found in the laminarian early sporophytes may be

consistent with the prediction that plants should have an increasing proportion of non-photosynthetic internal tissues as they grow older (Hellebust 1970). This might then affect the ability of older plants to absorb and utilize light for growth. Niihara (1975) has reported a higher rate of photosynthesis in younger ***Laminaria japonica*** than in older ones. Also, the reason may be found in the fact that the contribution of cell division to thickness as well as length and width is initiated as early sporophytes become polystromatic (Kain 1965). Growth rate might have declined due to increasing nutrient limitation as plants grew older, but frequent renewal of medium (every 3-4 days) probably eliminated this possibility.

The pattern observed for thallus morphology of early sporophytes of different ages at different irradiances shows that ***L. hyperborea*** maintained a rather widened form, whereas ***A. esculenta*** had an elongated form throughout the two developmental stages irrespective of irradiance. It is suggested that at least under continuous light, the traits of thallus morphology of these species are not affected by change of growth rate with age or variations in irradiance. In the case of ***L. saccharina,*** however, thallus shape must be correlated with growth rate, i.e. thallus became wider as the rate of width growth increased. Burrows (1964) reported that the shape of the base of ***L. saccharina*** frond appeared to be a function of growth rate. In spite of remarkable increase of width growth with age ***L. digitata*** seemed to sustain morphology of relatively elongated form compared with ***L. hyperborea,*** the widest thallus form among the species. This may be due to the fact that the overall growth of this species is inherently slow but constant and widespread.

Increase of growth rate sustained up to 128 μmol $m^{-2}s^{-1}$ under 7:17 light: dark condition could be an advantage at shallow depth in winter

for *A. esculenta* to outgrow the other species whose growth was light-saturated at a lower irradiance. Kain (1966) found about 70 μmol m^{-2} s^{-1} in an open water just above ELWS during winter. It is probable that although most sublittoral algal species are present all the year round (Kain 1960) they are less abundant in the winter because of poor light conditions and storms, and thus there may be more vacant rock available for settlement than in the summer (Kain 1966). Once *A. esculenta* colonizes a shallow depth through pre-empting the territory, therefore, the improved light conditions which follow will allow *A. esculenta* sporophytes a maximum growth rate at their very early stage, found in this study to be much higher in the long-day condition than that of other species in culture. It is possible that this may in part explain a dominance of *A. esculenta* at the upper margin of the sublittoral in some regions of Scotland (Kitching 1941) and a rapid growth of *A. esculenta* at 0.5-1 m depth even in the winter in Norway (Sundene 1962).

At irradiances below the saturation level, the rate of growth was proportional to MDI, i.e. the total quanta received per day, rather than to a particular daylength or irradiance. This has been shown to be the case for *Desmarestia aculeata* (Chapman and Burrows 1970) and for *Sacchorhiza polyschides* (Norton and Burrows 1969). The correspondence of growth rate to MDI also suggests a lack of a photoperiodic trigger (Murray and Dixon 1973) and that control of growth rate is by photosynthetic limitation through the interaction of irradiance and photoperiod (Garbary 1979). At irradiances greater than 57 μmol $m^{-2}s^{-1}$, however, growth was independent of MDI and an increased rate of growth was obtained only by an increase in daylength. This result may imply that under conditions above light saturation plants are not able to use all the light available for a particular MDI

(Chapman and Burrows 1970). It is clear that the enhanced growth achieved by increasing daylength at MDI greater than 10 μmol $m^{-2}s^{-1}$ was not attributed to a regulating effect of daylength mediated through interactions between temperature and enzyme activities (Hobson 1974) since variation in temperature during the study was minimal. The more sustained rates of carbon input in the longer irradiation conditions may have contributed to enhancing the growth rate to some extent.

In habitats with very low light levels, plants with broad and flat thalli reap the greatest energetic rewards because they have the greatest proportion of the cells in contact with light striking them and also can minimize self-shading (Dahl 1982, Hay 1986). Of the three species studied ***L. hyperborea*** might fit into this scheme, suggesting that its wider thallus compared with the other species enables this species to be better adapted to low light conditions. On the other hand, together with their somewhat translucent thallus at the early stage, the elongated thallus form may give ***A. esculenta*** an advantage of making efficient use of a favourable light environment. Early sporophytes of ***L. saccharina*** appeared to have morphological plasticity, possibly due to the fact that the morphological characteristics gradually develop throughout its life time (Norton et al. 1982). It was noticeable that at 13 μmol $m^{-2}s^{-1}$, i.e. sub-saturating irradiance for growth, long-day treatment allowed significantly more elongated form in ***A. esculenta*** and ***L. saccharina*** than the short-day treatment, whereas at saturating irradiances there was no difference in thallus form between the photoperiods. At the present there is no convincing evidence that it is due to a photoperiodic response which has not been known to occur at the early stages in ***Laminaria*** species (Lobban et al. 1985). But it is recommended that this aspect be investigated further. If a

photoperiodic response does exist perhaps the lack of response of thallus shape to different photoperiods at above saturating irradiances may be explained by postulating that the sensitivity of daylength perception mechanism was lessened due to the high irradiances (Breeman and ten Hoopen 1984).

The ability of very early sporophytes of ***L. hyperborea*** and ***L. digitata*** to make use of available light is limited by the existence of saturating levels, above which an increase in irradiance did not lead to a faster growth. For the same reason, under a similar MDI, longer period of lower irradiance produced a higher growth in both species than higher irradiance of short period. Kain (1965) also found that intermittent light (12:12 h light: dark) did not give faster growth than continuous illumination due to use of double irradiance in the light-dark cycle as that in the continuous light to produce the same total of quanta per day and the former irradiance was probably above the saturation level. However, ***L. digitata*** was found to require higher irradiance for faster growth with time. This was manifest in the fact that 24 day growth resulted in significantly longer length in the higher irradiance than in the lower irradiance within each period, a fact not been exhibited in 12 day growth. No such change in light requirement occurred in ***L. hyperborea*** during comparable periods of time. It is generally accepted that the relationship between seaweeds and their light demands changes according to the developmental stages (Lüning 1981a). In the case of relatively photophilic plants which can inhabit shallow water, a striking change in the light demands has been reported to happen in a very short time period (Fei and Neushul 1984, Fei et al. 1989, Hales and Fletcher 1989). In this respect, a switch in sensitivity to irradiance may be an advantage to ***L. digitata*** in adapting to a well-lit area better than ***L. hyperborea.***

Compared with *L. hyperborea, L. digitata* was short-survived in the dark. The ability of early stages of plants to survive in total darkness for long periods has been recorded in some brown algae and considered as an important ecological strategy to enable young plants to become established beneath a dense canopy until light condition improves (Kain 1964, 1965, 1969, Chapman and Burrows 1970, Moss and Sheader 1973). Kain (1969) found at least 80 day's survival in the dark of undeveloped gametophytes of four species (including *L. digitata*) of the Laminariales. However, in this study, mortality of young sporophytes of *L. digitata* in the dark appeared to be higher in a relatively short period compared with that of the gametophytes. The reason why there is a discrepancy in the ability to survive in the dark between gametophytes and early sporophytes may be postulated in three ways. Firstly, Kain (1969) stated 'although many of the spores died during the first few weeks a few were found to be capable of development after 80 days'. Although it was not clearly mentioned, the mortality of gametophytes during the first few weeks might have been comparable to 23% mortality of early sporophytes of *L. digitata* during 20 day dark incubation. Secondly, in the case of gametophytes, they had not been given any light pretreatment whereas early sporophytes had been grown for a time in light prior to being placed in the dark. In higher plants, the build-up of carbohydrates in light is known to impose a respiratory burden when plants are kept in long periods of darkness (Hutchinson 1967). At this stage, however, it is not certain that, as in land plants, the increasing respiratory burden possibly for sporophytes but not for gametophytes may explain the different resistances to darkness. Thirdly, physiological age might have played a role in governing different survival capacities in the dark (Antia and Cheng 1970). The difference in resistance to darkness between *L. hyperborea*

and *L. digitata* may be explained by the different sporing seasons of the species. In Britain, the reproductive period of *L. hyperborea* is clearly defined, from the end of September to early April whereas *L. digitata* sporophytes are mainly fertile in summer (Kain 1969). As early stages of *L. hyperborea* must become established in winter periods, it seems to be essential for plants to be able to withstand poor light conditions for survival. On the other hand, such physiological fitting may be less seriously needed for *L. digitata* than for *L. hyperborea,* considering that the former species can start its establishment in favourable light conditions of summer.

Faster growth in extremely reduced daylight conditions may give an advantage to *L. hyperborea* over *L. digitata* when resource for their competition is light. There is good evidence that tolerance to low irradiances may be the ultimate determinant of the lower limits of most seaweed species regardless of whether these limits are actually imposed by the physical environment or by competition with another species (Dring 1982). Together with the capability of higher survival in the dark, tolerance to shade seems to indicate a competitive advantage of *L. hyperborea* to *L. digitata* in the deep sublittoral. Some results gathered from field (Kain 1971b, 1976) showed that *L. digitata* rapidly colonized cleared areas within the sublittoral forest of *L. hyperborea,* but was gradually eliminated as the canopy was re-established. *L. digitata* has also been reported to be abundant down to 15-20 m where *L. hyperborea* is absent (Edelstein et al. 1970). These seem to imply that the difference in shade tolerance between *L. hyperborea* and *L. digitata* may be intensified when competition is operating.

As is often the case, if the saturating level for photosynthesis is higher than that for growth (Lüning 1981a), then plants may be producing short-term storage substances which they are unable to use

because growth is proceeding as fast as it can (Brown and Richardson 1968, Dring 1982). On the other hand, it is known that a dark period may cause vegetative growth at the expense of stored photosynthates (Shirley 1929). Foy and Smith (1980) have shown that growth efficiency of some blue-green algae was improved in light/dark cycle in comparison with continuous light. They explained this as being due to the photosynthetic production in the light periods in excess of the protein synthesis requirements being stored in the algae as carbohydrate which in turn was used to generate cell protein, DNA and chlorophyll a during the following dark period. Growth of some laminarian sporophytes achieved in the light-dark cycle as fast as in continuous light may, therefore, imply that at above saturating irradiances a dark period might provide cells with a by-pass for photosynthates to be used for metabolism and ultimately for growth which otherwise would only be accumulated to cause reduction in biosynthetic or photosynthetic apparatus of the cell (Dring 1982). Also, as the limitation of growth rate under continuous light by a light independent reaction was removed, the growth rate in the light-dark period might be determined by the photosynthetic capacity of the cell (Gibson and Foy 1983), hence light-saturated at a higher irradiance than in continuous light. On the other hand, the ratio of maximum growth rate under continuous light and 12:12 h light-dark cycle was 1.5:1 for ***L. hyperborea,*** 1.2:1 for ***L. saccharina,*** 1.1:1 for both ***A. esculenta*** and ***L. digitata*** compared with a ratio of photoperiods of 2:1, suggesting that ***L. hyperborea*** does not grow as fast in the light-dark cycle relative to continuous light compared with the other species. As it is not known that photosynthetic ability of ***L. hyperborea*** is lower than that of the other species, it does not seem to be due to a difference in the amount of photosynthates usable for growth during dark period. It has been

observed that *L. hyperborea* produces a small new phylloid during the half year in complete darkness (Lüning 1969). Raising a question, 'why should laminaran as a storage material be accumulated to a large extent in *L. hyperborea,* but not in *L. digitata* ?, Black (1950) postulated that lack of laminaran in *L. digitata* was related to its fast growth. Lüning (1981a) has pointed out that the deeper sublittoral species are well-adapted to survive periods of little or even no light due to their strategy of growth and building-up reserve materials while species of the upper sublittoral probably allocate more of the carbon fixed during the year to growth and reproduction than to reserve materials. Therefore, that insertion of dark period did not make any difference in *L. hyperborea* (a deep-sublittoral alga) compared with continuous irradiation might be due to its propitious property of keeping rather than consuming storage materials, whereas *L. digitata* (shallow water-dwelling species) seemed to maintain maximum growth rate in a break-even daylength (12:12 h light: dark) as fast as that in 24 h light by quickly using the accumulated photosynthate for growth. If this is so, as Lüning (1979) suggested, the reason why *L. digitata* does not persist in deeper water, whilst *L. hyperborea* does, may be partly explained by the species-specific storage capability.

Chapter 2. Effect of different spectral qualities of light

2.1. Introduction

In aquatic environments the light which penetrates a water column is highly variable in both irradiance and spectral quality. Total irradiance diminishes exponentially and some wavelengths such as red, orange and yellow wavelength in clear water are filtered out as a function of increasing depth, suspended particles and dissolved substances (Jerlov 1966). Consequently, blue and/or green wavelengths predominate at increasing depths. Perhaps due to these characteristics of underwater light climate, Engelmann's theory of complementary chromatic adaptation was put forward to explain adaptive mechanisms of algae to alteration of the energy distribution in visible light environment, i.e. one algal type of possessing the pigment to absorb the predominant wavelength of available light may have a competitive advantage over another (Kirk 1983).

On the other hand, in macroalgae, both experimental and theoretical evidence has been accumulated to indicate that chromatic adaptation does not occur and that all responses to light are to total irradiance (Dring 1981, Ramus 1983). However, as Dring (1990) pointed out, there is some exception in which chromatic adaptation might influence distribution of some seaweeds in a limited situation, for example, plants with thin thalli growing near the bottom of the photic zone, where light is the main growth-limiting factor. Meeson and Faust (1985) have found that growth rates of ***Prorocentrum minimum*** (Dinophyceae) responded to spectral quality at low irradiance but not at high irradiance. Considering the thin thallus and plausible underdevelopment of the pigment system of early sporophytes of species of the Laminariales, it seemed to be worthwhile to investigate if

there were any adaptive characteristics of this stage of plants to different spectral light regimes.

In brown algae, a large number of studies have shown notable differences in the morphogenetic response to different light qualities, for instance in 2-dimensional growth and hair formation of ***Scytosiphon lomentaria*** (Dring and Lüning 1975), in induction of gametogenesis of members of the Laminariales (Lüning and Dring 1972, 1975, Lüning and Neushul 1978, Lüning 1980), but little information is available on vegetative growth response to different light qualities (Dring and Lüning 1975, Müller and Clauss 1976).

Light of different wavelengths has also been reported to influence arrangement of chloroplasts in plants in different ways (Zurzycki 1980, Haupt 1982). Zurzycki (1955) has shown a relationship between the rate of photosynthesis and the chloroplast arrangement in low light. If this holds true, different growth rates in different light qualities due to chloroplast arrangement may not be unexpected.

The present study aimed to investigate the effect of different wavebands of light on growth of of laminarian early sporophytes and phaeoplast arrangement of ***L. hyperborea*** (the only species used) was observed.

2.2. Materials and Methods

2.2.1. Effect of narrow wavebands of light

The culture system consisted of a compartmentalized glass tank, the sides of which were blacked out, leaving small apertures through which a light beam could illuminate the inside of the compartment. This tank stood within a second glass tank, which was filled with tap water. A thermostat and water circulation system maintained the cultures within the inner tank at 10 ^{o}C. A projector was positioned opposite each compartment. The light source was the Voigtlander VP 200A projector with 24V 250W Halogen lamp and 4 Balzers interference filters (449, 545, 593 and 657 nm) one of which was inserted behind each projector lens to produce 10 nm half-band of narrow waveband of light. Irradiance was controlled by altering the voltage of the mains supply and maintained at 7 μmol $m^{-2}s^{-1}$ for ***L. hyperborea*** and 3 μmol $m^{-2}s^{-1}$ for ***A. esculenta*** and ***L. saccharina.*** Coverslips (18 x 18 mm, square form) bearing sporophytes were transferred to compartmentalized plastic boxes (100 x 100 x 18 mm). Each of the compartments, apart from the edges of the box, was just enough to accommodate one coverslip. Each compartment contained 4 ml of medium, renewed every 3-4 days so as to prevent nutrient depletion. At first, 6 coverslips were employed for each box, but later, the number was reduced to 4 coverslips, ensuring small variation in irradiances reaching the compartments. This lidded-plastic box was then sealed with a plastic lid and parafilm (Sigma) and set at the back wall of each aperture of the inner tank. The cultures were maintained for 8 days and harvested. The disadvantage of this system was that as the plastic

box was set up in an erect form, the upper part of each coverslip was often found to stand tightly against the wall of the plastic box, possibly affecting nutrient availability for young sporophytes growing there. But, as measurements were made for the largest plants on each coverslip, this possibility would have been ruled out. The fact that if one coverslip was contaminated, the others would not necessarily be affected seemed to be of practical use.

2.2.2. Effect of broad waveband of light

Cultures were also made under coloured fluorescent light (blue, green and red) combined with the corresponding colour of gelatin filters (Cinelux, Strand Lighting) in ventilated chambers. The details of irradiation systems have already been described in 'General materials and methods'. Two irradiances (5, 13 μmol $m^{-2}s^{-1}$) were employed for each light field. Duplicate or triplicate cultures were placed in each of 6 light fields. Cultures were made in petri dishes (60 mm diam., 13 mm high) filled with 20 ml medium and each containing 2-3 coverslips with adhering early sporophytes of a species of the Laminariales. The medium was changed every 3-4 days and harvesting was made 9-10 days later. Photon irradiance measured regularly did not differ more than ±10% over the experimental periods. Temperature was maintained at 12.5-14.5 °C for blue and green and 15-16 °C for red light. An experiment was made also to investigate the effect of a high irradiance (36 μmol $m^{-2}s^{-1}$) on growth. In the case of red light, however, it was not possible to control this in the chamber if more than 20 μmol $m^{-2}s^{-1}$ was produced. Therefore, the irradiance was attained with white fluorescent light (Polylux 4000) covered with a

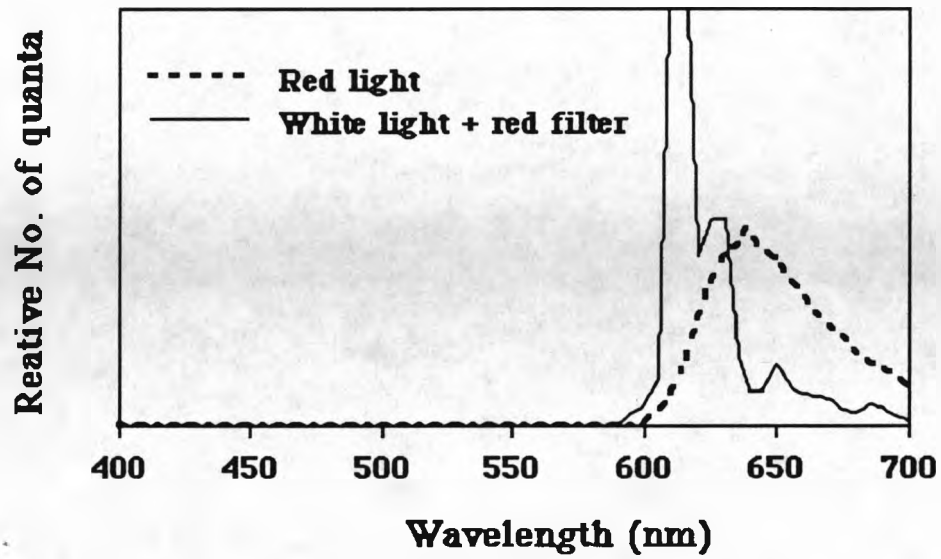

Fig. 2.1. Spectral distribution curves of red fluorescent tubes + filter and white fluorescent tubes (Polylux 4000) + red filter.

red filter (Cinelux No. 406) and used for further cultures. The spectral distribution of this series is shown compared with that of red fluorescent tube+red filter (Fig. 2.1).

2.2.3. Phaeoplasts arrangement of *L. hyperborea* in different irradiances of light qualities

Culture of ***L. hyperborea*** was made at different irradiances (5, 13 and 36 μmol $m^{-2}s^{-1}$) of different spectral light fields as described before. Continuous irradiation was done in order to prevent the initiation of endogenous circadian rhythms. After 10 days incubation in each light field, coverslips bearing sporophytes were harvested and fixed with 30% corn syrup. Staining was unnecessary since the thallus of early sporophytes is monolayered and translucent. The arrangement of phaeoplasts was checked under a microscope (Dialux 20EB, Leitz Wetzlar; x 1500) and photomicrographed.

2.3 Results

2.3.1. Effect of narrow wavebands of light

Relative growth rates of early sporophytes in narrow band light fields are shown in Fig. 2.2. *A. esculenta* did not show any significant difference ($p > 0.05$) in the mean growth rates between different light fields, while *L. hyperborea* and *L. saccharina* did (Table 2.1). For all the three species, growth rate was lowest in orange light (593 nm) among the light qualities (Fig. 2.2). In the *Laminaria* species, there was no significant difference in the growth rates between blue (449 nm) and green light (545 nm)-grown plants ($p > 0.50$), but the growth rate of red light (657 nm)-grown plants was lower than that of the former plants (Fig. 2.2; $p < 0.05$, S-N-K test).

2.3.2. Growth at different irradiances of broad waveband of light

The growth response to spectral light of different irradiances provided by fluorescent tubes plus filter series depended on the species (Fig. 2.3). In *L. hyperborea*, both light quality ($p < 0.001$) and irradiance ($p < 0.001$) significantly affected the growth rate and there was interaction between the factors (Table 2.2a). Both blue and green light allowed ca. 42-51% at 5 μmol $m^{-2}s^{-1}$ and 35-37% at 13 μmol $m^{-2}s^{-1}$ higher growth rate than red light at the corresponding irradiances. Also, growth rate in blue and green light increased by 42% and 37% respectively as irradiance increased from 5 to 13 μmol $m^{-2}s^{-1}$. In breaking down the interaction effects, it was found that between the

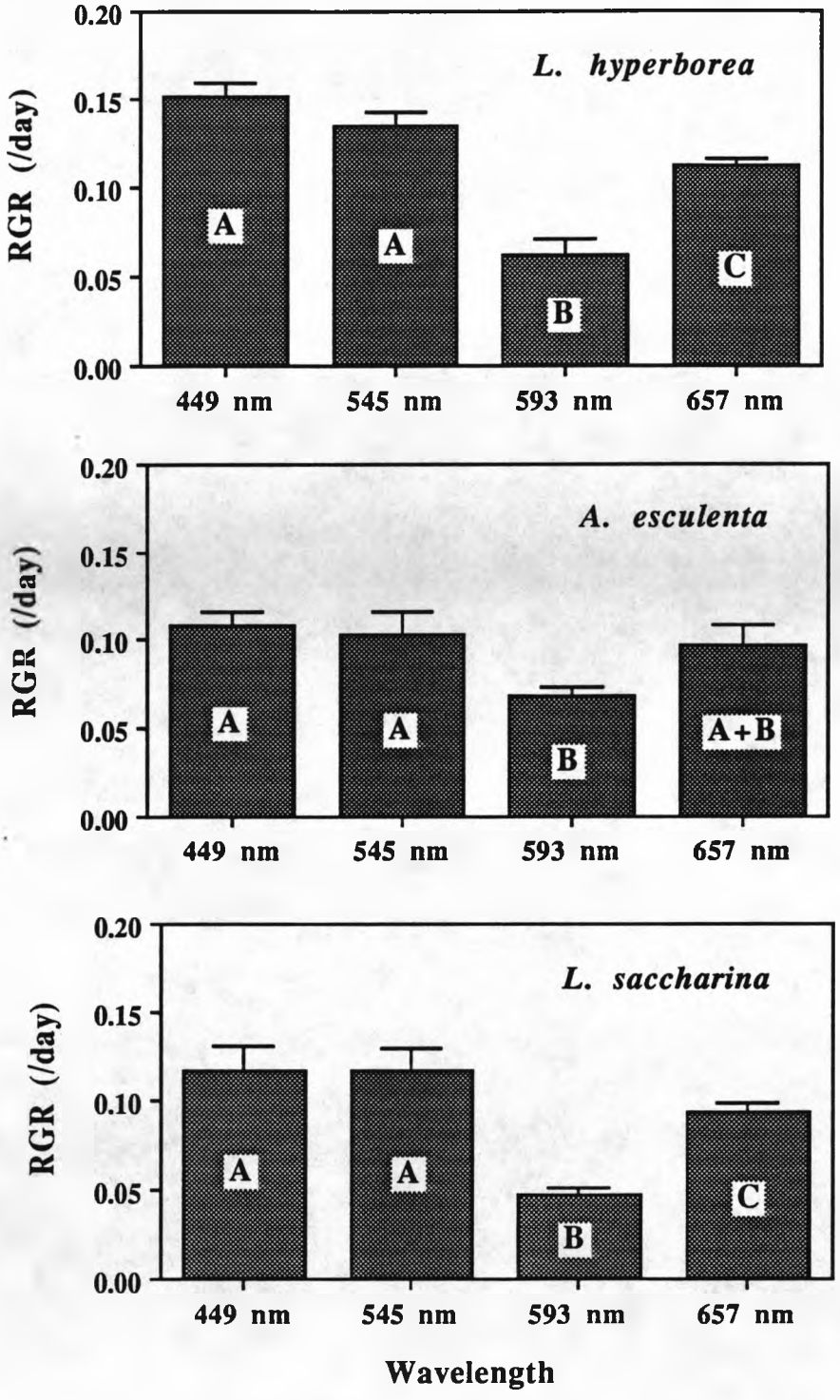

Fig. 2.2. Growth of early sporophytes of species of the Laminariales at 3-7 μmol $m^{-2}s^{-1}$ of narrow band of light. Mean RGR and one standard error are shown (n=4-6). Bars labelled with different letters are significantly different at p= 0.05 (S-N-K test).

Table 2.1. ANOVA table for RGR of laminarian sporophytes grown in narrow band of light. **0.01 ≥p> 0.001, ***0.001 ≥p, n.s. not significant.

(a) ***L. hyperborea***

Source	DF	SS	MS	F
Between groups	3	0.027	0.009	29.18***
Within groups	20	0.006	3.053×10^{-4}	
Total	23	0.033		

(b) ***A. esculenta***

Source	DF	SS	MS	F
Between groups	3	0.004	0.004	3.15 n.s.
Within groups	12	0.005	3.884×10^{-4}	
Total	15	0.008		

(c) ***L. saccharina***

Source	DF	SS	MS	F
Between groups	3	0.013	0.004	11.45**
Within groups	12	0.005	3.818×10^{-4}	
Total	15	0.018		

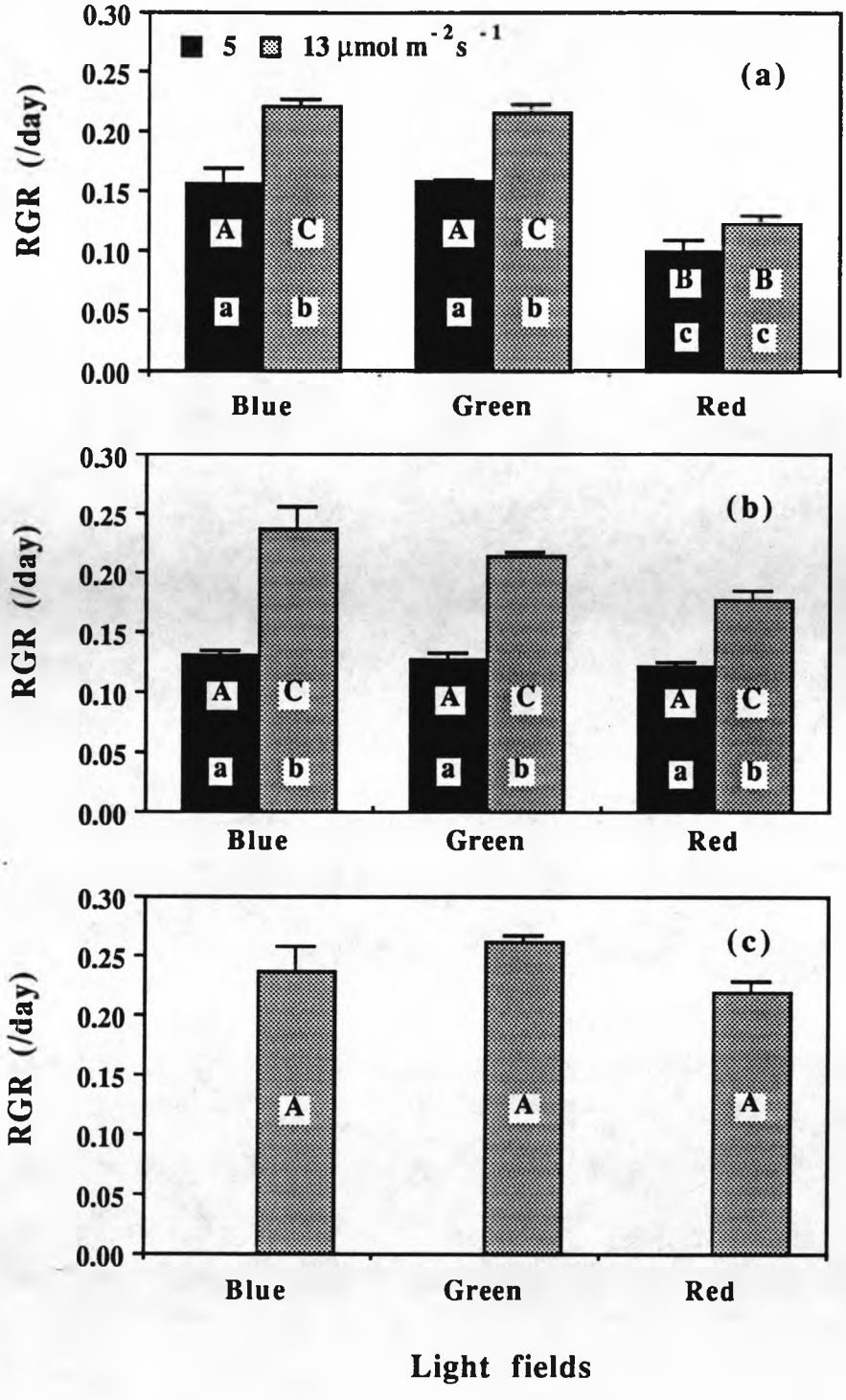

Fig. 2.3. Effect of different irradiances of broad band of light on the growth of laminarian early sporophytes. Mean RGR and one standard error (n=2-3) bars are shown. Capital letters inside the bars indicate significant differences at p= 0.05 among light qualities at an irradiance and small letters between irradiances in a light quality. (a) *L. hyperborea* (b) *A. esculenta* (c) *L. saccharina.*

Table 2.2. ANOVA table for RGR of laminarian sporophytes grown at different irradiances (5 and 13 μmol $m^{-2}s^{-1}$) of broad band of light. *0.05 >p≥ 0.01, **0.01 >p≥ 0.001, ***0.001 >p, n.s. not significant. In case of *L. saccharina,* ANOVA was performed on RGR at 13 μmol $m^{-2}s^{-1}$.

(a) *L. hyperborea*

Source	DF	SS	MS	F
Light quality (Q)	2	0.024	0.012	73.10***
Irradiance (I)	1	0.011	0.011	63.16***
Q x I	2	0.002	0.001	4.83*
Error	12	0.002	1.672×10^{-4}	

(b) *A. esculenta*

Source	DF	SS	MS	F
Light quality (Q)	2	0.004	0.002	7.31**
Irradiance (I)	1	0.031	0.031	123.33***
Q x I	2	0.002	0.001	4.16 n.s.
Error	12	0.003	2.534×10^{-4}	

(c) *L. saccharina*

Source	DF	SS	MS	F
Between groups	2	0.003	0.001	2.64 n.s.
Within groups	6	0.003	0.001	
Total	2	0.006	0.001	

factors the interaction was most pronounced in the red light field since there was no significant difference in the growth rates of plants grown in different irradiances ($0.10 > p > 0.05$).

The growth rates of ***A. esculenta*** were similar between different spectral fields, but plants showed from 82% in blue to 45% in red light higher growth at 13 than at 5 μmol $m^{-2}s^{-1}$ ($p < 0.001$, Table 2.2b). It was noticeable that increase in irradiance from the lower to the higher caused an increase of growth rate in ***A. esculenta*** twice as much as that in ***L. hyperborea*** under the comparable light qualities.

At 13 μmol $m^{-2}s^{-1}$, the growth rate of ***L. saccharina*** did not differ between spectral light fields ($p > 0.10$, Table 2.2c).

Growth rates of early sporophytes at 36 μmol $m^{-2}s^{-1}$ of coloured light are shown in Fig. 2.4. The ***Laminaria*** species did not differ in their growth rates under different light fields, but growth of ***A. esculenta*** seemed to be faster in blue and green than in red light ($0.05 > p > 0.01$, Table 2.3).

2.3.3. Phaeoplast arrangement of *L. hyperborea* in different irradiances of light qualities

Different patterns of phaeoplast arrangement were observed in ***L. hyperborea,*** between plants grown under different light fields (Fig. 2.5). In blue and green light, plants showed different arrangements of phaeoplasts at different irradiances. At 5 μmol $m^{-2}s^{-1}$, most phaeoplasts occupied the cell walls perpendicular to the light direction (face position) whereas at 13 μmol $m^{-2}s^{-1}$ a growing number of phaeoplasts were found at the side walls, parallel to the incident light (profile position). In red light, however, there seemed no difference in

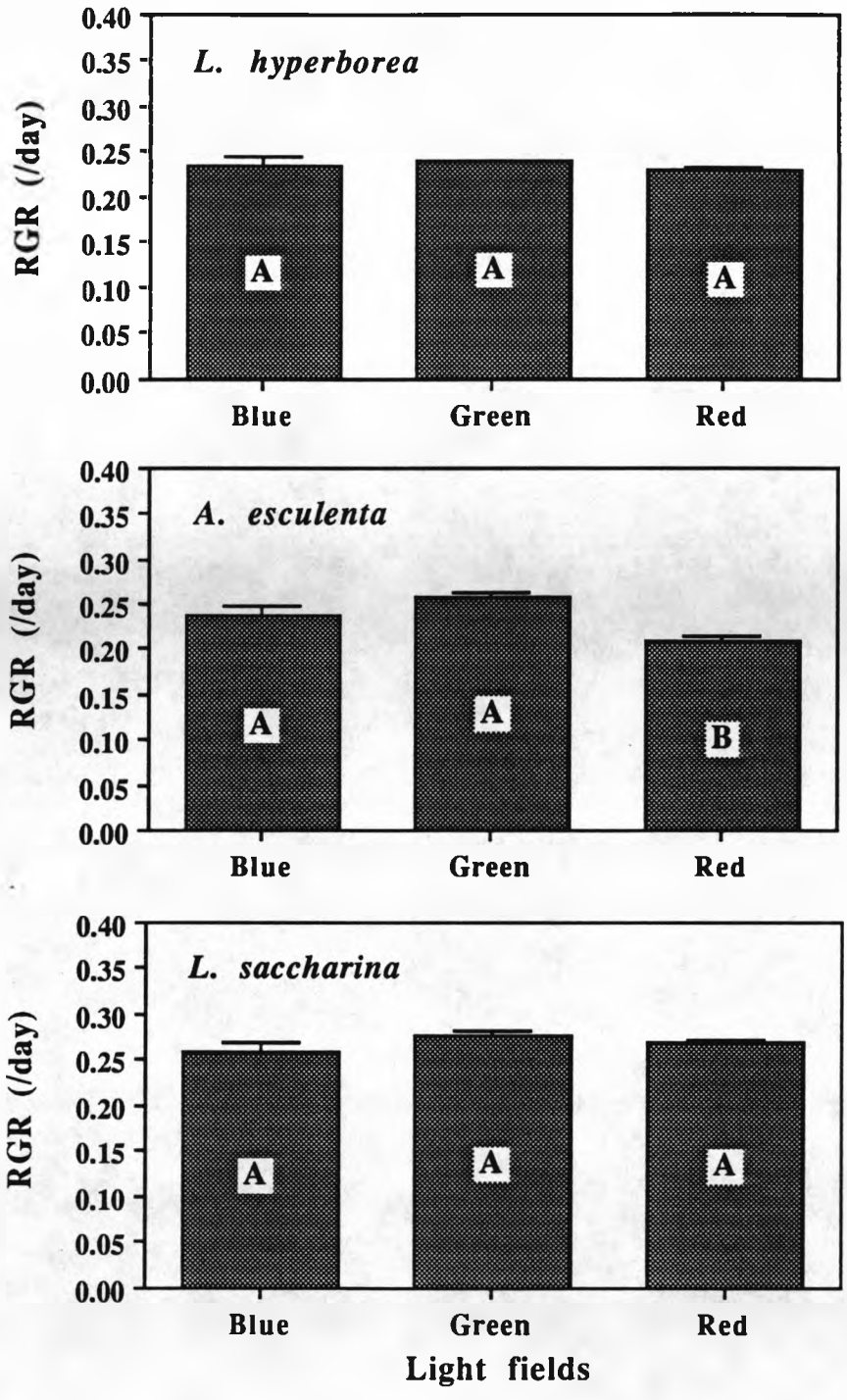

Fig. 2.4. Growth of laminarian early sporophytes at 36 µmol $m^{-2}s^{-1}$ of different spectral light. Mean RGR and one standard error (n=2-3) bars are shown and bars with the same letter are not significantly different at p= 0.05 (S-N-K test).

Table 2.3. AVOVA table for RGR of laminarian early sporophytes grown at 36 μmol $m^{-2}s^{-1}$ of different spectral light. *0.05 >p, n.s. not significant.

(a) ***L. hyperborea***

Source	DF	SS	MS	F
Between groups	2	1.742×10^{-4}	8.711×10^{-5}	0.73 n.s.
Within groups	6	0.001	1.192×10^{-4}	
Total	8	0.001		

(b) ***A. esculenta***

Source	DF	SS	MS	F
Between groups	2	0.004	0.002	11.25*
Within groups	5	0.001	1.557×10^{-4}	
Total	7	0.004		

(c) ***L. saccharina***

Source	DF	SS	MS	F
Between groups	2	0.001	2.653×10^{-4}	2.17 n.s.
Within groups	6	0.001	1.226×10^{-4}	
Total	8	0.001		

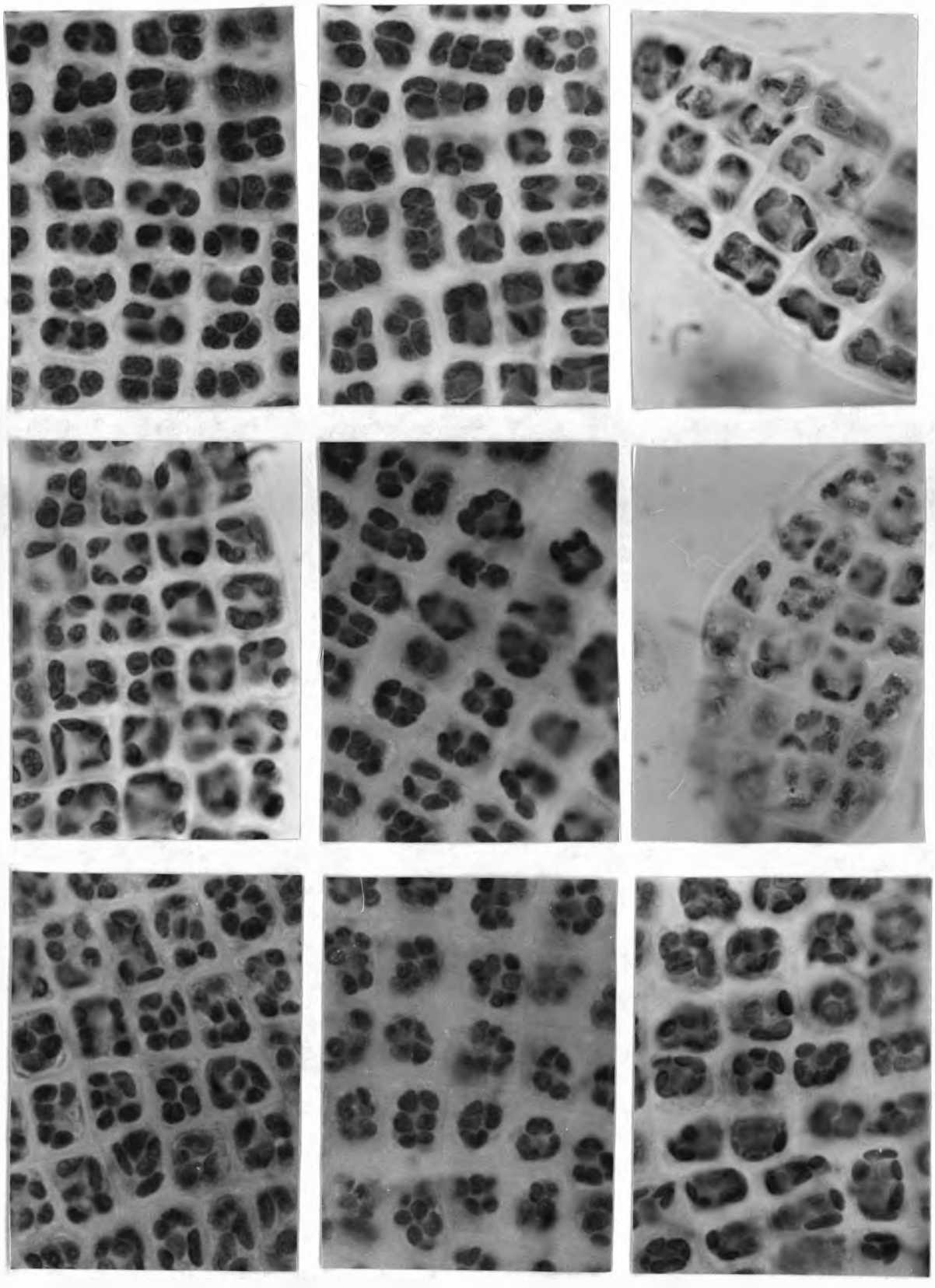

Fig. 2.5. Arrangement of phaeoplasts of ***L. hyperborea*** at different irradiances of different spectral qualities. Left to right (light quality): blue, green, red. Up to down (photon irradiance): 5, 13, 36 µmol $m^{-2}s^{-1}$.

the phaeoplast arrangements between plants grown at different irradiances (Fig. 2.5). The arrangement pattern of phaeoplasts in these irradiances of red light was similar to that at the higher irradiance of blue and green light. At 36 μmol $m^{-2}s^{-1}$, the arrangement of phaeoplasts in red light seemed to be similar to that at lower irradiances, whereas in the other light fields, there was no consistency in the phaeoplast arrangements, showing various arrangements even between the cells within a thallus.

2.4. Discussion

Brown algae contain a variety of photosynthetic pigments including chlorophylls and accessory pigments most of which mainly absorb radiation in the 400-550 nm range of wavelengths (Goedheer 1970) and this may define the spectral radiation which a species can use efficiently for photosynthesis and hence growth. Therefore, if growth in plants is entirely dependent on differences in the light-harvesting potential due to the distinct absorption of photosynthetically available radiation (PAR) by the pigments, higher growth in blue and green light of laminarian sporophytes would not be unexpected. First of all, the growth rate of early sporophytes of the ***Laminaria*** species was found to comply with the absorption spectrum (in ***L. saccharina,*** Halldal 1969) and the action spectrum of photosynthesis (in ***L. saccharina,*** thin thalli, Lüning and Dring 1985; low light-grown young sporophytes of ***L. hyperborea,*** Dring 1986) in that it is higher in blue (449 nm; 10 nm half band) and green waveband (545 nm) than either in orange (593 nm) or in red waveband (657 nm) at 3-7 μmol $m^{-2}s^{-1}$. Therefore, the growth rate of early sporophytes may be reasonably taken as representative of the ability to make use of light of different wavebands. As long as the growth irradiance was low (5 μmol $m^{-2}s^{-1}$), even in broad wavebands, plants showed much lower growth in red light (peak at 640 nm) than in the other light qualities. It could be postulated that fewer usable quanta for photosynthesis are emitted from red compared with the other light sources. Therefore, the relative number of usable quanta emitted from different light sources was calculated based on the action spectrum of photosynthesis in young sporophytes of ***L. hyperborea*** (Dring 1986) although the action spectrum may be different from that

Table 2. 4. Ratio of usable quanta emitted from different light sources (for a given photon irradiance).

Light source/light fields	Blue	Green	Red
	Ratio of usable quanta		
Coloured fluorescent tube + filter series	1.000	0.952	0.898
The same series as above for blue and green, but white light+red filter series for red light	1.000	0.952	0.830

of early sporophytes. Each value of the action spectrum in 5 nm intervals was multiplied by the number of quanta emitted from a light source at the corresponding wavebands, and the sum of these products for all wavelengths between 400 and 700 nm provided an estimate of the relative number of usable quanta. As seen in Table 2.4 , from the point of view of photosynthesis, blue and green light were both found to contain more usable quanta than red light for a given photon irradiance. Since the irradiance was equivalent in all the spectral irradiances, adjustment was made relative to the RGR in blue light simply by multiplying 1.05 for green light-grown plant and 1.11 for red light grown plants by the value of RGR in the corresponding light fields. The adjusted growth rate of ***L. hyperborea*** in red, however, remained slower ($p < 0.01$, Table 2.5) than that in the other light fields, suggesting that at a low irradiance red waveband may be absorbed or used for the growth with much lower efficiency. In contrast, in ***A. esculenta***, there was no change in the growth rate between different light qualities after the adjustment (Table 2.5). As far as differential effect of light quality on vegetative growth of plants is concerned, Dring and Lüning (1975) also found in ***Scytosiphon lomentaria*** a much lower growth rate (in terms of area) in red light than in blue or green light. Negligible growth in the radiation longer than 650 nm was reported for ***Dictyota dichotoma*** (Müller and Clauss 1976).

It is generally known that plants may respond to changes in the spectral composition of the light field either by altering the overall pigment content of the cells or by altering the balance between different photosynthetic pigments (Dring 1990). In this context, explanation of the similar growth rates between different light qualities at a given low irradiance shown by ***A. esculenta*** does not appear to be simple. Young sporophytes of ***L. hyperborea*** grown in different spectral light fields

Table 2.5. RGR of early sporophytes of *L. hyperborea* and *A. esculenta* at 5 μmol $m^{-2}s^{-1}$ of different spectral light. Mean ± standard errors and ANOVA table for the adjusted RGRs are shown (** 0.01 >p> 0.001, n.s. not significant). Values of different letter of superscript are significantly different at p= 0.05 (S-N-K test).

(a) *L. hyperborea*

RGR/light fields	Blue	Green	Red
Before adjustment	0.156 ± 0.013	0.158 ± 0.002	0.099 ± 0.009
After adjustment	0.156 ± 0.013[a]	0.166 ± 0.002[a]	0.110 ± 0.009[b]

ANOVA table

Source	DF	SS	MS	F
Between groups	2	0.005	0.003	11.36**
Within groups	6	0.001	2.386×10^{-4}	
Total	8	0.001		

(b) *A. esculenta*

RGR/light fields	Blue	Green	Red
Before adjustment	0.130 ± 0.005	0.126 ± 0.006	0.099 ± 0.009
After adjustment	0.130 ± 0.015	0.132 ± 0.006	0.135 ± 0.004

ANOVA table

Source	DF	SS	MS	F
Between groups	2	4.267×10^{-5}	2.133×10^{-5}	0.26n.s.
Within groups	6	4.873×10^{-4}	8.122×10^{-5}	
Total	8	0.001		

showed little pigment variation at low irradiances and few differences in the photosynthetic action spectra occurred (Dring 1986). If ***A. esculenta*** early sporophytes have a similar pigment system to that of young (1st year) ***L. hyperborea,*** no significant difference in the growth rate between plants grown in different spectral light fields may be interpreted to be the result of growth response of the former species to the same photon irradiance given for each light field. Also, that ***A. esculenta*** showed increase in growth rate with irradiance from 5 to 13 μmol $m^{-2}s^{-1}$, but little response to light qualities seems to lend direct support to the view that the growth is affected more by light quantity than by light quality.

Reasonable growth of ***A. esculenta*** sporophytes even in orange light in which the growth of ***Laminaria*** species was significantly slower than in the other light is hard to interpret, considering the lack of any prominent pigment to absorb the waveband (593 nm) in brown algae (Goedheer 1970, Jeffrey 1980, Dring 1990). On the other hand, Meeson and Faust (1985) found that the growth rates of ***Prorocentrum minimum*** (Dinophyceae) were highest in low level of blue-green light, where the pigment content and photosynthetic rates were lowest. They pointed out that it was ascribed to a differential influence of light quality on effectiveness of converting photosynthetic products into a new cell. Therefore, if the growth of ***A. esculenta*** is not the result of photosynthesis, it could be because orange light stimulates somehow a very efficient conversion of photosynthetic products into growth.

It is of interest to note that in ***L. hyperborea*** even an irradiance of 13 μmol $m^{-2}s^{-1}$ failed to enhance the growth rate in red light up to that in the other spectral light fields. The total amount of light which a cell can capture is dependent on two factors, (1) the amount of photosynthetic pigments, (2) the ability to absorb the ambient

wavelengths of light (Björkman 1973). Jeffrey and Vesk (1977, 1978) observed that in cells of the marine diatom ***Stephanopyxis turris*** grown in blue-green light, the cellular content of all the pigments was about twice that in cells grown under the same irradiance of white light. In some green algae, blue light stimulated chlorophyll formation (Senger 1987). However, the dinoflagellate ***Prorocentrum*** and the red alga ***Gracilaria*** had showed reduced chlorophyll concentrations after growing in blue light (Faust et al. 1982, Beer and Levy 1983). As there was only a minor influence of spectral qualities on pigment concentrations of young sporophytes of ***L. hyperborea*** at 5 or 20 μmol $m^{-2}s^{-1}$ (Dring 1986), the low growth rate of very early sporophytes of the same species in red light found in this study does not seem to be explained by the smaller amount of pigment. If the low growth rate is related with the amount of light captured, therefore according to the scheme of Björkman (1973), ***L. hyperborea*** may be lacking in ability to absorb or use the red waveband (peak at 640 nm) for growth at a very early stage, such as sporophytes younger than 10 days.

At 13 μmol $m^{-2}s^{-1}$, the growth rates of ***L. saccharina*** were similar between different spectral fields, whereas at 5 μmol $m^{-2}s^{-1}$ the growth seemed to be much slower in red than in blue or green light. It has been suggested that light quality can affect the metabolic processes which follow photosynthesis and contribute to growth (Kowallik 1982, Senger and Briggs 1981). The balance between protein and carbohydrate synthesis appeared to be inclined to carbohydrate synthesis in red light (Kowallik 1970, Clauss 1972). An accumulation of polysaccharides was observed in ***Dictyota dichotoma,*** following a reduction of the growth process in red light (Müller and Clauss 1976). Therefore, poor growth of ***L. saccharina*** in red light of 5 μmol $m^{-2}s^{-1}$ but not of 13 μmol $m^{-2}s^{-1}$ may have been due to a specific effect of a

narrow band of red light on growth processes at the lower light level. It could be because very low irradiance of red light favours carbohydrate formation which may be accompanied by a slowing of growth rather than protein synthesis which may lead directly to growth. The result suggests that in a low light level different light qualities may influence the growth through opening up specific channels for plants to use the photosynthates.

Alternatively, however, another possibility may be put forward to explain the enhanced growth rate at 13 μmol $m^{-2}s^{-1}$ of red light. Blue and green wavebands which produce faster rates of growth presumably saturate growth at a lower irradiance than the red waveband does. Therefore, beyond this level, increasing irradiance may not increase growth but cause an increase in growth rate in the red waveband. Thus, the growth rates at all wavelengths could catch up with those at the peaks and growth be independent of light quality.

To explain the lower growth of *A. esculenta* at 36 μmol $m^{-2}s^{-1}$ of red light than that at the same irradiance of blue or green light, it seemed to be necessary to introduce calculation of relative number of quanta emitted from light sources in the same way as done for low light treatment, supposing that the significantly lower growth in red light of *A. esculenta* than in the other light fields might have been due to the fact that the plants were actually given less number of quanta (13-17%) in the former (Table 2.4). When recalculation was made, the mean daily growth rate in red light was found not to be significantly different from that in green light and even higher than that in blue light (Fig. 2.6 and Table 2.6). On the other hand, the slightly lower adjusted growth rate in blue light at 36 μmol $m^{-2}s^{-1}$ might be due to light saturation of growth in the light field.

It is noticeable that at 36 μmol $m^{-2}s^{-1}$, no difference in the growth

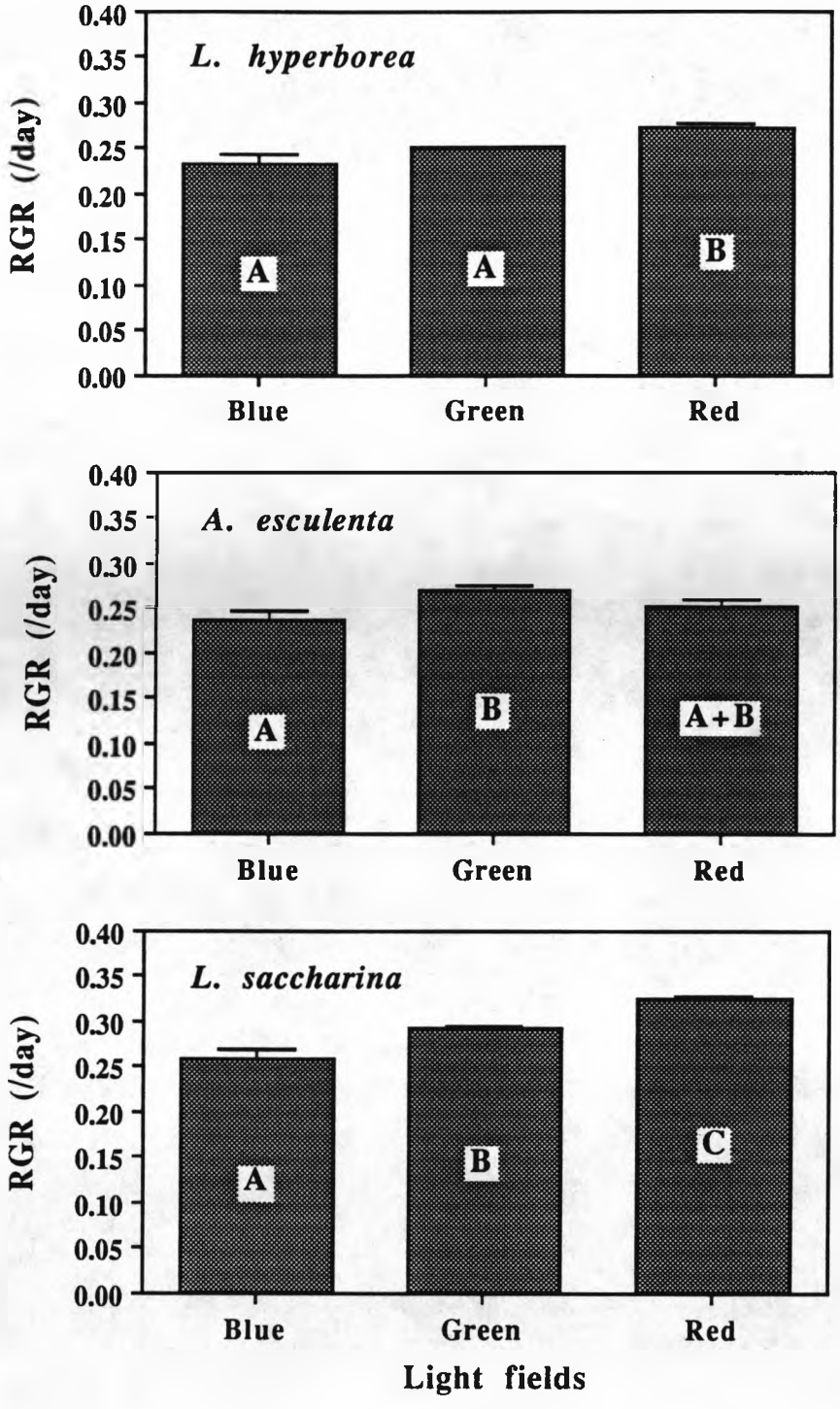

Fig. 2.6. Growth of laminarian early sporophytes at 36 µmol $m^{-2}s^{-1}$ of different spectral light. Mean value obtained from adjusted RGRs and one standard error bar is shown (n=2-3) and bars with the same letter are not significantly different at p= 0.05 (S-N-K test).

Table 2. 6. ANOVA table on adjusted RGR of laminarian early sporophytes at 36 µmol $m^{-2}s^{-1}$ of different spectral light. *0.05 >p≥ 0.01, **0.01 >p≥ 0.001, n.s. not significant.

(a) ***L. hyperborea***

Source	DF	SS	MS	F
Between groups	2	0.002	0.001	9.41*
Within groups	6	0.001	1.270×10^{-4}	
Total	8	0.003		

(b) ***A. esculenta***

Source	DF	SS	MS	F
Between groups	2	0.001	0.001	3.54n.s.
Within groups	5	0.001	1.849×10^{-4}	
Total	7	0.002		

(c) ***L. saccharina***

Source	DF	SS	MS	F
Between groups	2	0.007	0.003	26.40**
Within groups	6	0.001	1.238×10^{-4}	
Total	8	0.007		

rates of plants grown between different light qualities was found even in *L. hyperborea* (Fig. 2.4). This seems to be simply due to fact that growth might have begun to be saturated at 13 μmol $m^{-2}s^{-1}$ in blue or green light. On the other hand, when adjustment was made in consideration of the relative number of usable quanta, there was a difference in growth rate at 36 μmol $m^{-2}s^{-1}$ between the light qualities (Table 2.6) and a further test showed that growth in red light was significantly higher than that in the other light fields (Fig. 2.6). A similar pattern of growth response was also found in *L. saccharina* when they were grown at 36 μmol $m^{-2}s^{-1}$ of different spectral light fields. Accentuation of photosynthesis in red light compared with that in other light fields has been previously observed for red light-grown plants (*L. saccharina* gametophytes, Lüning and Dring 1975; *Scytosiphon lomentaria* sporelings, Dring and Lüning 1975). Also, Dring (1986) reported in *L. hyperborea* young sporophytes that there was little difference in the action spectrum of photosynthesis at low irradiances between different light qualities, but at high irradiance red light-grown plants exhibited more photosynthesis than did plants grown either in the other light fields or at lower irradiances under the red light. Therefore, high growth rate in red light may simply be the result of accentuated photosynthesis, highlighting the importance of interaction of both light quality and quantity in determining a growth.

Senn (1908, 1919) observed in *Dictyota dichotoma* that chloroplasts of the plant adapt to light conditions, i.e. orientation along cell membranes perpendicular to the incident light in response to dim light or arrangement parallel to the light direction in strong light. Since then, displacements of chloroplasts have been suggested to be a strategy of plants for varying light-harvesting (Zurzycki 1955, 1975). However, evidence of this is not substantial. Nultsch et al. (1981) have shown

that differences in absorption due to chloroplast movements to profile position could not account for a large reduction in photosynthetic rate of ***Dictyota dichotoma.*** In their study, the absorption difference between face (at low irradiance) and profile position (at high irradiance) was only by 6% whereas the rate of photosynthesis was reduced by about 75%. In ***L. hyperborea*** early sporophytes, the phaeoplast arrangement did not seem to be correlated with growth response. Profile position observed under red light of 5 μmol $m^{-2}s^{-1}$ may be tentatively consistent with the lower growth in the former light field since profile position would be a disadvantage in capturing light compared with face position in blue or green light. However, at higher irradiance, similar patterns of phaeoplast arrangement (profile position) between plants grown in blue and red light were associated with a markedly different growth rates, proving little relationship between light-harvesting for growth and phaeoplast arrangements. Additionally, under red light, the arrangement of phaeoplasts at 36 μmol $m^{-2}s^{-1}$ was very similar to that at either 5 or 13 μmol $m^{-2}s^{-1}$ although different light sources were used. This seems to indicate that red light cannot affect the arrangement of phaeoplasts. Light of wavelength above 500 nm has been reported not to induce chloroplast movement (Zurzycki 1980, Haupt 1982). Although some phaeoplasts showed different orientations within a single thallus, suggesting that the response may be at the level of the individual cells and not of the whole thallus, the change in the arrangement of phaeoplasts with irradiance observed in green light (peak at 530 nm) grown sporophytes of ***L. hyperborea*** may indicate that a further study would be necessary to understand the influence of light quality on the phaeoplast arrangement.

Chapter 3. Response to high irradiance of light

3.1. Introduction

Unrelated biophysical and biochemical processes, occurring on various time scales, may result in lowered photosynthetic rates at high irradiance levels and all these processes are collectively called 'photoinhibition' (Falkowski 1984). The adverse effects of intense illumination on photosynthesis have been well studied in algae (Harris 1978, Neale 1987). In macroalgae, Drew (1974) showed strong evidence for photoinhibition of ***L. hyperborea*** by daylight in shallow water. Kain and Dawes (1987) have also reported that there was more biomass in sporophytes of species of the Laminariales raised to shallow water from deeper water than in ones maintained in shallow water from the beginning, perhaps due to photoinhibition in the latter case. While photon irradiance and the time of exposure to a given irradiance is known to be an important factor governing the onset of photoinhibition (Belay and Fogg 1978, Samuelsson and Richardson 1982, Campbell et al. 1988), the quality of incident light has also been reported to affect photoinhibition (Nultsch and Agel 1986, Nultsch et al. 1987). Photoinhibition is certainly not a result of a single process. Some component of the Photosystem I complex (PS I) has been shown to be the site of most rapid damage or inactivation, but Photosystem II (PS II) has also been indicated as a primary target of photoinhibition (Larkum and Barrett 1983, Neale 1987).

On the other hand, there is a spectrum of responses with individual species being more or less able to tolerate high irradiances. Some algae can tolerate increasing photon irradiances up to full sunlight with no apparent photoinhibition of photosynthesis (Lewis et al. 1984) or growth (Jokiel and York 1984) after high light pretreatment. But the

extent to which adaptation is possible is genetically determined (Belay 1981, Powles 1984). While photosynthesis of ***L. hyperborea,*** known as deep water dwelling species, from shallow water was reduced under bright sunlight but to a lesser extent than was that from deeper water, there was no sign of reduction in photosynthesis of ***L. digitata,*** a shallow water inhabitant (Drew 1974).

Besides, in phytoplankton, photoinhibition occurs when organisms grown in low irradiance environments are suddenly exposed to higher irradiances (Neale 1987). A marine dinoflagellate, ***Amphidinium carterae,*** grown at 15 μmol $m^{-2}s^{-1}$, showed a significant photoinhibition when exposed to 350 μmol $m^{-2}s^{-1}$ (Samuelsson and Richardson 1982). In the field at shallow water depths, experimental removal of the kelp canopy in summer was found to damage the younger, subcanopy sporophytes (Wood 1987) and understorey red algae (Kain 1987). Flexibility in response to changes in irradiance may thus determine the tolerance of algae to high irradiance.

In this context, the implication of susceptibility to photoinhibition must not be overlooked for an understanding of algal ecology since it may bear a strong relation to the distribution of algae in time and space (Hellebust 1970, Lüning 1981a). The aim of this study was to investigate the susceptibility of laminarian early sporophytes to high irradiance either in artificial light or under sunlight and to predict if species-specific tolerance can be related to the vertical distribution of these species.

3.2. Materials and Methods

3.2.1. Effect of high irradiance of white light

Three culture dishes (60 mm diam.) containing 2-3 coverslips (13 x 13 mm) were placed either at 59 or 180 μmol $m^{-2}s^{-1}$ (±7%) of continuous light (Polylux 4000). The cultures were maintained under this condition for 8-10 days, after which they were harvested. Temperature varied from 12.0 to 14.5 °C during the experiments.
In another experiment, some sporophytes of ***L. hyperborea*** were grown under different light sources, namely Northlight and Polylux 4000. Irradiances employed were 55 and 110 μmol $m^{-2}s^{-1}$ (±10%) of continuous illumination. Plants were sampled after 8 days.

3.2.2. Effect of high irradiance of blue and green light

The same glass tank system as described in chapter 2 was used for this experiment. Sporophytes of ***L. hyperborea*** and ***L. saccharina*** were irradiated with 60 μmol $m^{-2}s^{-1}$ (±10%) of continuous blue and green light provided by Voigtlander VP 200A projector with 'Cinelux' filter system. The cultures were harvested 9 days later. Temperature was maintained at 14 ± 0.1 °C throughout the study.

3.2.3. Effect of transfer of low light acclimated plants to higher irradiance(s)

Since the first appearance of sporophytes duplicate cultures of ***L. hyperborea*** and ***L. digitata*** in petri dishes (100 mm diam., 10 mm high) each containing 2 coverslips were made at different irradiances of 19 and 55 μmol $m^{-2}s^{-1}$ (±10%) under Northlight (24 h illumination). The cultures were transferred to higher irradiances, i.e. from 19 μmol $m^{-2}s^{-1}$ to 55 and 135 μmol $m^{-2}s^{-1}$, from 55 to 135 μmol $m^{-2}s^{-1}$ respectively on day 8 and 20 for ***L. hyperborea*** and on day 7 and 14 for ***L. digitata.*** Plants were grown in the new light levels for a further 8 days and then harvest was made.

The same kind of experiment was repeated with ***L. hyperborea*** under Polylux 4000. Plants were grown at 15 and 63 μmol $m^{-2}s^{-1}$ for 8 or 16 days, after which they were transferred to higher irradiance(s), from 15 μmol $m^{-2}s^{-1}$ to 63 and 159 μmol $m^{-2}s^{-1}$ or from 63 to 159 μmol $m^{-2}s^{-1}$ (±25%).

An additional experiment was made under sunlight for early sporophytes of two species of the ***Laminaria*** (***L. hyperborea*** and ***L. digitata***) acclimated to three different irradiances for 12 days in the laboratory. Plants were exposed to sunlight in plastic boxes (100 x 100 x 18 mm) filled with 1.5 cm. The mean photon irradiance of sunlight was 512 μmol $m^{-2}s^{-1}$ at noon time (11: 25 A.M.-12: 35 P.M.) on 30 October, 1991 and variations in photon irradiances of solar radiation and temperature are shown in Fig. 3.1. After 30 min exposure, plants were cultured at 30 μmol $m^{-2}s^{-1}$ of continuous white light (Northlight) for 8 days before being examined for the survival under a microscope. Survival percentage was estimated by counting surviving sporophytes

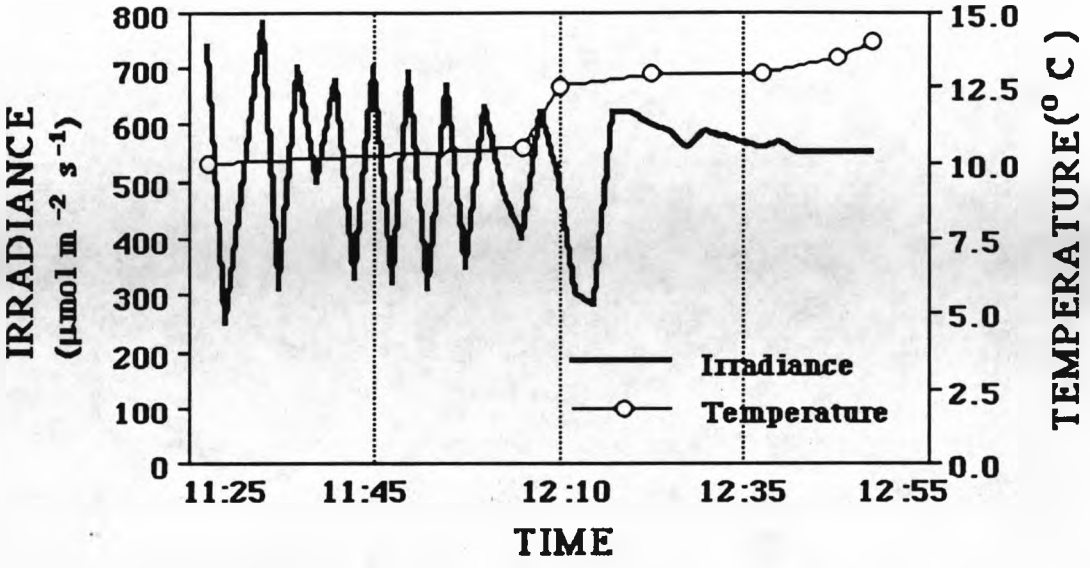

Fig. 3.1. Change in photon irradiance and temperature during the experiment conducted on 30 October, 1991 in open field (Port Erin, Isle of Man).

with over a half of their areas not completely white nor empty of cell contents.

3.2.4. Survival in strong sunlight

On 3 July, 1990 an experiment was set up to test the effect of sunlight transmitted through different coloured filters on 10 day-old sporophytes of ***L. hyperborea***. Coverslips bearing sporophytes were transferred to flat-bottomed crystallizing dishes (80 mm diam., 43 mm high) filled with 1 cm deep medium. Considering the air temperature (higher than 20 °C), the crystallizing dishes were immersed in the Plexiglass box (4 mm thick, 270 x 270 x 43 mm) filled with 5 cm deep tap water and then exposed to transmitted sunlight adjusted in its irradiance to 5% of full sunlight because of the limited transmittance of green filter. The mean irradiance was 1800 μmol $m^{-2}s^{-1}$ (±3%; open sky at 1: 45-2: 40 P.M.) and exposure time was 3 and 7 min. No lids were used for the dishes and temperature ranged from 16 to 20 °C during exposure to sunlight. For the control one dish with coverslips bearing sporophytes was wrapped with aluminium foil and remained in the water-containing box during the experiment. After exposure, the dishes were transferred to the laboratory and plants were cultivated in petri-dishes (100 mm daim., 10 mm high) filled with new medium under continuous white light of 30 μmol $m^{-2}s^{-1}$ (Northlight) for a further 8 days before an estimation of the survival was made.

On 13 Feburary, 1991 some sporophytes (10-14 day old) of ***L. hyperborea*** and ***A. esculenta*** in flat-bottomed crystallizing dishes were put out under sunlight for 20-60 min. In the case of ***A. esculenta*** some plants were also exposed to 50% reduced sunlight under a UV filter

(Strand Lighting; cutting at the radiation of wavelengths below 340 nm). The photon irradiance received was 750 μmol $m^{-2}s^{-1}$ (±6%) under direct sunlight and air temperature was 10.1±1.9 °C. Irradiated plants were cultivated in blue light of 13 μmol $m^{-2}s^{-1}$ at 12 °C for 8 days and then the survival was assessed.

On 30 October, 1991 two species of ***Laminaria*** (***L. hyperborea*** and ***L. digitata***) in plastic boxes (100 x 100 x 18 mm) filled with medium 1 cm deep were exposed to sunlight for 30-60 min (mean irradiance 512 μmol $m^{-2}s^{-1}$ at 11: 25 A.M.-12: 35 P.M.). Temperature was maintained at 12.9 ± 1.1 °C during exposure. The variations in photon irradiances of sunlight have already been shown in Fig. 3.1. After exposure, the plants were cultured for 8 days at 30 μmol $m^{-2}s^{-1}$ of continuous white light (Northlight).

3.3. Results

3.3.1. Effect of high irradiance of white light

Table 3.1 shows growth rates of laminarian sporophytes at 59 and 180 μmol $m^{-2}s^{-1}$ of continuous white light. ***L. hyperborea*** and ***L. saccharina*** exhibited a significant decline ($0.001 < p < 0.01$ and $p<0.05$ respectively) in growth rate at the higher irradiance in one experiment, but in another experiment there was no significant difference ($p> 0.05$) in growth rate between plants grown at the two irradiances although growth rate at the higher irradiance was slightly lower than that at lower irradiance. Also, in the former experiment, cultures of ***L. hyperborea*** grown at 168 μmol $m^{-2}s^{-1}$ under a UV filter (2A, Kodak; cutting radiation of wavelength less than 400 nm) showed a growth reduction similar to that at 180 μmol $m^{-2}s^{-1}$ without a filter (Table 3.2). For the other species, based on posteriori comparisons of means, there was no sign of significant decline in growth rate at 180 μmol $m^{-2}s^{-1}$.

When ***L. hyperborea*** sporophytes were grown under different light sources, there was no difference in growth rate attained by plants grown either at 55 μmol $m^{-2}s^{-1}$ or 110 μmol $m^{-2}s^{-1}$ in Northlight whereas in Polylux 4000 (Table 3.3) the growth rate at the higher irradiance was significantly lower ($p< 0.002$) than that at the lower irradiance.

3.3.2. Effect of high irradiance of blue and green light

When some early sporophytes of species of ***Laminaria*** were grown at

Table 3.1. Comparison of growth rates of early sporophytes at saturating and suprasaturating irradiances of white light (Polylux 4000 fluorescent tubes). Mean RGR and standard errors are shown (n=2-3). A F-value was obtained from ANOVA on RGRs for each experiment. *0.05 >p≥ 0.01, **0.01 >p≥ 0.001, n.s. not significant.

Species	Expt	Photon irradiance (μmol $m^{-2}s^{-1}$) 59	180	F
L. hyperborea	1	0.226 ± 0.008	0.205 ± 0.008	3.36 n.s.
	2	0.232 ± 0.005	0.165 ± 0.011	122.09**
A. esculenta	1	0.233 ± 0.017	0.239 ± 0.017	0.05 n.s.
	2	0.239 ± 0.004	0.250 ± 0.004	3.47 n.s.
L. digitata	1	0.185 ± 0.010	0.139 ± 0.020	4.20 n.s.
	2	0.225 ± 0.014	0.178 ± 0.011	7.25 n.s.
L. saccharina	1	0.290 ± 0.002	0.257 ± 0.009	13.46*
	2	0.238 ± 0.006	0.227 ± 0.021	0.28n.s.

Table 3.2. Effect of UV-filtering on growth of early sporophytes of *L. hyperborea* at high irradiance. Mean RGR ± standard errors (n= 2-3) and the ANOVA table are shown . Values of different letter are significantly different at p= 0.05 (S-N-K test). **0.01 >p≥ 0.001.

Photon irradiance (µmol $m^{-2}s^{-1}$)		
59	168 (+UV filter)	180
0.232 ± 0.005[a]	0.166 ± 0.001[b]	0.165 ± 0.011[b]

ANOVA table

Source	DF	SS	MS	F
Between groups	2	0.008	0.004	40.85**
Within groups	4	3.686×10^{-4}	9.130×10^{-5}	
Total	6	0.008		

Table 3.3. RGR of *L. hyperborea* sporophytes grown at two irradiances (55, 110 μmol $m^{-2}s^{-1}$) under different light sources and ANOVA table for the RGRs. Mean RGR and standard error is shown (n=3). *p< 0.05, n.s. not significant.

Light source/photon irradiance	55	110	F
Northlight	0.253 ± 0.015	0.249 ± 0.007	0.12 n.s.
Polylux 4000	0.235 ± 0.008	0.200 ± 0.001	18.29*

ANOVA table

(a) in Northlight

Source	DF	SS	MS	F
Between groups	1	4.817×10^{-5}	4.817×10^{-5}	0.12n.s.
Within groups	4	0.002	4.127×10^{-4}	
Total	5	0.002		

(b) in Polylux 4000

Source	DF	SS	MS	F
Between groups	1	0.002	0.002	18.29*
Within groups	4	4.173×10^{-4}	1.043×10^{-4}	
Total	5	0.002		

Table 3.4. RGR of laminarian sporophytes grown at 60 μmol $m^{-2}s^{-1}$ of blue or green light and ANOVA table for the RGRs. Mean RGR and standard error is shown (n=3-4). *p< 0.05, n.s. not significant.

Species/ Light fields	Blue light	Green light
L. hyperborea	0.204 ± 0.014	0.255 ± 0.009
L. saccharina	0.239 ± 0.005	0.264 ± 0.019

ANOVA table

(a) ***L. hyperborea***

Source	DF	SS	MS	F
Between groups	1	0.004	0.004	9.17*
Within groups	4	0.002	4.250×10^{-4}	
Total	5	0.006		

(b) ***L. saccharina***

Source	DF	SS	MS	F
Between groups	1	0.001	0.001	1.65 n.s.
Within groups	6	0.005	0.001	
Total	7	0.006		

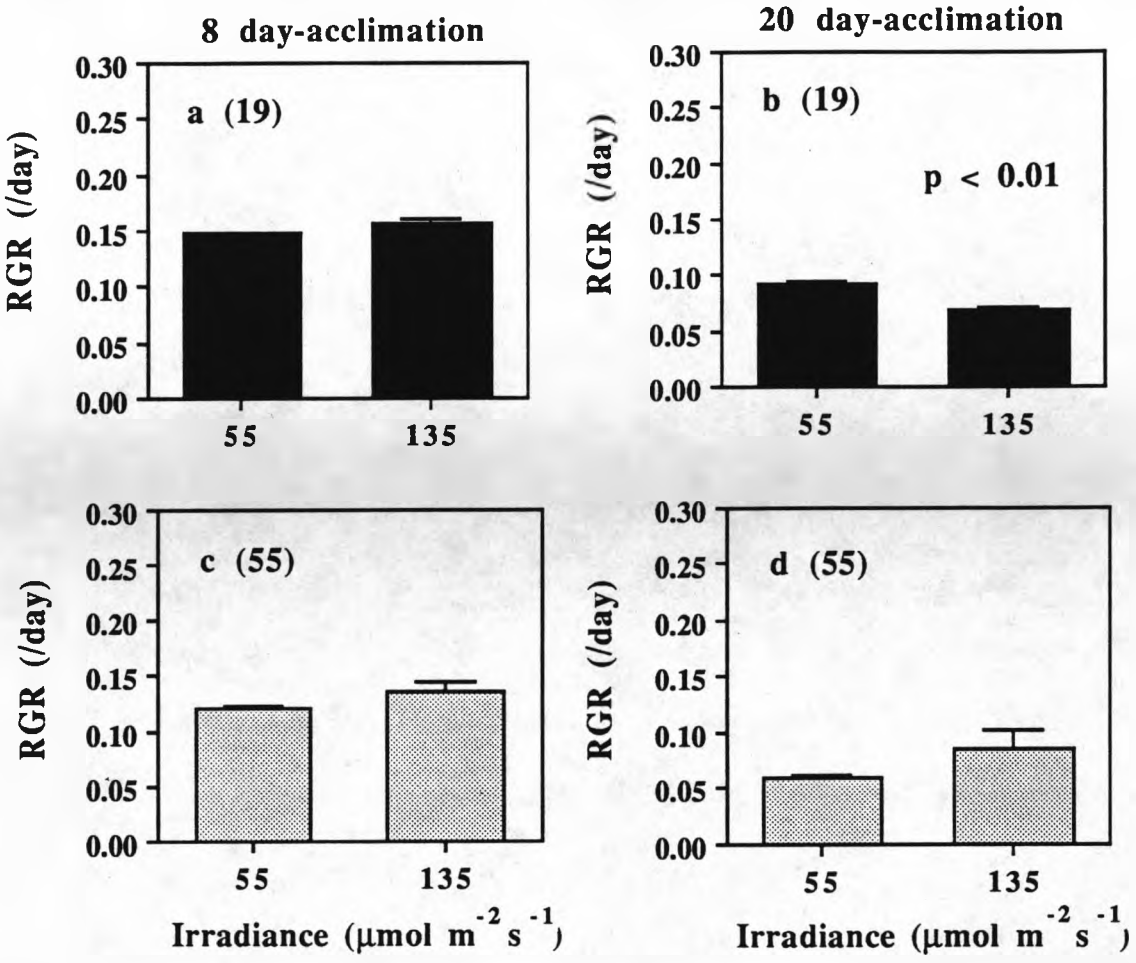

Fig. 3.2. Effect of transferrence to higher irradiance(s) on growth of *L. hyperborea* acclimated at different irradiances for different periods. Mean RGR and one standard error bar is shown (n=2). The values in brackets represent the acclimation irradiances under which plants had been grown before transfer was made. The acclimation period is indicated above each column and the degree of significance between means shown where it is significant. ANOVA table for the determination of significance is shown in Appendix 2. Light source was Northlight.

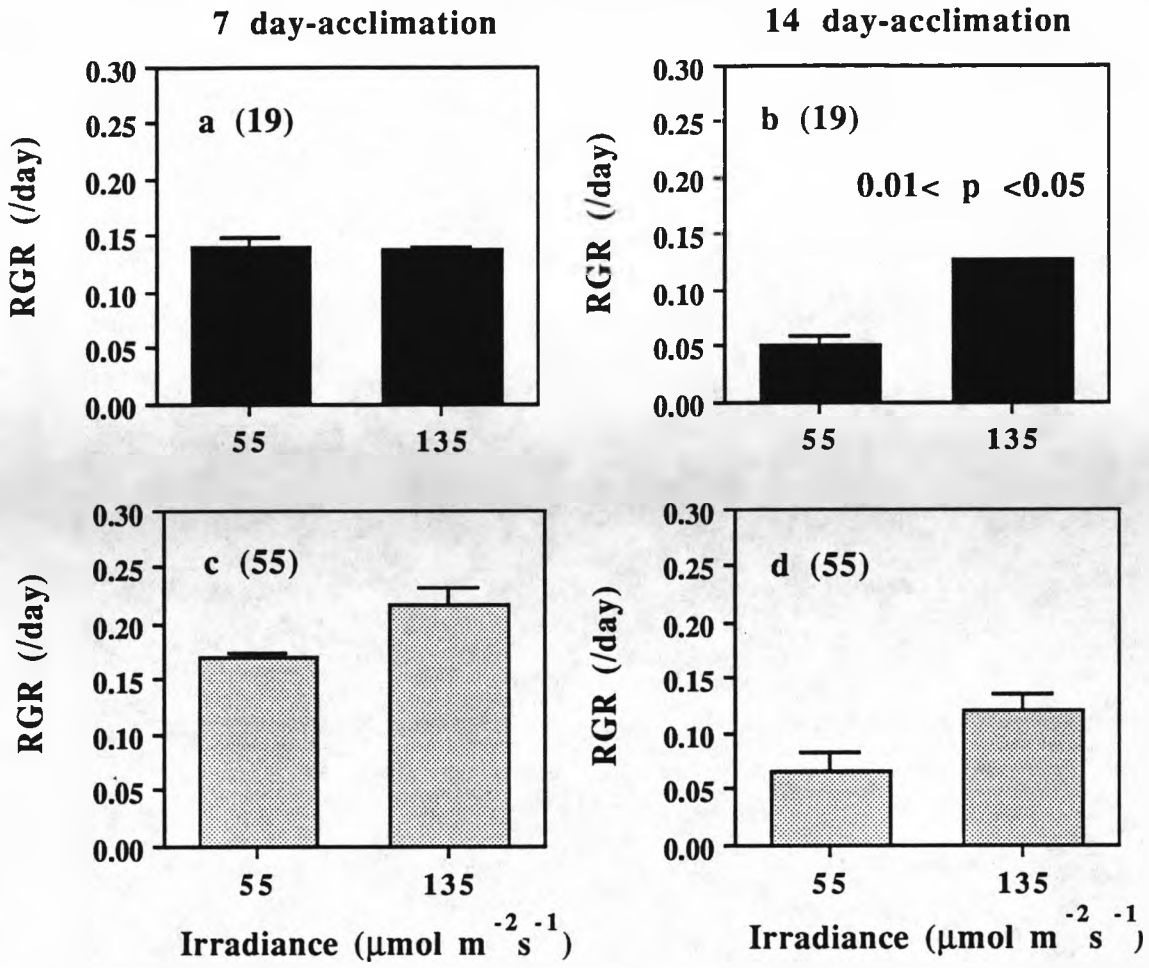

Fig. 3.3. Effect of transferrence to higher irradiance(s) on growth of *L. digitata* acclimated at different irradiances for different periods. Light source was Northlight. Refer to Appendix 3 for the ANOVA table and to Fig. 3.2 for the other details.

60 μmol $m^{-2}s^{-1}$, ***L. hyperborea*** grew more slowly ($p < 0.05$) in blue light than in green light, but in ***L. saccharina*** there was no difference in the growth rates between plants grown in blue and green light ($p > 0.20$; Table 3.4).

3.3.3. Effect of transfer of low light acclimated plants to higher irradiance(s)

Fig. 3.2 shows growth of ***L. hyperborea*** plants acclimated to low irradiances for different periods when they were transferred to higher irradiance(s). Following a transfer to 55 and 135 μmol $m^{-2}s^{-1}$ under Northlight, ***L. hyperborea*** plants acclimated to 19 μmol $m^{-2}s^{-1}$ for 8 days did not show any difference in the growth rates between the irradiances. However, growth rate of plants acclimated to the same irradiance for 20 days was lower at 135 μmol $m^{-2}s^{-1}$ than at 55 μmol $m^{-2}s^{-1}$ ($p < 0.01$; Fig. 3.2). In the case of plants acclimated to 55 μmol $m^{-2}\,s^{-1}$, growth rate did not seem to be affected by the acclimation period, showing a similar growth rate at both irradiances whether it was 8 day-acclimated or 20 day-acclimated plants (Fig. 3.2).

In ***L. digitata***, 7 day-acclimated plants to 19 μmol $m^{-2}s^{-1}$ responded in their growth rate to the higher irradiances similarly to 8 day-acclimated plants of ***L. hyperborea*** (Fig. 3.3). In 14 day-acclimated plants, however, the growth response to the higher irradiances was remarkably different from that of ***L. hyperborea,*** in that transfer to 135 μmol $m^{-2}s^{-1}$ resulted in a higher ($p < 0.05$) growth rate than to 55 μmol $m^{-2}s^{-1}$ (Fig. 3.3). As in ***L. hyperborea,*** sporophytes of ***L. digitata*** acclimated to 55 μmol $m^{-2}s^{-1}$ showed a similar growth rate at 55 μmol $m^{-2}s^{-1}$ and 135 μmol $m^{-2}s^{-1}$ irrespective of the acclimation period.

Under Polylux 4000 the change in growth irradiance of ***L. hyperborea*** from 15 μmol $m^{-2}s^{-1}$ to higher irradiances caused a contrasting difference in the growth rates between plants transferred to 63 and 159 μmol $m^{-2}s^{-1}$ depending on the acclimation period (Fig. 3.4). Sporophytes acclimated to 15 μmol $m^{-2}s^{-1}$ for 8 days did not show any difference in the growth rates when they were transferred to 63 an 159 μmol $m^{-2}s^{-1}$. On the contrary, plants acclimated to 15 μmol $m^{-2}s^{-1}$ for 16 days did not achieve any growth at 159 μmol $m^{-2}s^{-1}$ and even showed complete bleaching in their thalli while plants continued their growth when they were transferred to 63 μmol $m^{-2}s^{-1}$. In the plants acclimated to 63 μmol $m^{-2}s^{-1}$, growth rates was similar at 63 and 159 μmol $m^{-2}s^{-1}$ regardless of the acclimation period (Fig. 3.4).

When early sporophytes of ***L. hyperborea*** and ***L. digitata*** acclimated to three different irradiances for 10 days were exposed to direct October sunlight for 30 min, they differed in their survival percentages between species and depended on the acclimation irradiance within a species (Fig. 3.5). For plants acclimated to 8 μmol $m^{-2}s^{-1}$ of continuous white light, 61% of ***L. digitata*** plants survived sunlight whereas ***L. hyperborea*** showed only 8-11% survival percentages. When acclimated to 55 μmol $m^{-2}s^{-1}$, the survival percentage of ***L. hyperborea*** under sunlight was significantly higher than that of plants acclimated to 8 μmol $m^{-2}s^{-1}$ and it was not much lower than that of ***L. digitata***. For the both species the survival percentage of plants acclimated to 130 μmol $m^{-2}s^{-1}$ was not different from that of plants acclimated to 55 μmol $m^{-2}s^{-1}$.

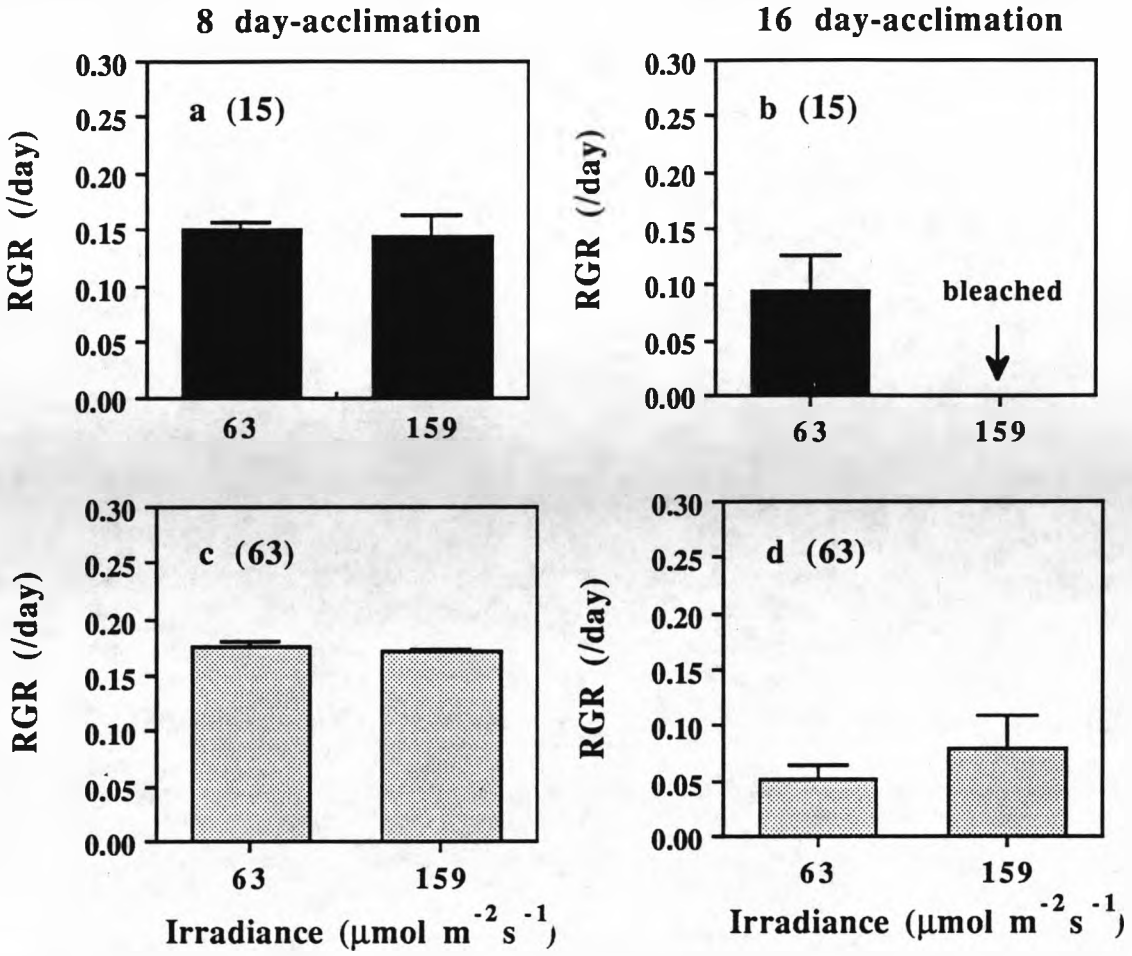

Fig. 3.4. Effect of transferrence to higher irradiance(s) on growth of *L. hyperborea* acclimated at different irradiances for different periods. Light source was Polylux 4000. Refer to Appendix 4 for the ANOVA table and to Fig. 3.2 for the other details.

3.3.4. Survival in strong sunlight

More than 85% of sporophytes of ***L. hyperborea*** (the only species tested) survived 3 min of in three light fields made under July sunlight and coloured filters (Fig. 3.6). The result obtained from a longer exposure period (7 min), however, showed that blue light had more detrimental effect (ca. 24% mortality), followed by red light (20%) and green light (13%) compared with 7% mortality in control.

Some early sporophytes (10-14 day-old) of ***L. hyperborea*** survived only 20 min of direct sunlight (0.90 mol m^{-2}; open sky in February), but not 40 min exposure. On the other hand, ***A. esculenta*** survived 40 min exposure in the same experiment, but not 60 min (Fig. 3.7). In ***A. esculenta,*** the survival percentage appeared to be closely related to total quanta received (Fig. 3.8). When exposed to either 350 µmol $m^{-2}s^{-1}$ for a longer period (under a UV-fiter) or 750 µmol $m^{-2}s^{-1}$ for a shorter period, for example, about the same percentage of sporophytes, 91-93% and 4-7% survived after receiving 0.84-0.90 mol m^{-2} and 1.68-1.80 mol m^{-2} respectively.

Two species of 10-14 day-old sporophytes differed in their resistance to direct October sunlight (Fig. 3.9). More than 90% of plants of ***L. hyperborea*** and ***L. digitata*** survived 30 min exposure (total quanta 0.90 mol m^{-2}; based on average photon irradiance of 512 µmol $m^{-2}s^{-1}$, $\pm$ 40% variation). When exposed to sunlight for 45 min a difference in the resistance between ***L. hyperborea*** and ***L. digitata*** in terms of survival percentage became apparent, the latter species showing a much higher survival (88%) than the former (13%). Sixty min exposure (1.84 mol m^{-2}) seemed to be almost lethal to ***L. hyperborea,*** but ***L. digitata*** survived 56%.

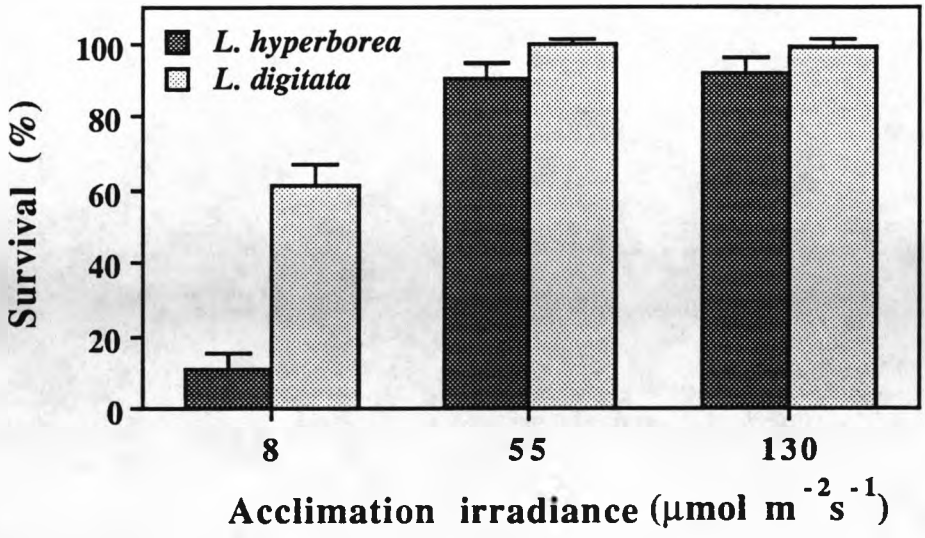

Fig. 3.5. Survival of two species of *Laminaria* under direct October sunlight after having been acclimated at different irradiances (24 h light, Polylux 4000) for 10 days. Vertical bars denote 95% confidence intervals (n=600).

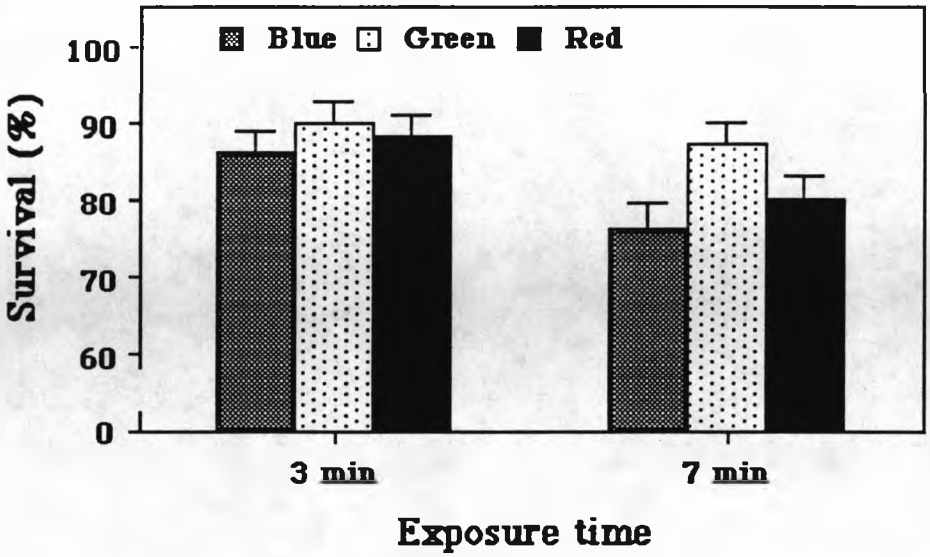

Fig. 3.6. Survival of early sporophytes of *L. hyperborea* under July sunlight transmitted through different coloured filters. Vertical bars are 95% confidence intervals (n=600). Control sample which was wrapped with aluminium foil and retained under sunlight during the experiment showed 7% mortality.

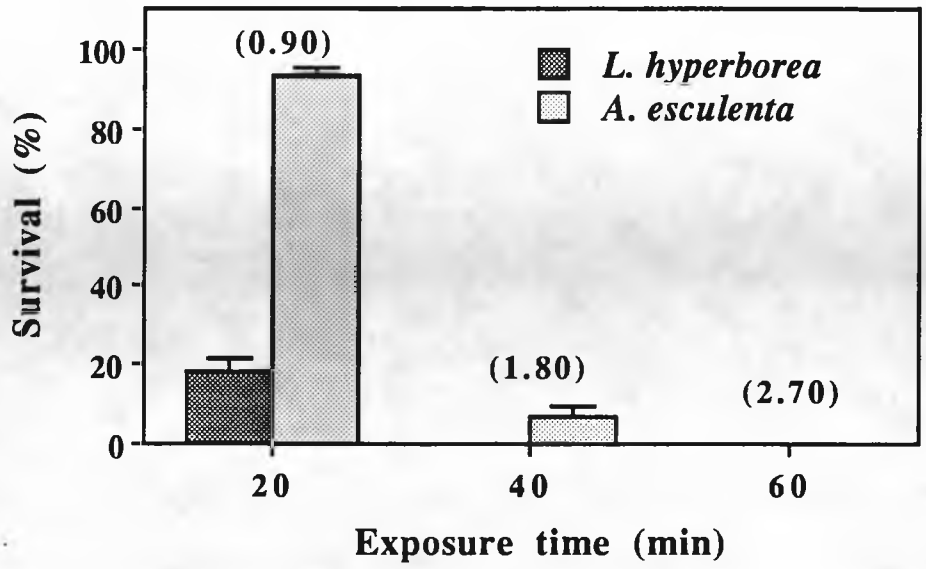

Fig. 3.7. Survival of early sporophytes of ***L. hyperborea*** and ***A. esculenta*** after different periods of exposure to sunlight. Vertical bars indicate 95% confidence intervals (n=600) and the values in brackets are total quantum doses (mol m^{-2}).

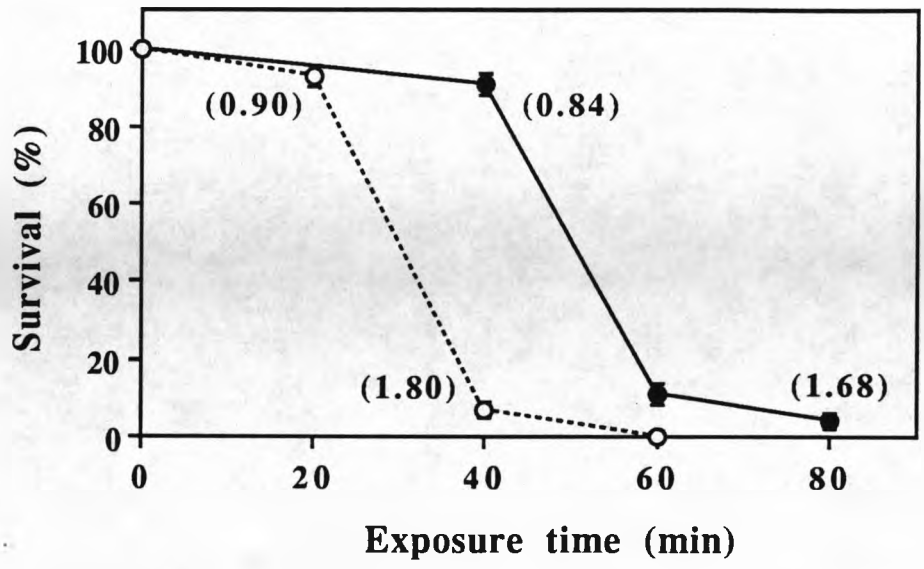

Fig. 3.8. Effect of different photon irradiances of sunlight on the survival of early sporophytes of *A. esculenta* (open circle 750 μmol $m^{-2}s^{-1}$, closed circle 350 μmol $m^{-2}s^{-1}$). The values in brackets are total quantum doses (mol m^{-2}) of similar magnitude made under different irradiances and exposure periods. Vertical bars indicate 95% confidence intervals (n=600).

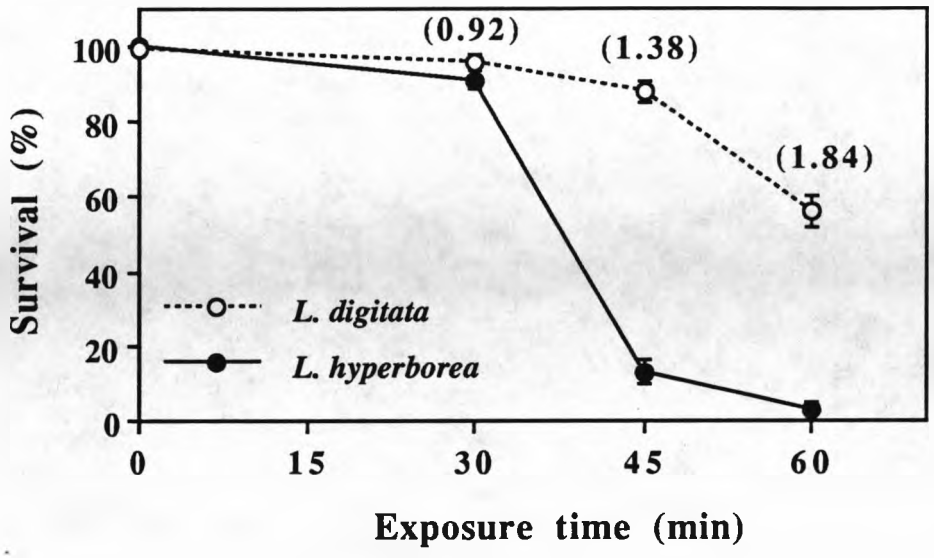

Fig. 3.9. Effect of October sunlight on survival of early sporophytes of *L. hyperborea* and *L. digitata.* Vertical bars denote 95% confidence intervals (n=600) and the values in brackets are total quantum doses (mol m^{-2}).

3.4. Discussion

After high-light exposure, light-saturated and light-limited rates of photosynthesis of some plants are reduced (see review by Neale 1987). This photoinhibition is well documented to occur at above 200-300 μmol $m^{-2}s^{-1}$ for phytoplankton (Harris 1978, Belay 1981). In brown algae, early stages of plants have been known to be sensitive to high irradiance, exhibiting a significant decline either in photosynthesis (***Macrocystis pyrifera*** gametophytes at less than 140 μmol $m^{-2}s^{-1}$, Fain and Murray 1982) or in growth (***Laminaria digitata*** gametophytes at 130 μmol $m^{-2}s^{-1}$, Fei 1985; ***Macrocystis pyrifera*** sporophytes at about 143 μmol $m^{-2}s^{-1}$, Fei and Neushul 1984; ***Sargassum muticum*** germlings at higher than 88 μmol $m^{-2}s^{-1}$, Hales and Fletcher 1989). In this study, of the four species, only ***L. hyperborea*** and ***L. saccharina*** showed a significant growth reduction at 180 μmol $m^{-2}s^{-1}$ although plants of a different genetic origin responded to the irradiance differently. Drew (1974) found that photosynthesis of ***L. hyperborea*** was rapidly reduced to zero in bright surface sunlight but ***L. digitata*** was completely unaffected, showing correspondence of the sensitivity to high levels of light with their ecological habitats, i.e. sublittoral for the former species and upper-sublittoral/ intertidal for the latter. However, ***L. saccharina*** populations from different light regimes exhibited variations in light-related traits which appeared to have a genetic basis (Gerard 1988). Ecotypic differentiation has been demonstrated in a number of macroalgal species in relation to a variety of environmental factors (see review by Russell 1986). Perhaps due to its existence over wide geographic and environmental ranges, ***L. saccharina*** has been the focus of several studies on ecotypic differentiation (Bolton et al. 1983, Gerard

1988, 1990). There is, however, no experimental basis in this study to explain the difference in the growth response to high irradiance between the two experiments in terms that ecotypically differentiated plants were used. It also seems to be premature at this stage to relate growth response of laminarian early sporophytes at 180 μmol $m^{-2}s^{-1}$ to the vertical distribution in the sea.

There has been a general awareness of the possibility that light sources of different spectral composition may cause different effects on the growth of certain algae (Kain and Fogg 1960, Epel and Krauss 1966). Accordingly, the adverse effect of Polylux 4000 on the growth of ***L. hyperborea*** early sporophytes at high irradiance compared with Northlight did not appear to be surprising, considering the differences in the emission spectrum as illustrated in 'General material & methods'. When a calculation of the usable number of quanta was made, taking into consideration the spectral response of ***L. hyperborea*** as well as number of quanta emitted from a light source, Polylux 4000 was found to contain more blue (3.6%) and green quanta (9.0%) than does Northlight (Table 3.5). Since the inhibitory effect of the former light source occurred at high irradiance, it may have been due to more quanta in the blue or green regions. Boney and Corner (1962) found in an experiment with fluorescent tubes as light sources, removal of only 1.6% of light from the green region of the spectrum prevented red algal sporelings of ***Plumaria elegans*** from severe growth inhibition. Therefore, a relatively larger increase in green portion than that in blue does not seem to imply that green light is likely to be a inhibiting factor to growth of early sporophytes of ***L. hyperborea*** in Polylux 4000. In this context, significantly lower growth rate in blue than that in green light at 60 μmol $m^{-2}s^{-1}$ may imply an adverse effect of excessive blue light on early sporophytes of ***L. hyperborea***. Blue light has been found to be

Table 3.5. Relative proportion of usable quanta distribution for two different light sources.

	Northlight (N)	Polylux4000 (P)	Difference (P - N)
400-500 nm	36.9 %	40.5 %	+ 3.6%
505-600 nm	34.1 %	43.1 %	+ 9.0 %
605-700 nm	29.0 %	16.4 %	- 12.6 %

inhibitory to growth of ***Euglena gracilis*** and to induce inhibition of cell elongation in excised wheat roots (Epel 1973). Irradiation with blue light resulted in the destruction of both components of cytochrome oxidase of ***Prototheca zopfii*** (Epel and Butler 1970). On the other hand, in ***Dictyota dichotoma***, effectiveness in inducing photoinhibition was much lower in blue than either in green or red light (Nultsch et al. 1987). However, as the quanta actively absorbed by photosynthetic pigments may be responsible for the inactivation (Jones and Kok 1966, Satoh 1970), high growth rate of ***L. hyperborea*** at lower irradiance of blue light (this study, chapter 2) may confirm the possibility of inhibitory effect of high irradiance of blue light on the growth of ***L. hyperborea*** at its early stage. Together with this, an experiment carried out under colour filtered sunlight seems to reinforce the evidence that the inhibitory or damaging effect was primarily attributable to the blue waveband. Comparatively high mortality in red filter transmitted sunlight may be explained when referring back to a previous result reported in chapter 2 in which ***L. hyperborea*** showed an increasing growth rate with increase in photon irradiance under red light, implying an active utilisation of this waveband by the early sporophytes. Therefore, for the same reason as in blue light, plants may be liable to be damaged by the red light at high irradiance. It has already been known in many algae that longer visible wavelengths contribute to pigment destruction (Hellebust 1970). Lower mortality in green light may suggest a protective role of green light-absorbing pigment, such as fucoxanthin. Shimura and Fujita (1975) have observed that in strong light, the excitation energy of fucoxanthin is less efficiently transferred to chlorophyll a , implying some other function of fucoxanthin at high irradiance, such as a protective function. Carotenoid pigments are known to exert a light-protecting

role, since excessive energy may be conducted from excited chlorophyll to carotenoid molecules (Jeffrey 1980, Anderson 1986).

It is well documented that the UV part of the spectrum can be photoinhibitory (see reviews by Halldal and Taube 1972, Worrest 1982). Subcanopy kelp sporophytes of ***Ecklonia radiata*** grown under sunlight without a UV-filter showed more severe tissue damage, photopigment destruction, reduced growth, and lower survivorship than those grown with a UV-filter (Wood 1987). However, in early sporophytes of ***L. hyperborea,*** there was a similarly significant growth inhibition even under a UV-filter. As seen from spectral distribution curve of Polylux 4000 (in 'General materials and methods'), only a small amount of UV is emitted from the fluorescent tube and the plastic lids used for culture dishes would have absorbed a good deal of UV. Therefore, it seems unlikely that UV caused the growth inhibition of ***L. hyperborea.***

The magnitude of a shift in irradiance is an important factor of a cell's ability to adapt successfully (Collins and Boyden 1982). Many species of phytoplankton respond to variations in photon irradiance by varying their cellular contents of chlorophylls and accessory pigments (Falkowski 1980). Such changes in pigmentation are macroscopic manifestations of changes in the molecular architecture of pigment-protein complexes which ultimately lead to changes in photosynthetic responses and growth rates (Falkowski and Owens 1980, Prezelin 1981). Some plants (sun plants) are known to have a genetically determined capability to increase the capacity for light-saturated photosynthesis gradually in response to an increased irradiance during growth, whereas others (obligate shade plants) have a very limited capability for adjustments (Björkman 1981). Therefore, photosynthetic capacity may also determine the extent of susceptibility to photoinhibition when the plants are transferred from low to higher irradiance. Differential

responses to high irradiance by ***L. hyperborea*** early sporophytes acclimated at 19 and 55 μmol $m^{-2}s^{-1}$ for 20 days may manifest photophysiological characteristics of this plant. In this species, plants acclimated to 19 μmol $m^{-2}s^{-1}$ seem to have a limited ability to adapt their photosynthetic capacity upward when transferred to 135 μmol $m^{-2}s^{-1}$, showing significantly lower growth rate at the latter irradiance than at 55 μmol $m^{-2}s^{-1}$. As Northlight used for this experiment had already been found not to be harmful at high irradiance in the previous experiment and plants acclimated for 8 days did not show any inhibition at 135 μmol $m^{-2}s^{-1}$, the growth inhibition found in plants acclimated to 19 μmol $m^{-2}s^{-1}$ for 20 days can be regarded as a real result due to the acclimation. On the other hand, plants acclimated to 55 μmol $m^{-2}s^{-1}$ did not show any growth inhibition when transferred to higher irradiance regardless of acclimation periods. The result simply appears to show that light response characteristics of a given species or individual can be strongly modified by the growth light regimes (Björkman 1981). Wilkinson (1974) found that populations of ***Eugomontia sacculata,*** from habitats differing in depths and turbidity, showed different tolerances to high irradiances. Photosynthesis in 20 m deep growing material of ***L. hyperborea*** was rapidly reduced in bright sunlight, while shallow water (3 m) material of the same species was more resistant (Drew 1974). Increased photosynthetic capacity in response to high light acclimation has been previously described for several species of macroalgae (Breeman and ten Hoopen 1984, Lapointe and Duke 1984, Gerard 1986).

Concerning the time to be taken to induce acclimation, studies on ***Porphyridium purpureum*** grown over a range from 10 to 180 μmol $m^{-2}s^{-1}$ showed that acclimation to the irradiance levels required several days or weeks (Levy and Gantt 1988). A dramatic change in the

composition of photosynthetic pigments occurred in the experimental algae after a week (Ramus et al. 1976, 1977). Variation in growth irradiance causes a large change of the light-harvesting apparatus and such adaptation is brought about by nucleus and ribosomal control of synthesis involving protein synthesis, which usually takes hours or days (Larkum and Barrett 1983).

Fucoxanthin often appears to increase less rapidly than chlorophyll a as either irradiance decreases or depth increases, resulting in low fucoxanthin/chlorophyll a ratio (Ramus et al. 1977, Dring 1986). Thus, considering the known protective function of fucoxanthin (Krinsky 1968, Shimura and Fujita 1975), insensitivity of plants acclimated at 55 μmol $m^{-2}s^{-1}$ to 135 μmol $m^{-2}s^{-1}$ may be in part ascribed to a higher ratio of fucoxanthin/chlorophyll than that of plants acclimated at 19 μmol $m^{-2}s^{-1}$ (Dring, personal communication). In Polylux 4000, the radiation of which had previously been found to be inhibitory to the growth of early sporophytes at high irradiance, even complete bleaching was shown for plants acclimated at 15 μmol $m^{-2}s^{-1}$ for 16 days when they were transferred to 159 μmol $m^{-2}s^{-1}$. Bulk bleaching of photosynthetic pigments occurs mainly at high irradiances and after longer times than those necessary for photoinhibition and impairment to the reaction centres (Satoh 1970, Abeliovich and Shilo 1972). Chlorophyll bleaching and photoinhibition are different processes. The primary event is an inhibition of the photosynthetic reaction and chlorophyll bleaching is a secondary reaction occurring only after photosynthesis is severely inhibited (Björkman 1981). This may explain why under Northlight only growth was inhibited but in Polylux 4000 bleaching happened.

On the contrary, the favourable response of low light-acclimated early sporophytes of *L. digitata* to high irradiance even after 14 day-

acclimation period seems to suggest that this species may have an inherent high ability to increase the capacity for effective utilization of high level of light. This property is similar to one of the sun-type characteristics which is estimated by photoprotection at high irradiance (Larkum and Barret 1983). Low light-acclimated gametophytes of an intertidal, sun-adapted species and a subtidal, shade-adapted species of ***Porphyra*** differed significantly in their resistance to photoinhibition (Herbert and Waaland 1988). Although the capability of chromatophore movement as a protective mechanism against high irradiance has been suggested (Nultsch and Pfau 1979, Nultsch et al. 1981) this cannot explain the difference in the sensitivity between low light-acclimated ***L. hyperborea*** and ***L. digitata*** to high irradiance since both species showed a similar transmittance change, following change in irradiance (Nultsch and Pfau 1979). Boney and Corner (1963) reported that the accessory pigments of a sublittoral red alga ***Brongniartella byssoides*** are mainly used for photosynthesis (energizing chlorophyll a indirectly), whereas those of an intertidal ***Antithamnion plumula*** are used for protection of photosynthetic systems against inhibitory light. Perhaps the same principle may be applicable to explain the high growth rate of low light-acclimated ***L. digitata,*** known as an intertidal/ sublittoral alga, at high irradiance, i.e. the role of accessory pigments adapted more to the function of protection than that of ***L. hyperborea,*** a mainly sublittoral alga.

Under sunlight (512 μmol $m^{-2}s^{-1}$ in October), plants acclimated to 8 μmol $m^{-2}s^{-1}$ showed significantly lower survival than those acclimated to either 55 or 130 μmol $m^{-2}s^{-1}$ in both species of ***L. hyperborea*** and ***L. digitata,*** confirming the previous result described above although the former species was more severely damaged than the latter. It seems evident that photoinhibition does not normally occur in early

sporophytes of canopy-forming species in the light regimes that they encounter in their natural environments. However, shadowing provided by the overlying thalli of parent plants could supply the early sporophytes growing underneath with sufficient opportunity to become acclimated to the available light condition. If some population of early sporophytes adapted to extended periods of greatly reduced irradiance is suddenly confronted with high irradiance in a natural setting, plants with high susceptibility to photoinhibition may be at an even greater selective disadvantage compared with plants otherwise. The result of this study suggests that ***L. hyperborea*** early sporophytes may be the former case compared with ***L. digitata.***

Significantly lower resistance of ***L. hyperborea*** to direct sunlight than that of ***A. esculenta*** and ***L. digitata*** may be connected with the fact that the first species is often absent from the eulittoral (Kitching 1941, Kain 1962, 1971b, Norton et al. 1977). Biebl (1952) found that intertidal species were more tolerant of high irradiance of sunlight than sublittoral algae, showing the resistance to be the typical characteristics of ecological resistance. On the other hand, the magnitude of quantum dose required to do lethal damage in early sporophytes was in general much higher than that reported in gametophytes of species of the Laminariales. The laminarian gametophytes of Californian species growing in the deeper sublittoral were killed after having received about 0.48 mol m^{-2} (corresponding to an exposure for 8 min to a photon irradiance of 1000 μmol $m^{-2}s^{-1}$; open sky in December; spores immersed in 1 cm deep enriched seawater; water temperature 12-18 °C) while the corresponding value was 0.90 mol m^{-2} in ***Egregia menziessi*** from the upper sublittoral (Lüning and Neushul 1978). On the other hand, under December sunlight at Helgoland (340-400 μmol $m^{-2}s^{-1}$), 50% of the gametophytes of ***L. hyperborea*** and ***L. digitata*** were killed

after 7.5-10.5 min (quantum dose 0.17-0.24 mol m^{-2}) and 30 min exposure (quantum dose 0.61-0.72 mol m^{-2}) killed over 90% of the gametophytes of both species (Lüning 1980). However, some early sporophytes (10 day-old) of the same species showed more than 90% survival percentage when exposed to even 0.92 mol m^{-2} (30 min exposure to 512 μmol $m^{-2}s^{-1}$) of October sunlight at Port Erin, Isle of Man . Additionally, 60 min exposure was almost lethal to ***L. hyperborea*** (3% survival percentage) whereas ***L. digitata*** survived about 50% in the same treatment, confirming its higher resistance to strong light than the former species. The reason why there is a discrepancy in the sensitivity to sunlight between gametophytes and early sporophytes may be inferred in two ways in spite of seasonal and experimental variations. Firstly, there may be an switch in the sensitivity to strong light between the different phases. Fei et al. (1989) showed that gametophytes of ***L. japonica*** could not tolerate an irradiance of 150 μmol $m^{-2}s^{-1}$ while sporophytes of 1-2 celled stage could tolerate more than 519 μmol $m^{-2}s^{-1}$. Phase-specific differentiation in light-related traits was also found in ***L. saccharina*** (Gerard 1990). Alternatively, higher temperature (about 13 °C) shown during the experiment in this study may in part explain the higher resistance of sporophytes than gametophytes exposed to low temperature (5 °C) during exposure to sunlight, reflecting some evidence that low temperature increases the sensitivity of photoinhibition. Steemann Nielsen (1942) found that exposure of ***Fucus serratus*** to a moderately high irradiance resulted in substantial photoinhibition when the alga was kept at 5 °C, but not when it was kept at 20 °C. In higher plants, exposure of single leaves of ***Phaseolus vulgaris*** to 2000 μmol $m^{-2}s^{-1}$ at 6 °C and normal air for 3 h resulted in a severe inhibition of quantum yield, but not at 12 °C (Powles et al. 1980).

As photodamage occurs as a secondary phenomenon after photoinhibition (Powles 1984), the findings described above may be used to infer differential sensitivity between gametophytes and sporophytes in terms of different temperatures used. However, lower survival in laminarian gametophytes of Californian species compared with that in early sporophytes in this study can not be explained by temperature effect since the temperature range in the former case was between 12 to 18 ^{o}C which is comparable to that in the present study.

Aside from its higher resistance to sunlight than ***L. hyperborea*** it is of interest to note that ***A. esculenta*** showed a reciprocity between survival percentage and total quantum dose, in other words, the survival percentage seemed to be independent of photon irradiance and the length of exposure. In a previous experiment conducted under fluorescent tubes, negligible mortality of early sporophytes of the same species was observed even in much larger quantum doses (140-150 mol m^{-2}) than those (maximum 2.52 mol m^{-2}, 100% mortality) in this experiment (Fig. 3.7). In the first place the possibility that no damage in fluorescent light might be due to the usual absence of UV in such light sources compared with sunlight was eliminated since the reciprocity was found between UV-filtered and -unfiltered conditions. Therefore, the reason may be found in the fact that in the previous experiment the quantum dose was given over long periods in lower irradiances (180 µmol $m^{-2}s^{-1}$ for 9-10 days). This may suggest that there must be after all an influence of photon irradiance itself in the form of a threshold below which no irreversible damage occurs (Lüning 1981a).

In the case of ***L. hyperborea,*** 0.90 mol m^{-2} killed more than 80% of the sporophytes in this experiment (Fig. 3.7), but only ca. 10% in another experiment already described above (Fig. 3.9). Five hypotheses

could be put forward to explain the discrepancy. Firstly, in this study, it was shown that early sporophytes of ***L. hyperborea*** differed in their sensitivity to high irradiance depending on the culture irradiance irrespective of the origin of materials, suggesting that the differential sensitivity to sunlight reported here might have been ascribed to the different pretreatment irradiation conditions. However, as plants in the two experiments were similarly pre-treated under almost an identical irradiance, it is unlikely that this hypothesis could explain such a remarkable distinction in sensitivity to sunlight between different experiments. Secondly, it may have been due to the difference in climatic factors, such as solar elevation, cloudiness etc. As the solar elevation diminishes, the ratio of blue to red wavelength in light decreases because of intensified removal of the more readily scattered, short wavelength light (blue) in the longer atmospheric path (Kirk 1983). However, there is no simple relationship between solar elevation and the spectral distribution of total irradiance because diminution in solar elevation may cause an increasing contribution of skylight rich in the shorter wavelength to total irradiance (Kirk 1983). Therefore, unless precise measurements of spectral distribution of sunlight were made, this could not be the certain reason for the differential sensitivity of ***L. hyperborea*** in different seasons. Thirdly, different genetic make-ups may have contributed to differentiate the sensitivity of early sporophytes to sunlight. Such a big difference in sensitivity between the populations, however, might not be expected unless plants came from different ecotypes. So far there has been no evidence of ecotypic differentiation in ***L. hyperborea*** (Kain, 1969). Fourthly, it must be noted that in the two experiments, post-treatment was made in different light, i.e. white light for the former experiment in which plants have shown 90% survival and blue light for the latter

resulting in 10% survival. Seemingly, blue light may have aggravated the possible damage of sunlight. However, as white light contained about a third of total quanta in the blue region (Appendix 6) and irradiance of white light employed was approximately three times that of blue light, it is dubious if a higher mortality in blue light treatment was due to the sensitivity of the damaged system to blue light. Finally, as seen in Fig. 3.1, there was a considerable fluctuation (40%) in photon irradiance (512 μmol $m^{-2}s^{-1}$) during the experiment in which higher survival was observed compared with that in the other experiment during which a relatively constant irradiance (750 μmol $m^{-2}s^{-1}$) was maintained (6% variation). The elimination of high irradiance photoinhibition is one of the most commonly reported effects of fluctuating irradiance (Harris 1978). This is because photoinhibition is a time-dependent process and relatively short exposures to high irradiance do not depress photosynthesis to the extent that it does in constantly high irradiance (Harris 1978). Therefore, the same explanation may be applicable to understand the considerable difference in the sensitivity of ***L. hyperborea*** to sunlight between the two experiments.

Chapter 4. Blue light photoreactivation

4.1. Introduction

Reversal of short wave ultraviolet (UV) damage by subsequent radiation of longer wavelengths is of almost universal occurrence in living organisms (Caldwell 1968, 1971). This phenomenon, known as 'photoreactivation', has mainly been studied in viruses, bacteria, fungi and higher plants (Dulbecco 1949, Kelner 1949, Bawden and Kleczkowski 1952, Caldwell 1968, Teramura 1982). The action spectra for far-UV (radiation of wavelength less than 300 nm) damage are known to be considerably different between systems, suggesting a variety of chromophores (Jagger 1964). However, Jagger (1964) has discussed the evidence that the major chromophore of far-UV consists of nucleic acids or proteins. As for the mechanism by which reactivation occurs, there is little information but the effective waveband in inducing photoreactivation has usually been reported to lie between 313 and 549 nm (Jagger 1958).

There is a wide range of response to 350-500 nm in plants from diverse taxonomic groups. The action spectra are found to show general similarity in the spectral range to that of photoreactivation, typically having their maximum around 370-380 nm and in the blue region within 400-500 nm (Hart 1988). For photoresponses of algae, blue light has been found to have differential effects in many aspects, e.g. enzyme effects, tropic response, induction of vegetative growth and reproductive activity and photoperiodic effects (Dring 1988). In this respect, the similarity of the spectral range for photoreactivation with that of blue light responses may suggest that the reactivation from UV damage is also one of the blue light-mediated responses.

Comparatively few studies on photoreactivation have been conducted with algae, although one of the very first reports was made in a brown alga ***Fucus furcatus*** Gardner on the reactivation of UV-induced delay of rhizoid formation (Whitaker 1942). Brown algae have been of particular interest where the blue light effects are concerned because the sensitivity is sometimes found to be quite different from the majority of blue light responses in other plants (Dring 1987).

In this context, the present study represents an approach to identify photoreactivation in early sporophytes of members of the Laminariales and determine the specific light requirements for the response.

4.2. Materials and Methods

4.2.1. Effect of various durations of UV

Gametophytes were grown on square coverslips (18 x 18 mm) in plastic petri-dishes (100 mm diam., 10 mm high) each containing 35 ml medium. The cultures were maintained under continuous light of 30-40 μmol $m^{-2}s^{-1}$ by Northlight white fluorescent tubes in a controlled temperature cabinet (12-15 °C). Sporophytes appeared in 8-14 days after inoculation and were treated experimentally after a further 10 days.

UV radiation was provided by 'TUV Germicidal Tubes' (Philips, 15 W). The output, 85 % of which is of wavelength 253.7 nm, is specified by the makers as 37 μW/ cm^2 at 1 m from the centre of a tube. An irradiation chamber was made of a white wooden box (530 x 390 x 280 mm) housing two germicidal tubes. The UV-tubes were switched on for 15-20 min to warm up before use. Unlidded crystallizing dishes (80 mm diam., 43 mm high), each containing 50 ml filtered seawater were laid 23 cm below the tubes in a water bath filled with tap water at a depth of 1.5 cm. Coverslips bearing early sporophytes were arranged in the centre of a dish lest some radiation should be cut down by the side of the dish during irradiation. Variation in temperature was less than 0.5 °C. Only plants in a healthy condition in terms of colour and cell pattern were used in experiments.

After different UV doses, laminarian early sporophytes were immediately transferred to petri-dishes, each filled with 35 ml medium and then cultured in continuous white light (32-36 μmol $m^{-2}s^{-1}$) for 8 days before the survival percentage was estimated. Some UV-irradiated sporophytes of *L. hyperborea* were also grown in nutrient-

unenriched seawater made by filtering (0.22 µm pore size) and autoclaving twice.

In higher plants, the most common symptoms after UV-irradiation are bronzing, scorching, glazing or chlorosis in leaves (Cline and Salisbury 1966, Teramura 1983). Following these findings, survival percentage was determined by counting normal sporophytes with more than half their thallus lacking any significant signs of colour change to white nor absence of cell contents.

4.2.2. Reactivation

Following UV irradiation, laminarian early sporophytes were maintained either in white light of 32-36 µmol $m^{-2}s^{-1}$ or in the dark for 8-10 days. The latter plants were cultivated in light for another 8 days after the dark incubation period.

In another experiment, early sporophytes of ***L. hyperborea*** were cultured at four different irradiances of continuous white light after exposure to a sublethal dose of UV irradiation.

4.2.3. Effect of different wavebands of light on reactivation

UV-irradiated laminarian sporophytes were cultivated at 10-11 µmol $m^{-2}s^{-1}$ in different wavebands of light for 8-10 days. Coloured fluorescent light tubes combined with Cinelux gelatin filters (Strand Lighting) were used. The light transmission through these combinations has already been shown in Fig. 0.1.

Some ***L. hyperborea*** sporophytes were exposed to various types of

irradiation, i.e. 12 h of blue, green or red light (10-11 μmol $m^{-2}s^{-1}$) followed by 12 h of white light (35-36 μmol $m^{-2}s^{-1}$) after UV exposure.

4.2.4. Relative effectiveness of blue quanta

To compare the relative effectiveness of blue and white light sources the manufacturers' data on spectral energy output between 400 and 700 nm and, in the case of blue light, spectral transmission of the blue filter were converted to quanta. The quanta in each of 5 nm wavebands were then expressed as a proportion of the total. The resulting curves were then multiplied by the action spectrum curves for the 2-dimensional growth responses of ***Scytosiphon lomentaria*** (Lyngb.) Link (Dring and Lüning 1975). The areas under these curves were then compared.

In connection with the above calculation, UV-irradiated sporophytes of the four species were treated with blue and white light in this proportion: 10-11 μmol $m^{-2}s^{-1}$ of blue and 32-36 μmol $m^{-2}s^{-1}$ of white (in some cases, 10-15 μmol $m^{-2}s^{-1}$ of white light was also used).

4.2.5. Blue quantum requirement

After exposure to a sublethal dose of UV irradiation, the plants were irradiated with 10-35 μmol $m^{-2}s^{-1}$ of continuous blue light for various periods up to 96 h. Individual coverslips bearing UV-irradiated sporophytes were then transferred to green light (15-18 μmol $m^{-2}s^{-1}$) at different times and cultured for 10 days.

4.3. Results

4.3.1. Effect of various durations of UV

The survival curves (Fig. 4.1) show an inverse relationship between duration of UV exposure and the survival percentage of laminarian sporophytes. As shown in Table 4.1, the relationship for each species was similar in two separate experiments ($p > 0.05$, Wilcoxon paired sample test). Up to 30 s, UV irradiation had no obvious effect on 10 day-old early sporophytes except for ***L. saccharina,*** showing similar survival in the irradiated plants to that of control (Fig. 4.1). After 60-90 s exposure, 30-80 % of plants died. Longer than 120 s UV irradiation was lethal to early sporophytes. ***L. saccharina*** was significantly ($p < 0.05$; Wilcoxon paired sample test) more sensitive than ***L. hyperborea*** and ***A. esculenta*** (Table 4.2).

When UV-irradiated sporophytes of ***L. hyperborea*** were cultured either in nutrient-enriched medium or -unenriched seawater, no difference was detected in the survival percentages (Table 4.3, $p \geq 0.05$; S-N-K test). As in the previous experiments, however, there were significant differences in survival percentage between the plants exposed to different doses of UV (Table 4.3).

4.3.2. Reactivation

In samples treated with white light immediately after 45-120 s UV exposure, survival percentages were much higher than those of the corresponding samples kept in the dark ($p < 0.05$, Fig.4.2). On UV-

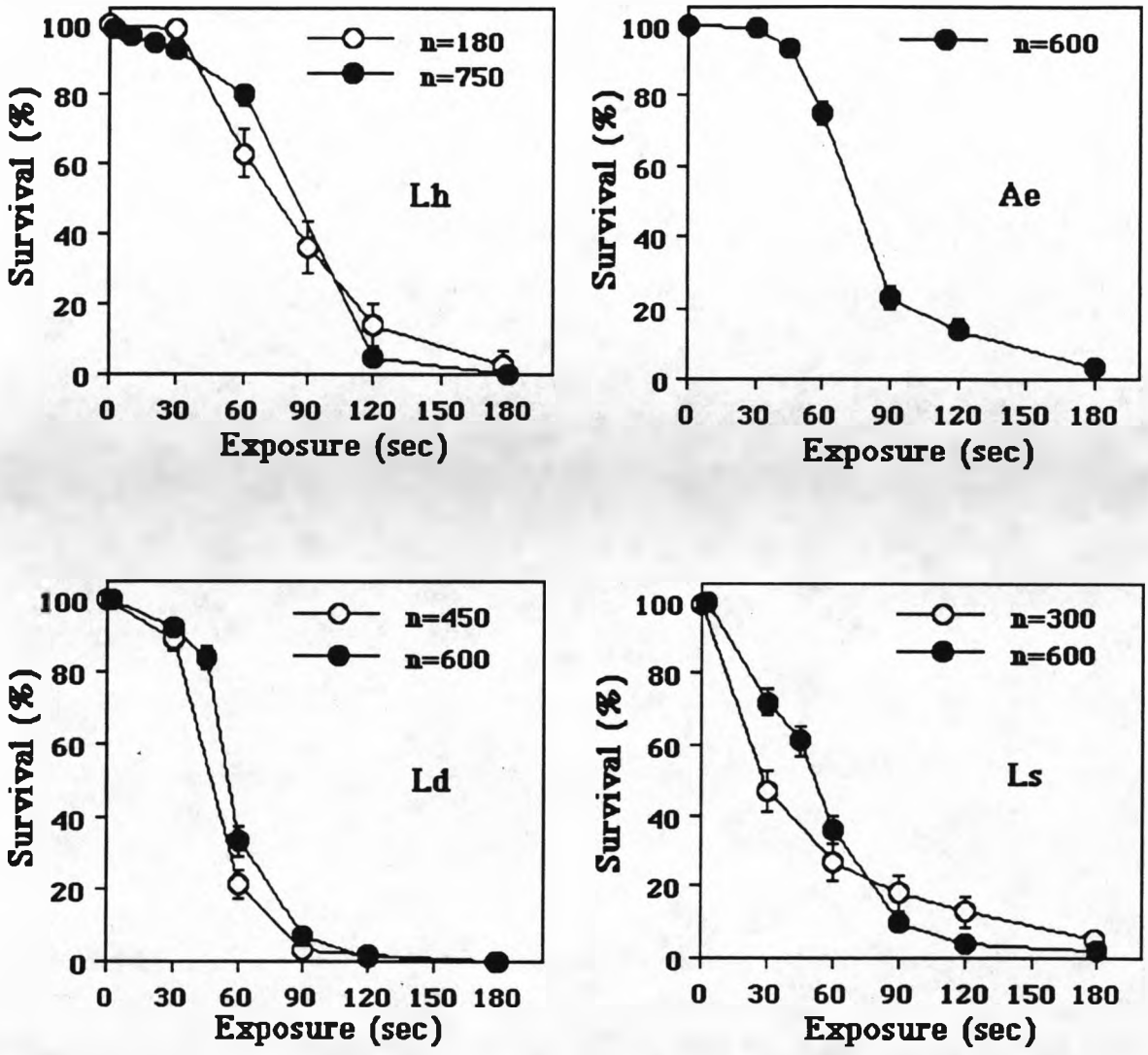

Fig. 4.1. Effect of duration of UV-radiation on survival of early sporophytes of members of the Laminariales. Circles of different pattern are survival values from two separate experiments. Vertical bars denote 95% confidence intervals. Lh: ***L. hyperborea,*** Ae: ***A. esculenta,*** Ld: ***L. digitata*** and Ls: ***L. saccharina.***

Table 4.1. Wilcoxon paired sample test of survival percentages from separate experiments for each species.

Ho: survival percentages in Expt 1 are the same as those in Expt 2.
Ho is rejected if either T_+ or T_- is less than or equal to the critical value, $T_{0.05(2),6}$.

	Sum of signed ranks		Conclusions
	T_+	T_-	$T_{0.05(2),6}=0$
L. hyperborea	14	6	Accept Ho
L. digitata	3	15	Accept Ho
L. saccharina	9	12	Accept Ho

Table 4.2. Wilcoxon paired sample test of survival percentages between different species. Lh: ***L. hyperborea,*** Ae: ***A. esculenta,*** Ld: ***L. digitata*** and Ls: ***L. saccharina.***

Ho: Survival percentages of the former species are the same as those of the latter species.
Ho is rejected if either T_+ or T_- is less than or equal to the critical value, $T_{0.05(2),6}$.

	Sum of signed ranks		Conclusions
	T_+	T_-	$T_{0.05(2),6}=0$
Lh vs Ae	9	12	Accept Ho
vs Ld	20	1	Accept Ho
vs Ls	21	0	Reject Ho
Ae vs Ld	20	0	Reject Ho
vs Ls	21	0	Reject Ho
Ld vs Ls	13.5	7.5	Accept Ho

Overall conclusion:

Ae Lh Ld Ls

Table 4.3. Comparison of survival percentages of UV-irradiated *L. hyperborea* between in nutrient-enriched medium and -unenriched seawater after different doses of UV exposure. Survival data were arcsine transformed prior to statistical analysis and mean and standard errors (in brackets, +/-) were produced from three replicates. Groups not sharing the same letter have significantly different survival percentages ($p < 0.05$, S-N-K test). ***$p < 0.001$, n.s. not significant.

Nutrient state\ Exposure (sec)	60	90	120
Enriched	63.1 (3.5/3.4)	35.7 (3.4/3.3)	14.0 (6.3/5.2)
Unenriched	73.0 (10.1/9.4)	30.3 (0.0/1.0)	17.7 (5.3/4.9)
Group	A	B	C

<ANOVA table for survival data>

Source	DF	SS	MS	F
Nutrient (N)	1	15.76	15.76	0.33n.s.
Exposure (E)	2	3196.44	1598.22	33.84***
NxE	2	67.69	33.84	0.72n.s.
Error		566.81	47.23	

irradiated sporophytes of ***L. hyperborea*** a comparison of the decay time for photoreactivation capability was made by retaining the plants in the dark for different periods (4, 8, 24 days) and then transferring to illuminate the plants with white light for 8 days. As shown in Fig. 4.3, survival percentage appeared to decrease as the length of dark incubation increased.

The effect of photon irradiance on survival of UV-irradiated sporophytes of ***L. hyperborea*** is shown in Fig. 4.4. Since no significant difference (at $p= 0.05$) was found between survival percentages at 35 and 60 $\mu mol\ m^{-2}s^{-1}$, reactivation saturation seemed to be saturated at the former irradiance.

4.3.3. Effect of different wavebands of light on reactivation

When photoreactivation was studied as a function of different wavebands in the same photon irradiance, it appeared that only blue light was highly effective, resulting in significantly higher survival in contrast to negligible survival observed either in green or red light (Table 4.4).

In 12:12 h of various types of irradiation, survival in 12:12 h blue: white light was found to be more than twice as great as that in 12 h green, red or darkness followed by 12 h white light (Fig 4.5). In the other three conditions, no significant difference was found among the treatments ($p> 0.05$; S-N-K test).

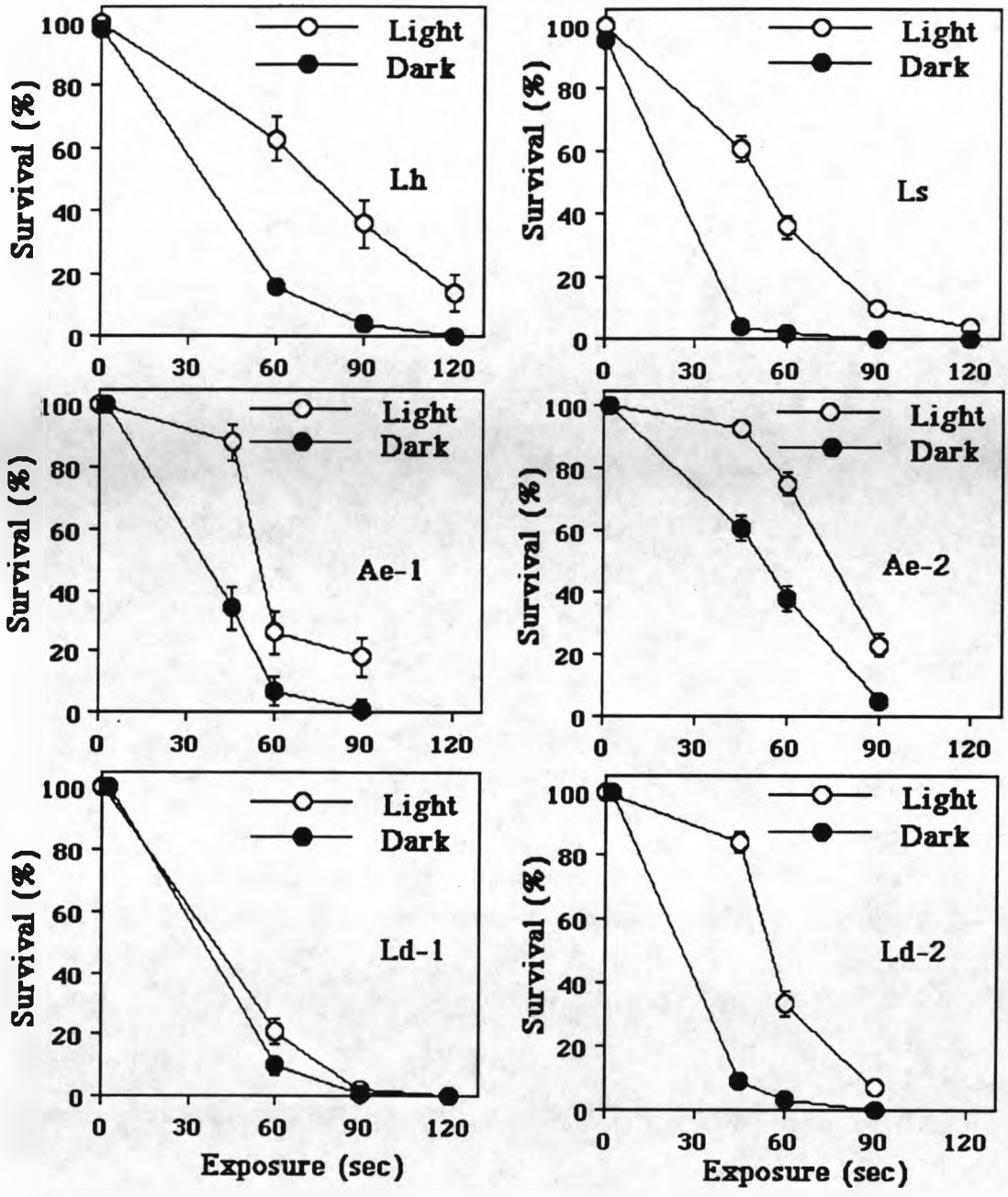

Fig. 4.2. Photoreactivation of early sporophytes irradiated by different durations of UV. Vertical bars indicate 95% confidence intervals. Lh: ***L. hyperborea,*** Ls: ***L. saccharina,*** Ae: ***A. esculenta*** and Ld: ***L. digitata.*** Different numbers beside the abbreviation of species name indicate different experiments.

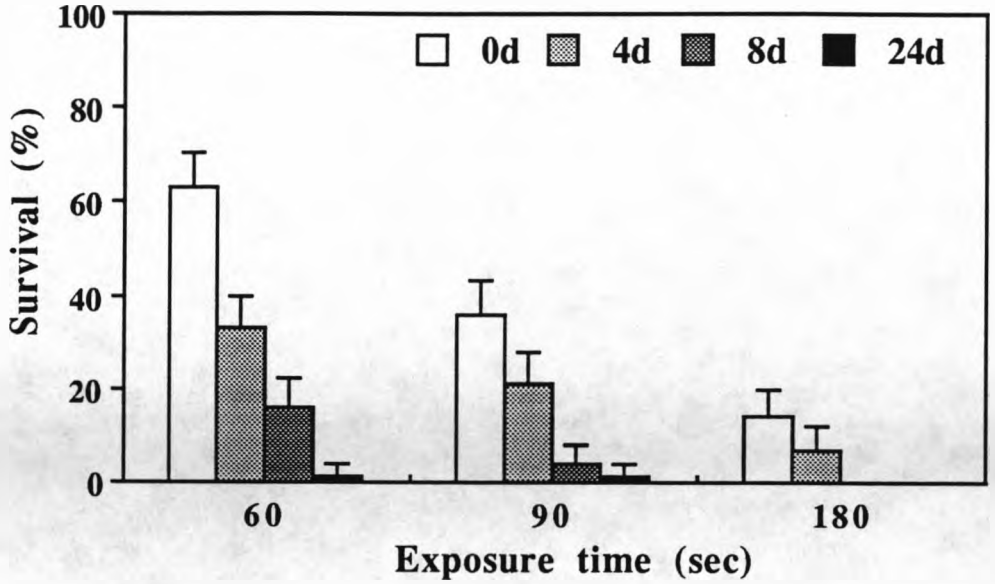

Fig. 4.3. Effect of length of dark incubation on survival of early sporophytes of *L. hyperborea* after different doses of UV exposure. Vertical bars indicate 95% confidence intervals (n=180).

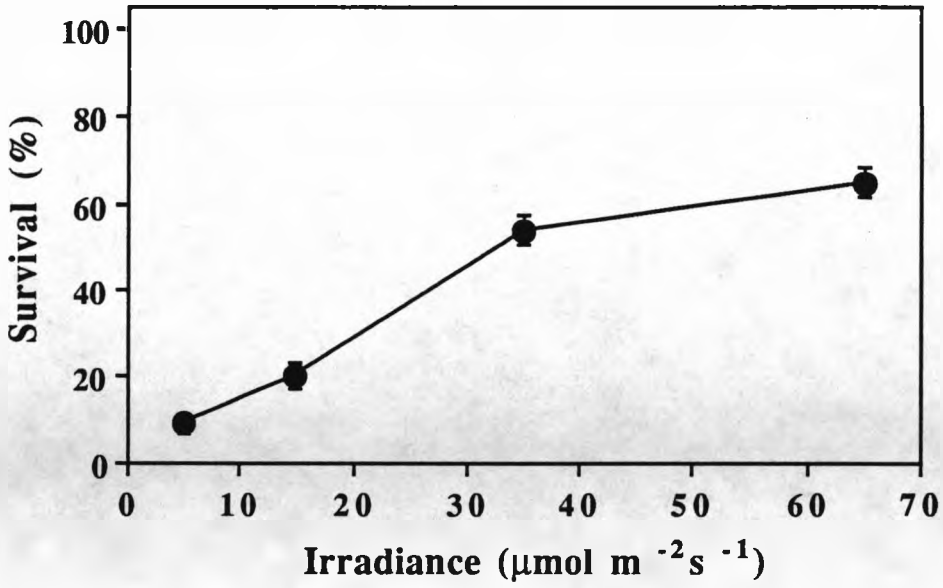

Fig. 4.4. Effect of photon irradiance on survival of UV-irradiated (90s) sporophytes of *L. hyperborea*. Plants were cultivated at four different irradiances of continuous white light (5, 15, 35, 65 μmol $m^{-2}s^{-1}$) for 8 days before survival percentage was estimated. Vertical bars indicate 95 % confidence intervals (n = 600).

Table 4.4. Comparisons of survival percentages of UV-irradiated early sporophytes of members of the Laminariales in different wavebands of light (10-11 μmol $m^{-2}s^{-1}$). Mean and standard errors (in brackets, +/-) were produced from three replicates. Values not sharing the same superscript in each species and exposure are significantly different at p=0.05 level (S-N-K test).

Species/Light fields (Exposure)	Blue	Green	Red	ANOVA F-value
L. hyperborea (90s)	51.9 (4.4/4.2)^{a}	0.3 (0.2/0.2)^{b}	0.1 (0.0/0.0)^{b}	278.35***
L. hyperborea (120s)	19.5 (5.5/5.1)^{a}	0.4 (0.4/0.2)^{b}	0.2 (0.0/0.0)^{b}	32.26***
A. esculenta (60s)	64.3 (3.5/3.7)^{a}	3.5 (0.8/0.8)^{b}	0.3 (0.2/0.2)^{c}	312.93***
L. digitata (60s)	46.9 (7.0/6.9)^{a}	0.5 (0.3/0.2)^{b}	0.1 (0.0/0.0)^{b}	95.51***
L. saccharina (60s)	24.9 (7.2/8.1)^{a}	0.3 (0.2/0.2)^{b}	0.1 (0.0/0.0)^{b}	22.26**
L. saccharina (90s)	13.4 (1.0/0.8)^{a}	0.4 (0.4/0.2)^{b}	0.2 (0.0/0.0)^{b}	133.27***

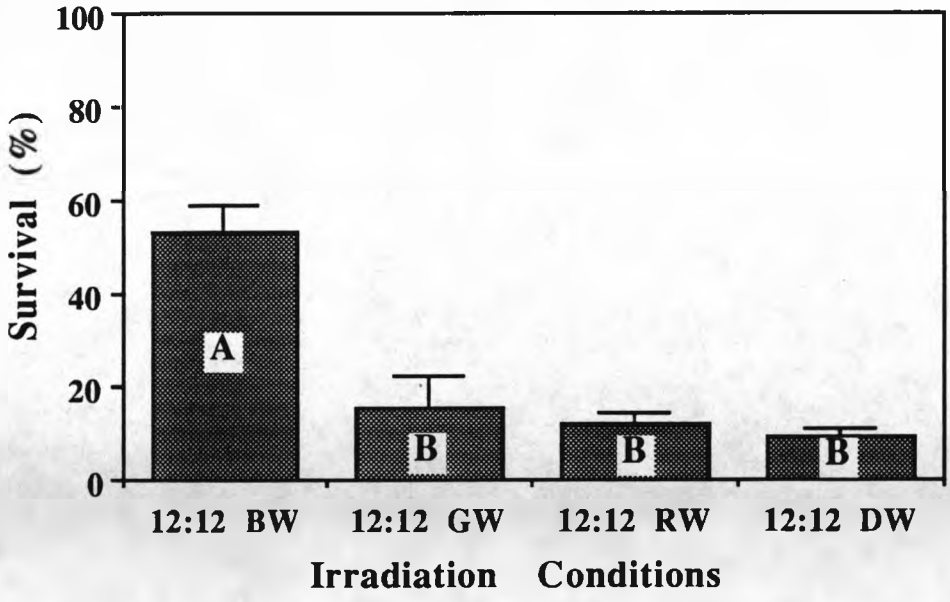

Fig. 4.5. Effect of various types of irradiation on survival of early sporophytes of ***L. hyperborea***. UV-irradiated (90s) plants were cultivated in 12 h of blue (B), green (G) or red (R) light (10-11 µmol $m^{-2}s^{-1}$) or darkness (D) followed by 12 h of white (W) light (35-36 µmol $m^{-2}s^{-1}$). Vertical bars show S.E. of the mean of three replicates (n= 200/rep.). Bars labelled with the same letter are not significantly different at p= 0.05.

4.3.4. Relative effectiveness of blue quanta

The spectral distributions of quanta from the two light sources between 400 and 700 nm are shown as equal area curves in Fig. 4.6. The proportions of blue quanta contained in blue and white light were calculated to be 71% and 21% respectively. The black-filled areas in Fig. 4.6 were derived from the product of these curves and the action spectrum curves for the 2-dimensional growth response of ***S. lomentaria*** (Dring and Lüning 1975). The area under the curve for blue light was 3.4 times that under white. A measured number of quanta of the blue light would therefore be 3.4 times as effective as the same photon irradiance of white light (of the types used) so similar effects might be expected from exposure to 3.4 times as much white light as blue. It is apparent from Table 4.5 that the effects on reactivation were similar when UV-irradiated sporophytes were treated with 10-11 μmol $m^{-2}s^{-1}$ of blue and 32-36 μmol $m^{-2}s^{-1}$ of white. In case of ***L. hyperborea*** and ***L. saccharina,*** again, both blue of 10-11 μmol $m^{-2}s^{-1}$ and white of 32-36 μmol $m^{-2}s^{-1}$ were approximately three times as effective in inducing photoreactivation as 10-15 μmol $m^{-2}s^{-1}$ of white light (Table 4.5)

4.3.5. Blue quantum requirement

Fig. 4.7 shows the results of experiments to determine the amount of blue light necessary for maximal photoreactivation of UV-irradiated early sporophytes. Saturation of the reactivation appears to occur when the total quantum irradiation reached about 4 mol m^{-2} for ***A. esculenta*** and ***L. saccharina*** and 6 mol m^{-2} for ***L. hyperborea.*** The

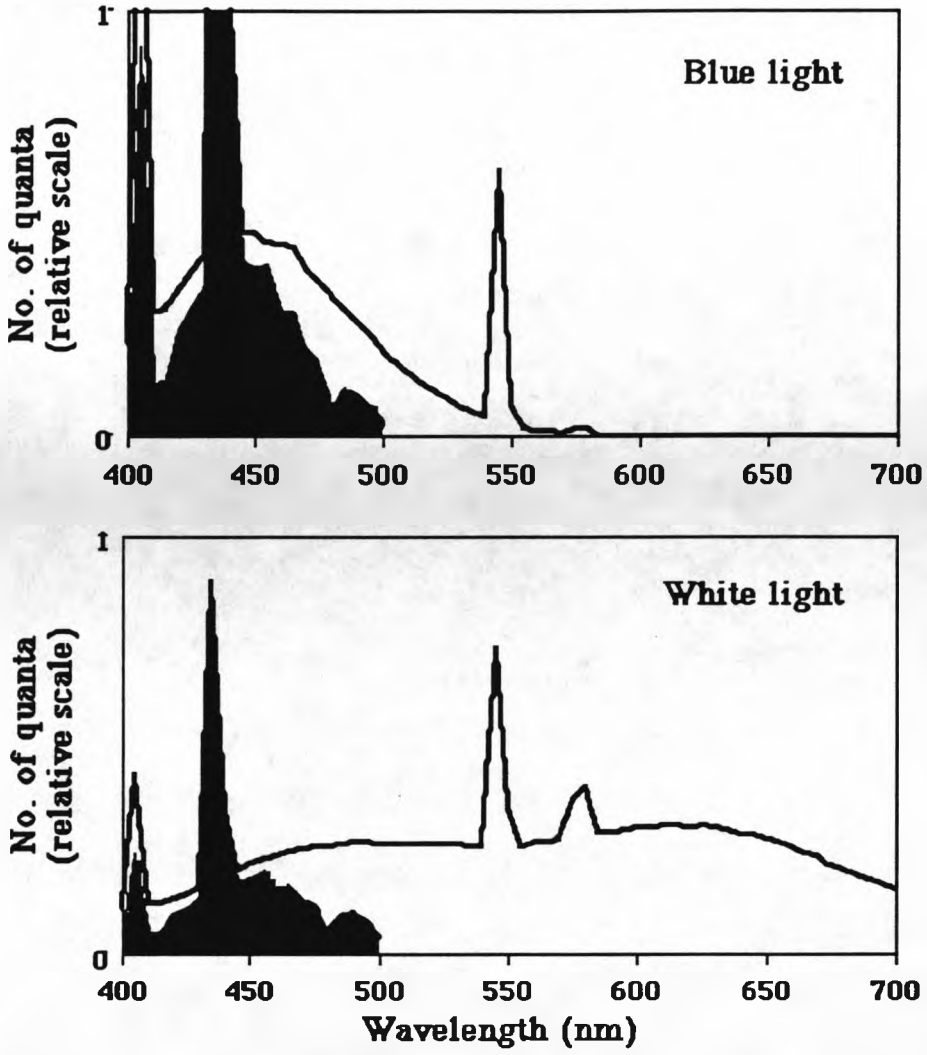

Fig. 4.6. Equal area curves of quanta within 400-700 nm (continuous line) and the product of these curves and that for the action spectrum of 2-dimensional growth of ***Scytosiphon lomentaria*** (black-filled). The blue fluorescent tube combined with a blue Cinelux filter and a Northlight tube were used for blue and white light respectively. See the text for details.

Table 4.5. Comparison of survival percentages of UV-irradiated sporophytes in different irradiation conditions (10-11 μmol $m^{-2}s^{-1}$ of blue light; 32-36 μmol $m^{-2}s^{-1}$ or 10-15 μmol $m^{-2}s^{-1}$ of white light). Three replicates were pooled to produce the mean and standard errors. Values sharing the same superscript are not significantly different in their survival percentages ($p > 0.05$, S-N-K test). ***$p < 0.001$, **$0.01 > P > 0.001$, n.s. not significant.

Species/Treatment (Exposure)	Blue (10)	White (35)	White (15)	ANOVA F-value
L. hyperborea (90s)	51.9 (4.4/4.2)^{a}	54.0 (4.8/4.9)^{a}	20.5 (1.4/1.4)^{b}	26.87***
A. esculenta (60s)	64.3 (3.5/3.7)	74.6 (3.0/3.1)		4.87n.s.
L. digitata (60s)	46.9 (7.0/6.9)	43.9 (6.8/6.9)		0.09n.s.
L. saccharina (60s)	24.9 (7.2/8.1)	35.7 (9.1/8.6)		0.76n.s.
L. saccharina (90s)	13.4 (1.0/0.8)^{a}	13.4 (1.1/1.1)^{a}	5.6 (1.3/1.8)^{b}	17.51**

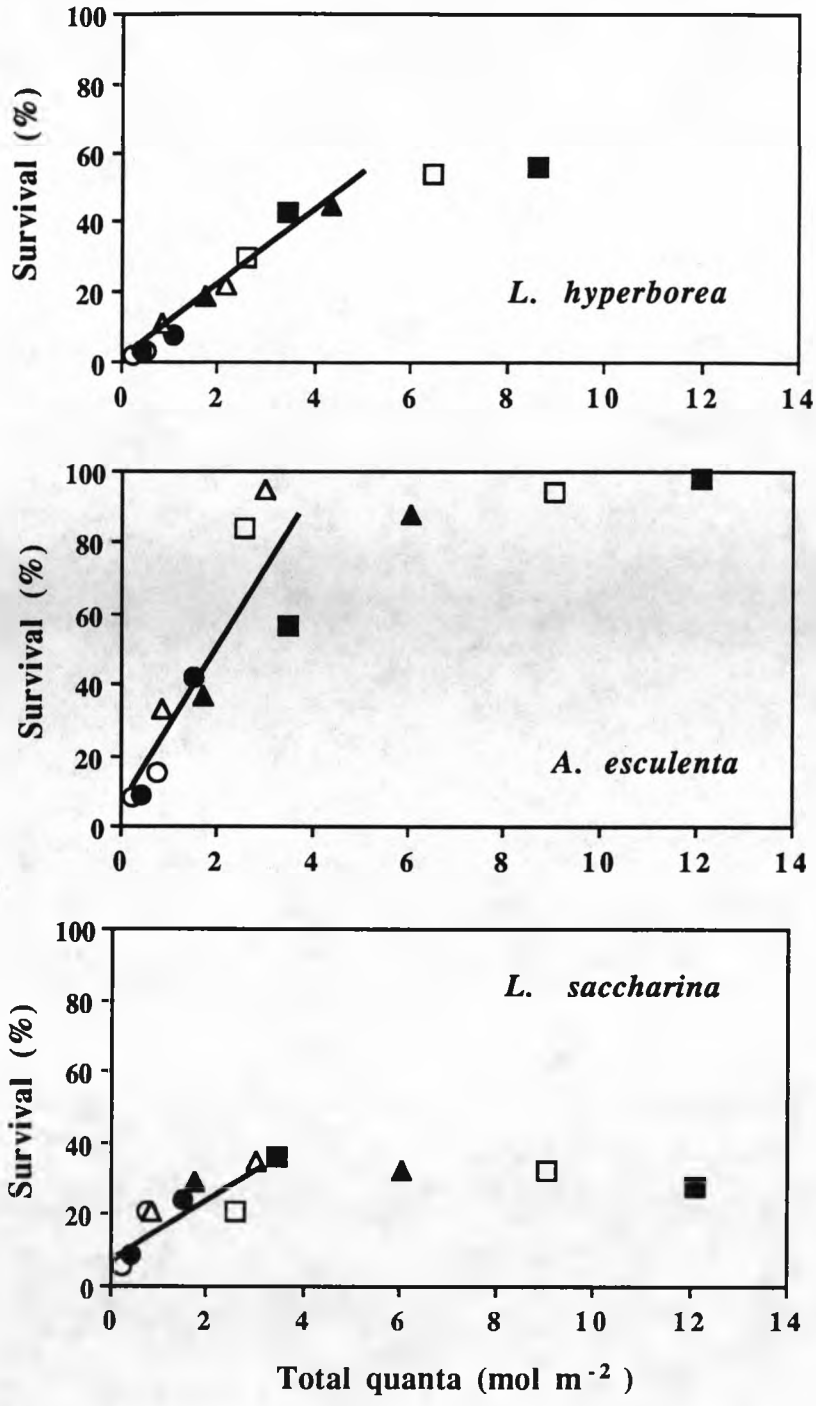

Fig. 4.7. Survival of UV-irradiated sporophytes (45s for *A. esculenta* and *L. saccharina*; 90s for *L. hyperborea*) after different periods up to 96 hr of blue irradiation (6 h- open circle, 12 h- closed circle, 24 h- open triangle, 48 h -closed triangle, 72 h- open square, 96 h- closed square). Regression line has the equation: $y = -1.56 + 11.60x$ ($r^2 = 0.98$) for *L. hyperborea*, $y = 5.11 + 23.11x$ ($r^2 = 0.80$) for *A. esculenta* and $y = 7.27 + 9.04x$ ($r^2 = 0.72$) for *L. saccharina*.

quantum requirements for a 50 % response calculated from the regression lines fitted to all non-saturating treatments were 1.2 mol m^{-2} for ***L. saccharina,*** 1.9 mol m^{-2} for ***A. esculenta*** and 2.5 mol m^{-2} for ***L. hyperborea.***

4.4. Discussion

Biebl (1952) has reported that UV irradiation of 1-4 min killed algae from both the littoral and the sublittoral. Although there is no way to compare irradiances, the sensitivity of laminarian early sporophytes does not seem to be very different from that found in those algae, showing high mortality by relatively short durations (60-180s) of UV irradiation. However, uncontrollable factors such as a variety of physiological state of plants due to different genetic make-ups and of experimental conditions found in separate cultures may in some way affect the sensitivity. Approximate comparisons of the sensitivity of members of Laminariales to UV irradiation seem to show that although sublittoral ***L. saccharina*** was more sensitive to UV than ***A. esculenta*** which can inhabit the lower eulittoral, the general difference in resistance to UV found in this study does not seem to bear any environmental relation for two reasons. First, UV radiations of wavelengths below 295 nm are completely depleted in nature (Caldwell 1971) and second, relative sensitivities to sunlight are found to be very different from those to UV (chapter 3). As Biebl (1952) pointed out, therefore, resistance to UV may be constitutional due to characteristics of the tissues of plants, and not conditioned by the environment.

Far-UV irradiation is known to be biologically effective principally because it is readily absorbed by proteins and nucleic acids (Giese 1964). In this study, it can be hypothesized for two reasons that the photochemical liability of UV damage for early sporophytes of members of Laminariales lies in nucleic acids. The first reason is that the photobiological effect of UV radiation is quite wavelength-dependent and the inactivation by UV of 253.7 nm is known to be

primarily restricted to nucleic acids (Caldwell 1971). The second is that while UV radiation is efficiently absorbed by both RNA and proteins, much higher doses must be absorbed before inactivation occurs (Caldwell 1981).

Nutritional states of post-treatment did not appear to influence survival of UV-irradiated sporophytes of ***L. hyperborea***, thus suggesting that nutritional conditions are not essential for the response leading to reactivation.

Photoreactivation is observed by comparing the survival of UV-irradiated plants either in light or in dark. Remarkable reactivation seems to occur in light until the plants are exposed to lethal dose of UV. The result may fulfill the dose reduction principle (Kelner 1949) by showing that plants cultivated in light after UV exposure act as though they had been given a much lower dosage of UV alone.

The ability to recover from UV damage appears to decrease with the dark incubation period, becoming almost zero in 24 days of incubation, irrespective of UV doses. This time lapse taken for complete loss of recovering ability and relatively high survival of 4 day-dark incubated plants after UV exposures may show that the primary effect of UV is not immediately lethal to the plants.

For reactivation only blue light was found to be effective. No discernible effect in the other photosynthetically active regions would seem to rule out the possibility that the response is attributed to differential effects of blue, green and red light either on growth or photosynthesis. Similar responses have been reported for other organisms such as blue-green algae (Van Baalen 1968, Yopp et al. 1979), ***Escherichia coli.*** (Kelner 1951), sea urchins (Wells and Giese 1950) and soybean leaves (Tanada and Hendricks 1953). Strangely, in ***A. esculenta*** a 60s UV treatment led to almost zero survival in green or in

red light (Table 4.4) whilst the same UV treatment allowed for about 40% survival in the dark (Fig. 4.2). This result may suggest that there is another way of rendering UV damage ineffective in this species, i.e. 'dark repair' which has been recognized to occur naturally in many cells (Jagger 1964). However, more information would be needed to come to any conclusion about this possibility.

For dependence of reactivation on photon irradiance of continuous white light, survival of UV-irradiated ***L. hyperborea*** was observed to be about 9% at 5 μmol $m^{-2}s^{-1}$ after 8-day culture. In cultures grown at 15 μmol $m^{-2}s^{-1}$, about half of the maximum survival percentage was found. Since reactivation seems to be very inefficient at the low irradiances, it would appear that there are only a small number of quanta available in low irradiances of white light. This supposition is in agreement with the fact that only 21% of the total visible quanta in white light is within the blue range (400-500 nm, Appendix 6). Therefore, the result may simply be due to insufficient blue quanta for the plants to be reactivated. Even in various irradiation conditions, UV-irradiated plants seem to respond to the amount of blue quanta received. Considering that 32-36 μmol $m^{-2}s^{-1}$ of white light is equivalent to 10-11 μmol $m^{-2}s^{-1}$ of blue light in terms of effective blue quanta, the similar survival percentage between these treatments, therefore, suggests that the extent of the response to light is independent of the other wavelengths present in the light fields and is governed solely by the blue light content.

Although this work on members of Laminariales did not include the determination of a detailed action spectrum for photoreactivation, it may be cogent to suppose a likely involvement of cryptochrome known to be ubiquitous in brown algae (Dring 1988). This idea is supported by the general similarity among the survival percentages of

UV-irradiated plants which have received nearly the same amount of effective blue quanta as calculated from action spectrum for two-dimensional growth of *S. lomentaria.*

Photomorphogenetic responses of brown algae to blue light have been of particular interest because of their high quantum requirement and long-term reciprocity which have not been reported in any other photobiological system (Dring and Lüning 1983, Dring 1987). Comparisons of the quantum requirements to obtain a 50 % response show that the quantity (1.2-2.5 mol m^{-2}) needed for photoreactivation in laminarian early sporophytes is similar to that previously reported for some other blue light responses (Table 4.6), placing this photoreactivation in a high quantum requirement group (Dring 1987). Similar long-term reciprocity is also reflected in the observation that the effect of blue light depends on the total quantum for up to 96 h, thus again suggesting that the role of blue light in this process is analogous to that in other photomorphogenetic responses of brown algae to blue light.

As for the mechanism of photoreactivation, Halldal and Taube (1972) stated that:

'Photoreactivation enzyme forms a complex only with UV-damaged DNA. The pyrimidine dimers-the most common legion in DNA-formed by UV radiations are split on absorption of photoreactivating light by the enzyme-DNA complex'

Recently, some enzymes have been isolated in ***E. coli.*** and ***Streptomyces*** and details of their activities studied (Galland and Senger 1988). Galland and Senger (1988) have pointed out that DNA-photolyases may play an important part in repairing far-UV induced DNA damage and chromophores of many DNA-photolyases are flavins or deazaflavins. In addition, Ruyters (1984) has reviewed that

Table 4.6. Comparison of high quantum requirements for a 50% response to blue light found in brown algae.

Species	Response	Quantum req. (mol m^{-2})	Ref.
Laminaria saccharina	Egg formation	2.0	Lüning & Dring (1975)
Macrocystis pyrifera	Egg formation	2.6	Lüning & Neushul (1978)
Scytosiphon lomentaria	Hair formation	2.0	Dring & Lüning (1975)
Scytosiphon lomentaria	2-dimensional growth	2.3	Dring & Lüning (1975)
Alaria esculenta	Photoreactivation	1.9	This study
Laminaria hyperborea	Photoreactivation	2.5	This study
Laminaria saccharina	Photoreactivation	1.2	This study

enzymes are certainly influenced by blue light either through coarse or fine control. If the same principle holds true, therefore, it seems reasonable to say that a blue light absorbing DNA-photolyase is likely to be involved in the blue light induced reactivation of UV-irradiated early sporophytes of members of the Laminariales and the flavoenzyme may be one of the blue light photoreceptors widespread in the brown algae. As the details of response may differ somewhat with different species, the nature of this speculated photoreceptor awaits better characterization by action spectrum determination.

General discussion

The aim of this study was to discover the physiological attributes of four species of laminarian early sporophytes towards radiation as an ecological factor and to attempt to predict their respective successes or failures in competition with each other in field.

The comparison of light requirements for growth showed that the saturating irradiance level is similar in all the species at the very early stage and as low as that for extreme shade plants. This may reflect firstly that light is not likely to be an ecologically differentiating factor under saturating conditions and secondly that photophysiological characteristics of very early stages of these species may be an adaptation to the dim light climate possibly encountered in an established kelp community.

Underwater photon irradiance is diminishing not only with depth but also with penetrating a canopy. Irradiance just above the rock amongst the kelp forest is severely reduced to 1-28% of that in open water at the same depth (Kitching 1941, Kain 1966). Therefore, under poor light conditions, greater tolerance to the shading conditions may be an operative factor to the successful habitation of the benthic environment. A culture study made in the dark showed that ***L. hyperborea*** was more tolerant of a long period of darkness than ***L. digitata.*** Whilst prolonged periods of total darkness will be of very limited occurrence in the field, situations of extremely low light are by no means infrequent, suggesting that this comparative experiment may indicate the potential survival of the species at very low irradiances, i.e. below the compensating point. Together with this, the higher growth rate of ***L. hyperborea*** than of ***L. digitata*** under reduced

levels of daylight in late autumn appears to confirm the likely success of ***L. hyperborea*** compared with ***L. digitata*** when competition is for light.

Seaweeds growing in different environments show morphological variability (Dahl 1971, Mathieson et al. 1981, Norton et al. 1981). Selection for efficient light capture has been an important factor affecting the external morphology of seaweeds (Hay 1986). Although light-related processes do not seem to cause morphological differences at the intraspecific level, thallus form of early sporophytes of different species expressed in terms of L/W ratio may manifest habitat-linked characteristics. When the susceptibility to herbivores or physical stresses are ignored, plants of translucent and elongated thallus form such as ***A. esculenta*** and ***L. digitata*** will procure a competitive advantage in well-lit area compared with plants with other types of thalli, by enabling the thallus to reach a favourable light. On the other hand, the broader thallus of ***L. hyperborea*** may confer on this plant an advantage in low-light environments since a greater proportion of the cells can be oriented perpendicular to the light source. Morphological plasticity found in ***L. saccharina*** presumably reflects its persistence over a wide range of habitats. A plant's response to the environment may arise quite early in its development, but also it may develop gradually throughout its life time or at least intermittently during periods of active growth (Norton et al. 1982). Therefore, long-term experiments would be required before it could be shown whether or not these initial differences in morphological development were reflected in the form of the adult thallus.

At levels of light below the saturating point the growth rate of laminarian early sporophytes is proportional to the total quanta received daily. As Chapman and Burrows (1970) stated, the close

correlation between the development (or growth) and total light energy available may be of general application to large sublittoral brown algae. It appears that this is an important facility for plants to utilse available quanta for growth in a habitat where light levels fluctuate constantly. Although all the species tested showed a similar growth response to total daily irradiance, there seems no contravention of the general ecological tenet that different species may have different ecological requirements. In this study, the ability of ***L. hyperborea*** and ***L. saccharina,*** sublittoral algae, to use available light was limited at lower MDI than that of ***A. esculenta,*** a species developing from the low eulittoral to upper sublittoral zone. Also, in long day conditions, the latter plants showed much higher growth rate. Once plants colonize vacant rock in winter, therefore, advent of spring may put ***A. esculenta*** at an advantage over the other species since increase in MDI will promote more rapid growth of the former species. However, experimental work in this study showed that although growth of early sporophytes in all the species used became slower with age, the rate of growth reduction was considerably faster in ***A. esculenta*** than in ***L. hyperborea*** or ***L. saccharina.*** When competing with the other species, the growth reduction of ***A. esculenta*** with age may be made up for by the ability to respond to more broad ranges of MDI but only in well-lighted environments. This might be one of the reasons why ***A. esculenta*** occurs mainly in shallow water, replaced by ***Laminaria*** populations in deeper water (Kitching 1941, Kain 1971b, Lewis 1971).

It has already been pointed out in many brown algal species that there is a sudden switch in light requirements and it is more striking at a particular stage than any other in the developmental stages (Norton and Burrows 1969, Fei and Neushul 1984, Fei 1985, Fei et al. 1989, Hales and Fletcher 1989). In this study, although the length of ***L. digitata*** at

75 μmol $m^{-2}s^{-1}$ was marginally greater than at 36 μmol $m^{-2}s^{-1}$ for the first 12 days, the length of the former far exceeded that of the latter after 24 days. In ***L. hyperborea,*** however, such difference was not detected. It is noticeable that the light demand of ***L. digitata*** for growth at the later stage is almost the same for that of ***L. saccharina*** adult plants reported by Fortes and Lüning (1980). The cause for this change in growth response to light may be explained by the assumption that small competing plants would reduce the light level encountered by the smallest stages, but as soon as the plants grow in length and extend above the turf, they would encounter higher light levels, and therefore, need the abilities to make efficient use of them (Fei and Neushul 1984). Frequent losses of canopy plants may also contribute to allowing higher light levels to the plants (Kain et al. 1976, Kirkman 1981).

In contrast to the other laminarian sporophytes, ***L. hyperborea*** did not grow faster in 12:12 h light: dark cycle relative to continuous light. The different growth pattern may not be due so much to differences in photosynthetic rates but to the way in which the photosynthates are used. From this study, it can be postulated that ***A. esculenta*** and ***L. digitata*** may channel most of their photosynthates into growth, and ***L. hyperborea*** rather into stored reserves. As light levels fall below the compensation point in deeper water during winter, stored materials may be necessary to sustain plants until spring. In this situation, ***L. hyperborea*** may have a competitive advantage over ***A. esculenta*** or ***L. digitata*** which has low energy reserves. In suggesting a control of physiological capability to use photosynthates over the behaviour of laminarian early sporophytes in field, however, a question arises as to why then ***L. hyperborea*** has a more southerly northern limit than the other species. In spite of the fact that at the extreme north limits, the available light may be much lower than that in more southerly range

during winter periods, and the capability of building up materials for winter periods should be of great advantage, ***L. hyperborea*** has the least penetration northwards among the species used in this study, being confined to the Norwegian coast (Kain 1969). It is known that ***L. hyperborea*** is the only species of the four to have a sporing period confined to winter in Britain (Kain 1969). If this holds true in the northern limit, this may explain the unlikely establishment of this species during dark winters despite the ability to use stored materials.

Different species of algae may have different systems for the utilization of light (Jones and Dent 1971). In this study, radiation of different spectral qualities revealed some differences in the growth responses between different species. It is of particular interest that ***L. hyperborea*** showed a significantly lower growth rate in red than in blue or green light at low irradiances. In young sporophytes of ***L. hyperborea*** (1st year plants), there was little difference in the action spectrum of photosynthesis between different light qualities at low irradiances (Dring 1986). If this is also the case for early sporophytes, different growth rates in different light qualities found in this study may have been due to an unbalanced relationship between photosynthesis and growth. The enhancement of protein synthesis by blue light and increases in the rate of carbohydrate synthesis by red light have been studied in some algae (Kowallik 1970, 1982, Clauss 1972, Müller and Clauss 1976, Senger and Briggs 1981). Likewise, it may be suggested that at low irradiance blue light leads to the production of protein and therefore directly to growth of early sporophytes, whereas red light leads to the production of carbohydrate which may just accumulate as a promise of future growth. According to Dring (1982), on the other hand, chlorophyll concentration increases during the growth and maturation of a seaweed thallus. French (1967) reported

that in a diatom (***Phaeodactylum tricornutum***), 16 day-old cultures contained a pigment absorbing one of the red wavebands not evident in 5 day-old cultures and there was a proportionate increase in chlorophyll a '680' relative to chlorophyll a '670' with age. Although 'age' in the cultures is not the age of an individual a slow development of chlorophyll system may be one of the candidates to explain a slower growth of early sporophytes of ***L. hyperborea*** in red light than in the other light fields. This may enable this species to achieve economies during the settling process in deeper water where most of the red waveband is cut off and blue and/or green wavebands are dominant, by making only the pigments to be used effectively and immediately at the prevailing light climate without paying too much protein cost for formation of chlorophyll. Seemingly, this is comparable to a chromatic adaptation, but can not be considered to be so since there was no difference in growth rate in all the spectral qualities at a higher irradiance, implying that the response is not independent of photon irradiances. Therefore, poor growth in red light of low irradiance compared with that in the other spectral light fields may be a byproduct made during the period of the early developmental stage of sporophytes of ***L. hyperborea.*** This hypothesis, however, must be in abeyance to be proven until a pigment extraction is performed on plants grown in different light qualities or a growth experiment is made at different irradiances under the same red light source (in this study, different red light sources were used for different photon irradiance treatments). The dependence of the growth rate of ***A. esculenta*** not on light quality but on photon irradiance is consistent with the so-called 'intensity adaptation' (Dring 1981, Ramus 1983). If the hypothesis of chlorophyll formation with age made for ***L. hyperborea*** is introduced, it appears that ***A. esculenta*** sporophytes may

have well developed pigment system at the very early stage compared with ***L. hyperborea.*** It is known that plants exposed to long periods of normal light rely to a much greater extent on chlorophyll a alone for photosynthesis (Boney and Corner 1962). In this context, ***A. esculenta*** seems to comply with the characteristics to put this plant at an advantage in the place where all the wavelengths are available such as in shallow water. Different growth responses to spectral qualities depending on photon irradiances shown by ***L. saccharina*** seem to be different from those of ***L. hyperborea*** in that the growth pattern of the former species in different light qualities was considerably different between a relatively small span of photon irradiances, i.e. at 5 μmol $m^{-2}s^{-1}$, growth rate in red light was much lower than that in the other light fields, but at 13 μmol $m^{-2}s^{-1}$ there was no significant difference in growth rates between the light fields. If early sporophytes of ***L. saccharina*** responded to spectral composition of light fields by altering the overall pigment contents as has been conjectured to be the case for gametophytes of the same species (Lüning and Dring 1975), it might be suggested that ***L. saccharina*** is very sensitive to the red waveband in response to changes in irradiance.

Marine plants that grow just above ELWS may be frequently exposed to irradiances above saturation (Dring 1982). Therefore, differential sensitivity towards high levels of light may be a major factor in algal distribution in the upper sublittoral (Lüning 1981a). In this study, of the four species, a limited population of ***L. hyperborea*** and ***L. saccharina*** showed a significant growth inhibition at a high irradiance. Furthermore, excessive blue light was found to be involved in the photoinhibition of growth of ***L. hyperborea.*** Considering that certain enzymes such as cytochrome and flavoprotein absorb light especially in the blue region, respiratory apparatus containing those enzymes seems

to be a likely target of photoinhibition (Epel 1973). Codd and Stewart (1980) also found that high irradiance of blue light inhibited the activity of ribulose biphosphate carboxylase. As Biebl (1952) suggested, on the other hand, the resistance to strong sunlight appeared to be the typical characteristics of an ecological resistance even for the very early stage of laminarian sporophytes. In this study, ***A. esculenta*** and ***L. digitata,*** shallow water dwelling species were much more tolerant of high irradiance of sunlight than ***L. hyperborea,*** a deeper water inhabitant. Unless early sporophytes are settled in open places, however, constant exposure to a direct sunlight would not always be predictable. Besides, for instance, in Isle of Man, low water springs occur in the early morning and evening, making sparser the possibility that plants encounter a high flux of photons. In overshadowed conditions where early stages of plants may start their establishment, therefore, the susceptibility of early sporophytes to high irradiance must be found in another context. In the sea, large changes in surface irradiance are sometimes damped by the shading effect of a canopy, but conversely, gaps opening or closing in the canopy sometimes results in large fluctuations in kelp forest irradiance (Gerard 1984). Laminarian early sporophytes may be acclimated to the irradiance constantly provided by the shade of a canopy and this may be maintained on a seasonal basis. It has also been known that the kelp communities can be compared with a monospecific stand of trees with an understorey of a few seedlings ready to develop once an opening appears in the canopy (Kirkman 1981). Therefore, a suitable break in the canopy may provide a chance for early sporophytes to be established by using higher irradiances. However, this study showed it unlikely that early sporophytes of ***L. hyperborea*** acclimated to very low irradiance can manage to grow when they encounter photon irradiance of more than

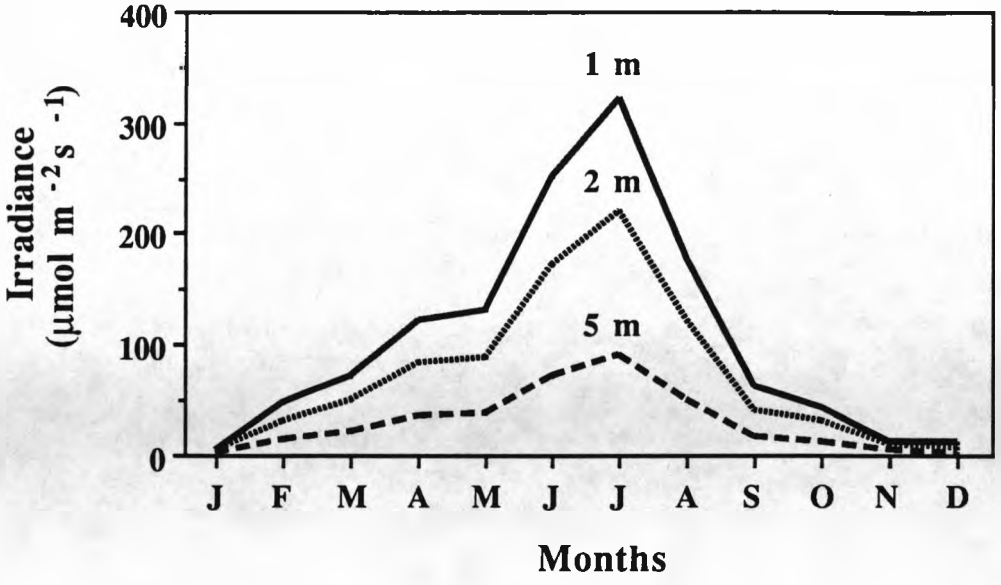

Fig. 5.1. Monthly means of recorded photon irradiance at different depths below LAT at Port Erin (replotting from Kain et al. 1976, using the original data). The irradiances at 1 and 2 m were calculated as percentages of those at 5 m, using Jerlov's (1966) transmittance data.

159 μmol $m^{-2}s^{-1}$. As seen in Fig. 5.1, this irradiance is reaching at 1 m depth below LAT in a spring period in Port Erin, Isle of Man (replotting from Kain et al. 1976, using the original data). Observing the vertical distribution in nearly all sites in the Isle of Man, Kain (1962) described that the upper limit of ***L. hyperborea*** varied from 0.9 above to 1.6 m below ELWS. As there is no remarkable difference between LAT and ELWS, concurrence of the two limits seems to suggest that a high sensitivity of low light acclimated plants to high irradiance may in part delimit the upper limit of ***L. hyperborea***. However, it can not be ruled out that within a limited range, ***L. hyperborea*** may have a potential to extend upwards on the shore, judging from an active growth of high light acclimated plants at the irradiance which was damaging to low light acclimated plants. In contrasts, ***L. digitata*** showed a fast growth even when the plants were transferred from low to very high irradiance, implying that early sporophytes are likely to replace gaps or openings in the canopy rapidly by use of high irradiance at shallow depth. This seems to agree with the field observation (Kain 1962) that the upper limit of ***L. digitata*** varied from ELWS to 2.5 m above it. Also, this light-related trait together with that previously found in this study, such as sustained growth rate with age, a sudden switch in sensitivity to high irradiance may suggest that ***L. digitata*** is able to hold its place at shallow water, baffling the possible penetration of ***L. hyperborea***. However, direct tests of these hypotheses in the field are required since it is improbable that the results of any experiments carried out in a laboratory condition can be directly applied to account for the behaviour of plants under natural conditions. In this context, it may be noteworthy that in a field experiment started in summer, initial canopy removal and successive weeding of ***L. digitata*** allowed ***L. hyperborea*** to extend its upper limit into ***L. digitata*** zone (Hill, personal

communication).

Contrary to the sensitivity to sunlight, that to UV radiation (253.7 nm) does not seem to bear any environmental relation. This may be not only because the resistance to UV is determined by different characteristics of the tissues of different species (Biebl 1952), but also because the chromophore to absorb UV is different from that of sunlight (Halldal and Taube 1972). While the typical photosynthetic pigments, such as chlorophylls and carotenoids are rather insensitive to far-UV irradiation (see reviews by Halldal and Taube 1972), liability of UV damage is primarily known to be nucleic acids (Caldwell 1981). Photoreactivation, known as a enzyme-mediated, light-dependent recovery from UV damage, was found to occur in laminarian early sporophytes. The results extend previous findings made on various other organisms to marine multicellular algae. In this process, only the blue waveband of the visible spectrum is effective and the role of blue light is analogous to that in other morphogenetic responses of brown algae to blue light in that there is a similarly high quantum requirement and long-term reciprocity. Supposing that the necessity of the repair system of DNA damaged by far-UV might have been abolished due to oxidation of the primitive earth and, therefore, the enzyme involved in the process may have acquired independent and diverse photoreceptor functions (Galland and Senger 1991), the similarity of blue light sensitivity in photoreactivation of laminarian early sporophytes to that in multifarious blue light responses reported in brown algae may not be unexpected.

In conclusion, it is suggested that light is of probable importance in determining the ecological distribution of early stage of laminarian species. Except at the extremes where tolerance is an ultimate factor

for a species to survive, for instance under direct sunlight, a species could be limited to a habitat where the light-related traits enable the plants to outcompete those of other species, hence ***A. esculenta*** and ***L. digitata*** to upper sublittoral and ***L. hyperborea*** to deep sublittoral. The result of this study does not seem to be enough to put ***L. saccharina*** either in shallow water group or in deep water one. This agrees with other reports about the plasticity of light-related traits in ***L. saccharina.*** To understand the ecology of species of Laminariales, however, it should also be kept in mind that other factors such as desiccation, temperature, grazing pressure and/or presence of epiphytes may operate in concert to determine the ultimate distribution of the species and the arena of competition may change during further developmental stages.

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Appendices

Appendix 1.

ANOVA table for fertility of ***L. hyperborea*** early sporophytes in different media. *** $p \leq 0.001$, n.s. not significant at $p = 0.05$.

<Expt. 1>

Source	DF	SS	MS	F
Between groups	3	1307.814	435.938	11.47***
Within groups	76	2888.541	38.007	
Total	79	4196.355		

<Expt. 2>

Source	DF	SS	MS	F
Between groups	3	949.168	316.389	10.43***
Within groups	76	2304.527	30.323	
Total	79	3253.694		

Appendix 2. ANOVA table for RGR of sporophytes of *L. hyperborea* transferred to 55 μmol $m^{-2}s^{-1}$ and 135 μmol $m^{-2}s^{-1}$ under Northlight. **0.01 >p≥ 0.001, *0.05 >p≥ 0.01, n.s. not significant at p= 0.05.

(a) Transfer after 8 day-acclimation to 19 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	9.025×10^{-5}	9.025×10^{-5}	3.57 n.s.
Within groups	2	5.050×10^{-5}	2.525×10^{-5}	
Total	3	1.407×10^{-4}		

(b) Transfer after 20 day-acclimation to 19 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	0.001	0.001	250.00**
Within groups	2	5.000×10^{-6}	2.500×10^{-6}	
Total	3	0.001		

(c) Transfer after 8 day-acclimation to 55 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	2.250×10^{-4}	2.250×10^{-4}	2.43 n.s.
Within groups	2	1.850×10^{-4}	9.250×10^{-5}	
Total	3	4.100×10^{-4}		

(d) Transfer after 20 day-acclimation to 55 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	0.001	0.001	2.54 n.s.
Within groups	2	0.001	2.562×10^{-4}	
Total	3	0.001		

Appendix 3. ANOVA table for RGR of sporophytes of ***L. digitata*** transferred to 55 μmol $m^{-2}s^{-1}$ and 135 μmol $m^{-2}s^{-1}$ under Northlight.

(a) Transfer after 7 day-acclimation to 19 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	2.50×10^{-7}	2.50×10^{-7}	0.003 n.s.
Within groups	2	1.89×10^{-4}	9.43×10^{-5}	
Total	3	1.89×10^{-4}		

(b) Transfer after 14 day-acclimation to 19 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	0.006	0.006	86.04*
Within groups	2	1.325×10^{-4}	6.625×10^{-5}	
Total	3	0.006		

(c) Transfer after 7 day-acclimation to 55 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	0.002	0.002	8.44 n.s.
Within groups	2	4.905×10^{-4}	2.452×10^{-4}	
Total	3	0.003		

(d) Transfer after 14 day-acclimation to 55 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	0.003	0.003	6.06 n.s.
Within groups	2	0.001	4.810×10^{-4}	
Total	3	0.004		

Appendix 4. ANOVA table for RGR of sporophytes of ***L. hyperborea*** transferred to 63 μmol $m^{-2}s^{-1}$ and 159 μmol $m^{-2}s^{-1}$ under Polylux 4000.

(a) Transfer after 8 day-acclimation to 15 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	3.600×10^{-5}	3.600×10^{-5}	0.10 n.s.
Within groups	2	0.001	3.730×10^{-4}	
Total	3	0.001		

(b) Transfer after 16 day-acclimation to 15 μmol $m^{-2}s^{-1}$

As naught growth rate was recorded of the plants transferred to 159 μmol $m^{-2}s^{-1}$ due to complete bleaching, ANOVA could not be performed on the data.

(c) Transfer after 8 day-acclimation to 63 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	1.225×10^{-5}	1.225×10^{-5}	0.55 n.s.
Within groups	2	4.450×10^{-5}	2.225×10^{-5}	
Total	3	5.675×10^{-5}		

(d) Transfer after 16 day-acclimation to 63 μmol $m^{-2}s^{-1}$

Source	DF	SS	MS	F
Between groups	1	0.001	0.001	0.68 n.s.
Within groups	2	0.002	0.001	
Total	3	0.003		

Appendix 5.

ANOVA table for (arcsine-transformed) survival percentage of UV-irradiated ***L. hyperborea*** in various types of irradiation (***$p \leq 0.001$).

Source	DF	SS	MS	F
Between groups	3	1627.242	542.414	18.16***
Within groups	8	238.915	29.864	
Total	11	1866.157		

Appendix 6.

Calculation of relative effectiveness of blue quanta in the two light sources. White-Northlight, Blue-blue fluorescent tube + Cinelux filter.

Wavelength	White-output	No of quanta	No/Total (NW)	Blue-output	No of quanta	No/Total (NB)	2-D Growth	2-D/79.98 (P)	NW * P	NB * P
400.000	34.250	13700.000	0.007	134.440	3361.000	0.013	32.680	0.409	0.003	0.005
405.000	131.520	53265.600	0.026	262.680	25217.280	0.098	44.720	0.559	0.014	0.055
410.000	38.305	14885.050	0.007	168.900	4475.850	0.017	34.400	0.430	0.003	0.008
415.000	35.820	14782.300	0.007	175.610	4565.860	0.018	36.120	0.452	0.003	0.008
420.000	39.045	16398.900	0.008	183.030	5216.355	0.020	58.480	0.731	0.006	0.015
425.000	42.470	18049.750	0.009	186.630	5785.530	0.023	63.640	0.796	0.007	0.018
430.000	46.580	20029.400	0.010	190.060	6462.040	0.025	67.080	0.839	0.008	0.021
435.000	253.450	110250.750	0.053	503.650	93175.250	0.363	79.980	1.000	0.053	0.363
440.000	94.530	41593.200	0.020	240.940	16824.860	0.065	73.980	0.925	0.019	0.060
445.000	56.170	24995.650	0.012	178.960	7337.360	0.029	69.660	0.871	0.011	0.025
450.000	59.595	26817.750	0.013	168.700	7338.450	0.029	67.080	0.839	0.011	0.024
455.000	61.650	28050.750	0.014	154.770	6964.650	0.027	71.380	0.892	0.012	0.024
460.000	63.705	29304.300	0.014	148.250	6893.625	0.027	62.460	0.856	0.009	0.018
465.000	65.760	30578.400	0.015	141.530	6793.440	0.026	53.320	0.667	0.010	0.018
470.000	66.445	31229.150	0.015	125.520	6087.720	0.024	42.140	0.527	0.008	0.013
475.000	67.130	31886.750	0.015	113.850	5578.650	0.022	37.840	0.473	0.007	0.010
480.000	66.445	31893.600	0.015	102.900	4990.650	0.019	17.200	0.215	0.003	0.004
485.000	67.815	32890.275	0.016	92.530	4580.235	0.018	30.100	0.376	0.006	0.007
490.000	68.500	33565.000	0.016	82.670	4133.500	0.016	30.100	0.376	0.006	0.006
495.000	67.130	33229.350	0.016	73.620	3607.380	0.014	25.800	0.323	0.005	0.005
500.000	65.760	32880.000	0.016	63.460	3046.080	0.012	12.900	0.161	0.003	0.002
505.000	65.075	32862.875	0.016	56.070	2863.325	0.010				
510.000	64.390	32838.900	0.016	47.570	2235.790	0.009				
515.000	63.020	32455.300	0.016	41.130	1891.980	0.007				
520.000	63.020	32770.400	0.016	34.930	1606.780	0.006				
525.000	62.335	32725.875	0.016	29.560	1344.980	0.005				
530.000	60.965	32311.450	0.016	24.080	1071.560	0.004				
535.000	59.595	31883.325	0.015	20.010	870.435	0.003				
540.000	59.595	32181.300	0.016	16.300	709.050	0.003				
545.000	165.770	90344.650	0.044	79.380	9604.980	0.037				
550.000	78.775	43326.250	0.021	17.810	1024.075	0.004				
555.000	57.540	31934.700	0.015	6.300	264.600	0.001				
560.000	58.910	32989.600	0.016	4.450	191.350	0.001				
565.000	58.910	33284.150	0.016	3.230	138.890	0.001				
570.000	59.595	33969.150	0.016	2.080	90.480	0.000				
575.000	78.775	45295.625	0.022	3.930	225.975	0.001				
580.000	84.940	49265.200	0.024	3.620	224.440	0.001				
585.000	61.650	36065.250	0.017	0.770	34.650	0.000				
590.000	61.650	36373.500	0.018	0.570	25.650	0.000				
595.000	61.650	36681.750	0.018	0.390	17.550	0.000				
600.000	62.335	37401.000	0.018	0.280	12.740	0.000				
605.000	62.335	37712.675	0.018	0.210	9.555	0.000				
610.000	62.335	38024.350	0.018	0.160	7.280	0.000				
615.000	61.650	37914.750	0.018	0.140	6.300	0.000				
620.000	60.965	37798.300	0.018	0.120	5.340	0.000				
625.000	60.965	38103.125	0.018	0.120	5.340	0.000				
630.000	59.595	37544.850	0.018	0.100	4.350	0.000				
635.000	58.225	36972.875	0.018	0.120	5.100	0.000				
640.000	55.485	35510.400	0.017	0.150	6.075	0.000				
645.000	54.800	35346.000	0.017	0.180	7.200	0.000				
650.000	52.060	33839.000	0.016	0.140	5.320	0.000				
655.000	50.690	33201.950	0.016	0.140	5.180	0.000				
660.000	48.635	32099.100	0.016	0.140	4.970	0.000				
665.000	45.210	30064.650	0.015	0.060	1.980	0.000				
670.000	43.155	28913.850	0.014	0.060	1.890	0.000				
675.000	39.045	26355.375	0.013	0.060	1.710	0.000				
680.000	36.305	24687.400	0.012	0.060	1.590	0.000				
685.000	34.250	23461.250	0.011	0.080	2.000	0.000				
690.000	32.195	22214.550	0.011	0.130	3.055	0.000				
695.000	29.455	20471.225	0.010	0.000	0.000	0.000				
700.000	27.400	19180.000	0.009	0.000	0.000	0.000				

Total	0.207	0.707

Abstract: Effect of visible and UV radiation on early sporophytes of species of the Laminariales

Light-related behaviour of early sporophytes of species of the Laminariales was investigated in laboratory culture.

The growth of four species (*Alaria esculenta, Laminaria digitata, Laminaria hyperborea* and *Laminaria saccharina*) was similarly light-saturated at about 30 μmol $m^{-2}s^{-1}$. The minimum irradiance for growth of *L. hyperborea* (the only species used) seemed to be less than 1-2 μmol $m^{-2}s^{-1}$. In all four species, there was a reduction in the growth rate with age.

The length/width ratios of thalli of *A. esculenta* and *L. digitata* were high, irrespective of irradiance, while that of *L. hyperborea* was lower. The thallus shape of *L. saccharina* seemed related to growth rate.

In 17:7 h light-dark cycle, the growth rate of early sporophytes increased with increased irradiance up to 57 μmol $m^{-2}s^{-1}$, beyond which there was no significant increase. In 7:17 h light-dark, however, *A. esculenta* showed a significant increase in the growth rate up to 127 μmol $m^{-2}s^{-1}$ (the highest irradiance used). In the short-day an increase above 10 mol $m^{-2}s^{-1}$ MDI (Mean Daily Irradiance) had no effect on the growth rate of *L. hyperborea* and *L. saccharina*, but in the long day there was a significant increase.

The ratio of maximum growth rate under continuous light and 12:12 h light-dark cycle was 1.5:1 for *L. hyperborea*, 1.2:1 for *L. saccharina* and 1.1:1 for both *A. esculenta* and *L. digitata*.

After 24 days, *L. digitata* but not *L. hyperborea* was found to require higher irradiance for faster growth with time. Compared with *L. hyperborea, L. digitata* was short-survived in the dark and showed a slower growth in extremely reduced daylight conditions.

L. hyperborea showed a significantly lower growth rate in red than in blue or green light at low irradiances but the growth rate of *A. esculenta* seemed to depend more on light quantity than on light quality. *L. saccharina* appeared to be sensitive to the red waveband in response to changes in irradiance.

In *L. hyperborea* (the only species tested), the growth response did not seem to be correlated with the arrangement of phaeoplasts.

Of the four species, a limited population of *L. hyperborea* and *L. saccharina* showed a significant growth inhibition at 180 μmol $m^{-2}s^{-1}$. Furthermore, excessive blue light was found to be involved in the photoinhibition of growth of *L. hyperborea*. *A. esculenta* and *L. digitata* were more tolerant of high irradiance of sunlight than *L. hyperborea*.

Early sporophytes of *L. hyperborea* acclimated to 13-19 μmol $m^{-2}s^{-1}$ for 14-20 days were growth-inhibited or photobleached with no sign of growth when they were transferred to 135-159 μmol $m^{-2}s^{-1}$. On the other hand, *L. digitata* showed a fast growth even when the plants were transferred from the low to the high irradiance. Neither species acclimated to 55-63 μmol $m^{-2}s^{-1}$ showed any inhibition in growth at 135-159 μmol $m^{-2}s^{-1}$. In addition, acclimation at 55 μmol $m^{-2}s^{-1}$ allowed *L. digitata* and *L. hyperborea* higher survival under direct sunlight of sublethal dose than did that at 8 μmol $m^{-2}s^{-1}$.

Exposure of UV-irradiated early sporophytes to visible light resulted in recovery from UV damage that would otherwise cause much higher mortality. For this photoreactivation, blue light was highly effective, whereas negligible reactivation was produced either in green or red light. The response in white light was proportional to the blue band it contained. The blue quantum requirement for 50% response was 1.2 mol m^{-2} for *L. saccharina*, 1.9 mol m^{-2} for *A. esculenta* and 2.5 mol m^{-2} for *L. hyperborea*.