LIGHT QUALITY EFFECTS ON CELLULAR ORGANELLES, METABOLISM AND FUNCTION OF ACID-BASE BALANCE IN *Ulva pertusa* KJELLMAN

アナアオサの細胞内小器官、代謝と酸・塩基平衡機能に及ぼす光波長の効果

BHASKARAN MUTHUVELAN

1998

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LIGHT QUALITY EFFECTS ON CELLULAR ORGANELLES, METABOLISM AND FUNCTION OF ACID-BASE BALANCE IN *Ulva pertusa* Kjellman

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INTRODUCTION

Seaweeds have been shown to reduce effectively the level of sea water eutrophication in integrated mariculture. In a series of studies, the genus *Ulva* have been reported as a biofilter (Ryther et al. 1975).

Ulva pertusa Kjellman (Ulvaceae, Chlorophyta) is a common species that grows on rock in the lower intertidal zone along almost the entire coast of Japan. This species also grows as a floating alga. The floating one, which has a thallus of yellowish green and also thinner than the wild type, is considered as a sterile mutant, because of its continuous vegetative development without forming any swarmers. And this floating type can grow faster than the wild type and also grows well during the summer when the other one disappears (Migita 1985).

With a view for future cultivation of this alga in the integrated mariculture system, studies have been carried out to determine the light intensity, temperature and salinity requirements for its optimal development (Floreto et al 1993), and also in a polyculture system with red sea bream and prawn *Penaeus japonicus* (Danakushumah and Hirata 1991, Hirata and Xu 1990). So far, the polyculture system was designed by floating *Ulva pertusa* in a net on the surface of the cage, but a lot of difficulties seemed certain to arise

during the typhoon and monsoon seasons. Since it is a floating alga, it would be better to cultivate in the bottom level of culture site. Regarding this, another factor which shows considerable variation in the natural environment of this depth is light quality, and its influence has never been investigated for this species.

Algal adaptation to different light intensities is known to vary the thylakoid components particularly the antennae size and number of photosynthetic complex. In contrast to this not much work has been done on the effect of light quality which is very important as they exist in different environments. Since this species is a member of Chlorophyta, it usually grows in the lower intertidal zone but hardly in the subtidal region.

From the above stand points, the present study was conducted to find out the solution of the following objectives: i) whether the light quality has considerable influence on the morphology and organelle development. If it was present, how it affects the development and morphogenesis of *Ulva pertusa*. ii) What do the physiological changes occur in *U. pertusa* during this treatment?. And the main aim was to reveal the biofilter ability through nitrogen uptake and

utilization at different light quality. The nitrogen in the form of nitrate (nutrient) or ammonia (mainly excretion) makes the aquaculture system more polluted and allows to grow numerous organisms as competitors. Regarding this, the analysis was extended to examine the nitrogen uptake from the medium, protein content, and nitrogen reductase activity in the *U. pertusa* cells. iii) Further investigation was extended to the interrelationship between *U. pertusa* with ambient environment (mainly CO_2, HCO_3^-, O_2, and pH are monitored). Then the roll of CO_2, and HCO_3^- as feed back functions and the inter link with pH are discussed.

CHAPTER I

LIGHT QUALITY EFFECT ON THE MORPHOLOGY AND DEVELOPMENT OF ORGANELLES IN *Ulva pertusa*

1. 1 Introduction

Algal adaptation to different light intensities is known to vary the thylakoid components particularly the antennae size and number of photo system complex. In contrast to this not much work has been done on the effect of different light quality which is very important as existing in different environments. So far it is understood that in marine plants light quality affects development (Luning and Dring 1973), morphogenesis (Muller and Clauss 1976), growth (McLachlan and Bidwell 1983), photosynthesis (Luning and Dring 1985), and also distribution (Tremblin et al. 1993), in which the blue light generally has a stimulating effect and red light, an inhibitory effect.

The overall process for the formation of plant biomass can be divided as follows: i) absorption of incident light by phytochrome as well as photosynthetic pigments, ii) splitting of water and formation of reduced NADP and ATP, iii) these products then act as substrates in reduction of CO_2 to simple carbohydrates, iv) conversion of carbohydrates to plant biomass, involving fixation of several organic elements, and v) maintenance of cell integrity (e.g., maintaining the chemical gradients across the membranes and replacing organic compounds that decay during growth). The maintenance of cell integrity process is sometimes regarded as separate from

growth but utilizes the same substrate pools (Markager 1993). In this chapter development of the cell organelles and variation at different irradiation quality have been observed by an electron microscope.

1. 2 Material and Methods

The species of *Ulva pertusa* was collected from Azuma town, Kagoshima Prefecture, Japan. Only healthy and clean fronds were used to avoid wound respiration (Bidwell and McLachlan 1985). The cut discs of the algal thalli were incubated for at least 20 h before preparation in the same medium as that of the experiments.

In this experiment each treatment for white, blue and red lights was conducted in triplicates. For this a total number of nine 3L Erlenmeyer flasks were used. Each flask was autoclaved with a 2.5L culture medium (sea water with 2% ESP medium, Provasoli, 1968), and plugged with silicon caps (Fig. 1). Three openings were made in each silicon cap: the first one was used as an air inlet for filtered 0.2 μm air (air moisture), the second one was covered with a cotton plug and used as an air outlet, and the third one was used as a node to collect water samples. To avoid the evaporation of the medium during the experiment, air moisture was applied. As shown in the Fig. 1, air was first passed through a 1L

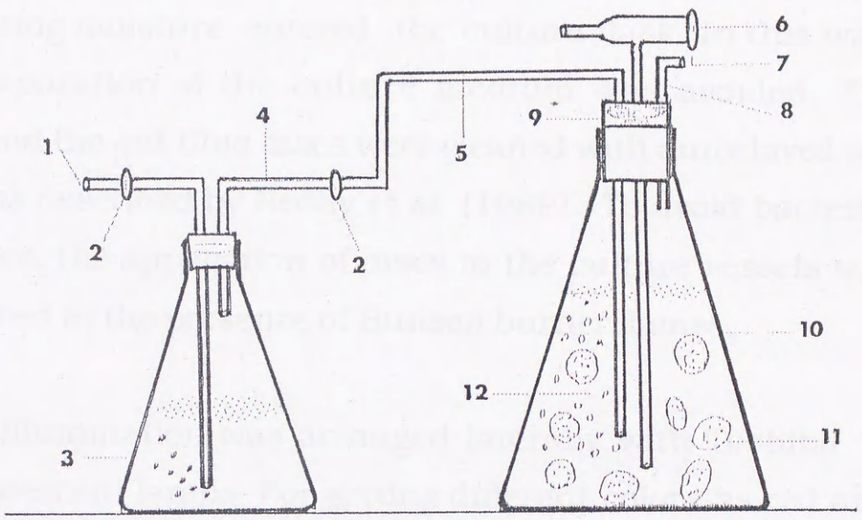

Fig.1. Illustration of experimental setup. 1, air inlet; 2, air filter; 3, autoclaved seawater; 4, air moisture outlet; 5, air moisture; 6, node for sampling(used only in later Chapter Ⅲ); 7, cotton plug; 8, air outlet; 9, silicon plug; 10, culture medium; 11, *Ulva* discs; 12, air bubbles.

Erlenmeyer flask, which contained autoclaved sea water. The inlet and outlet were capped with a 0.2 μm filter to avoid the passing of bacteria through air. Since the outlet of the 1 liter flask was connected to the inlet of the culture flask, only air containing moisture entered the culture flask. In this way, the evaporation of the culture medium was avoided. The thalli and the cut *Ulva* discs were cleaned with autoclaved sea water as described by Reddy et al. (1989). To avoid bacterial influence, the application of discs to the culture vessels was performed in the presence of Bunsen burner flames.

The illumination was arranged laterally with Toshiba 10 LD fluorescent lamps. For getting different colors as red and blue, the flasks were covered with respective color cellophane except the white light flask. The red one had isoquantic broad spectrum nanometers ranging from 600 to 700 nm and the peak transmission at 660 nm, and the blue one from 400 to 500 nm with a peak transmission at 430 nm. The transmitance of individual spectra was checked as described by Bird et al. (1981).

The experiment was conducted in a 14:10 h L: D photoperiod and the photon fluency rate for the three types of light quality was 55- 60 μmol m^{-2} s^{-1} (photosynthetically active radiation). Light intensity was checked and adjusted with a photometer (LiCor Model LI-185B) after the filter

(inside of culture flask). Aeration was given 24 h a day and the temperature was maintained at 25°C.

For observation of the ulrastructures, the samples were prefixed in 4% glutaraldehyde plus 0.1M cacodylate buffer with 0.25 M sucrose at pH 7.4 for 2 h at 4°C. The specimens were then washed three times with 0.1 M cacodylate buffer with 0.25 M, 0.12 M, and without sucrose at 4°C each for 30-45 min. They were post fixed for 10 h in 2% OsO_4 plus 0.1 M cacodylate buffer (pH 7.4) at 4°C. They were dehydrated through 25%, 50%, 70% and 80% ethanol each for 10 min at 4°C. The following immersion was continued at room temperature; 95% ethanol and 3 times in absolute ethanol for 20 min, then in absolute ethanol and QY-1 (1:1 and 1:3, respectively) each for 1 h and finally 2 times in QY-1 each for 30 min. The embedding was performed using an Epon mixture which contains EPON 812, 49.7 ml; DDSA, 29.4 ml; MNA, 23.8 ml; DMP-30, 1.5 ml. Sections were made by an ultramicrotome (LKB, Bromma 8800 Ultrome^R III) and stained with a saturated solution of 10% uranyl acetate in 50% ethanol for 15-30 min followed by 15 min in lead citrate (0.01 g in 10 ml of 0.1 M NaOH). They were observed at 100 KV under an electron microscope model H-700H (Hitachi Ltd).

Fig. 2. Morphological differences in cell organelles of *Ulva pertusa*. A, white light (WLC); B, blue light (BLC); C, red light (RLC). c, chloroplast; cw, cell wall; n, nucleus; p, pyrenoid; r, ribosome; s, starch; v, vacuole.

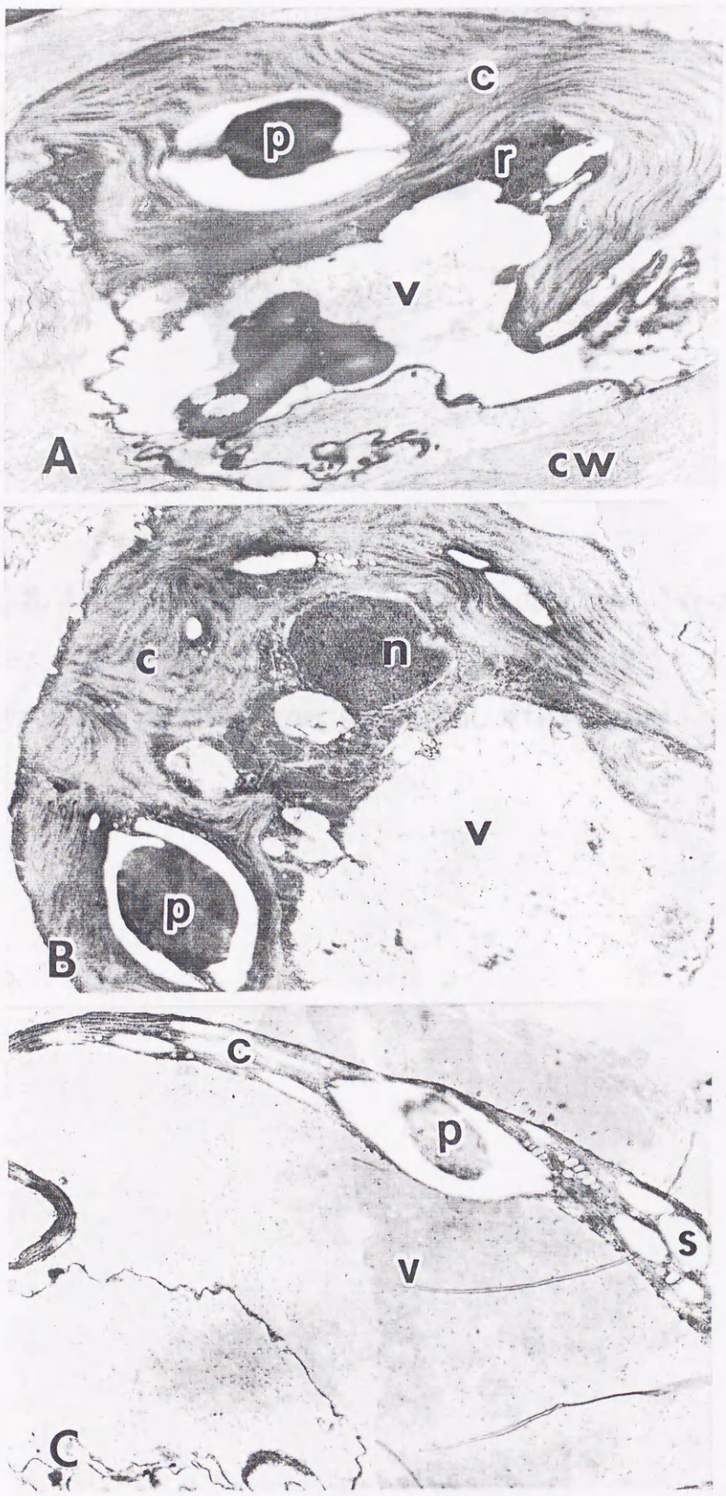
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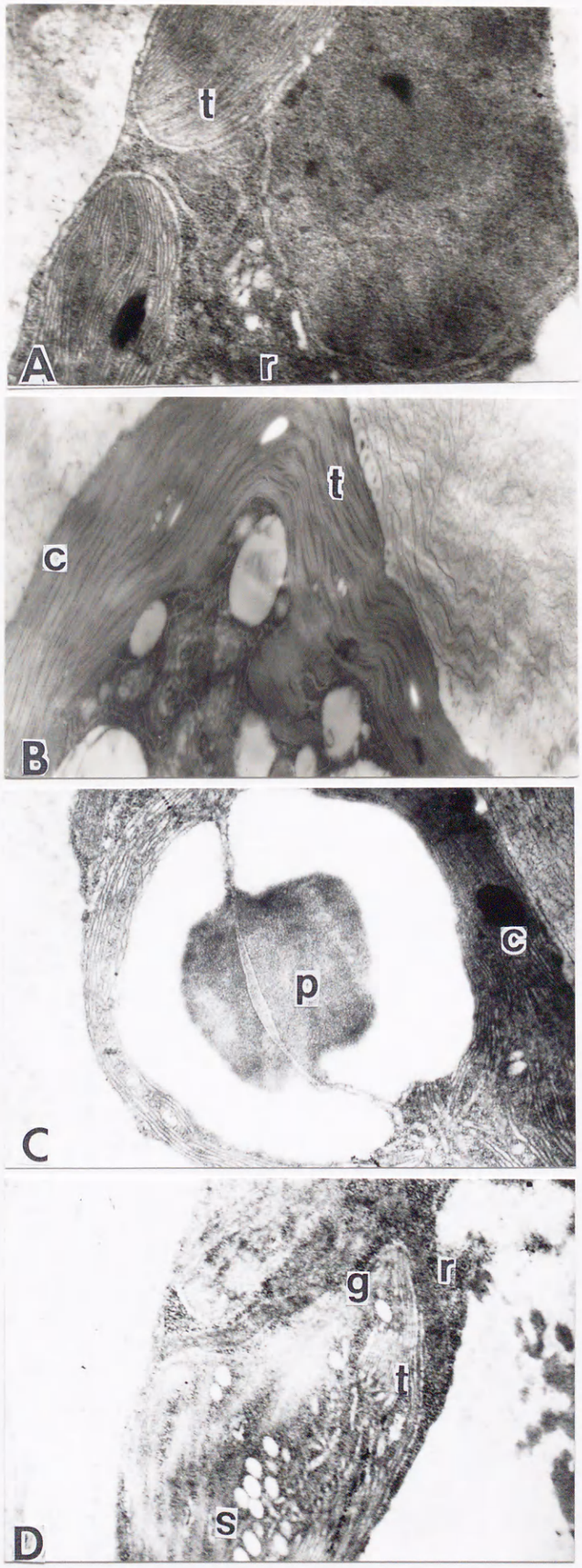
Fig. 3. Ultrastructures of cell organelles in *Ulva pertusa* under white light (WLC). c, chloroplast; g, grana; p, pyrenoid; r, ribosome; s, starch; t,thylakoid.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For quantitative measurements of sizes of cellular organelles on the negative of EM, a scale was put on respective length or breadth and the value was divided by its magnification. The measurement in μm was made through the length or breadth of organelles (in mm) divided by its magnification. For data, the analysis of variance (ANOVA) was applied to the groups. Then the mean values of the groups were subjected to *t*- test. Differences reported were as significant when $P < 0.05$ or $P < 0.01$.

1. 3 Results

1. 3. 1 Ultrastructures

From over 30 individual observations, the effects of light quality on the individual cell organelles of *Ulva* of white light culture (WLC), blue (BLC) and red radiation cultures (RLC) are shown in Figs. 2 - 6. The thalli of WLC clearly show well developed organelles (Fig. 2A). The chloroplast is clear with well developed thylakoids. The starch deposit in the chloroplast is less. The pyrenoid structures are clear with a dense protein body. At higher magnification, the thylakoids reveal two or three layered arrangement in most of the areas (Fig. 3A, B). The grana structures, which have not been found in most algae except in some chlorophycean members, are also observed in the *Ulva* grown under WLC conditions

Fig. 4. Ultrastructures of cell organelles in *Ulva pertusa* cultured under blue light (BLC). c, chloroplast; g, grana; n, nucleus; p, pyrenoid; r, ribosome; s, starch; t, thylakoid.

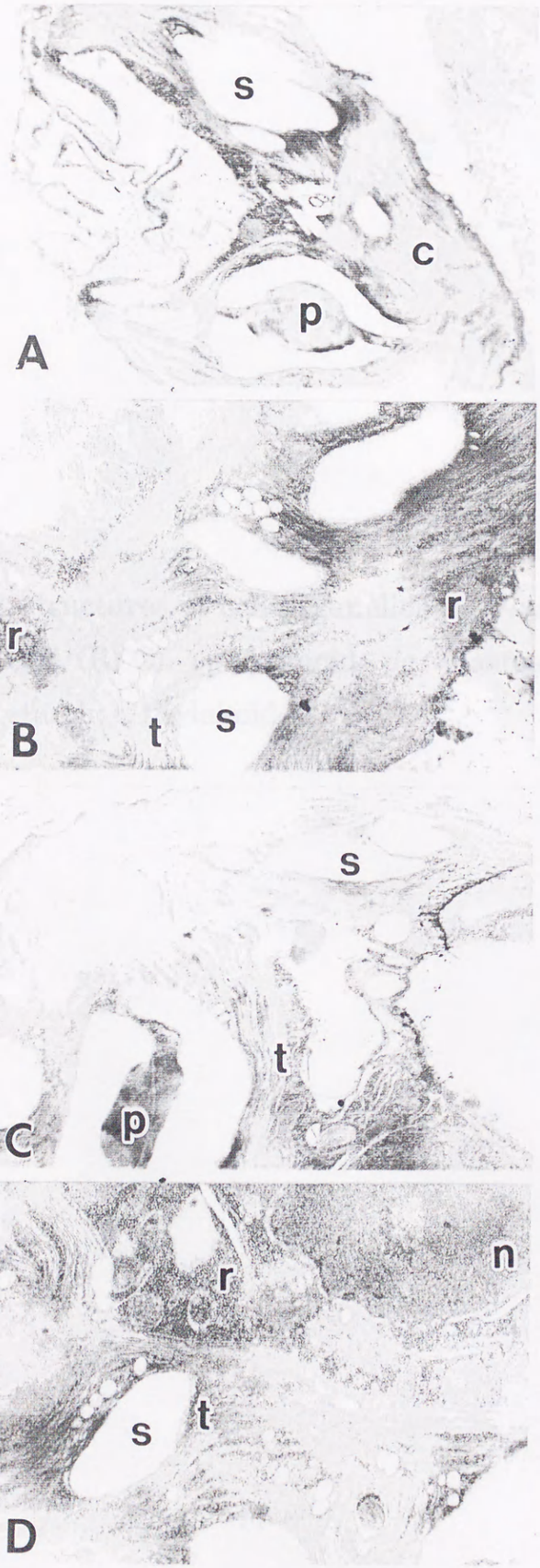
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Fig. 5. Ultrastructures of cell organelles in *Ulva pertusa* under red light (RLC). p, pyrenoid; pg, plastoglobuli; r, ribosome; s, starch; t, thylakoid.

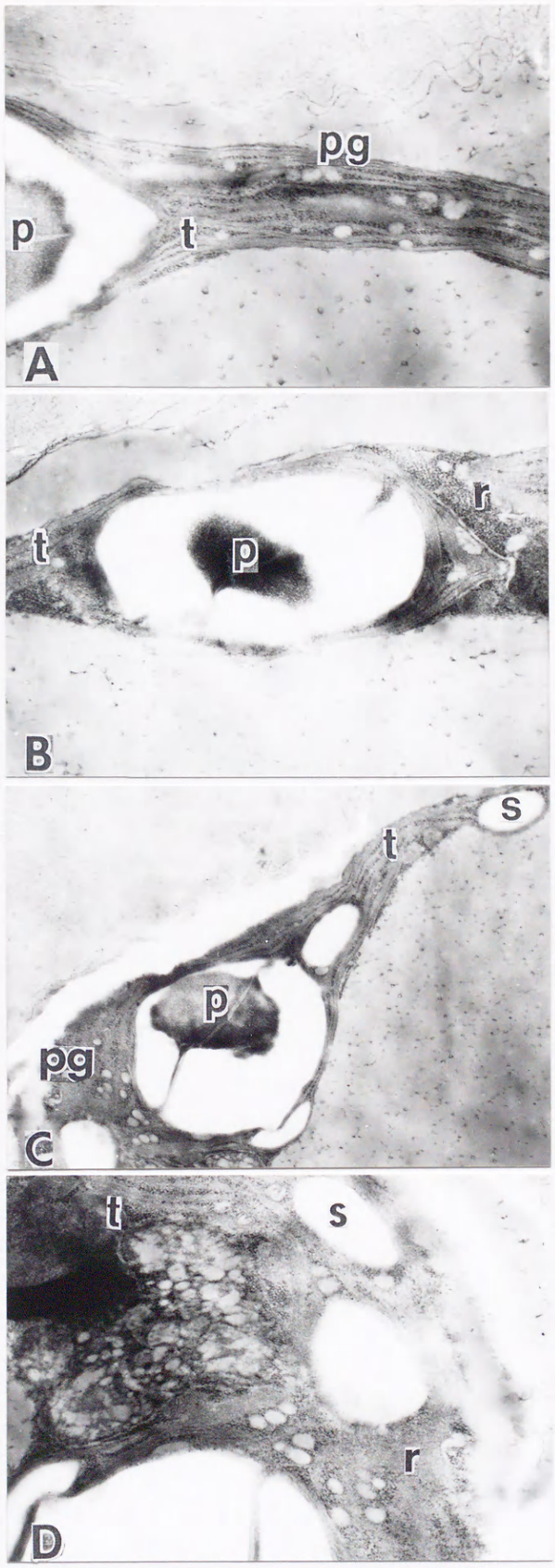
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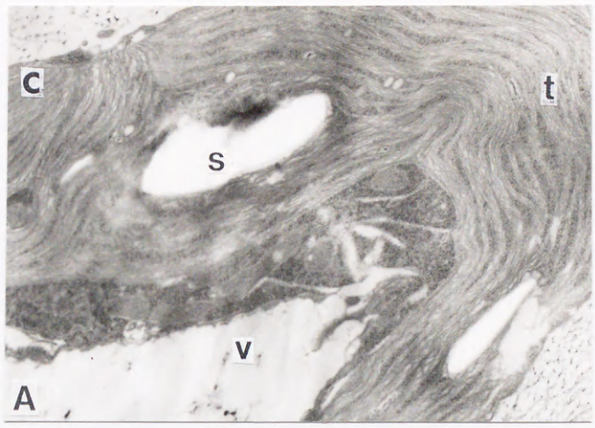
Fig. 6. Ultrastructures of chlorophyll in *Ulva* cells. A, white light culture; B, blue light culture; C, red light culture. c, chloroplast; thylakoids; n, nucleus; s, starch; r, ribosomes; v, vacuole.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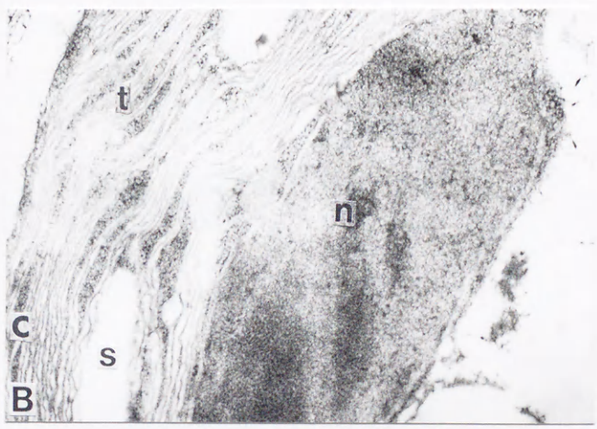
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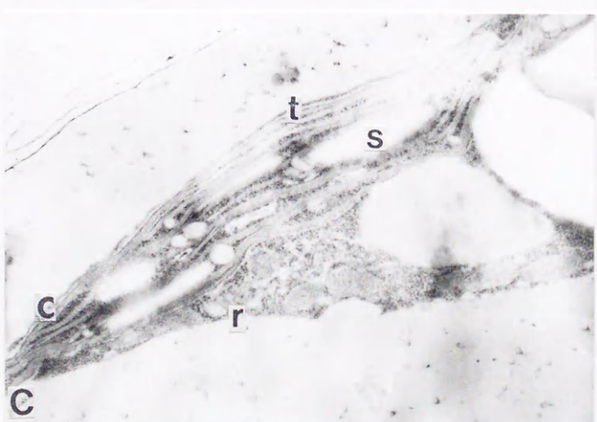
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(Fig. 3D). The density of the ribosome is very high along the outside of the chloroplast than the inner side.

In BLC, the cell organelles are found less prominent than those in WLC (Fig. 2B). The thylakoids are also as clear as in WLC but without much grana stacks (Fig. 4A-D). Apart from the thylakoids, dense ribosome and starch granules are evident in these cells and they are comparatively large in numbers than in WLC (Fig. 4B-D). The pyrenoid structure is similar to that in WLC.

In contrast to WLC and BLC, in RLC the chloroplast is not prominent and also reduced in size (Fig. 2C). After 15 days of red radiation application, the effect was seen on the damage of the chloroplasts. The thylakoid are less prominent and surrounded with dense ribosome (Fig. 5A-D). The pyrenoid is prominent and as clear as that in WLC and BLC. The number of starch grains is comparatively larger than in BLC. Large numbers of plastoglobule are also present in these chloroplasts.

The transmission electron microscopic evidence of white, blue, and red lights induced changes in the chloroplast thylakoid stocking, which is shown in Fig. 6. There was a more increased and well developed structure of cell organelles observed in WLC and BLC when compared to RLC. Enlarged

portions of the chloroplasts clearly show a developed thylakoid portion in WLC and BLC. In RLC, the thylakoid is mixed with dense ribosome and is not prominent. Furthermore, it is clearly observed with enlarged chloroplasts and greatly increased stacks of 3 thylakoid bands (lamella) in WLC. And in BLC, though the stacks of 3 thylakoid band portions are not well developed, the thylakoids are as clear as in WLC. On the other hand in RLC, the thylakoids are not clear like in BLC.

Thus the light induces ultrastructure changes of the chloroplast, not only on the magnitude but also on the architecture.

1. 3. 2 Quantitative Analysis

Quantitative changes in the size of cell and other organelles are presented in Table 1. This illustrates that the cells and organelles of BLC comparatively well developed than in RLC. Except nuclei, the sizes of cell, chloroplast and pyrenoid show significant differences in all three cultures. The WLC had highly developed cell organelles while RLC resulted in poor development.

Table 1. Quantitative changes of size of the cell and organelles in *Ulva pertusa* cultured under white (WLC), blue (BLC) and red (RLC) light conditions

Object site	WLC (in μm)		BLC (in μm)		RLC (in μm)	
	Av.	SD	Av.	SD	Av.	SD
Cell						
length	24.7	3.1	20.1	2.4	14.4	1.4
breadth	9.3	1.5	8.1	1.6	5.5	2.4
Chloroplast						
length	10.7	1.2	8.4	1.1	5.7	0.9
breadth	3.1	0.9	2.9	0.7	2.1	0.2
Pyrenoid with starch	2.9	0.1	2.8	0.1	2.1	0.2
Pyrenoid without starch	2.3	0.2	1.9	0.2	1.7	0.1

1. 4 Discussion

The results reveal that prolonged application of red light causes disorganization of the thylakoid. This could be due to the fact that the photon was even not enough to perform one hundred percent of the process of maintenance of cell integrity. On the other hand, blue light absorbed by phytochrome could run the carbohydrate, protein and respiratory cycles (Brinkman and Senger 1978 and Kowallik 1982). As a result, the cell integrity process performed well in BLC. Overall, the white light which has photon for the activation of both phytochrome and chlorophylls produces enough energy for optimal maintenance and growth. This suggests that the red part of the spectrum, though not by itself able to support the growth, is not inhibitory either to growth or to the maintenance of cell integrity. However, the photon is not enough to run other essential metabolic process. Earlier report of Lopez- Figueroa and Rudiger (1991), that nitrogen reduction is mostly stimulated by the blue spectrum, indicates that though the red light can run the photosynthetic process very well, the metabolic process and the morphological development mainly rely on the role of protein and lipid. The energy for the above said process may not be enough in the red light. And it might alter the products of photosynthesis in such a way as to halt growth and proceed the cell maintenance process with the limited

source of other substrates. It has been shown that such deficiency can not be compensated by the supplementation from sugar acetate or amino acids of the growth medium (Terborg 1965).

From the observations of morphology and cell organelle size, it is clear that the light quality plays a major role on the development of chloroplast, cell integrity and its architecture. In *U. pertusa* BLC condition produces better morphological structures than under RLC and such structures are comparable to the cells of WLC. Although a lot of research have been carried out in the past on the influence of light quality in algae, there are hardly few references available to inform the impact of light quality on the ultrastructure of these lower plants.

The effect of light quality on the chloroplast morphology illustrates that in RLC the thylakoid organization is not as clear as in BLC. Though a lot of attempt have been made on the light quality and algae, only few references are available with regard to light quality and the ultrastructural features of these lower plants. Land plants show morphological and physiological modifications depending on different light conditions (heliophytes and sciophytes) (Daubenmire 1974). In higher plants, so far it is well understood that blue light chloroplasts are similar in their prenyllipid composition,

photosynthetic activity and ultrastructure to sun type chloroplasts. Red light chloroplasts resemble those of shaded leaves and plants grown under high light intensities (Lichtenthaler et al. 1980). Both the primary development of the photosynthetic apparatus and its maintenance appear to require blue irradiation. In prolonged pure red light the thyllakoid membranes become disorganized (Vorkresenskaya 1972) and the photosynthetic capacity of algae (Terborgh 1966) and higher plants (Harnischfeger et al. 1974) is also affected. *Halimeda tuna* in a 6.0 m habitat has the morphology of shade-type plants (sciophyte) and in 0.5 m, *Halimeda* has sun-type (heliophyte) characteristics (Colombo and Orsenigo 1977). Unicellular marine algae contain the larger number of thylakoids per chloroplast in blue-green light compared to white light (Vesk and Jeffrey 1977) . On the other hand, in Cyanophyta it is observed that exposure to any spectral band (red, blue or green light), reduces the number of thylakoids compared to the cultures that are grown in white fluorescent light of the same light intensity (Albertano 1991). In our findings, the enlarged chloroplasts together with greatly increase stalks of 3 thylakoid bands (lamella) in WLC, clear development of thylakoid portions without lamellae in BLC. And finally less clear thylakoids with dense ribosome in RLC support the earlier report of Albertano (1991). The earlier finding in the diatom, *Chaetoceros*, of larger average cell size in blue light than in

the red is relevant to our present observations (Baatz 1941).

Overall, the present data indicate that the maintenance of cell integrity, which is considered as a separate process from the growth but utilizes the same substrate pools (reduced NADP and ATP), is the basic process for cell for the further division. That is because the better response of *Ulva pertusa* organelles has been observed under blue radiation condition than under red radiation.

CHAPTER II

LIGHT QUALITY INFLUENCE ON THE METABOLIC ASPECTS OF *Ulva pertusa*

2.1 Introduction

The problem of seawater eutrophication in integrated mariculture has been effectively reduced by some seaweeds. Among them, the genus *Ulva* is considered as the most effective because of its faster nitrogen uptake, and ease of cultivation and harvesting (Neori et al. 1991). Previous studies under field conditions, in the Nagashima Field Lab, Kagoshima prefecture, Japan, have shown that the sterile mutant *Ulva pertusa* grows free from contamination for long periods, and has an ability to remove the nitrogen (ammonia and nitrate) in a polyculture system with red sea bream (Hirata and Xu 1990) and prawn *Penaeus japonicus* (Danakusumah and Hirata 1991).

From the standpoint of future cultivation of these algae in the integrated mariculture system, preliminary studies have been carried out to determine the light intensity, temperature and salinity requirements for optimal development (Floreto et al. 1993). So far, the polyculture system is designed using floating *Ulva pertusa* in a net on the surface of the cage, but a lot of difficulties seem certainly to arise during the typhoon and monsoon seasons. Since it is a floating alga, it seems better to cultivate in the bottom level of the culture site. Regarding this, another factor which shows considerable variation in the natural environment of

this depth is light quality, and its influence has never been investigated for this species.

In marine plants, light quality affects growth (McLachlan and Bidwell 1983), photosynthesis (Luning and Dring 1985), and also distribution (Tremblin et al. 1993). Blue light generally has a stimulating effect, and red light, the opposite. Algae can adapt to changes in wavelengths of light by changing their cellular pigment composition (Kirk 1986). Light quality also significantly affects carbohydrate metabolism (McLachlan and Bidwell 1983), nitrate utilization (Corzo and Neil 1991) and chlorophyll synthesis (Lopez-Fiqueroa and Neil 1989). The aim of this work is to reveal the effect of blue and red light, in comparison to white light as reference, on the photosynthetic active pigments, growth, carbohydrate production and the nitrogen metabolism in *Ulva pertusa* cultivated in the laboratory.

2. 2 Material and Methods

2. 2. 1 Material

The species *Ulva pertusa* was collected from Azuma town, Kagoshima, Japan. The specimens were then either incubated immediately for the experiment or stored in the dark in natural sea water (5°C) until starting the experiment.

All the materials tested were used within three day of collection. Only healthy and clean fronds were employed. In order to avoid wound respiration (Bidwell and Mclachlan 1985), the cut sections of the algal thalli were incubated for 20 hours or more at the same temperature as in the experiment.

2. 2. 2 *Experimental Design*

A single factor experiment was conducted using a completely randomized block design, resulting each treatment in triplicates with a total of 9 sampling times. The parameters under the treatment were growth, contents of chlorophylls, carbohydrates and proteins and nitrate reductase activity. And the sampling of *Ulva pertusa* was made in alternative days for all parameters. For nitrate reductase activity, the sample was collected three times per day (ELP = early light period, at 7: 00; MLP = mid light period, at 13: 00; and DP = dark period, at 0: 00) in a light cycle started from 5: 00 to 19: 00 and the rest was under the dark. The experiment was conducted for 15 days under 25°C.

2. 2. 3 *The Culture Experiment*

The algal culture was carried out as described in Chapter I.

2. 2. 4 Measurements

Growth was measured as a percentage increase in the area of the frond discs cut from mature thalli (Plants 20 - 30 mm high). The discs were cut out from the expanded region of the fronds, avoiding the thin areas within 5 cm of the margin and the thicker areas near the center. A disc with a diameter of 1.8 cm was found to be the most successful. Specific growth rate (SGR) was expressed as an increment of area using the equation:

$$SGR = (\ln (Af/Ai) / t) \times 100$$

where, Ai is the initial area and Af is the area at the day of observation t (De Boer et al. 1978).

Nearly 2 g of fresh weight (FW) samples were used for the carbohydrate, protein and chlorophyll studies. And nine samples were collected from each treatment in every observation. All spectrometric analyses were made by a Hitachi, U-2000 double-beam spectrophotometer.

Carbohydrate content was determined by a phenol sulfuric acid method (Dubious et al. 1956), using glucose as a standard. For this, homogenized 0.02 or 0.20 ml of sample of *Ulva* was added with 5% phenol (20 or 200μl) and 1 ml of conc. H_2SO_4, and the mixure was incubated for 15 min at 37°C. Reading was made at a wavelength of 490 nm, and the

carbohydrate amount was calculated from the standard curve.

Protein was quantified by a Bio-Rad protein assay method (Bio-Rad 1979), using bovine gamma globulin as a standard. After homogenizing the sample, the assay was made with 1N NaOH. The extraction was made for 10 min at 100°C, later after treating with running cool water at room temperature it was centrifuged at 5000 rpm for 5 min. After collecting the supernatant the procedure was repeated for the residue three times. From this, 0.02 ml of sample was mixed with 5 ml of Bio-Rad solution and reading was made at 595 nm with the spectrometer. And the amount of protein was calculated with the standard curve.

Chlorophyll content was determined based on the method proposed by Hansman (1973). For the chlorophyll extraction first of all, the specimen was mixed with $MgCO_3$ and acetone (90%). After homogenization, it was centrifuged at 5000 rpm for 30 min and the clear supernatant was stored in the ice box. The procedure was repeated with the residue till extracting the whole amount of chlorophyll from the sample. And the observation was made at 645 and 665 nm.

2. 2. 5 Nitrate Reductase Assay

Nitrate reductase activity was assayed in situ by the procedure described by Maurino et al. (1986), as modified by Corzo and Neil (1991). The incubation mixture consisted of 0.1M sodium phosphate buffer, pH 8.0; 0.05 mM ethylene-diamide tetraacetate (disodium salt), pH 8.0; 0.01mM glucose; 30 mM KNO_3; and 0.1% (v/v) n-proponal. In each observation three samples of each treatment (0.16 g fresh weight each) were collected. And the observations were made three times in a day cycle, early (ELP), mid (MID) light periods and dark (DP) period, respectively. The tissue extract was made by placing the *Ulva* sample in a chilled mortar with ca. 1.5 g grinding sand and ground vigorously with a pestle while adding gradually 25 ml of ice cold phosphate buffer with 1 mM dithiothritol. The extract was centrifuged with a refrigerated centrifuge for 15 min at 15000 x g. And the clear supernatant was recovered and kept in the ice box for enzyme assay. From this, 4 ml of supernatant was taken in a centrifuge tube and added with 0.5 ml of 0.33 M potassium phosphate buffer (pH 8); 0.2 ml of 0.2 M KNO_3, 0.1 ml of 2 mM NADH, and 0.1 ml of 0.05 M $MgSO_4$ $7H_2O$. The control mixture was prepared in a separate test tube with the 0.1 ml of NADH replaced by 0.1 ml of potassium phosphate buffer. The sample was well mixed, covered with aluminum foil, and then incubated in a water bath at 25°C for 30 min. The reaction was stopped by adding 0.2 ml of zinc acetate and 3.4 ml of ethanol. After shaking the sample

again, it was centrifuged for 10 min at 10000 x g and the supernatants were decanted into fresh tubes. The absorbance was recorded at 543 nm. And the nitrite reduction was performed by the Cd-Cu method as described in the following Chapter III.

2. 2. 6 Statistics

Data were subjected to single factor ANOVA to understand the significant differences between treatment variation and experimental error (Gomez and Gomez, 1984). The average plus standard deviation at each sampling were obtained after the statistical treatment of the *F*-test against measured values of over three samplings from the triplicate flasks to reduce the measurement error ($P < 0.05$).

For red and blue lights these mean values of 15 days of one group were statistically compared to those of another group in order to examine significant differences between groups, using the regression analysis.

2. 3 Results

2. 3. 1 Growth, Carbohydrate and Protein Contents

During 15 days of the experiment, light quality had a significant effect on growth. The specific growth rate (SGR) varied highly in the white light culture (WLC), when compared to the blue (BLC) and the red light cultures (RLC). Fig. 7 shows that, the thallus growth was almost the same in RLC and the BLC during the first six days. After that growth under BLC was significantly higher than under RLC. Around 3.10% ($P < 0.01$) and 1.24% ($P < 0.05$) of SGR were obtained for the BLC and RLC, respectively. However in WLC, it was around 8.55% ($P < 0.001$).

Figures 8 and 9 show the evolution of carbohydrate and protein contents of *Ulva pertusa* cultivated under three light qualities. The carbohydrate content was about the same in RLC and BLC. On the other hand in BLC, the protein content was significantly higher than in RLC. Overall, the *Ulva pertusa* in WLC was found to have higher amount of carbohydrate and protein. With respect to the carbohydrate accumulation, in WLC it was remarkably higher (15.3 mg g^{-1} FW, $P < 0.01$)) than in BLC (3.9 mg g^{-1} FW, $P < 0.001$)) and RLC (4.4 mg g^{-1} FW, $P < 0.01$)). The highest increment in the carbohydrate content was observed in WLC. But in BLC and RLC the difference of increments was only 1.29 mg g^{-1} FW and 1.69 mg g^{-1} FW, respectively.

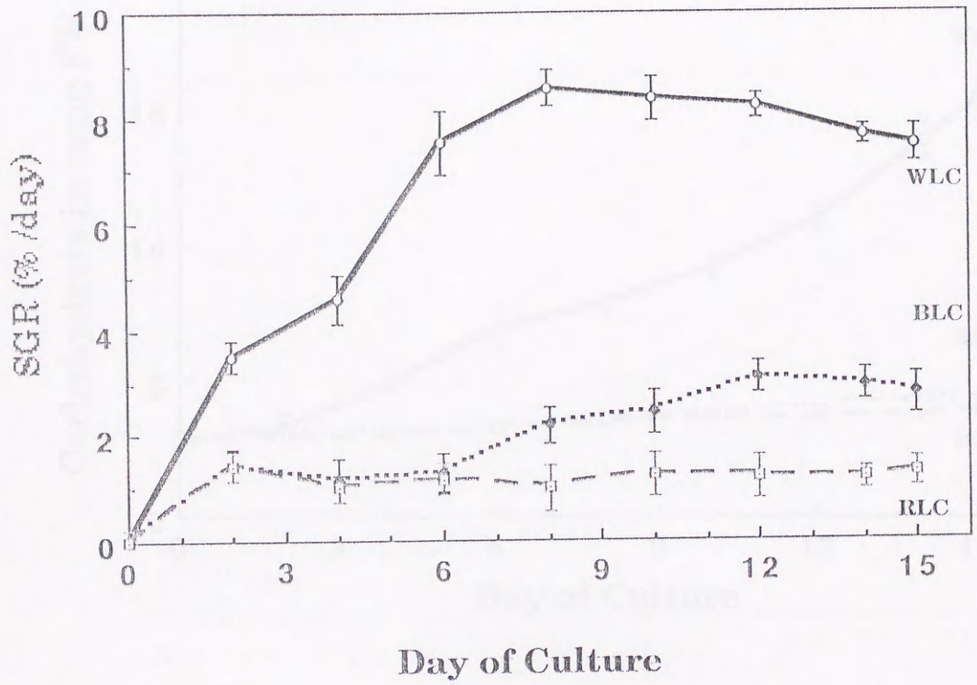


Fig. 7. Effect of light quality on the specific growth rate (SGR) of *Ulva pertusa*. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

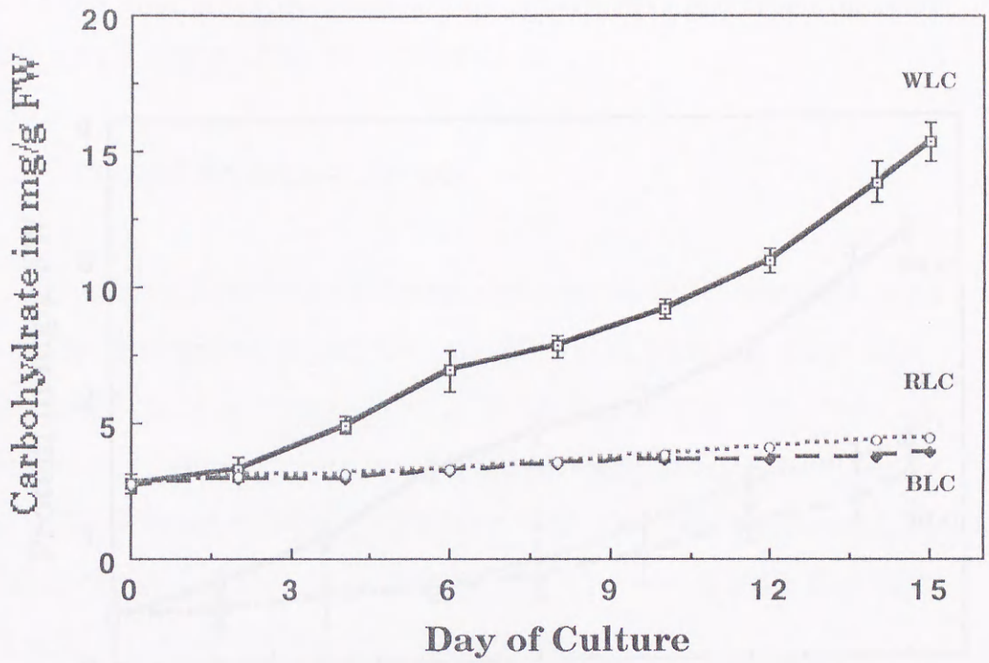


Fig. 8. Effect of light quality on the carbohydrate production of *Ulva pertusa*. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

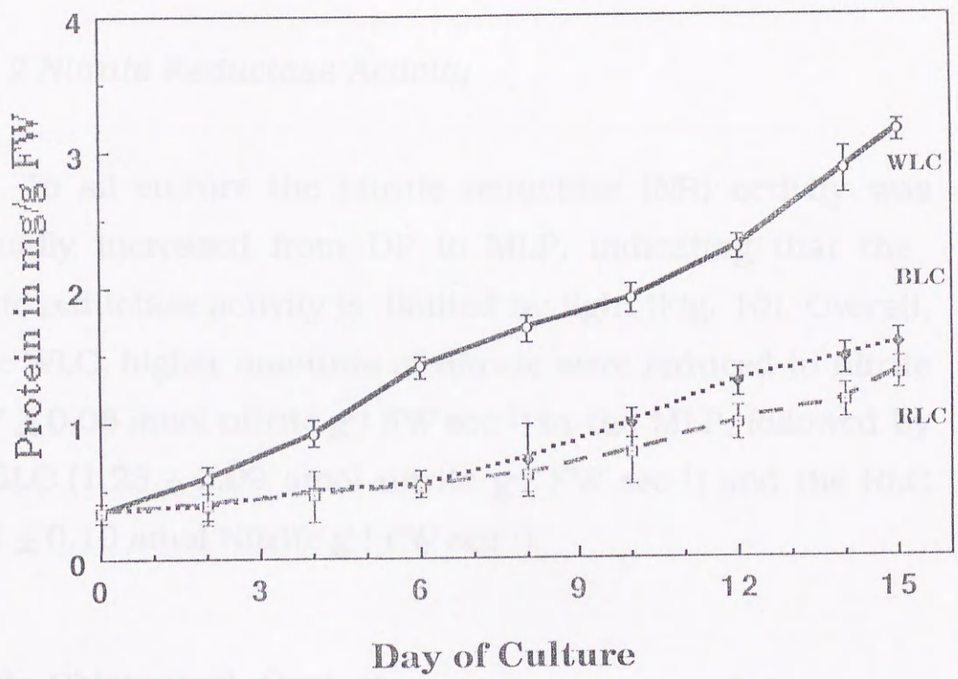


Fig. 9. Effect of light quality on the protein production of *Ulva pertusa*. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

In contrast to carbohydrates, protein was accumulated more (1.57 mg g^{-1} FW, $P < 0.001$) in BLC when compared to RLC (1.35 mg g^{-1} FW, $P < 0.01$). However, in WLC it was higher (3.13 mg g^{-1} FW, $P < 0.05$).

2. 3. 2 Nitrate Reductase Activity

In all culture the nitrate reductase (NR) activity was gradually increased from DP to MLP, indicating that the nitrate reductase activity is limited by light (Fig. 10). Overall, in the WLC, higher amounts of nitrate were reduced to nitrite (1.87 ± 0.08 μmol nitrite g^{-1} FW sec^{-1}) in the MLP, followed by the BLC (1.23 ± 0.09 μmol nitrite g^{-1} FW sec^{-1}) and the RLC (0.81 ± 0.10 μmol Nitrite g^{-1} FW sec^{-1}).

2 3. 3 Chlorophyll Content

Both Chl a and Chl b were higher in the thalli of BLC than RLC. Fig. 11 shows that the both chlorophyll were synthesized most in WLC, followed by BLC and then RLC. To obtain further evidence of the stability of the pigment system of *Ulva pertusa* cultured under three light qualities (WLC, BLC, RLC), the optical absorption in whole spectra was obtained through scanning spectrometry and compared (Figs. 12 & 13). In all 1, 5, 10, 15 days observations during

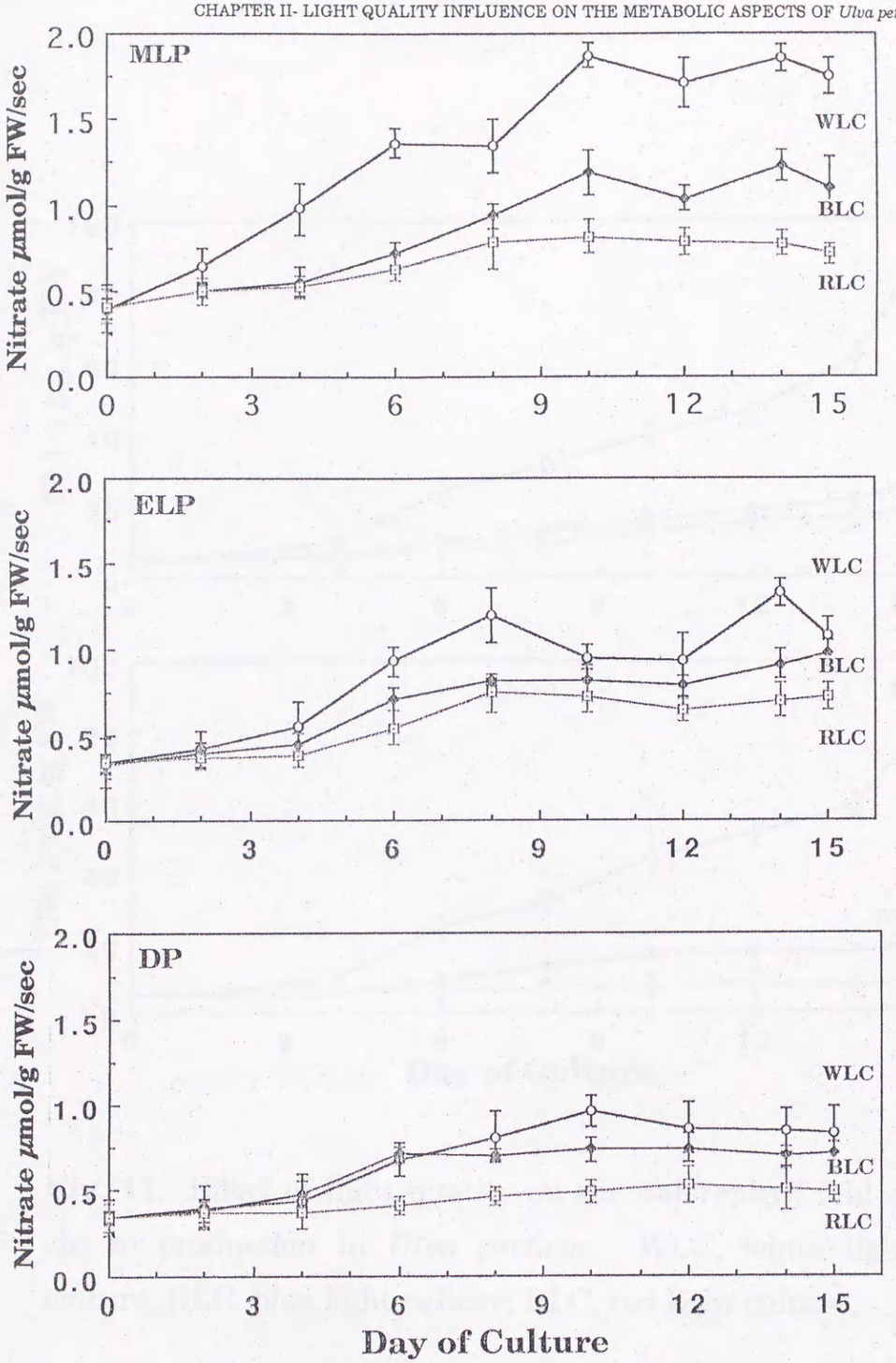


Fig. 10. Effect of light quality on the nitrate reductase activity of *Ulva pertusa*. MLP, mid light time, 13: 00; ELP, early light time, 7: 00; DP, dark time, 0: 00. WLC, white light culture; BLC, blue light -culture; RLC, red light culture.

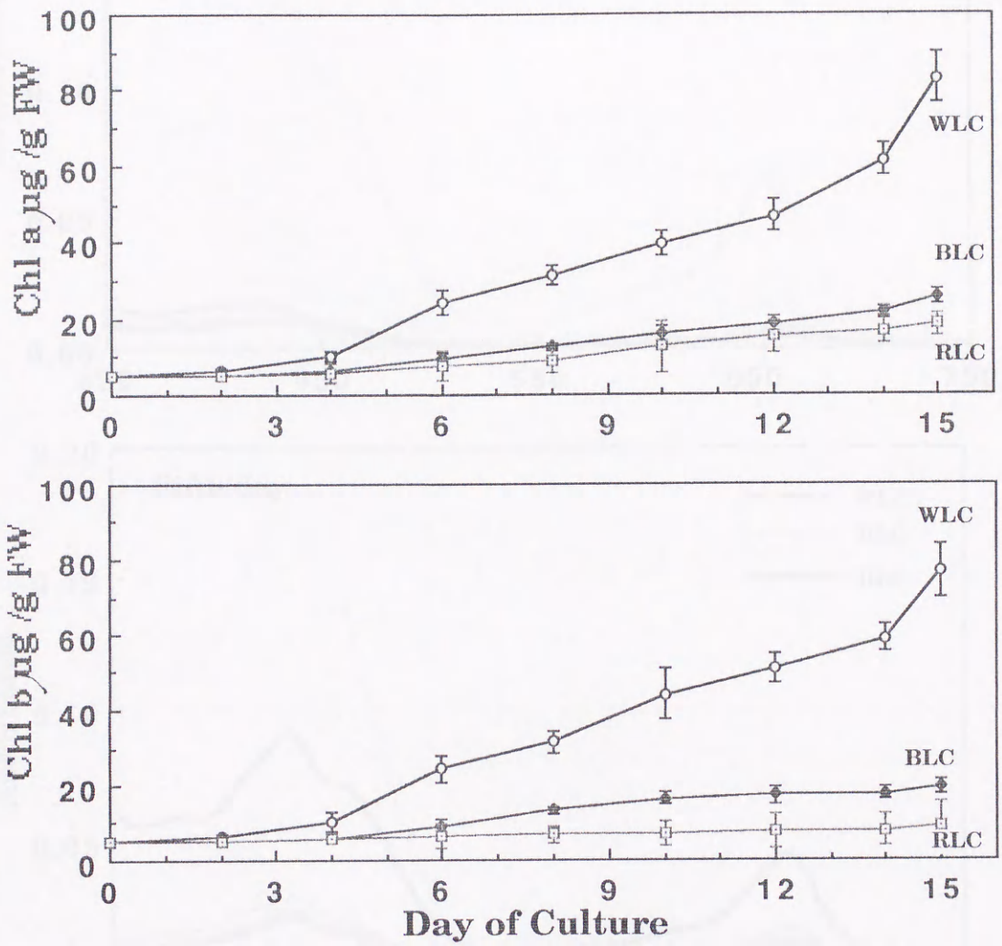


Fig. 11. Effect of light quality on the chlorophyll (chl a, chl b) production in *Ulva pertusa*. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

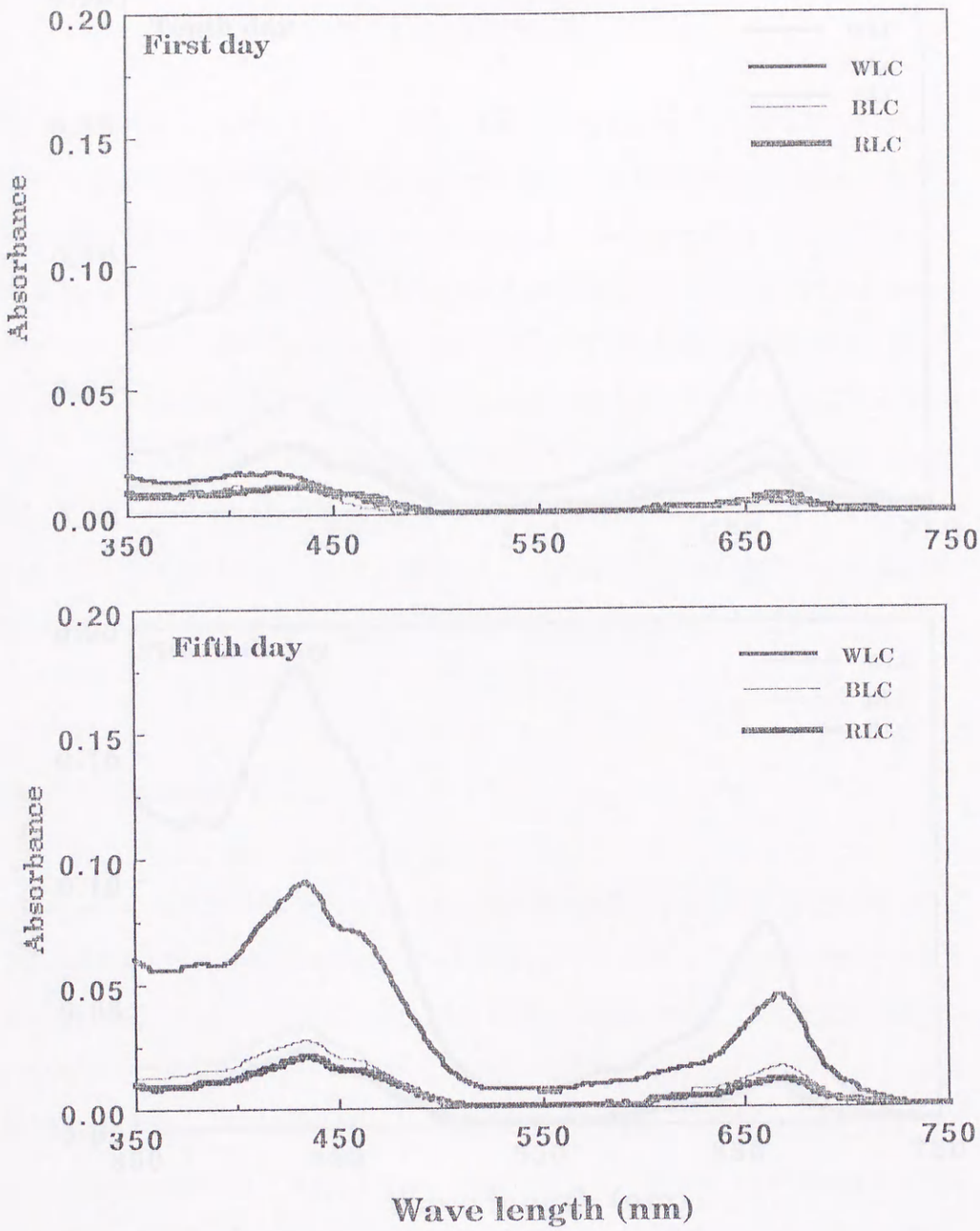


Fig. 12. Absorbtion spectra of pigments at different wavelengths on the first and fifth days of *Ulva pertusa* against light quality. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

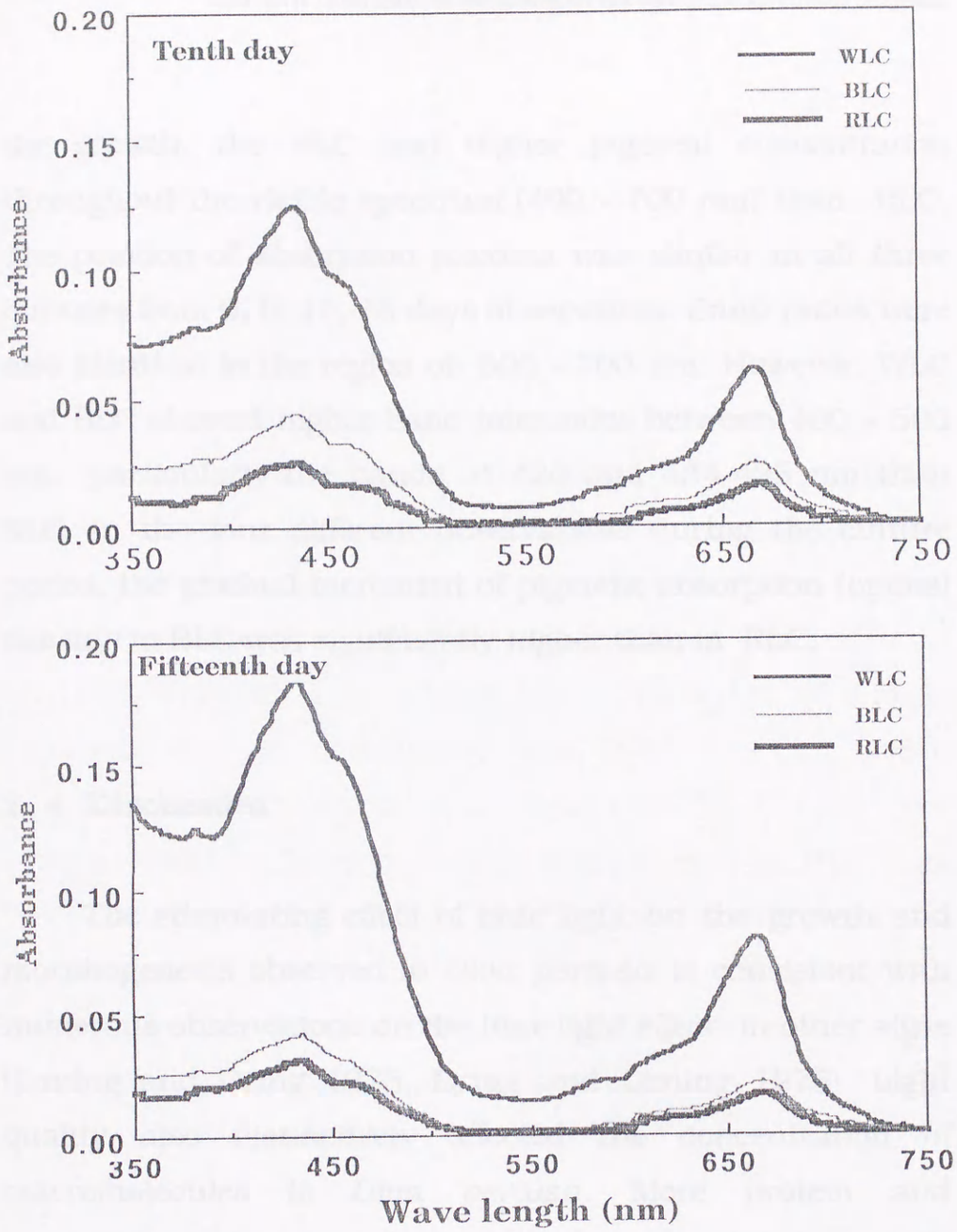


Fig. 13. Absorbtion spectra of pigments at different wavelengths on the tenth and fifteenth days of *Ulva pertusa* against light quality. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

the growth, the BLC had higher pigment concentration throughout the visible spectrum (400 - 700 nm) than RLC. The position of absorption maxima was similar in all three cultures from 0, 5, 10, 15 days observation. Band ratios were also identical in the region of 500 - 700 nm. However, WLC and BLC showed higher band intensities between 400 - 500 nm, particularly the bands at 420 and 434-435 nm than RLC. In the four different observations during the culture period, the gradual increment of pigment absorption (optical density) in BLC was significantly higher than in RLC.

2. 4 Discussion

The stimulating effect of blue light on the growth and morphogenesis observed in *Ulva pertusa* is consistent with numerous observations on the blue light effect in other algae (Luning and Dring 1973, Dring and Luning 1975). Light quality also distinctively affected the concentration of macromolecules in *Ulva pertusa*. More protein and comparatively less carbohydrate were accumulated in BLC than in RLC. The higher relative content of protein has been ascribed (Kowallik 1987) either to a blue light activation of protein biosynthesis, that would lead to a decrease in carbohydrate content by draining of degradation products of the BLC, or to a blue light enhancement of carbohydrate

degradation, delivering more intermediates for the synthesis of amino acids and protein. Furthermore, it has been shown that blue light activates and promotes new synthesis of pyruvate kinase (Kowallik and Schatzle 1980, Ruyters 1988) and phosphoenol pyruvate carboxylase (Kamiya and Miyachi 1975). High levels of these enzymes would result in a greater formation of organic acids and hence an increased synthesis of amino acids and protein. This metabolic response to the light quality has also been observed both in other higher plants and in green algae (Wild and Holzaptel 1980, Bird et al. 1981, Kowallik 1987, Dring 1988). Induction of protein synthesis due to continuous blue light has also been reported by Brinkmann and Senger (1978). Thus the comparatively lower carbohydrate content level in BLC may be due to the higher usage of carbohydrate for the metabolic activities of *Ulva* in BLC. The increased amount of protein content seems to be supported by the previous results; the organ development and chloroplast morphology in BLC have been better than in RLC.

The observation of higher nitrate reductase enzyme activity in the BLC with respect to the RLC is also consistent with previous results (Tischner and Lorzen 1979, Aparicio and Azuara 1984, Ninnemann 1987). Nitrate reductase activity is photo regulated via a blue light receptor and phytochrome in higher plants (Duke and Duke 1984,

Schuster et al. 1987). However, continuous blue light is more efficient than continuous red light at stimulating nitrate uptake and metabolism (Calero et al. 1980, Azuara and Aparico 1983, Corzo 1988). From the observations there are three interpretations that: i) blue light is capable of producing or activating more NR enzyme than red light, ii) that the availability of substrates for the NR enzyme is higher in the BLC than in RLC, iii) growth/ photosynthesis, thus metabolic demand for N is higher in BLC than in RLC.

The photo regulation of chlorophyll synthesis has been studied extensively in higher plants. Two sites of light regulation are known (Kasemir 1983). First, the level of synthesis of 5-amino levulinic acid appears to be indirectly controlled by phytochrome (Masoner and Kasemir 1975). Second, in etiolated angiosperms grown in the dark, the reduction of protochlorophyll to chlorophyll is strictly light dependent (Kasemir 1983). However, in lower plants, the photoreceptors controlling chlorophyll synthesis have been investigated to a lesser extent (Senger 1987). In general, it has been found that photomorphogenesis in lower plants is mainly controlled by a blue light photoreceptor (Humpback and Senger 1984), in *Chlorella vulgaris* (Kowallick and Schurmann 1984) and in *Ulva rigida* (Lopez-Figueroa and Neil 1989). In *Ulva pertusa*, the control of Chl a synthesis by

blue light seems similar to that observed in other green algae (Kowallik and Schurrmann 1984).

Senger (1987) proposed that a blue light photoreceptor controlled the physiological photorespiration in green algae. The present results also seem to confirm this suggestion.

CHAPTER III

LIGHT QUALITY INFLUENCE ON THE INTERRELATIONSHIP BETWEEN *Ulva pertusa* AND CULTURE MEDIUM

3. 1 Introduction

Seaweeds have been shown to reduce efficiently the level of seawater eutrophication in integrated mariculture (Ryther et al. 1975). In a series of studies, it was shown that some seaweed have yield and nutrient uptake capacity; adequate for cultivation and harvesting. Among those the genus *Ulva* has been reported as a biofilter (Cohen and Neori 1991, Parker 1981, Neori et al. 1991).

There have been several studies about the genus *Ulva*, reporting that they can grow well in both artificial media and waste water, showing a high capacity to take up nutrients mainly ammonia, nitrate, etc. (Duke et al. 1989, Fujita 1985, Rosenberg et al. 1984, Hirata and Xu 1990). Another important feature of this genus is that, like some other macrophytes, *Ulva* also can use HCO_3^- as a carbon source for photosynthesis (Ikemori and Nishida 1967).

In author's laboratory also, a floating sterile mutant of *Ulva pertusa* was successfully used as a biofilter in a feed back culture system with the prawn *Penaus japonicus* (Dhanakusumah & Hirata 1991) and also used as feed additive for the red sea bream from the feed back culture system (Hirata and Xu 1990). So far, the polyculture system was assigned with *Ulva pertusa* kept floating in a net on the

surface of the cage, but they were shown to be lot of difficulties handling the typhoon and monsoon seasons. Since it is a floating algae, it would be better if it could be cultivated in the bottom level of the culture site. With regard to this, another factor which shows considerable variation in the natural environment of this middle depth is light quality, and its influence has never been investigated so far for this species. Due to this, the following light quality experiment was conducted under laboratory conditions for this species: i) total nitrogen uptake, ii) CO_2 and pH fluctuations and iii) the HCO_3^- and CO_2 dynamics.

3. 2 Material and methods

3.2. 1 Material

The species *Ulva pertusa* (a floating sterile mutant, which has faster growth and is thinner than native *Ulva pertusa*), was collected from Azuma town, Kagoshima, Japan. The specimens were then either incubated immediately for the experimental tests, or stored in the dark in natural sea water (5°C), until being studied. All the materials tested were used within three days of collection. Only healthy and clean fronds were employed. In order to avoid wound respiration (Bidwell and McLachlan 1985), the cut sections of the algal

thalli were incubated for 20 hours or more at the same temperatures as in the experiment.

3. 2. 2 Experimental Design

A single factor experiment was conducted using a completely randomized block design, resulting in three treatments in triplicates. The observed parameters were growth, CO_2, O_2, HCO_3^-, pH and total nitrogen in the culture medium. The experiment was conducted for 15 days. The samples except growth and total nitrogen were collected three times in a day (ELP = early light period, at 7: 00; MLP = mid light period, at 13: 00; and DP = dark period, at 0: 00) in a light cycle started from 5: 00 to 19: 00 and the rest was under the dark. The total nitrogen was monitored every day and the growth was measured on alternative days.

3. 2. 3 The Culture System

General features were as described in Chapter I. Only the third opening was used in this experiment for water sampling.

3. 2. 4 Measurement

Growth measurement was already described in Chapter II.

Total nitrogen was measured as nitrite by the Cd-Cu method after persulphate oxidation of the sample (Grasshoff 1983). First of all the samples for the nitrate analysis were collected in glass bottles and stored in the refrigerator. And they were analyzed within 5 hours. For the analysis initially the Cu-Cd column reductor was prepared with copperized cadmium granules. The reductor was activated by passing about 250 ml of ammonium chloride buffer. After rinsing thoroughly with the buffer solution, the reductor was ready for use.

For the analysis of the sample, standard solutions were separately mixed with buffer (ammonium chloride) solution thoroughly and the bottle sample was placed under the aspirator tube from the reductor. First 30 ml was passed through the reductor column and the fraction was discarded. Again 30 ml was passed through the column and this one was collected for analysis. From this 20 ml was added with 1 ml of sulphanilamide after 1 min reaction, 1 ml of n-1-napthyl ethylene diamine reagent was added. After shaking well it left for 15 min till the azo dye color formation.

The absorbance was measured at 540 nm. This analysis was repeated three times for each sample and also for the blank and distilled water. And the nitrite was calculated with the formula as follows:

Nitrite μmol/ ml = F (As - Ab - At)

where As = the sample, Ab = the blank, At = the reading of distilled water and F = calibration factor.

The dissolved CO_2, and O_2 in the culture medium were measured through an automatic gas analyzer (ABL 330). To get total CO_2 first of all, the sample pH was reduced to 4.5 by using a buffer trisodium citrate dihydrate to get total CO_2. This total CO_2 was then measured with an automatic analyzer. The CO_2 and O_2 values were converted from mmHg of the raw data to mmol by using standard curves previously obtained from the preparatory experiments; CO_2 (mmol/l) = 4. 34 x CO_2(mmHg) - 27, O_2(mmol/l) = 0. 04 x O_2(mmHg) - 0. 78.

Finally, the HCO_3^- was calculated via CO_2, pH and the pK'1 by the equation proposed by Henderson-Hasselbalch (cf. Saruhashi 1970). The pH was measured with a pH meter (Yokogawa model pH 8.1).

In each treatment , for the CO_2, O_2, pH and HCO_3^-, three sets of triplicate experimental data were subjected to ANOVA *F*-test to confirm that there was no significant difference among the three sets. And the mean values of the triplicate were used to calculate the treatment mean and standard

deviation. They were presented in the tables and in the figures, the mean values were only plotted.

3. 3 Results

3. 3. 1 Growth.

As shown in Fig. 7, specific growth rate ≥(SGR) varied highly in white light culture (WLC) when compared with red light culture (RLC) and the blue light culture (BLC). In WLC, the highest SGR (8.55 ± 0.35) was observed on the 8th day of culture. But in the BLC and the RLC, the SGR was almost similar up to the 6th day, and after that it was higher in BLC than in RLC. The highest SGR for the BLC and RLC were 3.10 ± 0.28 and 1.24 ± 0.41% day^{-1}, respectively. However, the stimulatory effect of blue light on the growth in photosynthetically active low irradiance (60 μmol photon m^{-2} s^{-1}) was clearly understood during the 15 days of culture of *Ulva pertusa* in the continuous blue light application.

3. 3. 2 Total Nitrogen

Like specific growth rate, the nitrogen uptake by *Ulva pertusa* from the medium was faster in WLC than in BLC and RLC (Fig. 14). The BLC had higher total nitrogen uptake than the RLC. The uptake of total nitrogen was faster in the light

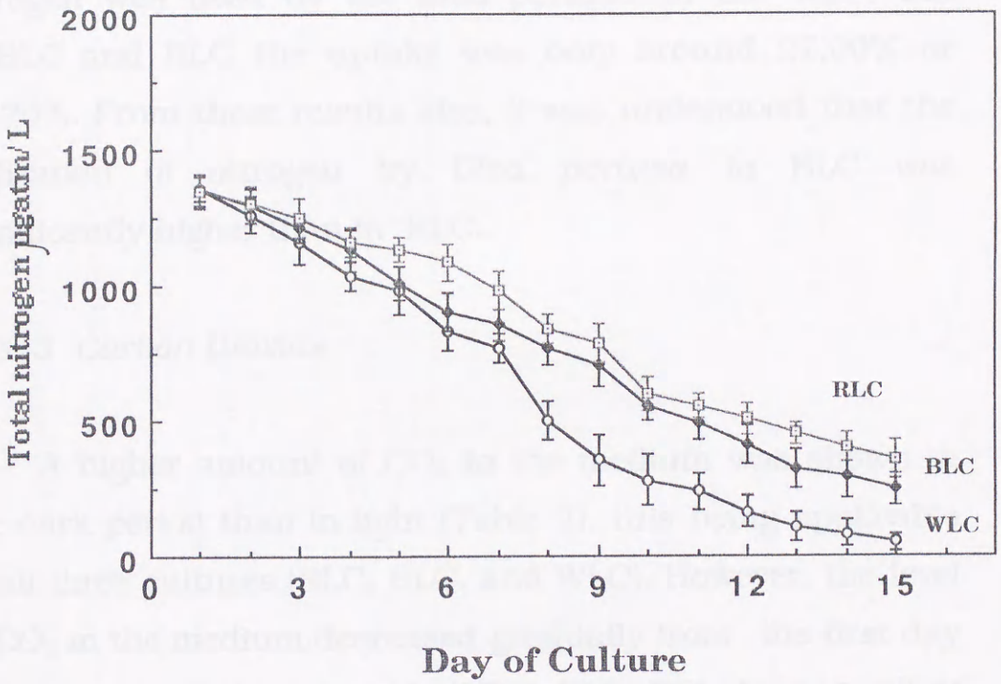


Fig. 14. Effect of light quality on the total nitrogen in the medium of *Ulva pertusa* culture. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

than the dark in all cultures. Nevertheless, there was not much variation in the data of the RLC and BLC in the dark. During the experimental period, around 42.10% of total nitrogen was used by the *Ulva pertusa* in the WLC, but in BLC and RLC the uptake was only around 27.00% or 16.70%. From these results also, it was understood that the utilization of nitrogen by *Ulva pertusa* in BLC was significantly higher than in RLC.

3. 3. 3 Carbon Dioxide

A higher amount of CO_2 in the medium was shown in the dark period than in light (Table 2), this being applicable to all three cultures (RLC, BLC, and WLC). However, the level of CO_2 in the medium decreased gradually from the first day of culture in all three periods (MLP, ELP, DP). Among all of them, as shown in Fig. 15, the lowest content of CO_2 was present in MLP in all cultures, where WLC had the lowest amount of CO_2 (0.016 mmol L^{-1}, $P < 0.01$).

3. 3. 4 Dissolved Oxygen

In contrast to CO_2, the O_2 level was higher in the light period than the dark. However, the significance in the difference of O_2 level in the light when compared to the dark was only observed in WLC. The difference was only a little in

Table 2. Effect of light quality on the CO_2 in the medium of *Ulva pertusa*. DP, dark time, 0: 00; ELP, early light time, 7: 00; MLP, mid light time, 13: 00

Days	Red light culture						Blue light culture						White light culture					
	MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)		MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)		MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)	
	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)	Av. ($\times 10^{-2}$)	SD ($\times 10^{-3}$)
1	3.9	3.2	4.8	3.0	5.4	2.0	3.8	3.2	4.4	3.1	5.0	3.0	3.3	2.3	4.0	4.6	5.4	6.0
2	3.9	2.1	4.6	1.0	5.0	4.0	3.8	2.1	4.0	4.0	5.0	4.0	2.9	2.1	4.0	3.5	5.0	4.2
3	3.8	2.1	4.4	2.0	4.8	1.0	3.6	1.4	4.0	4.0	4.7	4.0	3.0	3.2	3.6	2.1	5.0	4.1
4	3.6	2.1	4.0	3.0	4.8	6.0	3.6	6.0	3.6	4.1	4.7	6.0	2.6	4.1	3.5	3.2	4.6	3.0
5	3.3	2.2	3.8	3.0	4.3	3.0	3.2	3.0	3.6	3.2	4.7	3.0	2.6	3.0	3.3	4.1	4.3	1.0
6	3.3	1.2	3.7	2.1	4.0	1.0	3.2	2.0	3.6	0.1	4.3	2.1	2.5	2.0	3.3	2.1	4.1	2.0
7	3.2	1.2	3.8	0.1	4.0	3.0	3.0	1.0	3.3	0.0	4.2	0.1	2.5	0.0	3.0	3.3	4.0	0.0
8	3.0	3.0	3.6	2.2	3.8	2.0	2.9	2.1	3.2	2.0	4.2	2.1	2.6	2.0	2.8	3.2	4.1	2.2
9	3.0	2.0	3.5	4.2	3.8	5.0	2.8	5.0	3.3	5.0	4.0	5.0	2.4	5.0	2.4	3.5	3.6	5.5
10	3.0	5.6	3.6	6.1	3.7	3.1	2.8	3.1	3.3	3.0	3.9	3.2	2.0	3.0	2.4	3.0	3.7	3.0
11	2.7	3.0	3.5	1.0	3.8	2.0	2.6	7.0	3.3	7.0	4.0	7.1	1.8	3.1	2.3	7.0	3.6	7.0
12	2.7	7.1	3.4	4.0	3.8	1.0	2.6	2.1	3.2	2.1	3.9	4.1	1.8	4.1	2.4	4.0	3.3	4.0
13	2.7	4.0	3.2	4.0	3.7	4.0	2.4	1.2	3.0	4.2	3.7	4.0	1.7	0.0	2.0	4.0	3.0	2.0
14	2.6	0.2	3.0	2.1	3.7	0.0	2.4	3.0	2.9	2.2	3.6	0.0	1.6	0.0	1.8	0.0	2.9	1.0
15	2.6	4.1	3.0	3.0	3.7	3.0	2.4	4.0	2.8	3.0	3.6	1.1	1.6	1.0	1.6	1.0	3.0	3.0

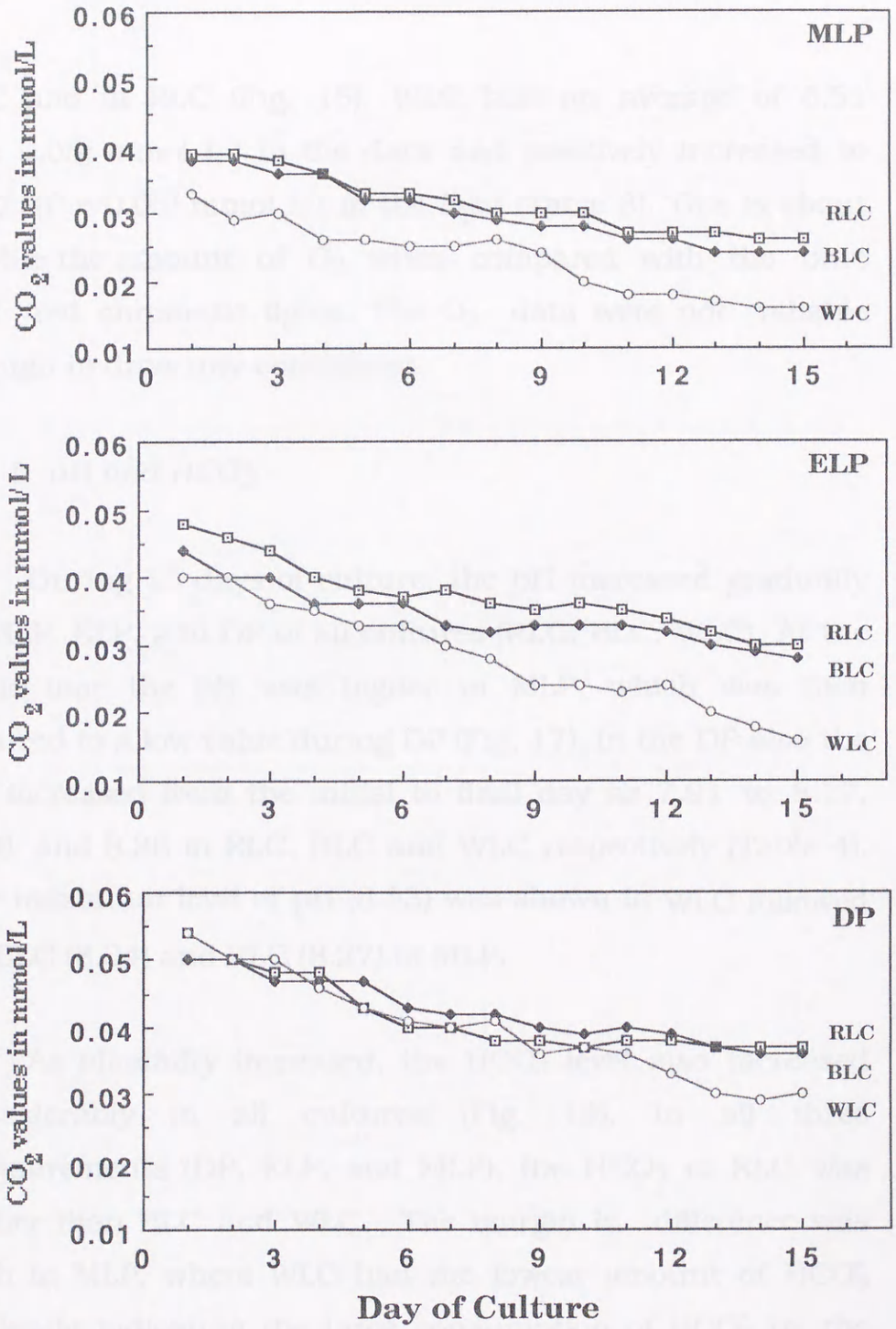


Fig. 15. Effect of light quality on the CO_2 in the medium of *Ulva pertusa* culture. MLP, mid light time, 13: 00; ELP, early light time, 7: 00; DP, dark time, 0: 00. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

BLC and in RLC (Fig. 16). WLC had an average of 6.51 ($P < 0.05$) mmol L^{-1} in the dark and positively increased to 6.67 ($P < 0.05$) mmol L^{-1} in the light (Table 3). This is about double the amount of O_2 when compared with the blue and red chromatic lights. The O_2 data were not reliable enough to draw any conclusion.

3.3. 5 pH and HCO_3^-

During 15 days of culture, the pH increased gradually in MLP, ELP, and DP of all cultures (RLC, BLC, WLC). At the same time the pH was higher in MLP, which was then reduced to a low value during DP (Fig. 17). In the DP also the pH increased from the initial to final day as 7.91 to 8.17, 8.20, and 8.36 in RLC, BLC and WLC respectively (Table 4). The maximum level of pH (8.53) was shown in WLC followed by BLC (8.34) and RLC (8.27) in MLP.

As alkalinity increased, the HCO_3^- level also increased considerably in all cultures (Fig. 18). In all three measurements (DP, ELP, and MLP), the HCO_3^- of RLC was higher than BLC and WLC. The margin in difference was high in MLP, where WLC had the lowest amount of HCO_3^- evidently indicating the large consumption of HCO_3^- by the *Ulva pertusa* in WLC. HCO_3^- was calculated of a high in

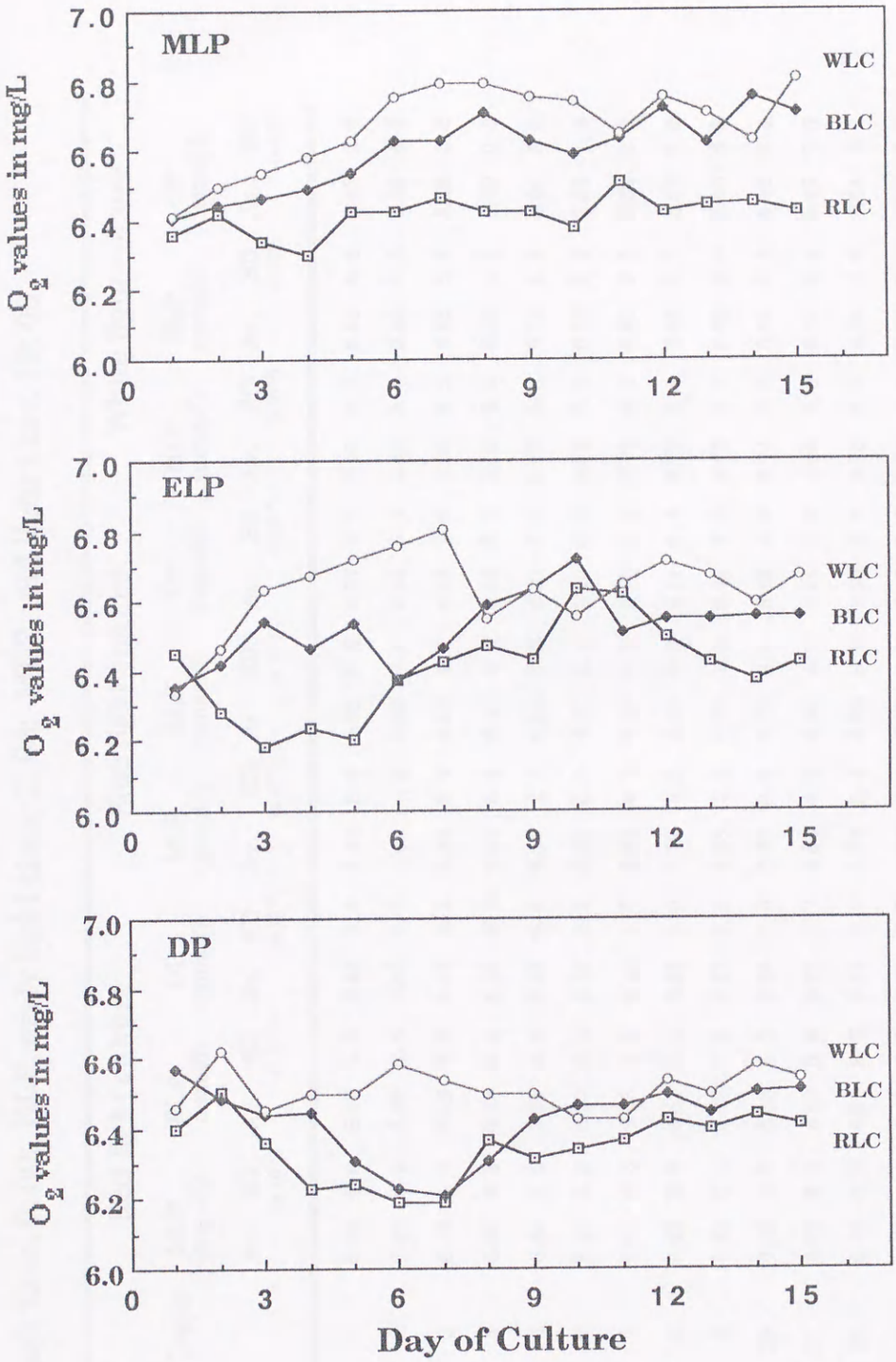


Fig. 16. Effect of light quality on the dissolved O_2 in the medium of *Ulva pertusa* culture. MLP, mid light time, 13: 00; ELP, early light time, 7: 00; DP, dark time, 0: 00. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

Table. 3. Effect of light quality on the O_2 in the medium of *Ulva pertusa* culture. DP, dark time, 0: 00; ELP, early light time, 7: 00; MLP, mid light time, 13: 00

Days	Red light culture						Blue light culture						White light culture					
	MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)		MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)		MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)	
	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)	Av.	SD ($\times 10^{-1}$)
1	6.36	2.0	6.45	5.2	6.40	5.4	6.41	5.2	6.35	5.2	6.57	4.7	6.41	6.4	6.33	5.5	6.46	2.5
2	6.42	0.0	6.28	3.6	6.50	3.3	6.45	3.6	6.42	0.3	6.48	3.7	6.49	2.4	6.46	7.5	6.62	6.3
3	6.33	5.4	6.18	2.2	6.36	2.4	6.46	2.2	6.55	4.3	6.44	2.6	6.54	6.3	6.63	6.4	6.45	2.5
4	6.29	4.6	6.23	6.4	6.23	5.2	6.49	6.4	6.47	3.6	6.45	6.5	6.58	4.1	6.68	4.5	6.50	0.3
5	6.42	1.5	6.21	2.4	6.24	6.3	6.54	2.4	6.54	7.5	6.31	5.5	6.62	6.3	6.72	6.4	6.50	3.5
6	6.42	2.2	6.37	6.3	6.19	3.3	6.63	6.3	6.37	6.4	6.22	5.3	6.75	7.5	6.76	5.5	6.58	1.3
7	6.47	4.3	6.43	4.1	6.19	3.7	6.63	4.1	6.46	6.5	6.20	5.2	6.79	6.2	6.81	6.3	6.54	3.3
8	6.42	2.5	6.47	6.3	6.37	9.6	6.72	6.3	6.59	0.6	6.31	5.4	6.79	5.4	6.55	3.3	6.50	7.5
9	6.42	0.1	6.43	7.5	6.31	3.3	6.63	7.5	6.63	1.5	6.42	6.5	6.75	3.7	6.63	2.5	6.50	6.3
10	6.38	0.0	6.63	6.2	6.34	7.6	6.63	6.2	6.72	9.6	6.48	5.6	6.74	6.2	6.56	6.5	6.42	2.4
11	6.51	5.2	6.62	5.4	6.37	3.7	6.64	4.2	6.51	4.7	6.47	3.6	6.65	4.6	6.64	5.4	6.42	2.5
12	6.42	6.2	6.50	3.7	6.42	9.6	6.72	5.4	6.55	6.2	6.50	8.6	6.75	6.5	6.71	4.6	6.54	5.2
13	6.45	4.2	6.43	6.2	6.40	5.5	6.62	7.4	6.55	4.2	6.45	6.5	6.71	4.1	6.67	7.5	6.50	5.2
14	6.45	0.5	6.37	4.6	6.44	5.6	6.75	2.2	6.55	1.2	6.50	3.7	6.62	3.3	6.59	4.5	6.58	6.3
15	6.42	0.0	6.43	6.5	6.41	4.3	6.71	5.2	6.56	3.2	6.51	7.5	6.80	5.2	6.67	6.5	6.55	3.7

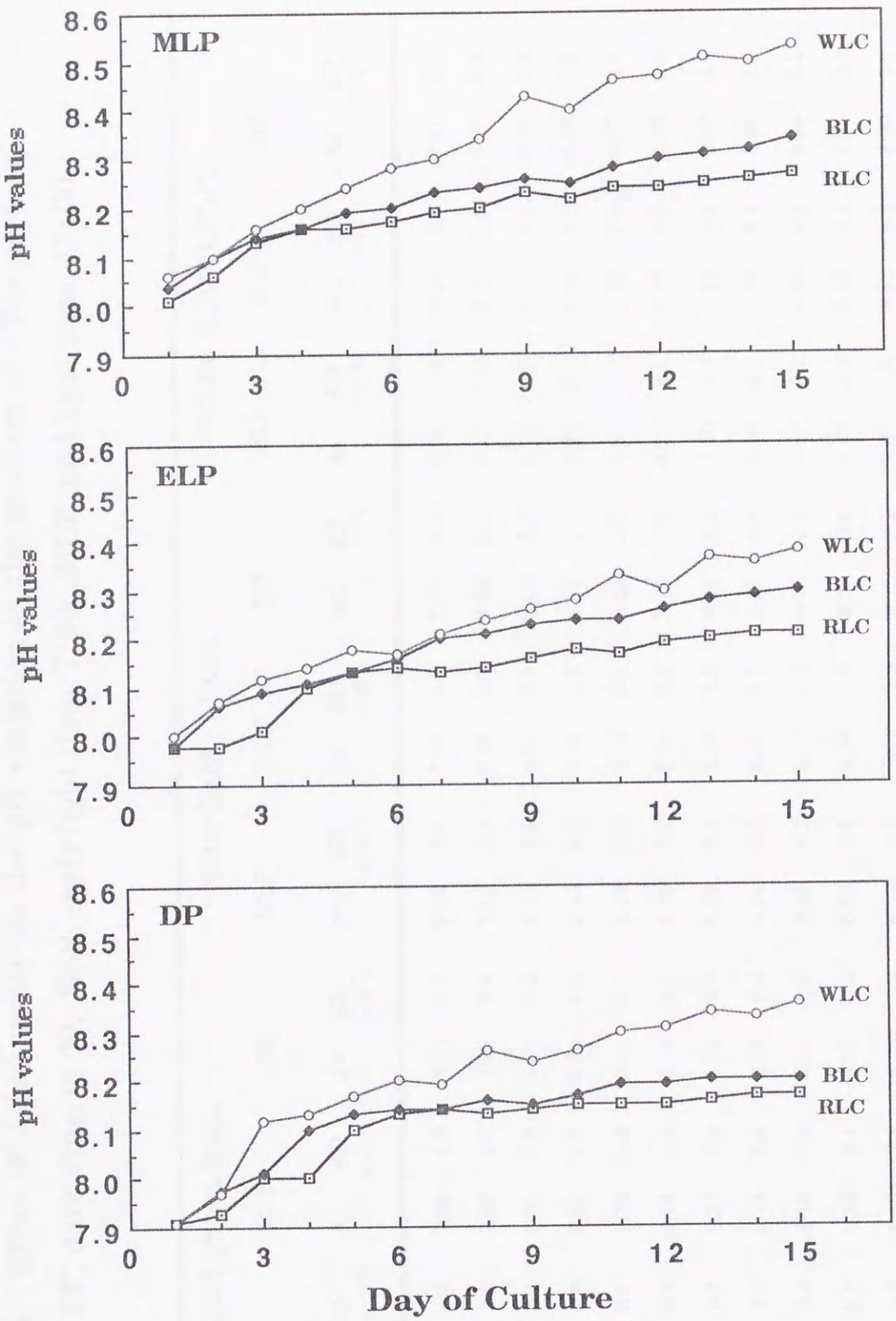


Fig. 17. Effect of light quality on the pH variation in the medium of *Ulva pertusa* culture. MLP, mid light time, 13: 00; ELP, early light time, 7: 00; DP, dark time, 0: 00. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

Table. 4. Effect of light quality on the pH variation in the medium of *Ulva pertusa* culture. DP, dark time, 0: 00; ELP, early light time, 7: 00; MLP, mid light time, 13: 00

Days	Red light culture						Blue light culture						White light culture					
	MLP		ELP		DP		MLP		ELP		DP		MLP		ELP		DP	
	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)	Av.	SD ($\times 10^{-2}$)
1	8.01	4.0	7.98	4.6	7.91	1.2	8.04	3.4	7.98	1.3	7.91	3.2	8.06	0.0	8.00	2.3	7.91	5.0
2	8.06	3.2	7.98	3.2	7.93	3.4	8.10	5.1	8.06	0.1	7.98	0.1	8.10	0.0	8.07	0.0	7.97	3.2
3	8.13	0.1	8.01	0.0	8.00	3.2	8.14	3.2	8.09	0.0	8.01	0.0	8.16	0.0	8.12	4.1	8.12	1.4
4	8.16	0.0	8.10	0.2	8.00	0.0	8.16	2.3	8.11	0.2	8.10	0.2	8.20	2.3	8.14	0.2	8.13	3.2
5	8.16	0.2	8.13	0.4	8.10	0.0	8.19	0.0	8.13	0.4	8.13	0.4	8.24	3.0	8.18	0.4	8.17	0.4
6	8.17	0.4	8.14	0.3	8.13	0.1	8.20	3.2	8.16	3.2	8.14	0.3	8.28	4.1	8.17	0.3	8.20	0.3
7	8.19	0.3	8.13	2.1	8.14	0.2	8.23	0.1	8.20	2.3	8.14	2.1	8.30	3.2	8.21	2.1	8.19	2.1
8	8.20	2.1	8.14	3.4	8.13	0.4	8.24	0.0	8.21	3.2	8.16	3.4	8.34	4.0	8.24	3.4	8.26	3.4
9	8.23	3.4	8.16	0.2	8.14	0.2	8.26	0.2	8.23	5.2	8.15	0.2	8.43	2.1	8.26	0.2	8.24	0.2
10	8.22	0.2	8.18	2.1	8.15	0.3	8.25	0.4	8.24	0.0	8.17	0.3	8.40	3.4	8.28	0.3	8.26	0.3
11	8.24	0.3	8.17	3.2	8.15	0.2	8.28	0.3	8.24	0.2	8.19	0.2	8.46	0.2	8.33	3.2	8.30	3.2
12	8.24	0.2	8.19	1.5	8.15	3.0	8.30	2.1	8.26	3.0	8.19	3.0	8.47	0.3	8.30	3.1	8.31	4.1
13	8.25	3.0	8.20	2.4	8.16	3.2	8.31	3.4	8.28	4.1	8.20	4.1	8.51	3.2	8.37	2.1	8.34	3.2
14	8.26	4.1	8.21	3.2	8.17	2.1	8.32	0.2	8.29	2.3	8.20	3.2	8.50	3.1	8.36	2.3	8.33	1.2
15	8.27	3.2	8.21	2.1	8.17	2.5	8.34	0.3	8.30	0.0	8.20	4.0	8.53	5.6	8.38	4.1	8.36	3.2

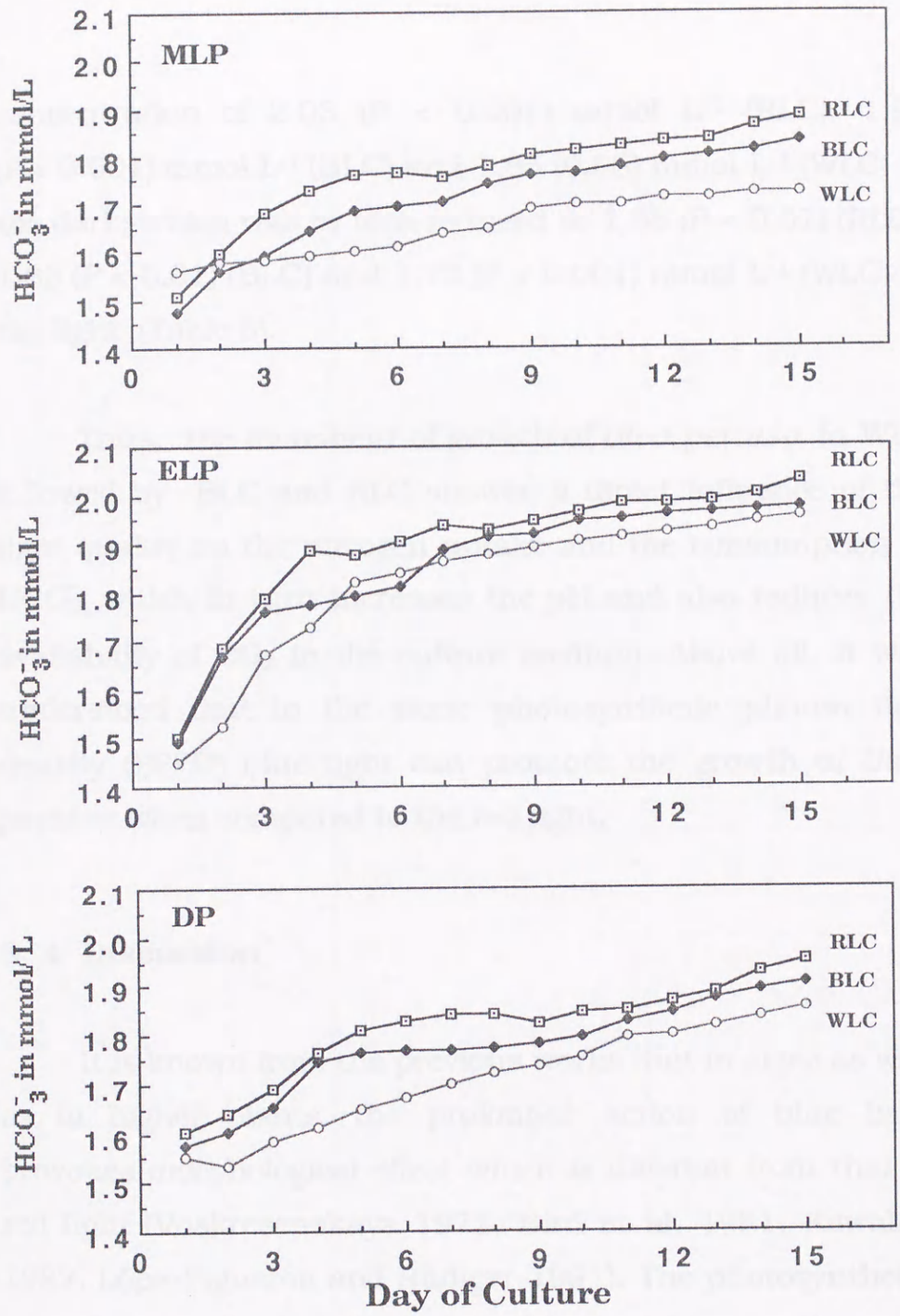


Fig. 18. Effect of light quality on the HCO_3^- variation in the medium of *Ulva pertusa* culture. MLP, mid light time, 13: 00; ELP, early light time, 7: 00; DP, dark time, 0: 00. WLC, white light culture; BLC, blue light culture; RLC, red light culture.

concentration of 2.03 ($P < 0.001$) mmol L^{-1} (RLC), 1.98 ($P < 0.001$) mmol L^{-1} (BLC) and 1.85 (0.05) mmol L^{-1} (WLC) in the dark; which was in tern reduced to 1.88 ($P < 0.01$) (RLC), 1.83 ($P < 0.01$) (BLC) and 1.72 ($P < 0.001$) mmol L^{-1} (WLC) in the light (Table 5).

Thus, the increment of growth of *Ulva pertusa* in WLC followed by BLC and RLC showed a direct influence of the light quality on the nitrogen uptake and the consumption of HCO_3^-, which in turn increases the pH and also reduces the availability of CO_2 in the culture medium. Above all, it was understood that in the same photosynthetic photon flux density (PPFD) blue light can promote the growth of *Ulva pertusa* when compared to the red light.

3. 4 Discussion

It is known from the previous works that in algae as well as in higher plants, the prolonged action of blue light provokes morphological effect which is different from that of red light (Voskresenskaya 1972, Bird et al. 1981, Kowallik 1982, Lópe-Figueroa and Rüdiger 1991). The photosynthetic experiment in the field may be problematic because it is very difficult to keep appropriate time scales and regulation (Harris 1986). Moreover, it is difficult to perform the light

Table. 5. Effect of light quality on the HCO_3^- variation in the medium of *Ulva pertusa* culture. DP, dark time, 0: 00; ELP, early light time, 7: 00; MLP, mid light time, 13: 00

Days	Red light culture						Blue light culture						White light culture					
	MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)		MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)		MLP (mmol/l)		ELP (mmol/l)		DP (mmol/l)	
	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)	Av.	SD ($\times 10^{-3}$)
1	1.51	4. 6	1.50	1. 2	1.60	5.2	1.48	5. 2	1.50	3. 7	1.58	5. 5	1.56	2. 1	1.45	0. 3	1.55	4. 5
2	1.60	2. 5	1.69	3. 6	1.64	3. 7	1.56	3. 2	1.67	2. 6	1.60	7. 6	1.58	6. 5	1.53	0. 4	1.53	3. 2
3	1.68	3. 7	1.79	3. 1	1.69	3. 7	1.59	6. 3	1.76	7. 5	1.65	6. 5	1.58	5. 2	1.67	0. 4	1.58	7. 4
4	1.72	9. 6	1.89	2. 3	1.77	4. 1	1.64	4. 6	1.77	3. 7	1.75	0. 6	1.59	4. 1	1.73	0. 6	1.61	3. 2
5	1.76	8. 6	1.88	4. 2	1.81	2. 4	1.69	0. 5	1.80	2. 6	1.76	0. 3	1.60	3. 7	1.82	0. 3	1.65	3. 2
6	1.76	6. 5	1.91	6. 5	1.83	1. 4	1.70	3. 2	1.81	2. 4	1.77	0. 2	1.61	7. 4	1.84	0. 2	1.67	6. 2
7	1.75	5. 5	1.94	8. 1	1.84	5. 2	1.70	3. 6	1.89	3. 7	1.77	0. 0	1.64	1. 2	1.86	0. 0	1.70	8. 9
8	1.78	0. 4	1.93	9. 1	1.84	3. 3	1.75	6. 3	1.92	6. 3	1.77	0. 2	1.65	3. 7	1.88	0. 2	1.72	9. 4
9	1.81	0. 6	1.95	7. 5	1.82	4. 6	1.76	5. 2	1.92	3. 2	1.78	0. 5	1.69	2. 4	1.89	0. 5	1.73	0. 5
10	1.82	0. 3	1.96	6. 5	1.85	4. 8	1.77	3. 3	1.95	1. 3	1.80	5. 3	1.70	1. 4	1.90	0. 3	1.75	0. 3
11	1.83	0. 2	1.99	3. 5	1.85	5. 8	1.78	4. 2	1.95	2. 3	1.83	6. 5	1.70	5. 2	1.91	0. 7	1.80	0. 7
12	1.84	0. 0	1.99	3. 7	1.87	6. 5	1.79	3. 7	1.96	0. 4	1.85	4. 2	1.71	6. 3	1.92	0. 4	1.80	0. 4
13	1.84	0. 2	1.99	2. 6	1.89	4. 8	1.80	3. 3	1.96	7. 4	1.87	6. 4	1.72	6. 3	1.93	0. 4	1.82	0. 4
14	1.87	0. 5	2.00	1. 5	1.93	2. 1	1.81	2. 1	1.97	5. 4	1.89	2. 5	1.72	6. 4	1.95	0. 0	1.84	4. 2
15	1.88	0. 3	2.03	7. 4	1.95	1. 3	1.83	2. 3	1.98	4. 2	1.91	4. 2	1.72	5. 4	1.95	0. 1	1.85	6 2

quality experiment in the field, where the energy utilization from the individual spectra can not be detected easily. In that case, the laboratory growth experiments are more suitable as they continued until the steady state of growth under controlled parameters.

Most likely the increment of pH in the light period may be due to the consumption of HCO_3^- by *Ulva pertusa* during photosynthesis. That is because seaweed can fix CO_2 with the help of RuBP carboxylase directly but in *Ulva* and a few other seaweeds, CO_2 incorporation is also done via a bicarbonate with the aid of carbonic anhydrase (Beer and Israel 1986). During this reaction OH^- is released, which in tern increases alkalinity of the culture medium.

Growth rate was considerably affected by all the controlled parameters. The stimulating effect of blue light on the growth of *Ulva pertusa* is comparable with other algae (Lüning and Dring 1973, Dring and Lüining 1975, Baghdadli et al. 1994). The highest growth rate of 8.55 ± 0.35% day^{-1} in WLC under the low irradiance of 60 μmol photon $m^{-2}s^{-1}$ in air equilibrated sea water is an appreciable one when compared to the previous field and lab experiments in *Ulva pertusa* (Hirata and Xu 1990, Matsukawa and Umebayashi 1987, Floreto et al. 1993;) and also as comparable with other growth rate experiments in the genus of *Ulva* (Lapointe and

Tenore 1981, Neori et al. 1991, Frost-Christan and Sand-Jensan 1990). The low light intensities were chosen for two main reasons: it is the condition more typical for many natural seaweed populations (Davison 1991); and to emphasize the light quality (blue, red) in low irradiance. As a result, the stimulatory effect of blue light on the growth of *Ulva pertusa* was thus evident even at this irradiance level.

Nitrogen is the primary material source limiting seaweed growth in the natural environment. This suggests that variation in seaweed growth rates should correspond to variation in N supply. N uptake capacity of seaweed (Vmax) is a direct function of a surface area to volume ratio (SA/V) (Rosenberg and Ramus 1984), whereas N storage capacity varies approximately inversely with SA/V (Duke et al. 1987). This suggests that the degree of coupling between seaweed growth rates and N supply may also be a function of SA/V. High SA/V species, which have high Vmax but low storage capacity, would have growth rates highly correlated with N supply, whereas low SA/V species with low Vmax would store N and have growth rates relatively independent of N availability (Rosenberg and Ramus 1982b). Thus, a seaweed's "functional form" (Littler and Littler 1980) may determine its ability to buffer nutrient variability. As described, in the present result also, since the *Ulva pertusa* had high SA/V, the growth rate is highly correlated with the

nitrogen uptake, especially in WLC and BLC. Similar findings were also reported in *Ulva* and other algae (Tischner and Lorenzen 1979, Fujita 1985, Henley et al. 1991, Lopéz-Figueroa and Rüdiger 1991) . Furthermore the result that blue light mediated nitrate uptake was also reported by Calero et al. (1980), Azuara and Aparicio (1983), and Duke and Duke (1984).

Most likely the increase of pH in the light period may be due to the consumption of HCO_3^- by *U. pertusa* during photosynthesis. That is because seaweeds can fix CO_2 with the help of RuBP carboxylase directly but in *Ulva* and few other seaweeds CO_2 incorporation is done via bicarbonate with the aid of carbonic anhydrase (Beer and Israel 1986). During this reaction OH^- is released, thereby increases the alkalinity of culture medium. In *Ulva pertusa*, the availability of this enzyme has been reported by Ikemori and Nishida (1967).

The low sensitivity of growth to increased O_2 concentration may also correspond to earlier reports on growth and photosynthetic experiments (Bidwell and McLachlan 1985, Beer and Israel 1986, Holbrook et al. 1988).

In conclusion, the increment of growth of *U. pertusa* in

WLC followed by BLC and RLC shows a direct influence on the nitrogen uptake and the consumption of HCO_3^- which in turn increases the pH and also reduces the availability of CO_2 in culture medium. Above all, it is understood that at the same PPFD blue light can promote the growth of *U. pertusa* when compared to the red light.

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSION

The floating sterile mutant of *Ulva pertusa* was grown in the laboratory under three light conditions. White light (as reference), broad band isoquantic red (600-700 nm) and blue (400-500 nm) light. The morphological variation of cell organelles in white (WLC), blue (BLC) and red light cultures (RLC) was studied by the electron microscopy after 15 days of light treatment with 14:10h light and dark photoperiod. The results indicate that:

(i) blue light is more efficient than the red light in developing the thylakoid architecture of individual cell organelles.

(ii) in WLC closely arranged, well organized thylakoids were seen, where as in BLC, although the numbers of thylakoid layers was similar to WLC and they were widely separated from each other. In contrast to this, in RLC the thylakoids were less prominent and were also densely covered with ribosome.

(iii) the cell maintenance (integrity) process, which has been most essential for the cell division and growth was positively controlled by the blue light.

(iv) the cell morphology of *U. pertusa* was relatively well developed in BLC than in RLC and also comparable to WLC.

On the metabolic aspects, the nitrogen uptake and usage in BLC were significantly far better than in RLC and comparable to WLC. From this it seems that the biofilter

mechanism is purely relay on the blue light irradiation. The results as follows:

i) the utilization of nitrogen in BLC is significantly higher than in RLC.

ii) the contents of chlorophyll, protein and nitrate reductase (NR) activity were significantly higher in BLC while carbohydrate content was slightly higher in RLC.

For the CO_2 remedy and feed back functions, the results are as follows:

i) a stimulatory effect of blue light on the growth at low irradiance (60 μ mol m^{-2} s^{-1}) was observed during the 15 days of culture.

ii) considerable CO_2 fluctuation in the light and dark periods was observed in all cultures, and it was higher in WLC, followed by the BLC and RLC.

iii) The consumption of HCO_3^- may have increased the pH level. As a result, it led to reduce air CO_2 in the medium.

The possible blue light induced the carbon metabolism and amino acids production are explained after Miyachi et al., (1979). As shown in the scheme (Fig. 19), the red light energy absorbed by phytochrome is used by chlorophyll's function to drive photosynthesis only. The overall process for the formation of plant biomass (growth) can be divided as follows:

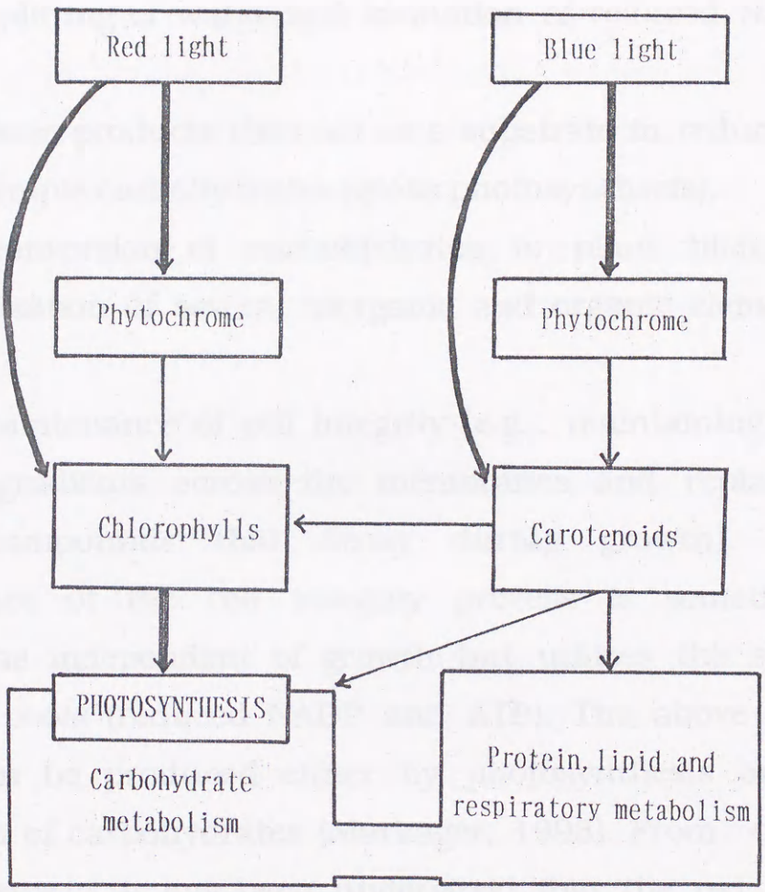


Fig. 19. Supposed routes of light quality influences on pigments and metabolism in *Ulva* cells. Routes except the bold lines are not yet completely proved.

(i) absorption of incident light by phytochrome and photosynthetic pigments.

(ii) splitting of water and formation of reduced NADP and ATP.

(iii) these products then act as a substrate in reduction of CO_2 to simple carbohydrates (gross photosynthesis).

(iv) conversion of carbohydrates to plant biomass, involving fixation of several inorganic and organic elements (N, P, S etc.).

(v) maintenance of cell integrity (e.g.., maintaining the chemical gradients across the membranes and replacing organic compounds that decay during growth). The maintenance of the cell integrity process is sometimes regarded as independent of growth but utilizes the same substrate pools (reduced NADP and ATP). The above said energy can be produced either by photosynthesis or by respiration of carbohydrates (Markager, 1993). From other relevant reports, it has been understood that the red light phytochrome only runs the calvin cycle and produces sugars. The prolonged application of red light resulted in disorganized thylakoid membrane and the light was not enough to maintain the cell integrity . On the other hand, as shown in Fig. 19, the blue light photoreceptor should run the calvin and TCA cycles, ultimately produce amino acids and fatty acids through blue light enhanced respiration (Kowallik, 1982). As a result, the cell integrity process should

be performed well. Overall, the white light, which has the energy for activation of both phytochrome and chlorophylls should produce good maintenance and the growth (Dring, 1988). The mechanism of photo controlled protein synthesis in *Ulva pertusa* has not yet been investigated. It could imply nitrate uptake as well as nitrate reduction and incorporation into amino acids and protein. The later process might be indirectly related to respiratory carbohydrate metabolism in general.

The acquisition of inorganic carbon by the common marine macroalgae *Ulva* is based on HCO_3^- utilization in combination with a system that concentrates CO_2 to the site of fixation via ribulose 1, 5-biphosphate carboxylase/ oxygenase (Rubisco) (Beer and Eshel 1983). One of the two ways described for utilizing HCO_3^- is by extra cellular dehydration followed by CO_2 uptake, whereas the other is by direct uptake of ionic form through plasma lemma. The first way depends on the extra cellular activity of carbonic anhydrase (CA) present in certain *Ulva* species (Bjork et al. 1993), while the function of putative anion transport was proposed to facilitate HCO_3^- uptake in another species (Drechsler and Beer 1991, Drechsler et al. 1993). In *Ulva pertusa*, the availability of this enzyme CA, has been reported by Ikemori and Nishida (1967). Except in the elevated CO_2 conditions (Adamec 1993), the uptake of HCO_3^- by those

plants that have the enzyme CA can be derived as follows (Gao et al. 1991):

$$HCO_3^- \xrightleftharpoons{CA} CO_2 + OH^- \qquad (1)$$

$$CO_2 + H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} (H_2CO) + O_2 \qquad (2)$$

In this case, the pH increases considerably, probably due to the release of OH^- ions (eq. 1). As alkalinity increases in the cultures, the HCO_3^- level also increases considerably. Due to this, dissolved CO_2 is hydrated from carbonic acids, which associates to bicarbonate (eq. 3, 4). As a result, in the culture media (CO_2) aq will reach an equilibrium again with that of bubbling gas pCO_2, by reducing the CO_2 from air (eq. 5)

$$(CO_2)aq + H_2O \rightleftharpoons H_2CO_3 \qquad (3)$$

$$H_2CO_3 \xrightleftharpoons{k} H^+ + HCO_3^- \qquad (4)$$

$$cpCO_2 \rightleftharpoons (CO_2)aq \qquad (5)$$

where c and k respectively indicate the solubility of CO_2 and dissociation constant whose values depend on the temperature and chlorinity of seawater. As calculated, the bicarbonate formation which should predominantly take place in the dark period resulted in lower pH than in the light, due to the release of H^+ ion (eq. 4).

The mechanism of the direct hydration and dehydration reactions of CO_2 in water can be written as follows (Johnson 1982):

$$\begin{array}{ccc} H^+ + HCO_3^- & \underset{k21}{\overset{k12}{\rightleftharpoons}} & H_2CO_3 \\ \uparrow k13 \quad k31 \downarrow & & \uparrow k32 \quad k23 \downarrow \\ CO_2 & & H_2O \end{array} \qquad (6)$$

It recognizes that CO_2 can react with water to form either HCO_3^- or H_2CO_3. The rate constant k_{12} and k_{21} are up to seven orders of magnitude greater than the hydration and dehydration rate constants. The two mechanisms shown above for the reaction of CO_2 with water can not, therefore, be separated. On the other hand, CO_2 also may react with OH^- ion:

$$CO_2 + OH^- \underset{kHCO_3}{\overset{kOH^-}{\rightleftharpoons}} HCO_3^- \quad (7)$$

Therefore, the rate law for the reaction of CO_2 in an air equilibrated condition is as follows:

$$\partial(CO_2)/\partial t = - (kCO_2 + kOH^- K_W/a_H)(CO_2) + (k_d a_H + kHCO_3)(HCO_3^-) \quad (8)$$

For this equation, K_W/a_H has been substituted for the activity of OH^- and K_W is the thermodynamic dissociation constant of water. In this case, the present results of pH, HCO_3^- and CO_2 dynamics in the medium, especially for WLC and BLC are much more correlated than the RLC. Moreover, if at certain pH levels (8.1<), there would be considerable CO_2 reduction in the medium, since *Ulva pertusa* may uptakes CO_2 from HCO_3^-. Furthermore the mechanism may be restricted to the algae which has the enzyme CA.

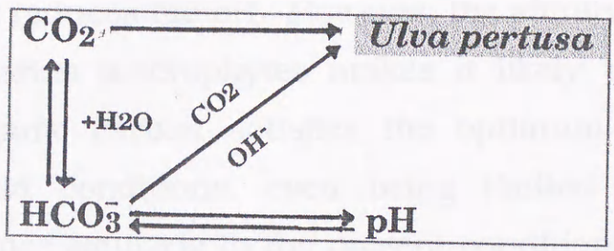
Utilization of HCO_3^- by marine macroalgae is not always a universal ability. There are exceptions of pure CO_2 users (Dromgoole 1978). Despite the importance of HCO_3^- as carbon source for photosynthesis and growth, especially in macroscopic plants, there are few studies for environmental conditions that mediate HCO_3^- use and few for direct

measurements of the affinity for HCO_3^- relative to CO_2. Among submerged macrophytes, the half saturation constant for CO_2 use $K_{1/2}$ (CO_2) is usually between 0.07 and 0.03 mM CO_2 under light saturated photosynthesis (Sand-Jenson 1983). This range in $K_{1/2}$ (CO_2) values, which is much higher than the equilibrated pool under light saturation, may be limited by the CO_2 supply; which may in turn allow the usage of HCO_3^- in certain macrophytes, as found in the present experiments.

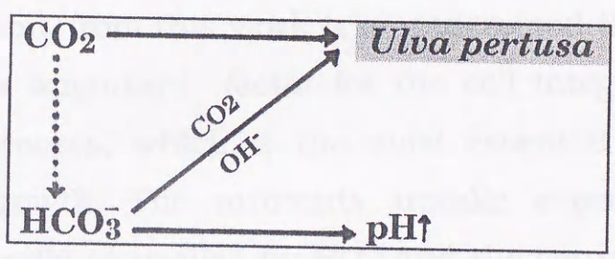
The low sensitivity of *Ulva pertusa* growth to the increased O_2 concentration also corresponded to other's growth and photosynthetic experiments (Colman 1984, Bidwell and McLachlan 1985, Beer and Israel 1986). In addition, among the marine macroalgae tested, many show the same insensitivity of photosynthesis to oxygen (Beer and Shragge 1987, Holbrook et al. 1988, Reiskind et al. 1988).

The possible interrelation between the observed parameters and the growth of *Ulva pertusa*, under different light quality is explained in Fig. 20. However, this scheme is more significant in the culture of WLC and BLC than RLC. During the light period, the usage of HCO_3^- by *Ulva pertusa*, increases the pH, may be due to the release of OH^- ion. As a result, HCO_3^- has a considerable effect in the increment of pH as well as in hydration of carbonic acids, which in turn slow

A . Both light and dark times



B. Light time



C. Dark time

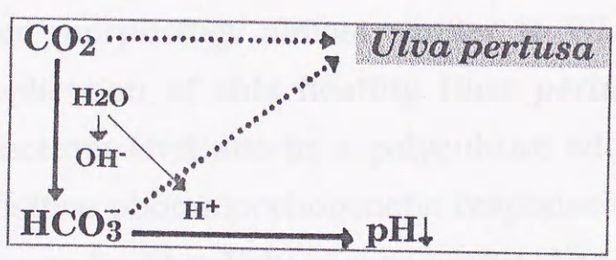


Fig. 20. Schematic representation of HCO_3^-, CO_2 and pH interactions in the light and dark times between *Ulva pertusa* and culture medium.

down by the photosynthetic usage of CO_2 considerably. On the other hand, in dark period, CO_2 involves in the hydration process and also reduces the pH. However, the affinity for the HCO_3^- of the marine macrophytes makes it likely that the supply of inorganic carbon satisfies the optimum growth under most field conditions, even being limited by the available irradiance similarly to the present conditions of the experiment.

In conclusion, from this work it is understood that blue irradiance is the important factor for the cell integrity (cell maintenance) process, which is the most essential for cell division and growth. The nutrients uptake especially of nitrogen is generally controlled by BLC and the consumption of HCO_3^- from the medium is higher in BLC than in RLC. Furthermore, it is only under blue light that thalli show development and morphology almost similar to WLC. So in the event of application of this floating *Ulva pertusa* as a biofilter in the bottom level site in a polyculture with fish in the field, the positive photomorphogenetic response obtained in the laboratory under blue light is very useful. And it seems to be related to the growth strategy of this algae in natural white light deficient conditions.

CHAPTER V

SUMMARY

1. The green algae *Ulva pertusa* distributing commonly in the intertidal zone has a floating type which exhibits vegetative development even in summer. Its absorptive function of nutrients seems useful as a biofilter for restraining the coastal eutrophication. However, this ability has not yet been studied in relation to irradiation condition of light.

2. For this reason, the present study was conducted to investigate the following articles: i) the light quality influence on the morphology and development of cell organelles in *Ulva pertusa*. ii) physiological changes that occur in *Ulva pertusa* metabolism during this irradiance, and iii) the biofilter function through nitrogen uptake and utilization at three light qualities. Further object was extended on the interrelationship between *Ulva pertusa* and environmental factors, mainly CO_2, HCO_3^-, O_2, and pH. The role of CO_2 and HCO_3^- as feed back function and the inter link with pH were discussed.

3. The experiment was conducted with a floating sterile mutant *Ulva pertusa*, in the laboratory for 15 days, under three light conditions: white light (as reference) WLC , broad band isoquantic red light (600-700 nm, peak transmission: 660nm) RLC and blue light (400-500 nm, peak transmission: 430 nm) BLC, at 55-66 μmol m^{-2} s^{-1} irradiance of 14L : 10D photoperiod at 25°C.

4. The morphological variations of cell organelles of *Ulva pertusa* in WLC, BLC and RLC were electron microscopically observed after 15 days of culture. The results have shown that the blue light is more efficient than the red light in the architecture of individual cell organelles. The cell maintenance (integrity) process, which is the most essential for the cell division and growth was positively controlled by the blue light. Furthermore, the cell morphology of *Ulva pertusa* was relatively well developed in BLC than in RLC and also comparable to WLC. The light quality affected the structure of the chloroplast morphology considerably. In WLC, thylakoids were arranged in 3 stalk bands (lamella), and in BLC also these were just as clear but without lamella. However, in RLC, the thylakoids were not prominent and were covered with dense ribosome.

5. On the metabolic aspects, the second experiment under the above condition treated successive samplings from incubating *Ulva* to deal with growth rate due to the increment measurement of leaf area, contents of carbohydrate, protein and chlorophylls. The nitrate reductase activity and nitrate content were measured.

6. The specific growth rate of WLC varied highly when compared to BLC and the RLC. The carbohydrate content was little higher in RLC than in the BLC. However, in the BLC,

the protein content was significantly higher than that in RLC. Overall, *Ulva pertusa* in WLC had the highest amount of carbohydrate and protein. And under the blue light the nitrate reductase enzyme had higher activity than in red light. The pigments including Chl a and Chl b were synthesized more in BLC than in RLC. The optical absorption of the whole spectra revealed that BLC had higher pigment concentration throughout the visible spectrum (400 - 700 nm) than RLC.

7. On the interrelationship between *Ulva pertusa* and the culture medium studies, from the successive samplings at the light and dark periods, such functions as the nitrogen uptake and acid-base balance with the medium were investigated under the same condition as above.

8. The measured specific growth rates for WLC, BLC and RLC were around 8.6%, 3.15% and 1.2% day^{-1}, respectively. The stimulatory effect of blue light on the growth in low irradiance (60 μmol photon m^{-2} s^{-1}) was shown during 15 days of culture. Around 42.1% of total nitrogen was used by the *Ulva pertusa* in WLC. But in BLC and RLC it was around 27.0% and 16.7%, respectively. However, the utilization of nitrogen by *Ulva pertusa* in the BLC is significantly higher than RLC. Considerable CO_2 fluctuation in the light and dark periods was shown in all cultures, and it was higher in WLC, followed by BLC and RLC. The pH increased gradually from

the initial day of culture, however, the pH values were higher in the light period than the dark in all cultures. The maximum level of pH (8.53) was measured in WLC followed by BLC (8.34) and RLC (8.27) in the light period. As alkalinity increases in the cultures, the HCO_3^- level also increased but it was only significant for WLC and the BLC.

9. In conclusion, from this work it is understood that compared to red irradiance (RLC), blue light (BLC) is the dominant factor for cell integrity (cell maintenance) process, which is the most essential for cell division and growth. The nutrients uptake especially of nitrogen is totally controlled by BLC and the consumption of HCO_3^- from the medium is higher in BLC than the RLC. Furthermore, it is only under BLC that thalli present a development and morphology almost similar to white light conditions, indicating the suitability for biofilter function of *Ulva*.

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