A−5.1.3 Acclimation of Marine Algae to the Enhanced UV−B (Final Report)

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Abstract The research is aimed to examine the UV−tolerance of seaweeds and the change of the tolerance in UV−irradiated seaweed bodies. Experimental cultivations of red algae were carried out with UV−irradiance only during light period in 12L:12D photoperiod. The experiments of *Chrysymenia wrightii* showed short term effects, which included the decrease of photosynthetic pigments and UV−absorbing components during the first light period, and the long term effects, which included the decrease of photosynthetic pigments, the increase of UV−absorbing components, and the lowered growth rate through the second light period. In same experiments of *Porphyra pseudolinearis*, however, the growth rate, which was lowered at once immediately after the first light period started, was recovered through the third or fourth light period when UV−absorbing components increased. Surveys of UV−absorbing components distribution in wild plants of *C. wrightii* showed that the apical portion contains the components with higher concentration than the basal portion. Experimental cultivation, with UV−irradiated seawater as culture medium, indicated that increase of nitrous acid concentration and other undetected changes in UV−irradiated seawater lowered growth rates of *C. wrightii* and *P. pseudolinearis*.

Key Words Seaweed, UV−B, Growth, Photosynthetic pigment, UV−absorbing component

1.Introduction

The stratospheric ozone layer is presumed to be depleted in the near future, by which enhanced UV radiation should reach to the surface of the earth. Especially, enhanced UV−B radiation ranging from 280 to 320nm in wavelength is feared to exert harmful influences on terrestrial lives. In the ocean, solar UV radiation was proved to penetrate at considerable rate up to 80%/m in clear seawater. [1)5)] The role of solar UV radiation in marine ecosystem is studied mainly about planktonic primary production and lower levels of consumption, or about benthic animals on tropical coral reefs. Studies about seaweeds suggest that UV radiation may inhibit the photosynthesis [3)7)8)9)] and be a potential factor determining the inhabiting depth. [9)15)] On the other hand, coral, phytoplankton, zooplankton, seaweed, and other marine lives were found to contain UV−absorbing components; mycosporine−like amino acids, [2)4)6)10)11)12)14)] which are discussed to be concerned with their UV−tolerance. Experimental cultivations of red algae with artificial UV−B irradiance indicated that enhanced solar UV−B radiation may affect some species to increase UV−absorbing components in their plant bodies. [16)]

2.Research Objective

The research was aimed to examine the UV−tolerance of seaweeds and the change of the tolerance in UV−irradiated seaweed bodies. Experimental cultivations of red algae, with artificial UV−B irradiance only during light period in 12L:12D photoperiod of visible light, were carried out to examine the course of changes in UV−B irradiated plants, especially on concentration of UV−absorbing components. The concentration of UV−absorbing components in wild plants of red algae were also surveyed and compared with results of the experimental cultivations. Because chemical changes of seawater in flasks used for cultivations were supposed to be produced by UV−B irradiance, their effects on cultivated red algae were examined by experimental cultivations.

3.Research Method

(1)The Course of Changes in Cultivated Plants of Red Algae Irradiated by UV−B

Several species of red algae were used for experimental cultivations. Cultivated tetrasporophytes of *Chrysymenia wrightii* originated from mature carposporophytes collected at sublittoral zone, and cultivated gametophytes (foliose thalli) of *Porphyra pseudolinearis*, through stock culture of sporophytes (conchocelis thalli), originated from gametophytes collected at supralittral to mediolittral zone. Both of them were cultured only with visible light irradiance.

Experimental cultivations with UV−B exposure were carried out in a culture chamber where controlled temperature and white−cool fluorescent illumination were available (Tab.1). Visible light illumination produced light−dark period with 24hour cycle. UV−B irradiance was supplied by a transilluminator (TL−33, UVP INC.; Fig.1) only during light periods. UV−B intensity was measured by a digital radiometer (UVX−31, UVP INC.; Fig.2). During cultivation, some of materials were sampled to estimate the growth on each schedule (Fig.3). In order to estimate concentrations of photosynthetic pigments and UV−absorbing components, absorption spectra ranging from 200 to 780nm were measured with ethanol extractions from sampled materials.

(2)Distribution of UV−absorbing Components in Wild Plants of Red Algae

Wild plants of *C. wrightii*, which forms grass field−like communities and grows in the manner of apical growth, were collected from a same community at 5 to 8m depth. Each of them were divided vertically into five layers (Fig.4), from which absorption spectra of ethanol extraction were measured to estimate concentration of UV−absorbing components.

(3)Effects of UV−irradiated Seawater on Cultivated Plants of Red Algae

Cultivated plants of *C. wrightii* were cultured under only visible light with Provasolli's enriched seawater [13] (PES) as culture medium, which was irradiated by a UV transilluminator only during light period of 12L:12D photoperiod during fixed numbers of preceding days (Tab.2). The growth was followed every seven days during cultivations.

Nitrous acid concentration was proved to increase in UV−irradiated seawater when it was previously enriched (Tab.3; Teduka, personal communication). Then, cultivated plants of *P. pseudolinearis were cultured* with seawater as culture medium of five treatments about periods of UV−irradiance and opportunities of enrichment (Tab.4). Experimental cultivations with "Nitrite−modified PES" as culture medium, which was added with sodium nitrite instead of sodium nitrate in the PES treatment, were also carried out.

4.Result

(1)The Course of Changes in Cultivated Plants of Red Algae Irradiated by UV−B

The experimental cultivation only under visible light indicated that cultivated plants of *C. wrightii* may grow more rapidly at 20 to 25 ℃ than at 10 to 15 ℃ (Fig.5). Growth of plants cultivated under UV−irradiance was inhibited through the second light period in comparison with that of plants irradiated only with visible light (Fig.6). Optical densities of chlorophyll indicated by absorbency at 664.4nm decreased immediately after the start of the first light period, and was kept at that lower level during cultivations. Optical densities of UV−absorbing components indicated by absorbency at 321.2nm, which decreased at once during the first light period, increased through the second light period (Fig.7).

Growth rates of *P. pseudolinearis* under UV irradiance, which declined at once during the first and second light periods, was recovered and in the increasing trend through the third or fourth light period (Fig.8). Optical density ratios of UV-absorbing components to chlorophyll, indicated by absorbency at 665.6nm and 335.6nm respectively, was in the increasing trend through the third or fourth light period, although they decreased at once during the first and second light periods (Fig.9).

(2)Distribution of UV-absorbing Components in Wild Plants of Red Algae

Absorption spectra from vertical five layers of *C. wrightii* had several peaks, which indicated optical densities of chlorophyll and UV-absorbing components at 664.8nm and 321.2nm respectively (Tab.5). Peaks in the range of visible light were almost same in every layer. However, absorbency of peaks in the UV range, especially at 305.6nm and 321.2nm, were higher in apical portions than those in basal portions.

(3)Effects of UV-irradiated Seawater on Cultivated Plants of Red Algae

Cultivated plants of *C. wrightii* grew at higher rates in 0 to 1 day UV-irradiated PES than those in 3 to 7 days UV-irradiated PES (Fig.10). Cultivated plants of *P. pseudolinearis* grew also at higher rate in 0 to 1 day UV-irradiated PES than those in 3 to 6 days UV-irradiated PES (Tab.6). Plants cultivated in seawater enriched after UV-irradiated, where nitrous acid concentration was not considered to increase, grew at higher rates for the first 3 days than those in seawater enriched before UV-irradiated where nitrous acid concentration was considered to increase, although their growth rates declined after the third day of cultivations. Growth of *P. pseudolinearis* cultivated in "Nitrite-modified PES" was inhibited in comparison with those in ordinary PES (Tab.7).

5.Discussion

Previous experiments suggested that some species of red algae may increase their UV-absorbing components in their bodies under UV radiation.[16] Because of those results, it was considered to be important for UV-exposure experiments and surveys of UV-absorbing components in plants how materials experienced UV radiation before experiments or surveys. On the other hand, because UV-absorbing components were discussed to be concerned with UV-tolerance of marine lives, some species of red algae were supposed to have an ability for acclimation to the enhanced UV-B.

In this study, experiments of *C. wrightii*, irradiated by UV-transilluminator only during light period in 12L:12D photoperiod, showed short term effects of UV-B, which included the decrease of photosynthetic pigments and UV-absorbing components during the first light period, and long term effects, which included the decrease of photosynthetic pigments, the increase of UV-absorbing components, and the lowered growth rate through the second light period. In same experiments of *P. pseudolinearis*, however, growth rate, which was lowered at once immediately after the first light period started, was recovered through the third or fourth light period when UV-absorbing components increased. Because of these results, *P. pseudolinearis* inhabiting supralittral to mediolittral zone may have an ability for acclimation to the enhanced UV-B.

Because wild plants of *C. wrightii* forms grass field-like community at sublittoral zone, UV intensity in their micro environment can change vertically and relatively by self-shading. Because they grow in the manner of apical growth, their meristem can be exposed to relatively intensest UV-radiation. UV-absorbing components in wild plants of *C. wrightii* were revealed to be contained in apical portions with higher concentration than those in basal portions. These results suggested that wild plants of *C. wrightii* may change the concentration of UV−absorbing components in a response to the vertical change of UV intensity in their micro environment or as a protection of their meristem.

In many cases of UV−exposure experiments of marine lives, materials are stored into quartz glass vessels with seawater and irradiated by UV−radiation. Chemical changes of seawater in vessels are supposed to be produced by UV−irradiance. In this study, UV−irradiated seawater was proved to inhibit growth of *C. wrightii* and *P. pseudolinearis*. Effects of the enhanced UV−B on seaweeds may include seawater−intermediate systems in addition to direct effects.

Nitrous acid concentration was proved to increase in UV−irradiated seawater which was previously enriched (Teduka, personal communication). Experimental cultivations of *P. pseudolinearis* with treatments of seawater as culture medium, which were enriched before and after UV−irradiance, indicated that the inhibitory effects on seaweeds may include the increase of nitrous acids and other undetected changes in UV−irradiated seawater. These chemical changes of seawater may be produced by the enhanced UV−B in tide pools, seaweed forests, or embayments where seawater mass is relatively easy to be stilled.

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Table.1 Conditions for experimental cultivations of red algae with UV-irradiance.

Material	Temp. ℃	Visible light μ W/cm²	UV light μ W/cm²	Photoperiod	Culture medium
Chrysymenia wrightii	20	1050	200	12L:12D	PES
Porphyra pseudolinearis	20	900	230	12L:12D	PES

Table.2 Conditions for experimental cultivations of red alga *Chrysymenia wrightii* with UV-irradiated seawater as culture medium.

	Temp. ℃	Visible light μ W/cm²	UV light μ W/cm²	Photoperiod
Seawater preparation	20	1050	200	12L:12D
Cultivation of plants	20	1050	0	12L:12D

Table.3 Changes of nitrous acid concentration in UV-irradiated seawater as culture medium.

No.	Treatments	Nitrous acids
1	Enrichment → Visible and UV Irradiance for 7days → ------ →	Increasing
2	------ → Visible and UV Irradiance for 7days → Enrichment →	not Increasing
3	Enrichment → Only Visible Irradiance for 7days → ------ →	not Increasing
4	------ → Only Visible Irradiance for 7days → Enrichment →	not Increasing

Table.4 Conditions for experimental cultivations of red alga *Porphyra pseudolinearis* with UV-irradiated seawater in five treatments as culture medium.

	Enrichment	Temp. ℃	Irradiance days	Visible light μ W/cm²	UV light μ W/cm²	Photoperiod
Seawater preparation	Before UV	20	0	1050	200	12L:12D
	Before UV	20	1	1050	200	12L:12D
	Before UV	20	3	1050	200	12L:12D
	Before UV	20	6	1050	200	12L:12D
	After UV	20	6	1050	200	12L:12D
Cultivation of plants	−	20	−	1050	0	12L:12D

Table.5 Optical densities of photosynthetic pigments and UV-absorbing components (OD Subst. / g wet weight) in vertical five layers of wild plants of red alga *Chrysymenia wrightii*

Layer *	Wave Length (nm) 260.4	280.4	290.8	305.6	321.2	416.0	431.6	618.0	664.8
1	21.9	18.7	17.8	15.0	12.0	14.8	15.3	2.2	9.4
2	18.9	17.0	16.1	13.9	11.8	12.8	13.2	2.1	8.7
3	22.0	20.3	19.8	18.5	16.2	14.8	15.3	2.5	10.5
4	22.3	20.9	21.1	20.8	18.1	13.8	14.2	2.3	9.7
5	22.8	21.5	21.7	21.3	18.7	16.8	17.4	2.7	11.4

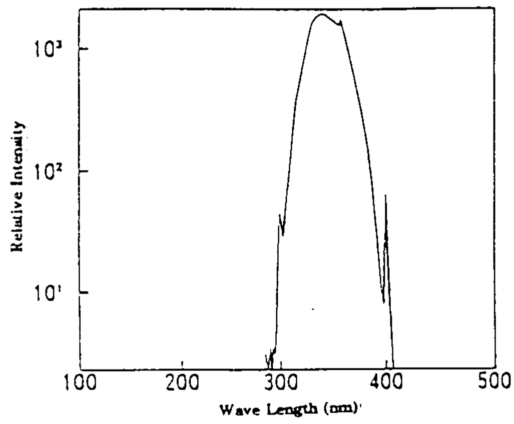
* See Fig.4

Table.6 Thallus area increasing rates (%/day) of red alga *Porphyra pseudolinearis* cultivated in UV-irradiated seawater in five treatments as culture medium.

Irradiance days	Erichment	Cultivating Period 0-3day	3-6day	6-9day	0-9day
0	Before UV	27.93	24.43	16.68	23.01
1	Before UV	31.53	29.18	22.22	27.64
3	Before UV	25.64	23.12	12.32	20.36
6	Before UV	22.14	19.17	15.44	18.92
6	After UV	28.30	19.00	16.17	21.16

Table.7 Thallus area increasing rates (%/day) of red alga *Porphyra pseudolinearis* cultivated in ordinary PES and "Nitrite-modified PES" as culture media.

Culture Medium	Cultivating Period 0-3day	3-6day	6-9day	0-9day
Ordinary PES − 1	33.41	29.23	20.19	27.61
Ordinary PES − 2	31.10	29.30	23.77	28.06
Nitrite-modified PES − 1	25.17	19.67	16.96	20.60
Nitrite-modified PES − 2	26.05	25.19	14.86	22.04



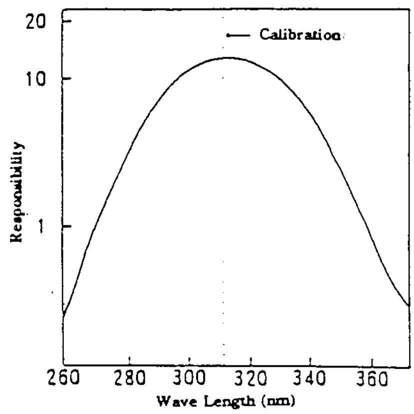


Fig.1−2 UV−irradiance used for experiments. Left; wave composition of transilluminator, TL33, UVP Inc. Right; responsibility of digital radiometer, UVX−31, UVP Inc.

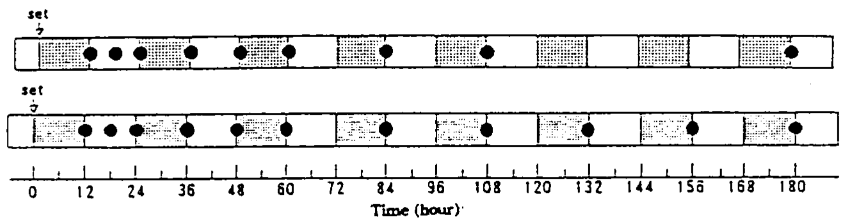


Fig.3 Schedules for UV−exposure experiments of red algae *Chrysymenia wrightii* (upper) and *Porphyra pseudolinearis* (lower). □; light period. ▒; dark period. ● ; sampling.

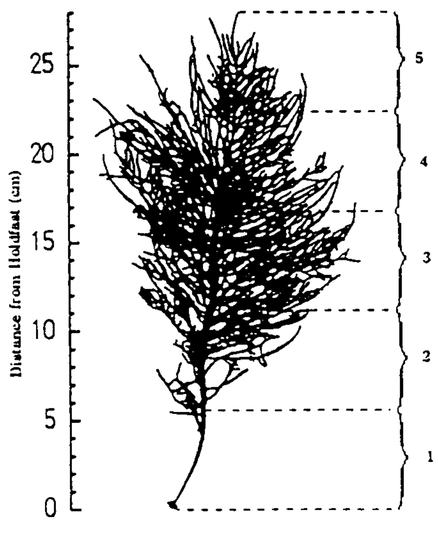


Fig.4 Layers in wild plants of red alga *Chrysymenia wrightii* to estimate UV−absorbing component distribution.

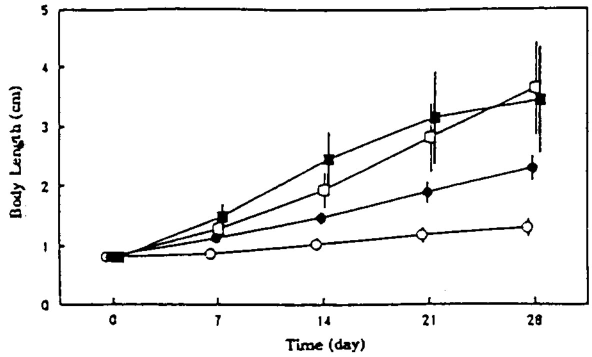


Fig.5 Growth of red alga *Chrysymenia wrightii* cultivated only under visible light. ○; 10 ℃. ●; 15 ℃. □; 20 ℃. ■; 25 ℃.

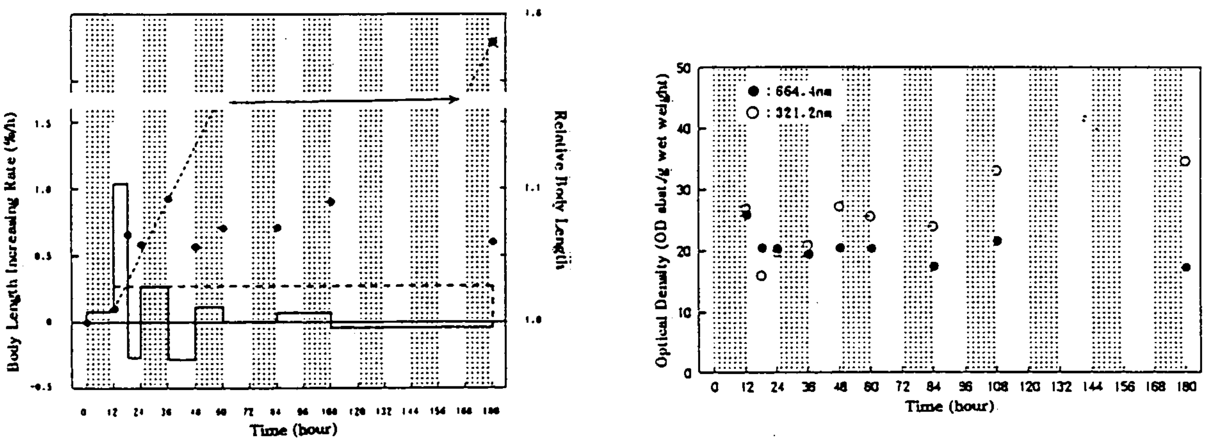


Fig.6-7 Growth of red alga *Chrysymenia wrightii* irradiated with UV transilluminator only during light period in 12L:12D photoperiod. Left; relative change of body length (●) and increasing rate of body length (line); doted lines indicating data at 20 ℃ from Fig.5 as a reference. Right; concentrations of chlorophyll and UV-absorbing components indicated by 64.4nm and 321.2nm, respectively.

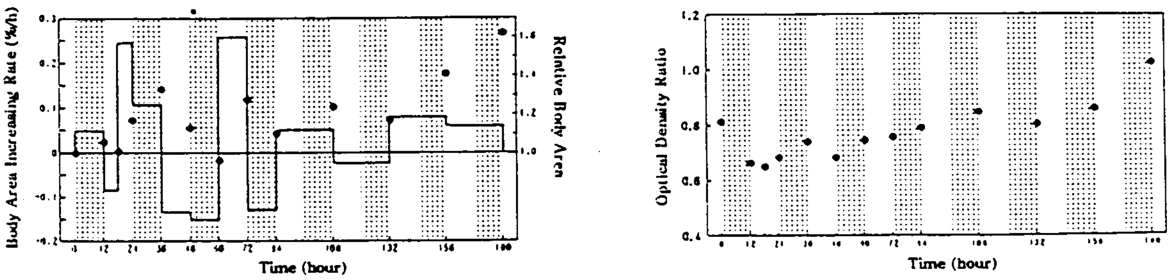


Fig.8-9 Growth of red alga *Porphyra pseudolinearis* irradiated with UV transilluminator only during light period in 12L:12D photoperiod. Left; relative change of body length (●) and increasing rate of body length (line). Right; concentration ratios of UV-absorbing components (335.6nm) to chlorophyll (665.6nm).

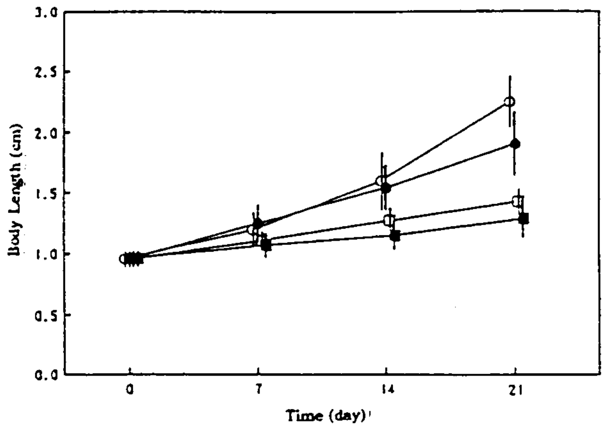


Fig.10 Growth of red alga *Chrysymenia wrightii* cultivated in UV−irradiated Provasolli's enriched seawater. ○ ; 0day UV−irradiated seawater. ● ; 1day UV−irradiated seawater. □ ; 3days UV−irradiated seawater. ■ ; 7days UV−irradiated seawater.