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The Effects of Copper on the Early Stages of *Undaria pinnatifida* (Harv.) Suringar (Laminariales, Phaeophyta) under Temperature-Irradiance Gradient

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The effects of copper were investigated in the economic brown alga *Undaria pinnatifida* under the temperature-irradiance gradient. On the meiospore settlement and germination, there was not significant effect of irradiance. However, the effects of temperature and copper concentration were significant, and the interaction between temperature and copper treatment was prominent. Copper treatment at high temperature was more detrimental to the gametophyte development. The growth of gametophytes was retarded under suboptimal temperature range and higher copper concentrations. The optimum condition of the gametophyte growth was 14°C and 50 μE $m^{-2}s^{-1}$. Irradiance became significant at the later stages of gamtophyte development. The chlorophyll contents and the electron transport activities in sporophyte were significantly reduced under 100 μg Cu l^{-1} added samples.

Key Words: copper, developmental stage, temperature-irradiance gradient, *Undaria pinnatifida*.

INTRODUCTION

Metallic elements are found in living organisms, where they play a variety of roles. They may be structural elements, stabilizers of biological structures, components of control mechanisms, and most of all, enzyme activators or components of redox systems. Some metals are essential elements and their deficiency results in impairment of biological functions.

The uptake of heavy metals show various effects in algae. At certain concentration levels, biological minor, or trace elements may increase the growth rate (Huntsman and Sunda, 1980). In the case of non-biological heavy metals, organisms are tolerant to some concentration, depending on the conditions of the organism and the form of the metal (Lepp, 1981). But at higher concentration levels, the organisms are likely to be affected in their growth, photosynthesis, mitochondrial respiration and other general metabolic pathways. Moreover, these effects can lead to death.

One of the most commonly observed effects of heavy metal poisoning is a change in cell size or morphology. This has been observed in a wide variety of organisms. The coupling of cell growth and cell division in *Dunaliella tertiolecta* (Davies, 1976); interruption of cell separation, cytoplasmic changes in color and contents, distrup-

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tion, cytoplasmic changes in color and contents, distruption of chloroplast intergrity and dispersion (Thomas *et al.*, 1980), morphological abbreviation (Sunda and Guillard, 1976) in phytoplankton species, and abnormal growth pattern and chloroplast structure (Brinkhuis and Chung, 1986) have been observed.

Though there are still uncertainties about the process involved in trace metal accumulation by brown seaweeds, their abilities to concentrate trace metal ions from the surrounding seawater make some seaweeds useful indicators of marine trace metal pollution (Bryan, 1983; Bryan and Hummerstone, 1973; Haug *et al.*, 1974; Myklestad *et al.*, 1978). Other macrobenthic green and red algae also have been considered as indicators (Andryushchenko and Polikarpov, 1974; Burrows, 1971; Edwards, 1972; Preston *et al.*, 1972; Foster, 1976; Fuge and James, 1974; Melhuus *et al.*, 1978; Seeliger and Edward, 1977; Seeliger and Knak, 1982). Moreover, there are differences between species in affinity for particular elements (Black and Mitchell, 1952), and many species show seasonal changes in metal concentrations, which may result, at least partially, from seasonal variation in growth (Fuge and James, 1974).

It is generally accepted that copper is essential for metabolic processes, as well as being toxic to a diverse range of biological systems, even in minute quantities. Trace amounts of copper are essential for algae and copper has particular significance for plastocyanin. There is no conclusive evidence for copper limitation in natural waters, but copper may be toxic to some phytoplankton species (Huntsman and Sunda, 1980; Jackson and Morgan, 1978; Morel and Morel-Laurens, 1983; Steeman-Nielsen and Wium-Andersen, 1970, 1971).

The effects of copper can be differentiated under the various environmental conditions such as temperature and irradiance (Cedeno-Maldonado and Swader, 1972; Greenfield, 1942; Kanazawa and Kanazawa 1969; Steeman-Nielsen and Wium-Andersen, 1971). The growth of other laminarian plants was affected by such environmental factors (Lee and Brinkhuis, 1988). Therefore, it is useful to investigate the effects of copper under the temperature-irradiance gradient.

The present study elucidates the copper effects on *Undaria pinnatifida* under the various temperature-irradiance gradient. Because this species has been cultivated as an important economic species for a long time, the identification of the relationship among temperature, irradiance and copper concentration will provide valuable informations on the management of aquaculture as well as the coastal ecosystem analysis.

MATERIALS AND METHODS

General Culture Procedure: A ripe portion of sporophyll with mature sori was cut from *Undaria pinnatifida* plants and wiped clean with 5% NaOCl (v/v in filtered seawater) and further rinsed with membrane filtered (Gelman) seawater. Cleaned sorus materials were allowed to dry in the dark for 3 hours prior to meiospore release, and immersed in seawater to make meispore suspension. The concentration of meispore suspension was adjusted to 50×10^4 cells ml^{-1} with 0.2 μm filtered seawater. The number of meiospores was counted using a hemacytometer.

Fifty microliters of the suspension were inoculated onto glass microscope coverslips (22 × 22 mm). Six-celled tissue culture wells were used as culture vessels. Immediately after inoculation, the cell wells were transferred to temperature-irradiance gradient plates of the types used by Halldal and French (1958), Edwards and Van Baalen (1970), Yarish *et al.*, (1979) and Siver (1983). A thermal gradient (6, 10, 14 and 18°C) was set by heating and cooling opposite ends. At a right angle to the thermal gradient, a photon fluence rate gradient (20, 50 and 80 μE m^{-2} s^{-1}) was imposed by five, 40 watt, cool-white fluorescent bulbs. The

photoperiod was 12:12 h LD.

After the spore settlement onto coverslips (12 h after inoculation), coverslips were transferred to cell wells containing 10 m*l* enriched seawater medium. The seawater was 0.45 μm filtered, uv-treated 4 hours with continuous stirring, and then 0.2 μm filtered again for sterilization before nutrient and copper agumentation. The media were enriched with 883 μM NO_3^- (as $NaNO_3$) and 36 μM PO_4^{-3} (as NaH_2PO_4) (Sunda and Guillard, 1976), then supplemented with diluted copper sulfate stock solution. All glassware surface were acid cleaned and Milli-Q water soaked before use. Copper was supplemented, and the concentration of copper in the media were 5, 10, 50 and 100 $\mu g l^{-1}$. The media were siphoned off and changed weekly. Af forty eight hours after the inoculation, percentage germination was determined on the basis of germ-tube production by scoring 100 meiospores selected in each cell well. Sporophytes, female gametophytes were scored under the criteria used by Kanda (1936). Ten female gametophytes of each sample were haphazardly selected, regardless of fertility, and measured for length to monitor the gametophyte growth.

Development stage analysis: Several significant stages can be identified during the incubation period of 3 weeks. They can be defined as six steps (Chung and Brinkhuis, 1986):

Stage I: Meiospore settlement without germ tube production

Stage II: Meiospore settlement with germ tube production

Stage III: Early stage of developing gametophyte (the movement of cytoplasmic contents toward germ tube and the increase in cell size)

Stage IV: Later stages of developing gametophyte (morphogenesis)

Stage V: Mature gametophytes

Stage VI: Sporophyte stage

One hundred plants were scored for stage development in each coverslip. If a particular stage represented more than 50% of the population, the development status of that was described as representing that stage.

Culture of young fronds: On February 1989, 5 month-old sporophytes (54.7 ± 13.3 cm in length and 11.6 ± 5.4g in wet weight) were collected from a commercial kelp farm in Kori. The symmetrical blade segments (*ca.* 30 cm^2 in blade area) or discs (3.2 cm in diam.) centered by midrib 25 cm above holdfast were cut off and acclimated for a week in culture vessels, which were kept in an environmental shaker. The culture vessels contained 200 m*l* seawater media which had been uv-irradiated, 0.2 μm membrane filtered, and then enriched with nitrate and phosphate as mentioned above. Each acclimation vessel held 2 blade segments and 3 discs, and was kept at 12°C, under 30 $\mu E\ m^{-2}s^{-1}$ with 12L:12D photoperiod. The incubation set-up was constantly stirred in a rotary shaking motion. The media were changed every other day.

After a week of acclimation, all the segments and discs were collected and ramdomly transferred to 200 m*l* capacity culture dishes whose copper concentration were augmented to 5, 10, 50, 100 $\mu g l^{-1}$ in the environmental shaker. The environmental conditions of the culture vessel were kept the same as the acclimation one, except the copper concentration.

After 7 days of incubation, hole punches (9 mm in diam.) were taken from the segments and discs for chlorophyll contents and oxygen evolution. Three discs of 9 mm diameter were homogenized in 90% acetone with the addition of two drops of saturated $MgCO_3$ solution. After extraction, the samples were centrifuged and the supernatant was analyzed. The relative concentration were calculated from the equations of Jeffrey and Humphrey (1975). At the same time, oxygen evolution of five small discs (6.8 mm in diam.) from each treatment samples was monitored using the Clark type oxygen meter (YSI 53 Oxygen monitor) in 2.5 m*l* of the enriched control seawater as a buffer solution at 10°C. The tungsten lamp (100 V/300 W) with the C-4

type blue filter (Kenco Co.) was used as the light source of about 3400 lux. The electron transport activities were calculated from the equation of Binder and Bachofen (1976).

Statistical analysis: All data sets used in statistical analyses were first tested for normality and homogeneity of variances. The G-test and the Kolmogorov-Smirnov test were applied for normality tests. Bartlett's, F_{max} or log-anova tests were applied for homogeneity tests. If either condition was not fulfilled, variables were transformed to meet the assumptions of the analysis. Three-way analysis of variance (ANOVA) was used to examine the significance of copper concentration, temperature and photon fluence rate responses. All statistical tests follow descriptions given by Sokal and Rohlf (1981). The level of significance for comparisons was at least $p=0.05$, unless otherwise noted.

RESULTS

Meiospore germination: After release, meiospores lost their motilities and rapidly attached to substrata provide. Meiospores germinated under the temperature range tested (6-18°C), and the photon fluence rate range tested (20-80 μE $m^{-2}s^{-1}$). In the control media, germination assessed at 48 hours after inculation ranged from 19-34%, and there was significant temperature effect on the percent germination (Fig. 1). Germination was greatly suppressed under the highest temperature of 18°C and the lowest temperature of 6°C. Generally, the percent germination was maximal at 10°C.

All the copper treatments at the temperature 6, 14, and 18°C depressed germination (Fig. 1). However, the meiospore germination was not affected by copper treatment at 10°C. ANOVA revealed that both temperature and copper treatment were significantly affected meiospore germination, and temperature was accounted for the greatest proportion of the variance (Table 1). However, there was no significant effect of photon fluence rate on germination. ANOVA also showed that the interaction of temperature and copper concentration was significant.

Gametophyte development: The developmental stages of the plants under 50 μE m^{-2} s^{-1} treatment were examined on Day-3, 9, 18, 26, 32 and 43. Identification of the development was based on the 6 stage steps described earlier. The Day-3 observation showed meiospores with germ tubes were less than 50%, representing Stage I in all treatments (Fig. 2)

In control media, the representative stage at Day 9 was the meiospore with germ tubes in all temperature treatment except under 18°C. The highest temperature clearly supressed the development, and most of the development stopped at Stage II at that temperature throughout the observation period, even though there was successful germination of meiospores. The early stage of gametophytes appeared on the 18th day, which can be identified by the movement of cytoplasmic content to the germ tube and the increase in cell size. Up to the 18th day, all the cells in the different temperature of 6, 10, and 14°C showed the same developmental pattern. On the 26th day, more than 50% of the female gametophytes at 10 and 14°C went morphogenesis. However, plants at 6°C showed somewhat delayed development not showing progress till the 43rd day. On the 32nd day, one-celled oogonia began to appear at 10 and 14°C. Though the plants at 10 and 14°C began to produce a few zygotes and sporophytes on the 43rd day, most of the plants remained at mature gametophyte stage.

Copper treatment revealed different effects on the developing stages of gametophytes depending on temperature. Copper treatment at high temperature of 14°C was more detrimental to the plants at all copper treatment compared to 6 and 10°C; meiospores settled with germ tubes on the 18 Day, but most of the meiospore did not go further development in 5, 10, and 50 μg Cu l^{-1}. The meiospores in 100 μg Cu l^{-1} showed mortality, which was defined as empty cells. At

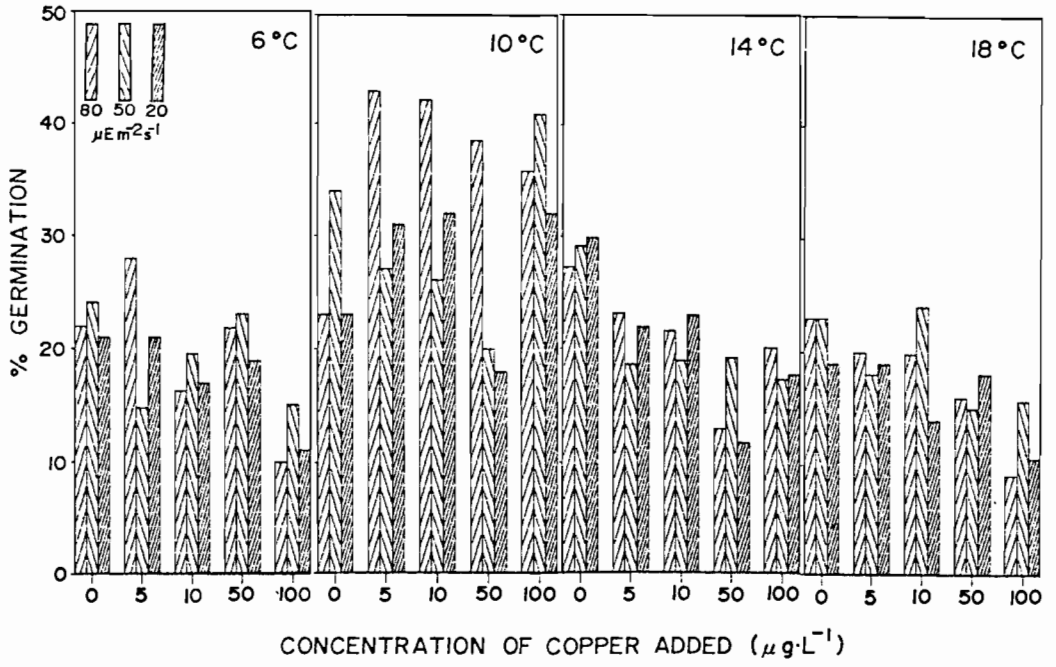

Fig. 1. Percent germination of spores *vs.* copper concentration and temperature in *Undaria pinnatifida.* Germination was determined by the presense of germ tube 48 hr after spore inoculation. Bars represent % germination of each photon fluence rate.

Table 1. Analysis of variance (Three-way ANOVA with replication) table for effects of irradiance, temperature and copper treatment on the germination success (%) of meiospores of *Undaria pinnatifida.* Germination success is percentage of meiospores having germ tubes 48 hours after inoculation. Data were arc sine of square root transformed prior to ANOVA

Source	DF	SS	MS	F	
I (Irradiance)	2	82.308	41.154	2.63	ns
T (Temperature)	3	1519.957	506.652	32.44	***
Cu (Cupper treatment)	4	343.337	85.834	5.49	***
I×T	6	130.872	21.812	1.40	ns
I×Cu	8	220.818	27.602	1.77	ns
T×Cu	12	677.127	56.427	3.61	**
I×T×Cu	24	374.792	15.616		

ns: not significant at alpha = 0.05 level, **: $0.001 < p \leq 0.01$, ***: $p \leq 0.001$

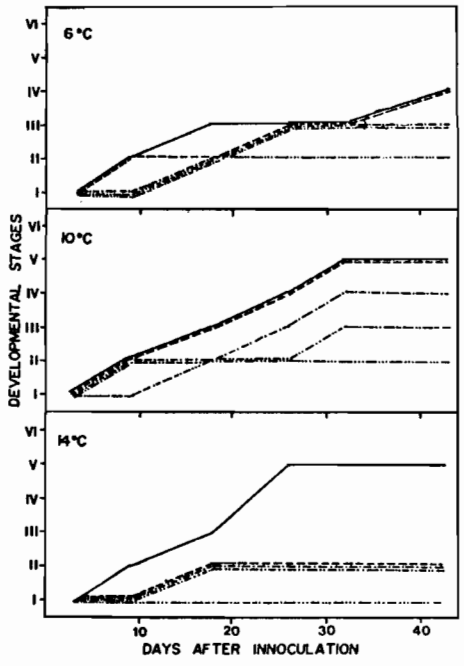

Fig. 2. Developmental stages of meiospore settlement and gaemtophyte in *Undaria plannatifida:* Control, —; 5 μg Cu l^{-1}, - - -; 10 μg Cu l^{-1}, —·—; 50 μg Cu l^{-1}, —··—; 100 μg Cu l^{-1}, ——.

10°C, the plants in the control, and 5 μg Cu l^{-1} media showed the same development pattern. Other plants in higher copper concentrations (10 and 50 μg Cu l^{-1}) showed retarded development, and they did not show maturity until 43 days after the meiospore inoculation. At 6°C, the plants in the control and 5 μg Cu l^{-1} showed similar development pattern, though there was some delay in 5 μg Cu l^{-1}. Most plants in 10 and 50 μg Cu l^{-1} remained in the early stage of developing gametophytes until the end of experiment. The plants in 100 μg Cu l^{-1} showed settlement with germ tubes, but they did not developed to gametophytes throughout the experimental period at all.

Female gametophyte growth: The length of female gametophytes was measured on the Day-18, 26, 32 and 43 after meiospore inoculation. If there was no female gametophytes observed in some treatment, germinating meiospores were measured instead.

The three observations up to Day-32 showed that there was no significant effect of photon fluence rate on female gametophyte growth (Fig. 3). However, the female gametophyte growth was significantly affected by temperature and copper treatment. The female gametophyte growth was maximal at 10°C, and lowest at 14°C, except the control plants grown under 50 μE m^{-2} s^{-1}. At that treatment, female gametophyte grew at best at 14°C. The length of female gametophyte reached 76.7 ± 21.4 μm on the 18th day, 153.0 ± 32.2 μm on the 26th day, and 302.2 ± 37.8 μm on the 32nd day. Copper did not affect the female gametophyte at 6°C. However, the female gametophyte growth was significantly depressed in 5, 10, 50, and 100 μg Cu/l^{-1} media at 10 and 14°C. ANOVA clearly showed that the interaction of temperature and copper treatment was significant (Table 2).

The last observation on the 43rd day showed that all three factors, photon fluence rate, temperature and copper treatment had significant effects on the female gametophyte growth.

Table 2. Analysis of variance (Three-way ANOVA with replication) table for the effects of irradiance, temperature and copper treatment on the measurement of female gametophytes of *Undaria pinnatifida* thoughout the experimental period. Data were log-transformed prior to ANOVA. Only data for 6, 10 and 14°C were used.

Source	DF	SS	MS	F	
Day-18					
I (Irradiance)	2	1.762	0.881	1.11	ns
T (Temperature)	2	34.670	17.335	21.89	***
Cu (Cupper treatment)	4	28.056	7.014	8.86	***
I×T	4	3.229	0.807	1.02	ns
I×Cu	8	7.322	0.915	1.16	ns
T×Cu	8	19.545	2.443	3.08	*
I×T×Cu	16	12.665	0.792		
Day-26					
I (Irradiance)	2	3.168	1.584	0.90	ns
T (Temperature)	2	67.263	33.632	19.12	***
Cu (Cupper treatment)	4	63.298	15.825	9.00	***
I×T	4	5.304	1.326	0.75	ns
I×Cu	8	12.151	1.519	0.86	ns
T×Cu	8	33.849	4.231	2.41	ns
I×T×Cu	16	28.147	1.759		
Day-32					
I (Irradiance)	2	5.487	2.744	1.30	ns
T (Temperature)	2	88.758	44.379	21.09	***
Cu (Cupper treatment)	4	73.530	18.382	8.74	***
I×T	4	8.470	2.117	1.01	ns
I×Cu	8	22.023	2.753	1.31	ns
T×Cu	8	57.568	7.196	3.42	*
I×T×Cu	16	33.661	2.104		
Day-43					
I (Irradiance)	2	34.742	17.371	4.35	*
T (Temperature)	2	51.817	25.909	6.48	**
Cu (Cupper treatment)	4	118.761	29.690	7.43	**
I×T	4	71.846	17.962	4.49	*
I×Cu	8	32.807	4.101	1.03	ns
T×Cu	8	40.125	5.016	1.26	ns
I×T×Cu	16	63.942	3.996		

ns: not significant at alpha = 0.05 level, *: $0.01 < p \leq 0.05$, **: $0.001 \leq 0.01$, ***: $p \leq 0.001$

The growth of female gametophyte was depressed under 20 μE $m^{-1}{}_{s^{-1}}$, compared to ones grown under 50 and 80 μE m^{-2} s^{-1}. Copper treatments were accounted for the greatest pro-

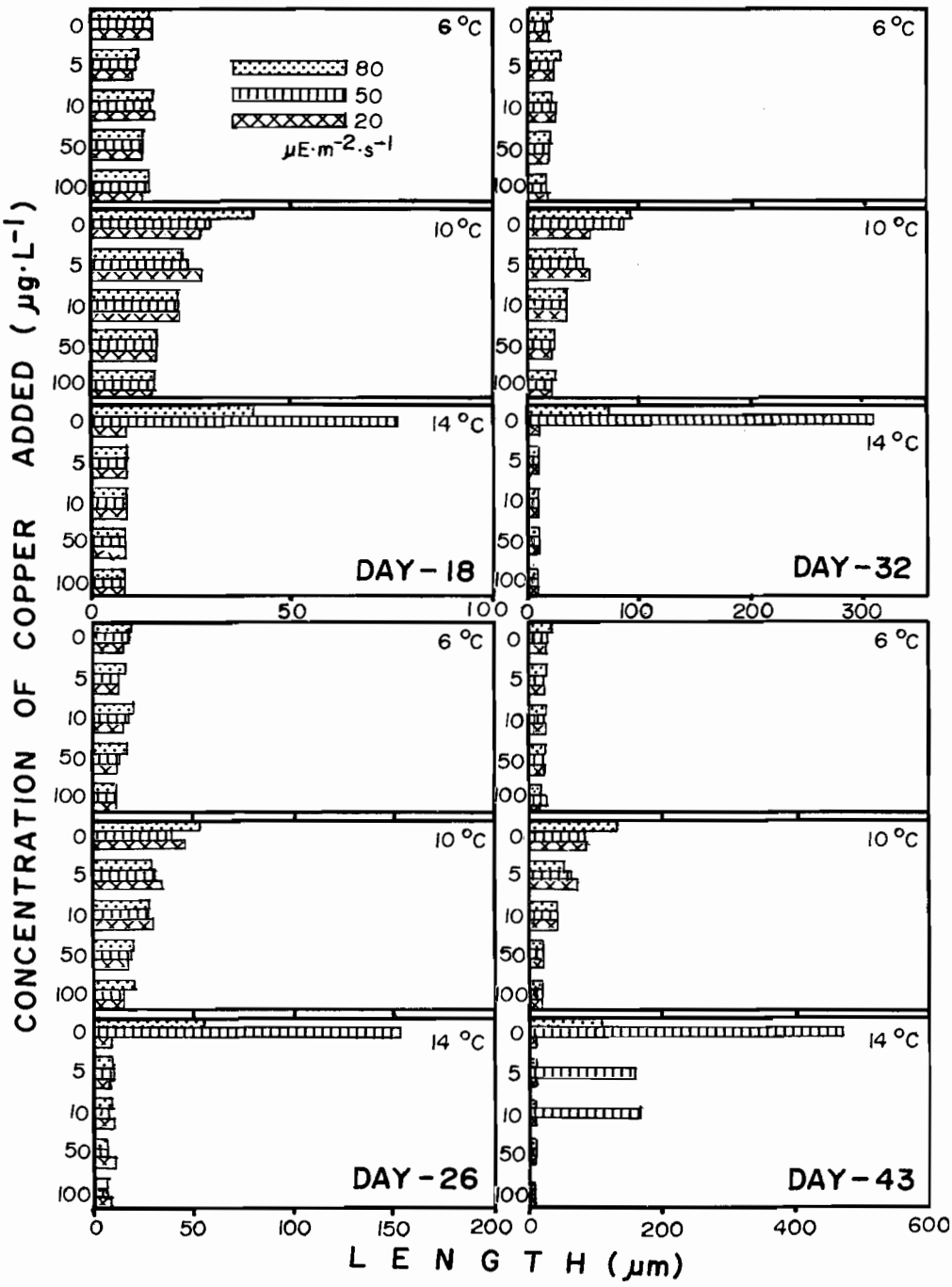

Fig. 3. Length measurement (μ m) of female gametophytes *vs.* copper concentration and temperature in *Undaria pinnatifida* throughout the experimental period. Bars represent average length of female gametophytes under each photon fluence rate.

portion of the variance. Also the interaction of photon fluecnce rate and temperature was significant. The length of female gametophyte reached $467.1 \pm 55.2\ \mu m$ for control plants grown under 14°C and 50 $\mu E\ m^{-2}\ s^{-1}$ treatment.

Chlorophyll content and oxygen evolution: During the experimental period, sporophytes in 100 μg Cu l^- media exhibited chlorosis and disintegration at the distal and marginal blade protions. Table 3 showed the total chlorophyll contents of each treatment. Total chlorophyll of the plants in 100 μg Cul^{-1} media were significantly different from those plants in other at the alpha = 0.05 level.

Slopes and activities of electron transport of each treatment the shown in Fig. 4 and Table 4. The highest activity was monitored in the 5 μg Cu l^- added samples. However, there was not significant differences among the control and copper treatments except 100 μg Cu^{-1} supplemented one. However, the slope showed a decreasing trend with increasing copper concentration above 5 μg Cu l^{-1}. The slopes could be normalized based on the chlorophyll content per surface area. The ratios of activity (slope) to the chlorophyll content are shown in Table 3. Plants under 100 μg Cu l^{-1} treatment showed significantly reduced total chlorophyll contents and oxygen evolution rates.

Table 3. Total chlorophyll contents of the control and copper treated *Undaria pinnatifida* sporophytes after 7 days

Treatment	Total chlorophyll contents (mg chl./cm² surface area)	
Control	13.65 ± 2.62	(100%)
5 $\mu g\ l^{-1}$	12.92 ± 1.26	(94.6%)
10 $\mu g\ l^{-1}$	12.34 ± 2.54	(90.4%)
50 $\mu g\ l^{-1}$	12.05 ± 1.52	(88.3%)
100 $\mu g\ l^{-1}$	10.37 ± 0.47	(76.0%)

Table 4. The rates of oxygen evolution and their normalized electron transport activities in *Undaria pinnatifida* after 7 days of copper treatments

Treatment	Regression Coefficient (mV/min)	Intercept (mV)	Normalized activity u eq electron hr mg chl/cm²
Control	1.18 ± 0.014	18.52	0.684 ± 0.008
5 $\mu g\ l^{-1}$	1.22 ± 0.097	18.17	0.748 ± 0.059
10 $\mu g\ l^{-1}$	1.14 ± 0.033	17.99	0.731 ± 0.021
50 $\mu g\ l^{-1}$	1.03 ± 0.014	19.30	0.677 ± 0.009
100 $\mu g\ l^{-1}$	0.81 ± 0.010	20.81	0.618 ± 0.008

DISCUSSION

Metals are concentrated from the water by factors of $10^3 - 10^4$ in brown algae (Bryan, 1983; Davies, 1978; Phillips, 1977). The site of metal accumulation has not been fully understood, although there is some evidence that physodes may contain concentrates of the metals (Lignell *et al.*, 1982; Pellegrini, 1980). Some Phaeophyta are able to tolerate relatively high levels of heavy metals (Bryan, 1983; Lignell *et al.*, 1982). Although cadmium, lead and mercury are considered generally to be the most dangerous pollutants for human, copper as a micronutrient is assumed to have considerable importance for marine organisms in some areas by virtue of its abundance.

In the present study, there were signs of delayed meiospore germination and gametophyte development, and inhibition of female gametophyte growth during the course of the experiment using copper concentration up to 100 μg Cu l^{-1}. There have been several studies describing growth inhibition by metals in seaweeds (see Rai *et al.*, 1981; for review). Strömgren (1979, 1980) presented the reduction in growth rate by metal application in some fucoid algae. It is known that the copper concentration range of 25-75 $\mu g\ dm^{-1}$ delayed the development of sporophyte formation up to 13 days in *Laminaria hyperborea* (Hopkins and Kain, 1978).

It is known that the role of copper in photosynthetic organisms depends greatly on its concentration. It is well established that copper is a micronutrient for plants. Copper is an essen-

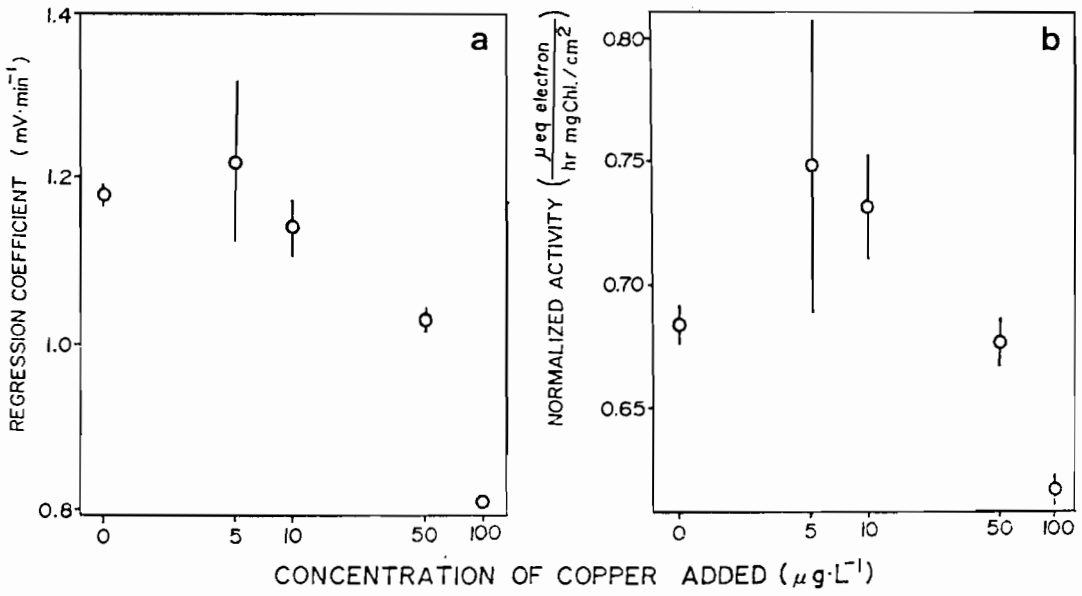

Fig. 4. Comparison among the regression coefficients (a) and the normalized electron transport activities (b) in *Undaria pinnatifida* sporophytes.

tial component of the electron transport system of chloroplast, plastocyanin in algae and higher plants (Katoh *et al.*, 1962; Walker, 1953). The microalga *Scenedesmus acutus* had optimum growth at a copper supply of 0.1 to 1 μM. Its growth was reduced to 50% of the normal rate and chlorophyll was bleached when the medium was depleted of copper (Sandmann and Böger, 1980). However, concentrations of copper above 0.1 μM are increasingly toxic to photosynthesis in *Chlorella vulgaris* (Greenfield, 1942; Habermann, 1969). The concentration required for 50% inhibition was about 2 μM of cupric sulfate in the green alga *Ankistrodesmus falcatus* (Shioi *et al.*, 1978). In the present study, in *Underia pinnatifida* at 5 μg Cu l^{-1}, which was the lowest copper concentration tested, there was no conclusive evidence for copper limitation.

The concentrations applied in the present study were similar to those in the natural environment. Copper is present in the open ocean at a concentration of approximately 1.0 μg l^{-1} (Brewer, 1975). However, concentrations of copper have been reported up to 230 μg l^{-1} in near shore waters; New York Bay, 2.7-65 μg l^{-1} (Waldhaur *et al.*, 1978), La Roisiere, U.K., 17.4-60.2 μg l^{-1} (Romeril, 1977), Norwegian Fjord, 3.77-77.0 μg l^{-1} (Stenner and Nickless, 1974, 1975), La Rabida, Spain, 107 μg l^{-1} (Stenner and Nickless, 1974), Ras Beirut, Beirut, 100-230 $\mu g l^{-1}$ (Shiber and Shatila, 1979). These coastal areas are inhabited by kelps. Therefore, plants can be highly susceptible to the metal pollution.

In the present study, each stage seemed to have quite different response to copper, and temperature was the most important factor in determining the degree of inhibition. Especially higher temperature of 14 and 18°C resulted in more detrimental effects, compared to low temperature of 6°C. The effects of heavy metal on the plants depend on several factors. Most of all, the temperature-dependent biological activity may change the rate of the metal uptake. Munda (1979) showed enhanced Zn uptake in *Fucus virsoides* and *Enteromorpha prolifera* caused by higher temperature, but the increase was not linear. He also found that the accumulation of Zn and Cd into the vegetative parts of *Fucus spiralis* thalli was temperature dependent and enhanced

at higher temperatures (Munda, 1986). Klump (1980) showed that arsenic uptake increased in direct proprotion to increasing temperature in *Fucus spiralis* and *Ascophyllum nodosum*. Also low temperature reduced the uptake of Cd in *Chlorella*, reflecting a decreased binding efficiency and metabolism in tissue (Hart *et al.*, 1979). Presumably, increased detrimental copper effects in *Undaria pinnatifida* at the higher temperature range may be attributed to the enhanced metal uptake in higher temperature in the present study.

Light has been considered as another important factor in the metal uptake in algae. Greenfield (1942) showed that the toxicity of *Chlorella vulgaris* cells mostly retarded the dark reaction rate and had a lesser inhibitory effect on the light stage of photosynthesis. Bachmann and Odum (1960) reported no measurable uptake of Zn in the dark, though definite uptake took place in the light in marine benthic algae. The degree of inhibition was lower at low light intensity than at high light intensity. (Cedeno-Maldonado and Swader, 1972). Rice and Lapointe (1981) showed that there was remarkable decrease in algal Fe, Cd and Rb with increasing light level in *Ulva*. In the present study, the effects of copper did not appeared in the meiospore germination, gametophyte development and growth in earlier stage under the photon fluence rate ranged from 20 to 80 μE $m^{-2}s^{-1}$. However, it was notable that last measurement on the 43rd day showed the irradiance had significant effects on the growth of female gametophyte. Presumably, light became important in the later stages of gametophyte growth.

In laminarian plants, it is known that complexity of the triggering gametophytic development, growth and gametogenesis involves combination of numerous factors (see Kain, 1979; for review). Also laminarian gametophytes have been characterized as extreme shade plants with regard to the influence of photon fluence rate on photosynthesis and vegetative growth. Generally, the light saturation of photosynthesis or vegetative growth occurred at the low irradiance below 20 μE $m^{-2}s^{-1}$ (Bolton and Lewitt 1985; Cosson, 1975; Kain, 1964; Lüning, 1981; Lüning and Neushul, 1978). Considering light and temperature interaction effects on the gametophyte development and juvenile sporophyte growth, most of the variation was attributable to primarily temperature not the irradiance (range 5-120 μE $m^{-2}s^{-1}$) in the previous study of *Laminaria saccharina* (Lee and Brinkhuis, 1988). Conclusively, *Undaria pinnatifida*, one of laminarian plants, may have low light requirement, and did not show any differentiation in photon fluence rates depending on copper concentration.

Compared to the early development of gametophytes, chlorophyll contents and the oxygen evolution of sporophyte fronds were not affected at the copper concentrations up to 50 μg Cu l^{-1}. Though there was decreasing trends in the chlorophyll contents and electron transport activities with increasing copper augmentation, it was not significant except 100 μg Cu l^{-1} treatment. The significant copper effects occurred only at the highest concentration of 100 μg l^{-1} in the present study. It is known that marine plants exudes substances (Sieburth, 1969; Moebus and Johnson, 1974; Pregnall, 1983). They may form complexes with metals that regulate the free metal ions and ionic complexation and speciation (McKnight and Morel, 1980), and make the medium suitable for growth in algal species reducing the toxic effects (Steemann Nielsen and Wium-Andersen, 1971). Further research is required to define the effects of exudation on copper treatment in *Undaria pinnatifida* and other aquatic plants.

Kang and Ko (1977) reported that in *Undaria pinnatifida* the optimum temperature for meiospore germination was 17-20°C, and germination was not successful at the temperature higher than 27°C. Once germinated, gametophytes could grow at temperatures up to 23°C. Gametogenesis occurred at the temperature lower than 20°C, and gametophytes

did not mature at the temperature higher than 23°C. The present study showed optimum temperature for germination was 10-14°C, and even if the germination occurred, gametophyte did not grow at 17°C. The optimum condition for the gametophyte growth was 14°C and 50 μ E m^{-2} s^{-1}. Genrally, the temperature requirement in present study was lower compared to Kang and Ko (1977). This is comparable to the report that seasonal temperature and photon fluence rate requirement in gametogenesis of *Laminaria saccharina* were different throughout the year and generally these requirements were parallel to the concurrent environmental conditions (Lee and Brinkhuis, 1988). The sours matrial used in the present study was collected in winter, but those in Kang and Ko (1977) in late spring and summer. Further research is required to elucidate seasonality in gametogenesis in this species.

Distributional ranges of seaweed species are governed by survival of adult plants and their ability to successfully reproduce (Druehl, 1981; van den Hoek, 1982a, b). The present informations of the effect of copper can be applied to the ecosystem level. The effect of copper on the early stage of meiospore germination and gametogenesis *Undaria pinnatifida* may decrease the total primary production in the kelp-based system due to failure in the recruitment and decreased growth, and produce potential deleterious impacts on the kelp aquaculture.

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