식물학회지 제33권 제2호
Korean J. Bot. 33(2) : 141−146

Effects of Temperature and Salinity on Germination and Vegetative Growth of *Enteromorpha multiramosa* Bliding (Chlorophyceae, Ulvales)

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海產 綠藻 털가지파래(*Enteromorpha multiramosa* Bliding)의 發芽와 生長에 대한 溫度와 鹽分度의 效果

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ABSTRACT

Germination and vegetative growth of *Enteromorpha multiramosa* Bliding from Pyoson, Cheju Island were investigated in laboratory under various combinations of temperature (5-25 °C) and salinity (8-48‰). Percent level of germination was relatively high at all combinations of the two factors. The highest value among the combinations was revealed at 15 °C and 32‰. Dry weight also was fairly high at all levels of combination with maximum value at 20 °C and 32‰. Analysis of variance for germination and growth was completed respectively and polynomial prediction models were constructed. F ratio revealed that all factors had a significant effect ($p<0.001$) on percentage of germination and dry weight, and their interactions also were significant ($p<0.001$), although the F ratio of interactions was far less than that for either the separate effect of temperature or salinity. Response surface of polynomial equation represented that temperature influenced less than salinity on germination, while it effected remarkably on vegetative growth, so that *Enteromorpha multiramosa* was kept to visible macrothalli from winter to spring in Cheju Island.

INTRODUCTION

The intertidal region of rocky shores is subject to fluctuations of various environmental factors. Seawater temperature is one of the principal factors to fluctuate seasonally and to determine geographic distribution of marine macroalgae (Cambridge *et al.*, 1984; Setchell, 1920; van den Hoek, 1975, 1979, 1982a, b; Yarish *et al.*, 1984). van den Hoek (1982a) proposed that the boundaries of geographic distribution in benthic algal species could be defined according to limiting temperature, either for growth and reproduction during favourable growing season or survival during adverse season. Salinity also can be one of the most important environmental factors next to temper-

This work was partly supported by a Hanyang University Faculty Research Grant.

ature to affect the horizontal distribution of benthic marine algae. Its influence, however, is local rather than global, as salinity gradients are more pronounced in coastal waters, where fresh water discharge counteracts the relatively stable oceanic condition (Bird and McLachlan, 1986).

Enteromorphas are most common green algae in lower tidal zone of Cheju Island. Environmental factors may affect their germination and vegetative growth. Numerous field and culture studies related to germination or vegetative growth of *Enteromorphas* were reported in relation to various physico-chemical factors including light, temperature and nutrients (Woodhead and Moss, 1975; Ohno *et al.*, 1981; Pandey and Ohno, 1985). However, the combined effects of temperature and salinity have scarcely been attempted.

The object of this investigation, therefore, is to identify relative effects of temperature and salinity on germination and vegetative growth of *Enteromorpha multiramosa* under laboratory conditions. The data were statistically analyzed, and response surface was generated. The results are discussed in relation to seasonal presence or absence of the macrothalli at the areas investigated.

MATERIALS AND METHODS

Enteromorpha multiramosa Bliding was collected in May, 1989, at a tide pool in lower intertidal zone of Pyoson, Cheju Island (33° 19′ 00″N, 126° 51′ 00″W). The materials were rinsed several times with sterile seawater and transferred to pyrex Petri dishes containing sterile seawater. After two to three days, the plants were cultured in pyrex culture dishes (7 cm × 7 cm) containing 200 ml PES medium (Provasoli, 1966) until maturation. Experiments were started with zoospores, which were obtained with small apical fragments kept at 15 °C, 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ (40 W cool-white fluorescent lamps, Philips) and 16:8 daily photoperiod, as follow.

Experimental design. Two-way analysis of variance factorial design experiment was run concurrently. A complete factorial design experiment was adopted with temperature and salinity in each representative at six levels: temperature from 0 to 30 °C in 5 °C intervals, and salinity of 8, 16, 24, 32, 40, and 48‰ under 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity and 16:8 daily photoperiod. The temperature differences were prepared by 150 cm × 45 cm and 2.5 cm thick aluminum temperature gradient plate. The salinity differences were obtained by diluting seawater with distilled water, or concentrating natural seawater at 70 °C, prior to enrichment. The culture medium was renewed once a week to minimize the change of salinity, pH, or depletion of the nutrients.

Germination. In culture vessels (disposable multiple well plates, Corning, 25810-6) containing 22 mm × 22 mm cover glass and 10 ml PES medium a dilute suspension of zoospores (0.1 ml) was inoculated and subjected to various conditions of temperature and salinity for microscopic examination of the spore germination. Cover glass was examined under the microscope at × 100 magnification. The numbers of settled zoospores and their germination were counted in ten fields of view chosen at random. Germination was taken as initiation of the peg-like rhizoidal cell from the spores. The level of germination was expressed as a percentage of the total numbers of spores settled, and counted at the end of 2-week culture.

Vegetative growth. The growth experiments were conducted with approximately 0.2 cm long seven-day-old germlings inoculated simultaneously into the pyrex culture dishes (7 cm × 7 cm). Their growth was assessed by measuring dry weight at the end of 4-week culture. The plants were rinsed with distilled water and dried at 70 °C for 24 h. Dry weight was measured on an average of 50 plants with 0.01 mg accuracy, while the percentage of germination was estimated by a mean of about 200 plants.

Statistical analysis. For two-way analysis of variance (*ANOVA*) and regression analysis of a second order polynomial on percentage of germination and dry weight with the PC "SAS" (SAS Inst. Inc., Release 6.03) package utilizing an IBM personal computer was adopted. Response surface graphs were fitted to the data by a second order polynomial equation. In constructing these graphs, "Surfer" (Golden Software, Inc., Version 4.08), a surface generating package available, was used.

RESULTS

Germination. The germination of *Enteromorpha multiramosa* from zoospores under 30 different combinations of temperature and salinity was given in Table 1. This result represents the percentage of germination in response to temperature and salinity on an aver-

Table 1. Germination (%) of *Enteromorpha multiramosa* under various combinations of temperature and salinity. Each value is a mean of three replicates

Temp. (°C)	Salinity (‰)					
	8	16	24	32	40	48
5	6.7±3.1	7.0±1.7	19.3± 7.0	18.7± 6.1	23.3±11.0	14.0±3.5
10	15.3±8.5	34.3±8.0	72.7±10.8	77.7±12.0	85.0± 9.6	15.7±8.1
15	28.3±4.9	57.0±7.6	84.3± 6.0	87.0±18.2	77.0±13.1	17.3±5.5
20	8.0±4.0	24.0±6.3	81.0±14.4	84.0±13.5	72.3± 7.8	20.3±9.2
25	11.3±4.6	40.0±5.0	43.0± 3.6	70.7±10.7	47.3± 8.3	8.7±3.1

age of three times experiments. Zoospores liberated at the same time did not germinate synchronously when kept under controlled condition of temperature and salinity. The level of germination (%) was relatively high at all combinations of the two factors. The lowest value was at 5 °C and 8‰, whereas the highest at 15 °C and 32‰.

The result of *ANOVA* for percentage of germination data was given in Table 2. The coefficients for a polynomial response surface were shown using variable combination level of temperature and salinity (Fig. 1). The model ($R^2 = 0.94$) containing temperature, salinity and their interaction accounted for about 94% of the total sum of squares, with salinity being the most important. Temperature and salinity accounted for 80% of the total sum of squares. In order to facilitate a comparison among the two factors, their relative contribution to the sum of squares explained by the analysis of variance model was evaluated in comparison F ratio, which revealed that all factors had a significant effect on germination of *E. multiramosa*. Temperature influenced less than salinity on germination. Their interaction was significant, although the value of F ratio was far less than that for either temperature or salinity.

Table 2. Analysis of variance (*ANOVA*) table for the effects of temperature and salinity on germination (%) of *Enteromorpha multiramosa*

Source	df	Sum of squares	Mean square	F	p
Temperature	4	20551.600	5137.900	67.87	<0.001
Salinity	5	44086.889	8817.378	116.48	<0.001
Interaction	20	11292.000	564.600	7.46	<0.001
Model	29	75930.489	2618.293	34.59	<0.001
Error	60	4542.000	75.700		
Total	89	80472.489			

The effects of temperature and salinity on germination of the alga could be represented as a series of response surfaces where percentage of germination was on the vertical axis, and that of temperature and salinity were on the horizontal axes (Fig. 1). Regression analysis was used to obtain a least square fit of a second order polynomial for each surface. Both the linear and quadratic effects of temperature and salinity acting individually had a significant effect ($p<0.001$), and the combined effect of temperature and salinity was also significant ($p<0.001$).

The percentage of germination decreased with both decreasing and increasing either temperature or salini-

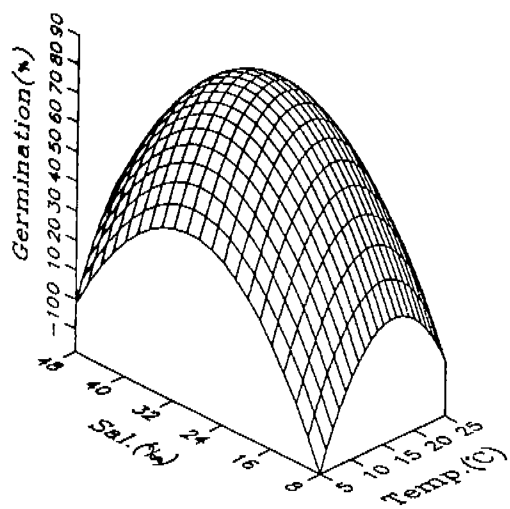

Fig. 1. Response surface of germination (%) as a function of temperature and salinity. The surface represents the least squares fit of the polynomial: $S = -a_0 + a_1X + a_2Y - a_3X^2 - a_4Y^2$, where S = percentage of germination, X = temperature and Y = salinity. $a_0 = 114.714444$, $a_1 = 10.540762$, $a_2 = 7.568423$, $a_3 = 0.320159$ and $a_4 = 0.12792$. All coefficients are significant at $p<0.001$ level.

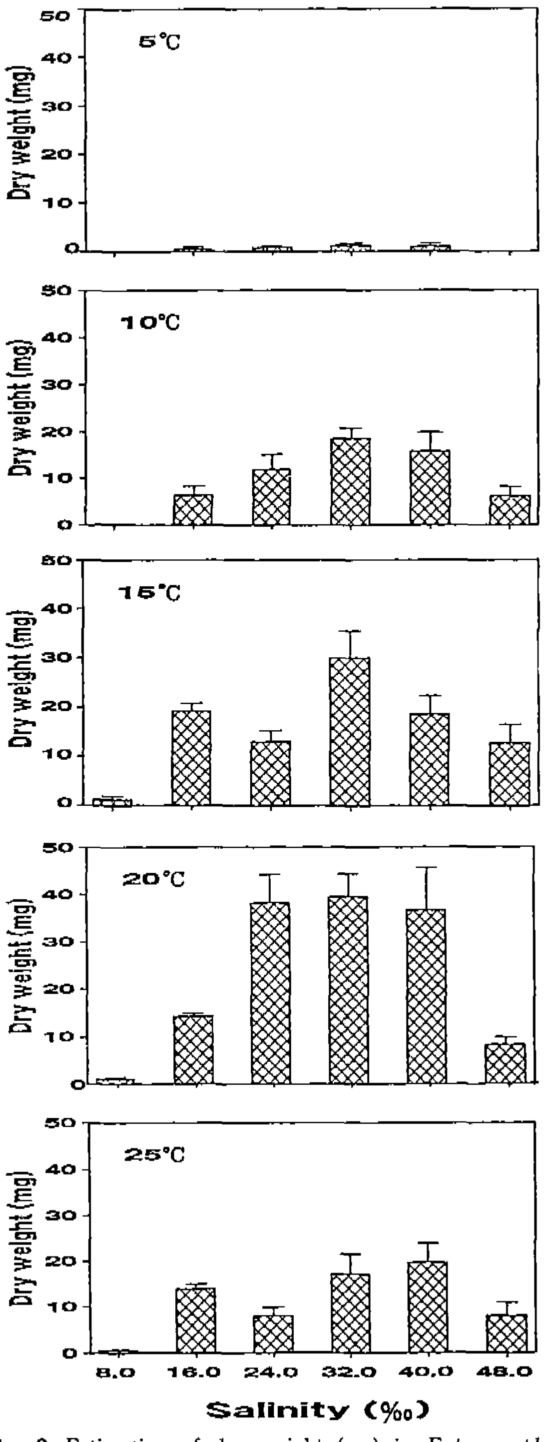

Fig. 2. Estimation of dry weight (mg) in *Enteromorpha multiramosa* under various combinations of temperature and salinity. Each bar is a mean of three replicates. The vertical lines at the top of each bar indicate standard deviation.

Table 3. Analysis of variance (*ANOVA*) table for the effects of temperature and salinity on dry weight (mg) of *Enteromorpha multiramosa*

Source	df	Sum of squares	Mean square	F	*p*
Temperature	4	4971.252	1242.813	120.06	<0.001
Salinity	5	4305.457	861.091	83.18	<0.001
Interaction	20	2899.444	114.972	14.00	<0.001
Model	29	12176.153	419.867	40.56	<0.001
Error	60	621.113	10.352		
Total	89	12797.266			

ty in such that the lowest point on the surface lay at 5 °C and 48‰,while the highest at 15 °C and 24‰. The inverse U-shaped trough of salinity tilled downwards more steeply than that of temperature due to its greater effect on germination (Fig. 1). Without considering temperature the lowest point of salinity lay at 8‰ on this response surface. Germination of *E. multiramosa* was reduced at the both low and high levels of temperature and salinity, respectively. The salinity had a stronger effect on germination of the alga than the temperature.

Vegetative growth. The factorial experiments on dry weight of *E. multiramosa* at 30 different combinations of temperature and salinity after 4 weeks culture were shown in Fig. 2. Dry weight production was fairly high at all combinations of the both two factors except for the combinations with 5 °C or 8‰. A comparison of the individual bar indicated that optimum temperature for the growth of *E. multiramosa* lay at 20 °C, whereas the growth was under severe stress at 5 °C. The dry weight exhibited highest growth near 32‰ at all temperature attempted, except for 25 °C, where the optimum salinity lay at 40‰.

Analysis of variance on dry weight data revealed that temperature, salinity and their interaction were significant ($p<0.001$), showing temperature to have the largest F ratio, although the F ratio for their interaction was far less than that for either temperature or salinity (Table 3). The model ($R^2 = 0.95$) containing temperature, salinity and their interaction accounted for about 88% of the total sum of squares. Both the temperature and salinity accounted for about 66% of the total sum of squares.

The data of dry weight production was represented graphically as response surfaces (Fig. 3). The response surface for dry weight production is least square fit of a

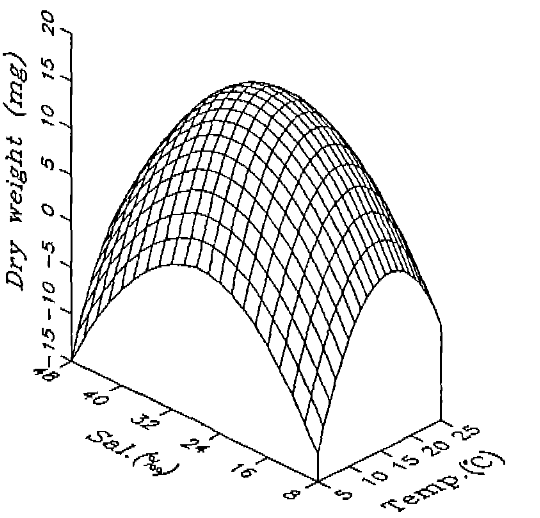

Fig. 3. Response surface of dry weight (mg) as a function of temperature and salinity. The surface represents the least squares fit of polynomial: $S = -a_0 + a_1X + a_2Y - a_3X^2 - a_4Y^2$, where S = dry weight, X = temperature and Y = salinity. a_0 = 42.219556, a_1 = 3.858927, a_2 = 2.125068, a_3 = 0.116508 and a_4 = 0.037212. All coefficients are significant at $p<0.001$ level.

second order polynomial to the factorial experiments data. On both the linear and quadratic effects of two environmental factors acting individually were significant ($p<0.001$), and those of interaction also did significant ($p<0.001$). Temperature and salinity effected markedly on dry weight. Optimum condition for dry weight production in *E. multiramosa* was near 20 °C and 32‰. The surface droped from maximum at 20 °C to below the zero line at 5 °C. The inverse U-shaped trough of temperature tilled downwards more steeply than that of salinity due to the greater effect of temperature on dry weight production.

DISCUSSION

The effects of temperature and salinity on germination and vegetative growth have been reported for many algae, but most of them were studied on phytoplankton. However, a few of them were for benthic macroalgae (Yarish and Edwards, 1982; Vandermeulen, 1986; Hales and Fletcher, 1989). Yarish and Edwards (1982) explained that growth of germlings and adults in laboratory culture among cross gradients of salinity and temperature was correlated to their distribution in the field. Vandermeulen (1986) found optimum condition for *Colpomenia peregrina* to be 20 °C and 30‰, whereas Hales and Fletcher (1989) reported it for germling of *Sargssum muticum* to be 25 °C and 34‰.

Woodhead and Moss (1975) found that the germination rate of zoospores of *Enteromorphas* was more than doubled by a raise of temperature from 10 °C to 20 °C. Optimum conditions for growth of *Enteromorpha prolifera* and *E. intestinalis* were reported to be 10-16 °C and 12-19‰ (Pandey and Ohno, 1985).

Enteromorpha multiramosa Bliding grows abundantly during winter and early spring in shallow waters along rocky shorelines of Cheju Island, Korea. Fertile plants become only a few cm high and much ramified with mostly opposite but often verticillated at right angles to main axes. The cells are characteristically large in dimension (Bliding, 1963). The field observation indicates that *E. multiramosa* exhibits a macrothalli stage occurring during during winter and spring seasons at the investigated area.

Two major environmental factors which may affect germination and subsequent vegetative growth can be temperature and salinity. In laboratory culture, our results indicate that the zoospores of *E. multiramosa* are able to germinate successfully under a wide range of temperature and salinity conditions, and the vegetative growth proceeds over a broad range of temperature and salinity from 5 to 25 °C and 8 to 48‰ (Table 1, Fig. 2). They both exhibit the optimum condition between 10-25 °C and 24-40‰, respectively. Lower and higher ranges of temperatures are inhibitive to the germination and vegetative growth. Those ranges of salinity also retards the rates of germination and growth.

F ratio through *ANOVA* reveals that all factors have a significant effect ($p<0.001$) on germination (%) and dry weight, and their interaction, too. Regression analysis of the data produces a second order polynomial expression. Both linear and quadratic effects of temperature and salinity either separately or in concert are significant on germination and growth. Response surface is a convenient way to visualize physiological responses to temperature and salinity. Polynomial equations represent statistical rather than empirical models (Graham *et al.*, 1985), and show the effects of both temperature and salinity on germination and growth of *E. multiramosa*. Salinity has a stronger effect on germination, but that on growth is temperature. The present results exhibit that *E. multiramosa* can survive poorly below 10 °C and above 25 °C of temperature.

E. multiramosa was abundant at Cheju Island during the winter and spring seasons in macrothallus state. This may be caused by salinity which rarely drops below 26‰, and by the seawater temperature rarely exceeded 16 °C (Anonymous, 1986), so that salinity and temperature conditions may not be stressful to the plants. However, macrothalli of *E. multiramosa* were scarce or very rare during summer season in Cheju Island, when the mean high temperature of seawater becomes 27 °C. Therefore, temperature would be the main physical factor which keeps *E. multiramosa* to form visible macrothalli during winter and spring seasons in Cheju island.

According to our experiment, *E. multiramosa* predicted to have somewhat a high optimum of temperature and salinity compared with other species of *Enteromorpha* to be comfirmed (Woodhead and Moss, 1975; Ohno *et al.,* 1981; Pandey and Ohno, 1985).

적 요

제주도산 해산 녹조 털가지파래 (*Enteromorpha multiramosa* Bliding)의 발아 및 생장에 대한 온도와 염분도의 효과를 요인실험 (factorial experiment)으로 조사하였다. 발아율과 건중량은 각각 15℃와 32‰ 및 20℃와 32‰에서 가장 높았다. 분산분석 결과, 이들 온도와 염분도의 각 요인과 복합요인의 효과는 고도의 유의성을 보였으며, F 값은 발아에는 염분도가, 생장에는 온도가 최대였다. 또한, 다항회귀식에 의한 표면반응 (surface response)에서도 발아에는 염분도가, 생장에는 온도가 가장 크게 효과적임을 확인할 수 있었다.

REFERENCES

Anonymous. 1986. Mean oceanographic charts of adjacent sea of Korea. Fish. Res. Dev. Agency, Pusan, Korea. 186 pp.

Bird, C.J. and J. McLachlan. 1986. The effect of salinity on distribution of species of *Gracilaria* Grev. (Rhodophyta, Gigartinales): An experimental assessment. *Bot. Mar.* **29:** 231-238.

Bliding, C. 1963. A critical survey of European taxa in Ulvales. Part I. *Op. bot. Soc. bot. Lund.* **8:** 1-160.

Cambridge, M., A.M. Breeman, R. van Oosterwyk and C. van den Hoek. 1984. Temperature responses of some North Atlantic *Cladophora* species (Chlorophyceae) in relation to their geographic distribution. *Helgoländer Meeresunters.* **38:** 349-363.

Graham, J., J.A. Kranzfelder and T.A. Martin. 1985. Light and temperature as factors regulating seasonal growth and distribution of *Ulothrix zonata* (Ulvophyceae). *J. Phycol.* **21:** 228-234.

Hales, J.M. and R.L. Fletcher. 1989. Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. IV. The effect of temperature, irradiance and salinity on germling growth. *Bot. Mar.* **32:** 167-176.

Ohno, M., O.P. Mairh, V.D. Chauhan, A. Tewari, R.M. Oza, H.V. Joshi, R.S. Pandey and P.S. Rao. 1981. Mass cultivation of green alga *Enteromorpha* on the coast of Okha. *Rep. Usa Mar. Biol. Inst. Kochi Univ.* **3:** 51-59.

Pandey, R.S. and M. Ohno. 1985. An ecological study of cultivated *Enteromoprha*. *Rep. Usa Mar. Biol. Inst. Kochi Univ.* **7:** 21-31.

Provasoli, L. 1966. Media and prospects for the cultivation of marine algae. *In,* Culture and collection of algae. *Eds.* Watanabe, A and A. Hottori. Jap. Soc. Plant Physiol. Tokyo. pp. 63-75.

Setchell, W.A. 1920. The temperature interval in the geographical distribution of marine algae. *Science, N.Y.* **53:** 187-190.

van den Hoek, C. 1975. Phytogeographical provinces along the coast of the northern Atlantic Ocean. *Phycologia* **14:** 317-330.

van den Hoek, C. 1979. The phytogeography of *Cladophora* (Chlorophyceae) in the northern Atlantic Ocean, in comparison to that of other benthic algal species. *Helgolander Meeresunters.* **32:** 374-393.

van den Hoek, C. 1982a. Phytogeographic distribution groups of benthic marine algae in the North Ocean. A review of experimental evidence from life history studies, *Helgoländer Meeresunters.* **35:** 153-214.

van den Hoek, C. 1982b. The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biol. J. Linn. Soc.* **18:** 81-144.

Vandermeulen, H. 1986. Growth of *Colpomenia peregrina* (Phaeophyceae) in culture: Effects of salinity, temperature and daylength. *J. Phycol.* **22:** 138-144.

Woodhead, P. and B. Moss. 1975. The effects of light and temperature on settlement and germination of *Enteromorpha. Br. Phycol. J.* **10:** 269-272.

Yarish, C., A.M. Breeman and C. van den Hoek. 1984. Temperature, light, and photoperiodic responses of some Northeast American and West European endemic rhodophytes in relation to their geographic distribution. *Helgoländer Meeresunters.* **38:** 273-304.

Yarish, C. and P. Edwards. 1982. A field and cultural investigation of the horizontal and seasonal distribution of estuarine red algae of new Jersey. *Phycologia* **21:** 112-124.

(Received April 19, 1990)