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Growth Characteristics of Five Microalgal Species Isolated from Jeju Island and Four Microalgal Stock Strains in Hatchery

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Five microalgal species isolated from the Jeju coast and four microalgal stock strains in hatchery were cultured in order to investigate their adaptation to extreme changes in environmental factors such as salinity, water temperature, and nutrients. In case of salinity variation, *Nitzschia* sp. of Bacillariophyceae, *Isochrysis galbana* of Haptophyceae and *Tetraselmis gracilis* of Prasinophyceae showed optimum growth at the low salinity of 20 and 25 psu. *Amphora coffeaeformis* and *Chaetoceros simplex* of Bacillariophyceae, and *Pavlova lutheri* of Haptophyceae adapted well at the relatively high salinities of 30 and 35 psu. However *Phaeodactylum tricornutum* of Bacillariophyceae and *Chlorella* sp. of Chlorophyceae showed euryhaline property. In case of water temperature variation, most of all the species studied were inhibited at 10°C. *C. simplex, Nitzschia* sp., *P. tricornutum, Chlorella* sp. and *T. gracilis* grew well at above 20°C. *A. coffeaeformis, I. galbana* and *P. lutheri* adapted also at the high temperature of 30°C. Each microalgal strain showed different growth rates and its maximum biomass. Generally microalgal populations from the Jeju coast grow well in relatively high salinity and high water temperature. Their growth were inhibited at low water temperature, but not likely affected at low salinity. This study indicates that the microalgal populations could not be affected by abnormally low salinity phenomena, which have happened occasionally around the west Jeju coast in summer and have led macrobenthic animals to mass mortality.

Key Words: abnormally low salinity, biomass, environmental change, growth curve, growth rate, microalgal culture

INTRODUCTION

Microalgae play an important role in the marine ecosystem as a primary producer which contribute to maintain the biological production and eventually to enhance the fishery production. Some species are used as live feed for shellfish and other invertebrates in farms (Loosanoff and Davis 1963; Ryther and Goldman 1975; Park and Hur 2000). Some species are also used for industrial purpose to eliminate organic matters in waste disposal through gas exchange (Huguenin 1974). Meanwhile some harmful microalgal blooms cause damages by massive degradation of water quality and by toxin production.

The physiology of microalgae is affected by physicochemical factors such as water temperature, salinity, light intensity, nutrient concentration, and pH (Thomas 1966). It, however, is very hard to identify the influence of environmental factors on the microalgae directly in nature. Thus physiological experiments are generally applied to understand the mechanism of environmental impacts on microalgal populations through laboratory culture studies (Park *et al.* 1993; Chang *et al.* 1998; Cho *et al.* 1998).

Jeju Island is located off the southern coast of Korea and famous for gastropod aquaculture. However a massive water flow of the Changjean (Yangtze) River has frequently reached the southern coast of the island in the summer season (Hyun and Pang 1998) and taken place an abnormally low salinity phenomenon which has caused fatal damages to useful commercial organisms such as gastropod and fish in the farm of Jeju Island (Suh *et al.* 1999). This study was designed to get information on microalgal growth at the unexpected environmental changes in terms of salinity, water temperature, and nutrient concentration based on microalgal culture study isolated from the Jeju coastal waters and some feed microalgae for aquaculture.

MATERIALS AND METHOD

Isolation and culture of microalgae

Five microalgal species including 4 species of

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Table 1. Characteristics of species used in the batch culture

Species name	Ecological type	Cell length (μm)	Sampling site	Alternative factor
Bacillariophyceae				
Achnanthes longipes Agardh	benthic	50	Topdong	
Amphora coffeaeformis Kützing	benthic	30	Topdong	temp., sal.
Chaetoceros simplex Ostenfeld	pelagic	5-7	B.M.H.*	temp., sal., N:P ratio
Nitzschia sp.	tychopelagic	20	Sungsan	temp., sal.
Phaeodactylum tricornutum Bohlin	benthic	60	Sungsan	temp., sal.
Chlorophyceae				
Chlorella sp.	pelagic	10-12	B.M.H.*	temp., sal.
Prasinophyceae				
Tetraselmis gracilis (Kylin) Butcher	pelagic	10-15	Gangjung	temp., sal.
Prymnesiophyceae				
Isochrysis galbana Parke	pelagic	8-10	B.M.H.*	temp., sal., nutrients
Pavlova lutheri (Droop) Green	pelagic	10-12	B.M.H.*	temp., sal.

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Table 2. Mean physical and chemical factors at Jungmun coast (1997. 7-1998. 6) and culture conditions of media in this culture study

	Water Temp. (°C)	Salinity (psu)	pH	T-N (ppm)	PO_4-P (ppm)	SiO_2-Si (ppm)	N:P
Sea water	18.9	33.67	8.15	6.55	0.38	7.15	15.0
Media	-	-	8.50	13.57	34.38	32.80	14.9

Bacillariophyceae and 1 species of Prasinophyceae were isolated from attached plates in farm and in natural seawater (Table 1), and maintained in unialgal culture at conditions described in Table 2. Four microalgal stock strains including 1 species of Bacillariophyceae, 1 species of Chlorophyceae, and 2 species of Haptophyceae in Bukjeju Marine Hatchery were used (Table 1). All species were identified using microscope (Zeiss Axioplan II) and SEM (Hitachi S2460N).

A single species was isolated with capillary pipette using inverted microscope and transferred to culture chamber after washing (Stein 1973). The isolated microalgae were cultured in the Erdschreiber EV media (Føyn 1934) for growing, and then subcultured to f/2 media (Guillard and Ryther 1962) for mass culture. Seawater was filtered through GF/F (Whatman 47 mm) after aging for over two months, and used for culture media after autoclave. The N:P ratio of culture media was controlled as 14.9 and the nutrient concentration was consisted of two times total-nitrogen, five times silica and ninety times phosphorus, which were higher concentration than the adjacent seawater of Jeju coast (Table 2). For each experiment we averaged the environmental factors such as water temperature, salinity, total nitrogen, phosphorus, and silica in order to simulate the culture condition with the in situ environmental condition, whose data were collected in the southern coast of Jeju Island from July 1997 to June 1998 (Table 2).

The effect of salinity, water temperature, and nutrients were surveyed for the growth characteristics of each microalgal species. Salinity was controlled at 20, 25, 30, 35 psu for most algal taxa, but *Chlorella* sp. was incubated in the wider range of 0, 10, 20, 30, 40 psu at the same water temperature and same nutrient concentration in the both case. The water temperature was also controlled in the condition of 10, 20, 30°C. The pH of 8.5 and nutrient concentration were same in all the treatments.

For the effect of nutrient concentration in *Isochrysis galbana* culture, the f/2 medium dilution conditions were adjusted at 100%, 50%, 25% and 0% in the same condition of 20°C, 25 psu, pH 8.5. In order to know the effect of N:P ratio for *Chaetoceros simplex*, the ratio was controlled at 1:1, 8:1, 16:1, 32:1 in the same condition of 20°C, 35 psu, pH 8.5 per each experiment. For the N:P effect experiment, $Na_2SiO_3 \cdot 6H_2O$ (sodium silicate) and vitamin B_{12} were added. All culture experiments were done with white fluorescent light under the irradiance of 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ at 12:12 L:D photo cycle.

Biomass and growth rate measurement

Microalgal chlorophyll a concentration (μgChl-$a \cdot l^{-1}$) was measured as the biomass component by the extrac-

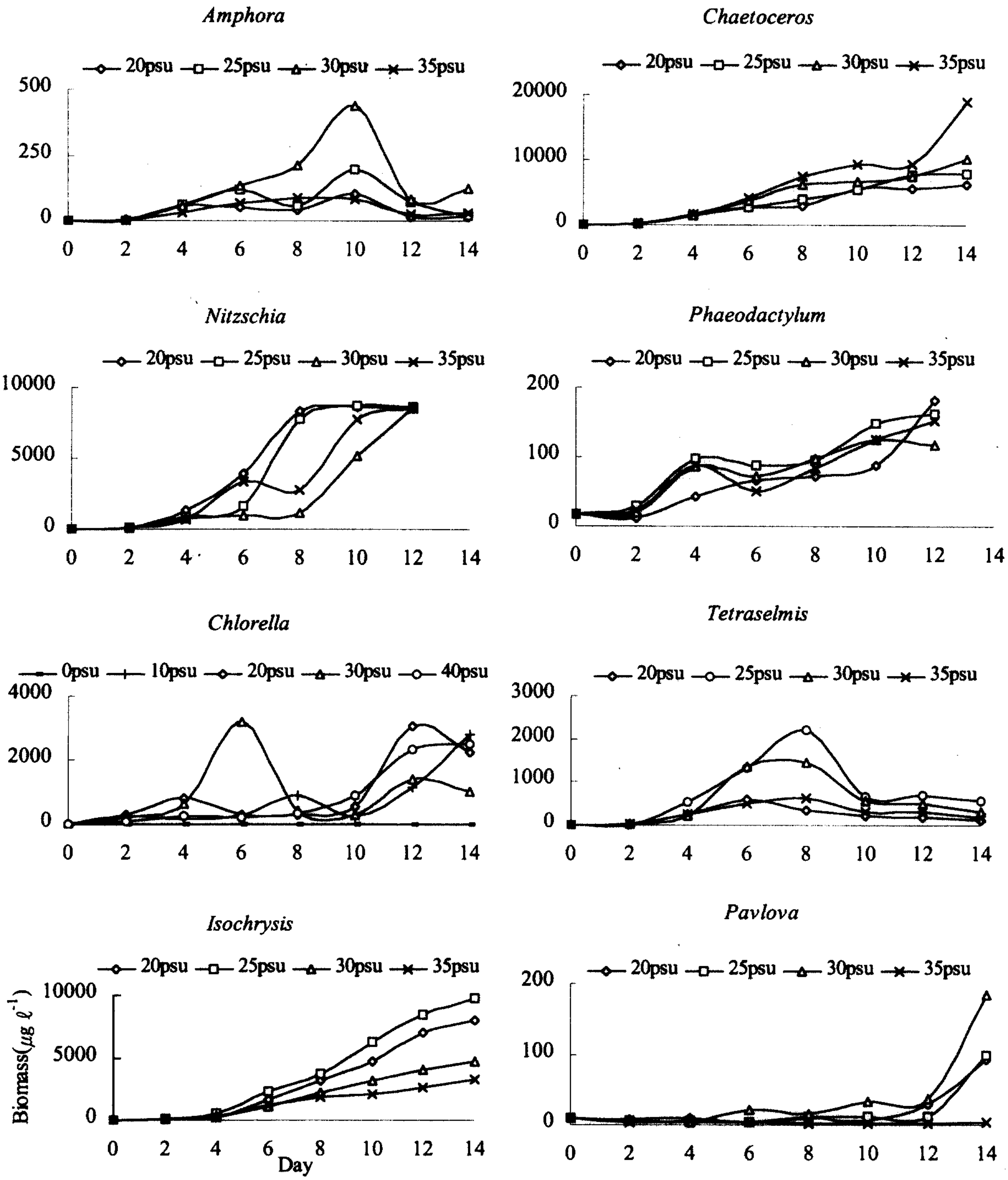


Fig. 1. Patterns of growth curves of microalgal species at different salinities (culture conditions: water temperature, 20°C; pH, 8.5; light intensity, $I = 180\ \mu E \cdot m^{-2} \cdot s^{-1}$, L:D = 12:12).

tion method (Parsons *et al.* 1984) using spectrophotometer (Shimadzu UV-1201). Growth rate can be expressed as doubling time (t_d) and specific growth rate (μ). We use the specific growth rate (μ) calculated from biomass increase per unit time in this study (Pirt 1975).

$$\mu\,(\text{day}^{-1}) = \frac{\ln (X_1/X_0)}{t_1 - t_0}$$

where X_0 and X_1 are quantitative expression of the biomass of cells given usually in terms of chlorophyll *a* concentration at beginning (t_0) and at end (t_1) of selected time interval during incubation.

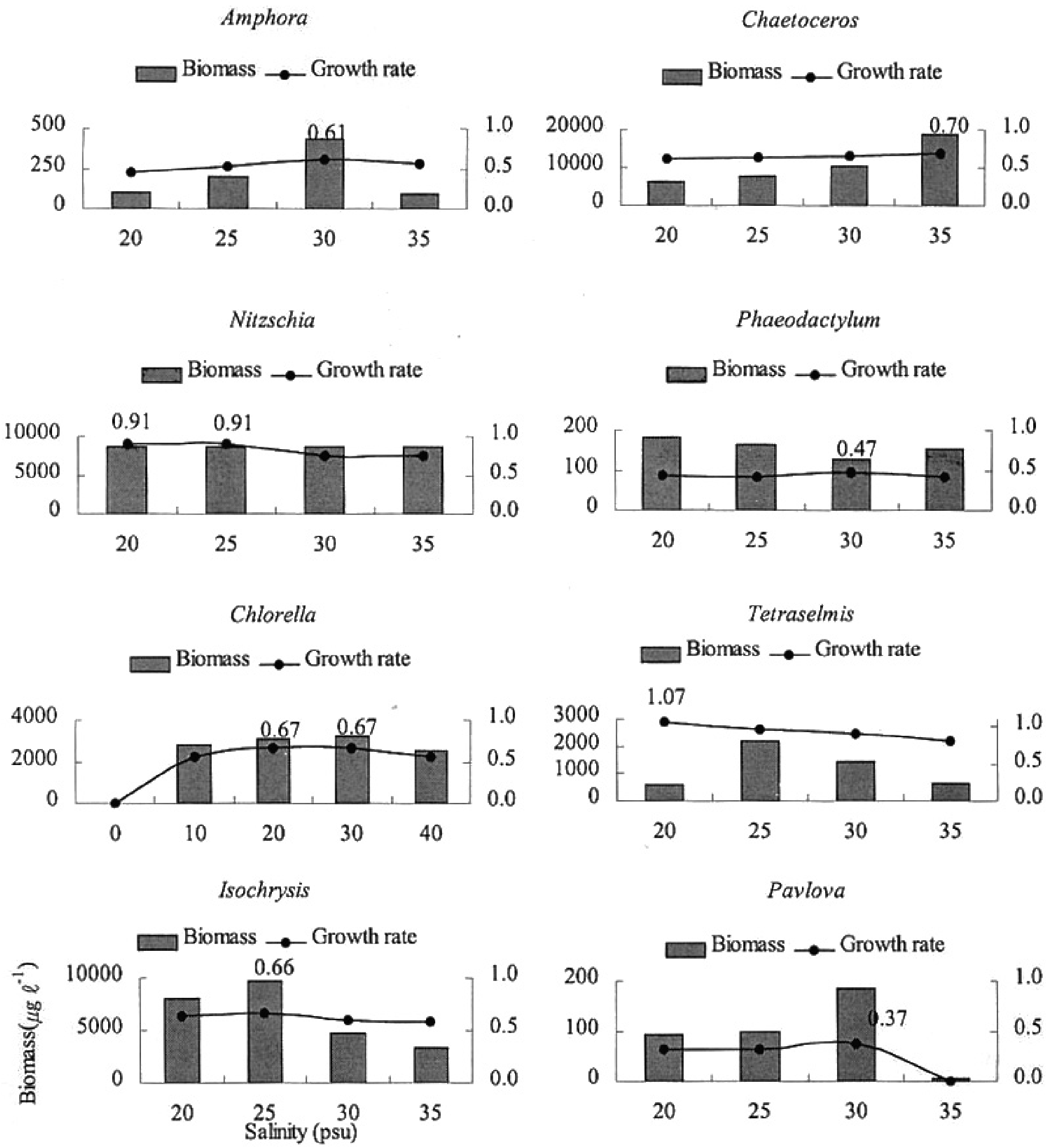


Fig. 2. Comparison of growth rates (μ) and maximum biomass (μgChl-*a* $\cdot$ l^{-1}) at different salinities.

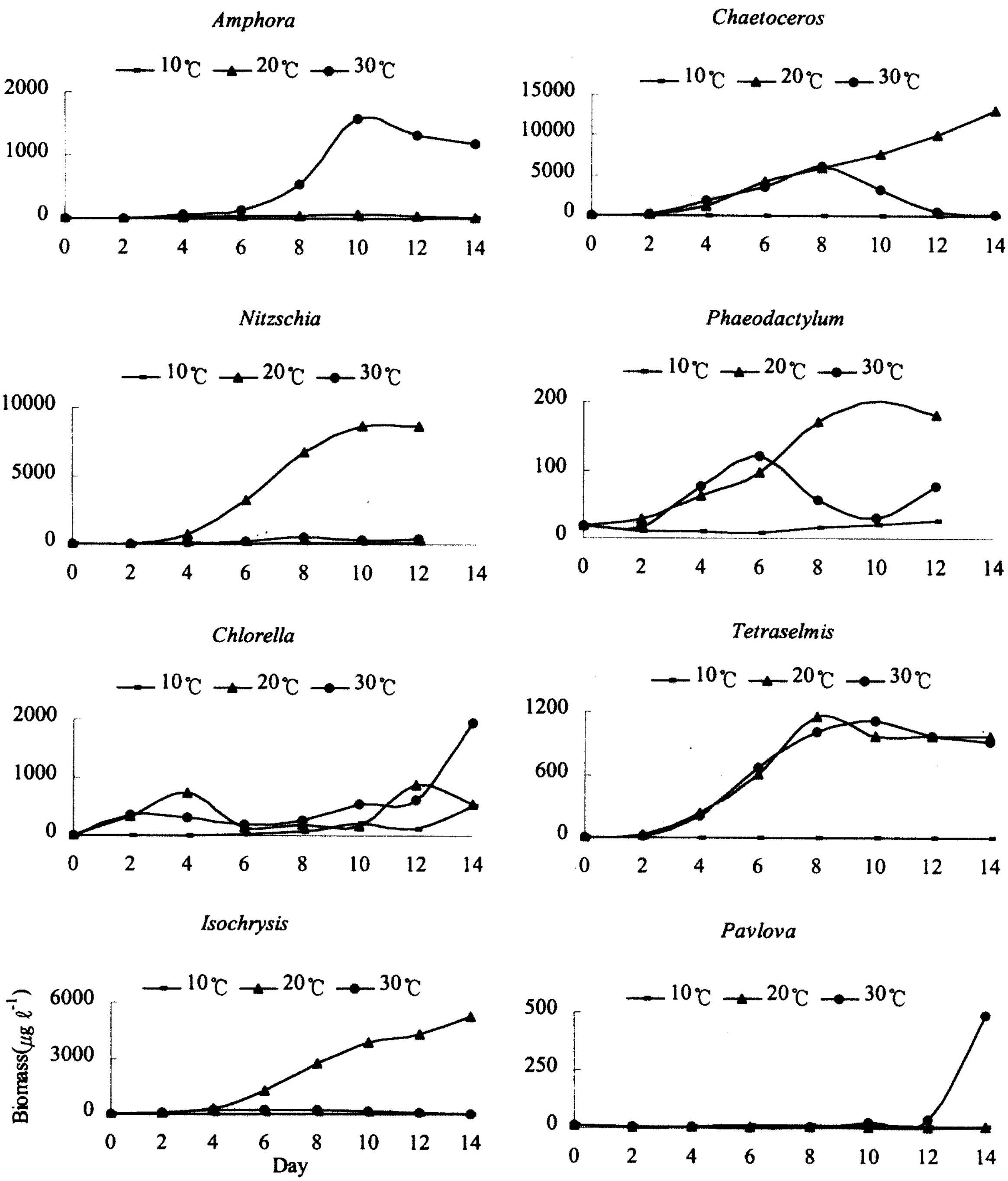
Primary productivity

For primary productivity 2 μCi $NaH^{14}CO_3$ (sodium bicarbonate) per each subsample of 80 m*l* captube were added and mixed well, then cultured for 2 hours in the light and dark condition, simultaneously. After culture, the samples were filtered with GF/C (Whatman 25 mm), and the filter paper moved into 20 m*l* scintillation vial. After acid fuming was done to get rid of excess ^{14}C, a cocktail solution (Aquasol NEN) of 15 m*l* was added, and then measured for photosynthesis activity using liquid scintillation counter (Packard Tri-carb-2700 TR). Primary productivity was calculated by carbon activity using external standard method (Parsons *et al.* 1984).

RESULTS

Growth characteristics on the salinity

Generally the sharp increase of growth curve of each species appeared in the second day. The patterns in salinity treatments were different (Fig. 1). *A. coffeaeformis, C. simplex, P. tricornutum,* and *T. gracilis* showed the lowest growth at 20 psu, while *Nitzschia* sp. and *Chlorella* sp. exhibited the highest growth at 20 psu (Fig. 1). *I. galbana*



Amphora

Chaetoceros

Nitzschia

Phaeodactylum

Chlorella

Tetraselmis

Isochrysis

Pavlova

Fig. 3. Patterns of growth curves of microalgal species at different temperatures (culture conditions: salinity, 35 psu; pH, 8.5; light, I = 180 $\mu E \cdot m^{-2} \cdot s^{-1}$, L:D = 12:12).

and *P. lutheri* represented the highest growth at 25 and 30 psu, respectively, but they showed the lowest growth at 35 psu (Fig. 1). The growth patterns of *I. galbana* in each salinity treatment were similar but with different rates during the 14 days of incubation period.

P. tricornutum and *Nitzschia* sp. had biomass maxima at 20 psu (Fig. 2), *I. galbana* and *T. gracilis* at 25 psu, *A. coffeaeformis* and *P. lutheri* at 30 psu, and *C. simplex* at 35 psu, respectively (Fig. 2). *Nitzschia* sp. and *Chlorella* sp. grew well to all ranges of salinity controlled in this study.

The maximum growth rate of each species ranged from 0.37 to 1.07 per day in salinity treatment (Fig. 2). The growth rates varied within a similar range in each species, but *Chlorella* sp. and *P. lutheri* recorded lowest growth rate at 0 psu and 35 psu, respectively (Fig. 2). We

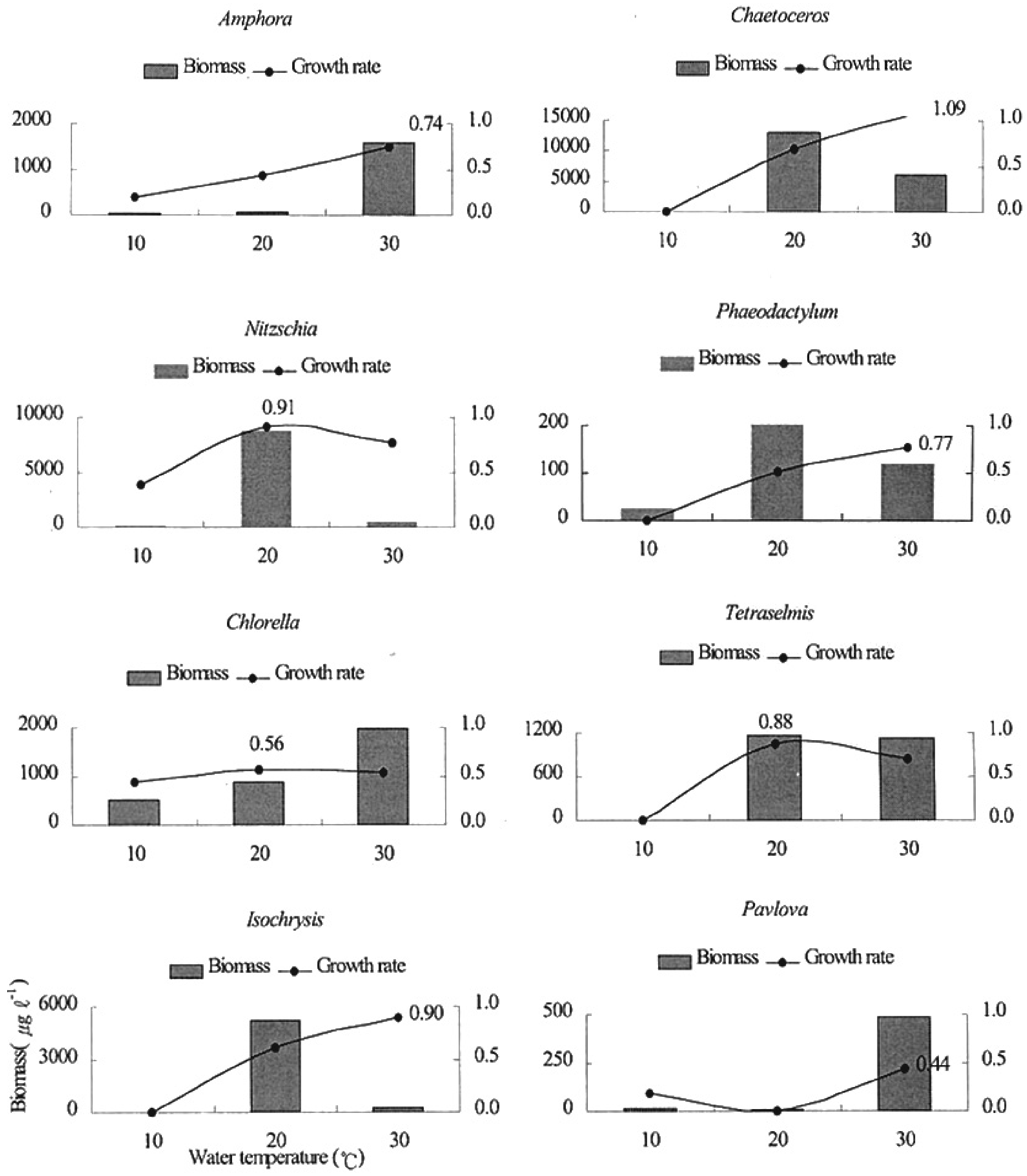


Fig. 4. Comparison of growth rates (μ) and maximum biomass (μgChl-*a* $\cdot$ l^{-1}) at different temperatures.

speculate that the salinity variation may not affect the growth rate of each taxa, even though the maximum biomass changed slightly by salinity. This means that abnormally low salinity occurred in summer season is not likely to demage the microalgal populations in the study area.

Growth characteristics on the water temperature

The patterns of growth curve of eight species were different according to water temperature conditions (Fig. 3). At 10°C, all microalgal taxa did not grow, and their cells lost pigments and degraded. *C. simplex*, *Nitzschia* sp., *P. tricornutum*, *Chlorella* sp., *T. gracilis* and *I. galbana* showed the exponential growth at 20°C (Fig. 3), but the initiation of the exponential growth were different

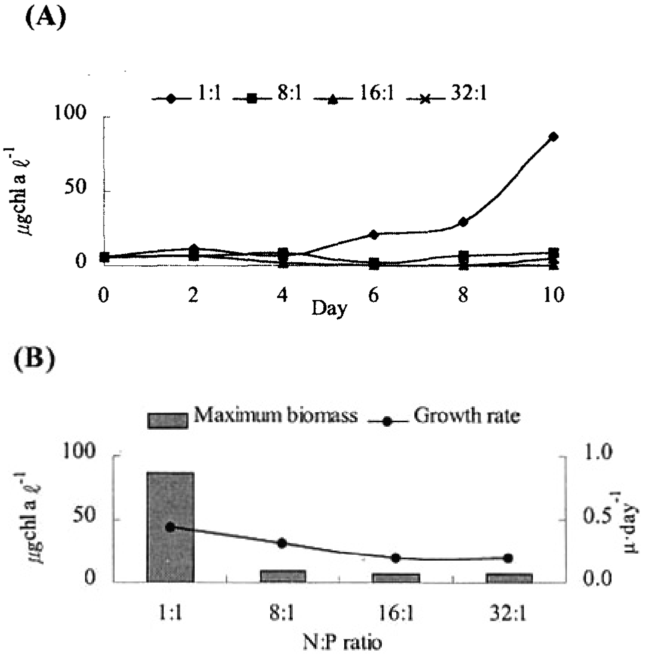


Fig. 5. (A) Patterns of growth curve of *Chaetoceros simplex* at different N:P ratios (culture conditions: water temp., 20°C; pH, 8.5; salinity, 35 psu; light intensity, I = 180 $\mu E \cdot m^{-2} \cdot s^{-1}$, L:D = 12:12). (B) Comparison of growth rate (μ) and maximum biomass ($\mu gChl\text{-}a \cdot l^{-1}$) of *Chaetoceros simplex* at different N/P ratios.

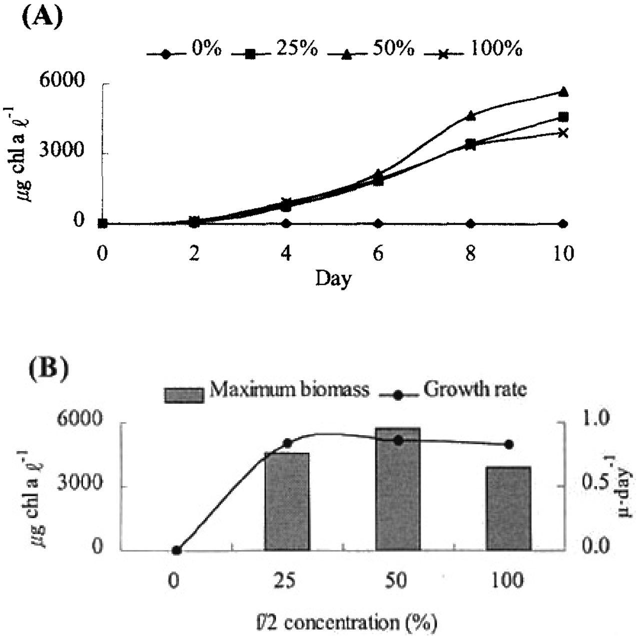


Fig. 6. (A) Patterns of growth curve of *Isochrysis galbana* at different f/2 medium dilutions (culture conditions: water temp., 20°C; pH, 8.5; salinity, 25 psu; light intensity, I = 180 $\mu E \cdot m^{-2} \cdot s^{-1}$, L:D = 12:12). (B) Comparison of growth rate (μ) and maximum biomass ($\mu gChl\text{-}a \cdot l^{-1}$) of *Isochrysis galbana* at different f/2 medium dilutions.

according to species. *A. coffeaeformis* and *P. lutheri* exhibited high growth at a high temperature of 30°C (Fig. 3), which means they are likely to be a warm water species. *Nitzschia* sp. and *I. galbana* grew only at 20°C, and *A. coffeaeformis* and *P. lutheri* only at 30°C, which means they have possibly a very narrow tolerance range on temperature.

The maximum growth rate ranged from 0.44 to 1.09 per day, and the maximum biomass of each species were formed at 30°C for 5 species and at 20°C for 3 species (Fig. 4). The maximum biomass, however, appeared at 20°C in the case of *C. simplex, Nitzschia* sp., *P. tricornutum, T. gracilis* and *I. galbana*. Whereas *A. coffeaeformis, P. lutheri* and *Chlorella* sp. showed the maximum biomass at 30°C. All the diatoms tested exhibited the maximum biomass at 20°C except for *A. coffeaeformis*, and the maximum growth rate at 30°C except for *Nitzschia* sp.

Growth characteristics on the nutrients

The N:P ratios of 8:1, 16:1, and 32:1 did not affect the patterns of growth curve of *Chaetoceros simplex*, but it was different at the ratio of 1:1 (Fig. 5A). The growth rate decreased as the N:P ratio increased or as the phosphorus content decreased (Fig. 5B). The maximum biomass exhibited highest at the 1:1 ratio, but were low at the rest of other ratios (Fig. 5B).

The best growth of *Isochrysis galbana* was obtained at 50% of f/2 medium, and followed by 25%, 100% in order. However it did not grow at 0% (Fig. 6A). The rates of 50%, 25% and 100% were not significantly different (Fig. 6B). The concentration of growth media affected the maximum biomass of *I. galbana*. The highest biomass was found at 50% (Fig. 6B). The 50% dilution of f/2 media showed better growth than that of 100% f/2 medium.

Primary productivity and photosynthesis rate

Table 3 shows primary productivity, photosynthesis rates and chlorophyll biomass of each microalgal species in this study. *A. coffeaeformis* was highest with 1009.9 $mgC \cdot m^{-3} \cdot hr^{-1}$ in primary productivity as well as with 833.7 $\mu gChl\text{-}a \cdot l^{-1}$ in chlorophyll biomass, but had low photosynthesis rate of 1.21 $mgC \cdot mgChl\text{-}a^{-1} \cdot hr^{-1}$. However *Chlorella* sp. exhibited high primary production of 915.2 $mgC \cdot m^{-3} \cdot hr^{-1}$ as well as the highest photosynthesis rate of 5.89 $mgC \cdot mgChl\text{-}a^{-1} \cdot hr^{-1}$, even though

Table 3. Primary productivity (PP), photosynthesis rate, chlorophyll biomass of each microalgal species in this culture study (culture conditions: water temp., 20°C; salinity, 32 psu; light intensity, 180 $\mu E \cdot m^{-2} \cdot s^{-1}$)

Species name	Chl-*a* ($\mu g \cdot l^{-1}$)	PP ($mgC \cdot m^{-3} \cdot hr^{-1}$)	Photosysnthesis rate ($mgC \cdot mgChl\text{-}a^{-1} \cdot hr^{-1}$)
Achnanthes longipes	148.7	366.8	2.47
Amphora coffeaeformis	833.7	1009.9	1.21
Chaetoceros simplex	12.6	16.2	1.29
Nitzschia sp.	656.1	896.1	1.37
Chlorella sp.	155.4	915.2	5.89
Tetraselmis gracilis	468.6	566.8	1.21
Isochrysis galbana	705.9	477.3	0.68
Pavlova lutheri	319.5	486.5	1.52

its chlorophyll biomass was very low with 155.4 $\mu gChl\text{-}a \cdot l^{-1}$. *A. longipes* was relatively high in the photosynthesis rate in spite of being low in chlorophyll biomass (Table 3). *C. simplex* was lowest with 12.6 $\mu gChl\text{-}a \cdot l^{-1}$ and 16.2 $mgC \cdot m^{-3} \cdot hr^{-1}$ in chlorophyll biomass and primary productivity, respectively. The photosynthesis rate of *I. galbana* was lowest with 0.68 $mgC \cdot mgChl\text{-}a^{-1} \cdot hr^{-1}$ in this experiment, but this species represented a second highest biomass and an average primary productivity (Table 3).

DISCUSSION

Abnormally low salinity phenomena happened in 1996 and 1998 summer due to the massive water flow of Changjean (Yangtze) River from China. The salinity was recorded around below 26 psu in the west Jeju coast, where the minimum salinity lowed to 20 psu (Hyun and Pang 1998). Some macrobenthic animals suffered from severe salinity stress due to such an abnormally low salinity and led to mass mortality (Suh *et al.* 1999). This study, however, indicates that the low salinity did not affect the microalgal populations; nevertheless, it caused a severe damage to benthic animals. Several species studied in this study showed rather better adaptation to salinities at 20 and 25 psu than to normal salinity of above 30 psu. *P. tricornutum* was adapted well to wide salinity range with high growth rate and biomass showing euryhaline characteristics in this study. This species has been frequently reported in the coastal waters and occasionally offshore of Korean waters (Lee and Huh 1983; Shim and Park 1984).

All the species in this experiment did not grow in a low temperature at 10°C, or bleached and died. The microalgal abundance around Jeju Island generally decreased in winter which is affected by water temperature (Lee *et al.* 1989, 1990). *Chlorella* sp. and *T. gracilis* grew well at 20 and 30°C in this study, which means these species are eurythermal. However, many species used in this study did grow better at 20°C than at 30°C. Most of all phytoplankton populations in Jeju Island coastal waters may grow well in spring and fall rather than in summer (Lee *et al.* 1995). From this experiment we learn the microalgae of Jeju Island show a seasonal fluctuation which is a result of the adaptation to water temperature, and the results are coincided with some results about adaptation to water temperature in other culture experiments (Bunt 1968; Thomas 1966).

Growth of microalgae at different N:P ratios showed different patterns of adaptation. Phosphorus is likely to be a limiting factor for *C. simplex*, which grew best at the ratio of 1:1. However, the microalgae in the nutrient experiment represented low biomass compared with those in other experiments of salinity and water temperature. *I. galbana* showed different patterns of growth to different f/2 medium dilutions. It grew better in f/4 (50% dilution) than in f/2 (100%) medium. This suggests that the microalgae has been adapted to wide range of f medium concentration.

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