Intraspecific Variation in the Temperature Niche Component of the Diatom *Skeletonema costatum* from Korean Coastal Waters

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Final biomass yields (peak optical density) and growth rates (divisions/day) of seven clones of *Skeletonema costatum* from Korean coastal waters were measured to understand their intraspecific variations in the light intensity niche component under 25℃ condition. Daily growth rates of 6 of 7 *S. costatum*, clones were maximum at 6000 lux while that of YS4, a neritic clone, was maximum at 9000 lux. The final biomass yields of 4 of the 7 *S. costatum* clones were maximum at the lowest light intensity of 2000 lux. Minimum final biomass yields were found at 9000 lux in all the *S. costatum* clones other than an estuarine clone, HDC9. The intraspecific variations of the mean growth rate and mean final biomass yield under each of the three different light intensity in terms of the coefficient of variation were not greater than 10% in any of the 7 *S. costatum* clones.

Key words : intraspecific variation, diatom clones, *Skeletonema costatum*, temperature niche, growth rate, biomass yield

Introduction

The lowest taxonomic category for most studies on physiological ecology of phytoplankton is species level (McGowan, 1971). Thus, various clones in a single species are assumed to have totally the same characteristics. Notable genetic differences between clones of the same species have long been demonstrated in many organisms (Haldane, 1946; Jain and Bradshaw, 1966), and recently even in phytoplankton (Conte et al., 1995; McQuoid and Hobson, 1995; Bell, 1990, 1991; Brand, 1981, 1982, 1991; Brand et al., 1981; Myrphy and Guillard, 1976; Guillard and Ryther, 1962). In a review of relevant reports on genetic variation in marine phytoplankton species Brand (1991) concluded that the genetic variability among the clones within a population is not so large as to preclude the use of one clone as somewhat representative of the population. Genetic differentiation among spatially and/or temporally separated populations, however, is large enough to recognize more than one representative clone for a phytoplankton species (Brand, 1991). Here we estimated the genetic differences among seven *Skeletonema costatum* clones from West Coast and Jinhae Bay, Korea to understand the intraspecific variation in the temperature niche component of those spatially close clones.

Materials and Methods

Clonal cultures of the marine diatoms at Kunsan National University were used for the present study on the subspecies-level characterization of phytoplan-

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Table 1. The origin of the clonal cultures

clone	species	location	date	isolator
KES10	*Skeletonema costatum*	Estuarine Weir of Kum River	06/02/94	W. Yih
H17	*Skeletonema costatum*	Estuarine Weir of Kum River	06/02/94	G. Y. Hahn
NC-37	*Skeletonema costatum*	Tidal pool, Naecho-Do, Kunsan	07/20/93	W. Yih
HDC9	*Skeletonema costatum*	inner Daechon Harbour	08/12/94	G. Y. Hahn
BRS1	*Skeletonema costatum*	Near Boryong E1. Power Plant	05/17/94	W. Yih
J9	*Skeletonema costatum*	inner Jinhae Harbour	07/04/93	W. Yih
YS4	*Skeletonema costatum*	off Tae Ahn peninsula	02/19/94	W. Yih
HYS4	*Thalassiosira* sp.	outer Kum River Estuary	07/08/94	G. Y. Hahn

kton cells. Among the 110 clones of marine phytoplanktow 7 clones of *S. costatum*, a major dominant diatom species in Korean west coast all the year round, and 1 clone of *Thalassiosira* sp. (Table 1) were selected as experimental cultures. Each clonal culture was established by single cell isolation (Guillard, 1973) before enrichment. Basal seawater for culture media was collected from the surface layer of 'Station 14' (36° 10.8′ N, 126° 09.2′ E) near Sochongdo, Yellow Sea in July 1992 (Yih et al., 1994; Yih, 1993). Basal seawater and each stock solution were separately autoclaved. The 32‰ basal seawater was enriched with 10^{-4}M $NaNO_3$, 10^{-5}M NH_4Cl, 10^{-5}M NaH_2PO_4, 10^{-4}M Na_2SiO_3, 10^{-5}M EDTA, 10^{-6}M Fe-EDTA, 10^{-7}M $ZnSO_4$, 10^{-7}M $MnSO^4$, 10^{-9}M $CuSO_4$, 10^{-8}M $CoSO_4$, 10^{-9}M biotin, 10^{-7}M thiamine, and 10^{-8}M vitamin B_{12} (Brand, 1990). For the experiments 20×150mm glass culture tubes with autoclavalbe caps (Sigma, USA) were used after a cleaning procedure. The procedure includes cleaning with detergent, repeated rinses, soaks of diluted HCl and distilled water, and final rinsing with the sterilized basal seawater. The experimental cultures were incubated at three different temperatures (5, 15 and 25℃), and a light intensity of 2000 lux was maintained with 12 : 12 L : D cycle. New bach culture was established sequentially by transferring an inoculum from each batch culture at its early stationary phase. After 2~3 generations of acclimation the actual measurements of each culture were done during the following 3~6 generations. Optical densities at 665 nm of five replicates of each of the 24 experimental cultures (3 temperature by 8 clones) were measured at the same time each day with a UV/VIS spectrophotometer. Maximal growth rates were determined from the rate of optical density increases, and the peak optical density in a single generation was recorded as the final biomass yield of each generation.

Results and Discussion

The coefficients of variation among the replicate maximal growth rates of *S. cosatum* cultures were 9~24% with its mean of 16.0% (Table 2), and the coefficients of variation among the mean maximal growth rates of the seven *S. costatum* clones at each of the 3 temperatures were calculated to be less than 7% (Table 4). Mean maximal growth rates of HYS4, a *Thalassiosira* clone, were less than half of those in other 7 *S. costatum* clones whose mean maximal growth rates ranged from 0.44 up to 0.71 divisions/day (Table 2). The observed increases in mean maximal growth rates at 25℃ as compared with the growth rates at 5℃ seems to be rather smaller than those in other studies under saturating light (Li, 1980). The experimental light intensity of 2000 lux which simulates the in-situ light-limiting condition of the coastal waters of Korean west sea (Choi and Shim, 1986) may be a growth-limiting condition (Eppley, 1972; Aruga, 1965) for the *S. costatum* cultures in the pre-

Table 2. The mean maximal growth rate (divisions/day) of each clone with its 95% confidential interval and coefficient of variation among replicate measurements

clone	T(℃)	mean maximal growth rate	95% confidence interval	*C.V. (%)	number of replicates
KES10	5	0.45	0.421~0.479	10.4	10
KES10	15	0.61	0.570~0.655	17.6	25
KES10	25	0.64	0.592~0.685	17.8	23
H17	5	0.47	0.434~0.505	15.0	15
H17	15	0.61	0.563~0.655	19.2	25
H17	25	0.70	0.665~0.731	12.2	25
NC37	5	0.44	0.422~0.468	9.2	12
NC37	15	0.55	0.513~0.580	13.9	20
NC37	25	0.62	0.574~0.674	16.9	17
HDC9	5	0.47	0.426~0.506	16.8	15
HDC9	15	0.57	0.532~0.617	19.0	25
HDC9	25	0.64	0.600~0.671	14.2	25
BRS1	5	0.44	0.385~0.494	18.9	9
BRS1	15	0.62	0.579~0.657	14.7	21
BRS1	25	0.69	0.643~0.742	18.1	25
J9	5	0.54	0.472~0.613	24.0	13
J9	15	0.63	0.598~0.668	14.3	25
J9	25	0.71	0.657~0.754	17.5	25
YS4	5	0.45	0.424~0.480	11.4	13
YS4	15	0.52	0.481~0.557	18.5	25
YS4	25	0.66	0.616~0.700	16.3	25
HYS4	5	0.27	0.223~0.320	25.7	8
HYS4	15	0.38	0.349~0.417	17.6	15
HYS4	25	0.37	0.327~0.415	25.0	17

*C.V.(%) means the coefficient of variation in percentage.

sent study. None of the experimental clones failed to grow under 5℃ condition, and the mean maximal growth rates increased rather linearly with temperature increase in all the *S. costatum* clones (Fig. 1). This result demonstrates the similarity in the temperature niche components of the *S. costatum* clones under 2000 lux condition, and also implies that the extremely low in-situ light intensity rather than the low water temperature be responsible for the minimum *S. costatum* biomass of Korean west coast in winter (Yih and Shin, 1994; Choi and Shim, 1986). The recorded minimum of 0.44 divisions/day under 5℃ (Table 2) support the Eppley's suggestion on the better suboptimal tolerance of marine phytoplankters than their tolerance in temperatures above the optimum (Eppley, 1972). Two groups of *S. costatum* clones with different temperature optima can be seen in Fig. 1. Linear increase in growth rate at 5~25℃ interval is evident in the first group (Fig. 1. A.), while the linearity is modified by the abrupt lessening of its slope at 15~25℃ interval in the second group (lower graph in Fig. 1. B.).

The coefficients of variation among the replicate final biomass yields of *S. costatum* cultures were 5~11%

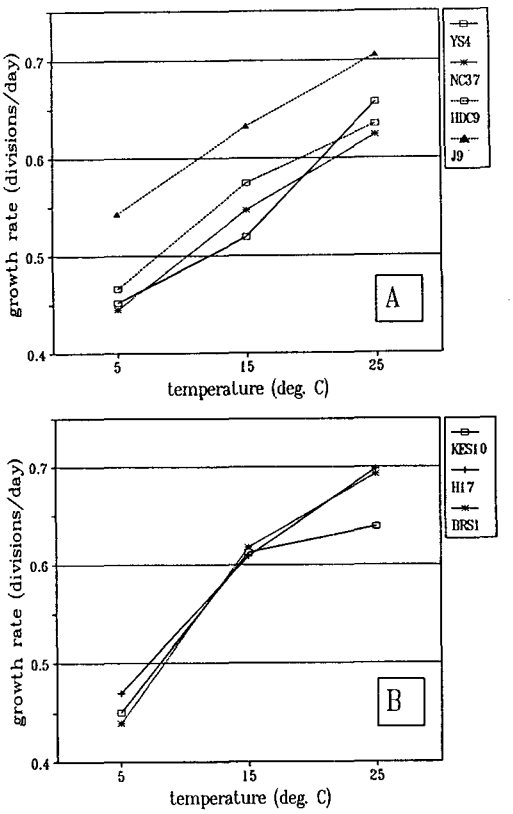

Fig. 1. The mean maximal growth rates of the seven *Skeletonema costatum* clones at 5, 15 and 25℃ under 2000 lux. Refer Table 2 for the statistics of the original measurements.

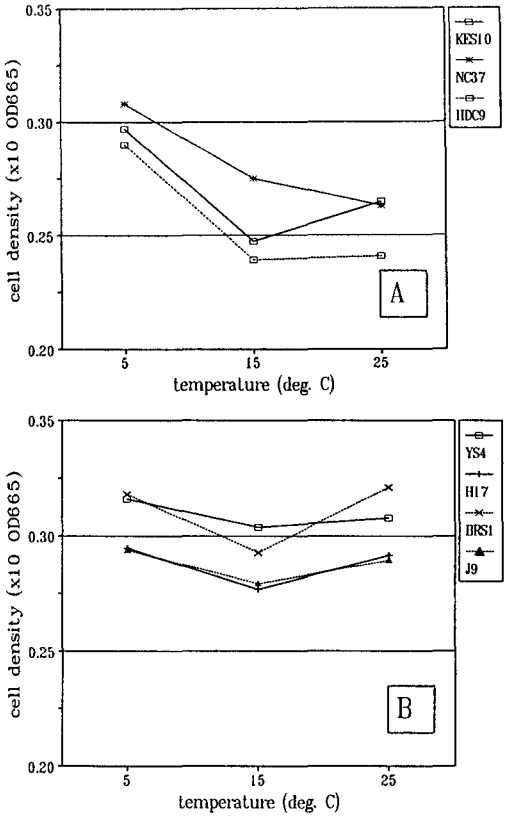

Fig. 2. The mean final biomass yields of the seven *Skeletonema costatum* clones at 5, 15 and 25℃ under 2000 lux. Refer Table 3 for the statistics of the original measurements.

with its mean of 8.4% (Table 3), and the coefficients of variation among the mean final biomass yields of the 7 *S. costatum* clones at each of the 3 temperatures were calculated to be less than 10% (Table 4). Mean final biomass yields (×10 optical density at 665 nm) in HYS4, a *Thalassiosira* clone, were 60~70% of those in other 7 *S. costatum* clones whose mean final biomass yields ranged from 0.239 up to 0.321 (Table 3). The variation of the mean final biomass yields under the 3 different experimental temperatures within a single clone was calculated to be far less than the interclonal variations of the temperature-averaged mean final biomass yields (Table 3). Thus, the notable intraspecific variation of final biomass yields was demonstrated clearly. Except BRS1, a clone from a thermal effluent point of Boryong Electric Power Plant, and HYS4 (a *Thalassiosira* clone), highest final biomass yields were recorede at 5℃ in all the other *S. costatum* clones (Table 3). In KES10, NC 37 and HDC9 (Fig. 2. A.) the final biomass yields at 5℃ was far greater than those at 15 or 25℃, while the final biomass yields at both 5 and 25℃ were only slightly different in other *S. costatum* clones (Fig. 2. B.). The latter group may have well adapted to wider range of temperature than the former group.

Although the coefficients of variations of the two measured parameters (mean maximal growth rate and mean final biomass yield) among the seven *S. costatum* clones were less than 10%, their temperature niche components enabled us to separated them into

Table 3. The mean biomass yield (×10 optical density at 665 nm) of each clone with its 95% confidential interval and coefficient of variation among replicate measurements

clone	T(℃)	mean biomass yield	95% confidence interval	*C.V. (%)	number of replicates
KES10	5	0.207	0.287~0.307	5.4	10
KES10	15	0.247	0.239~0.256	8.8	25
KES10	25	0.265	0.257~0.273	7.9	25
H17	5	0.295	0.281~0.308	9.0	15
H17	15	0.277	0.267~0.287	9.3	25
H17	25	0.292	0.283~0.300	7.5	25
NC37	5	0.308	0.298~0.318	6.0	14
NC37	15	0.275	0.267~0.283	6.2	20
NC37	25	0.263	0.253~0.273	9.2	23
HDC9	5	0.290	0.275~0.305	10.2	15
HDC9	15	0.239	0.230~0.248	9.5	25
HDC9	25	0.241	0.232~0.249	8.9	25
BRS1	5	0.318	0.302~0.334	8.2	10
BRS1	15	0.293	0.281~0.305	10.5	25
BRS1	25	0.321	0.311~0.331	8.1	25
J9	5	0.294	0.280~0.308	9.2	15
J9	15	0.279	0.267~0.291	11.0	25
J9	25	0.290	0.280~0.299	8.6	25
YS4	5	0.316	0.306~0.326	6.0	15
YS4	15	0.304	0.293~0.314	8.8	25
YS4	25	0.308	0.299~0.316	7.0	15
HYS4	5	0.173	0.158~0.188	11.5	7
HYS4	15	0.174	0.166~0.182	9.6	15
HYS4	25	0.172	0.163~0.181	12.6	20

*C.V.(%) means the coefficient of variation in percentage.

Table 4. Statistics on the mean maximal growth rates (divisions/day) and the mean final biomass yiels (×10 OD_{665}) of the seven *Skeletonema costatum* clones

T(℃)	statistics	mean maximal growth rate	mean final biomass yield
5	range	0.44~0.54	0.290~0.318
	average	0.47	0.303
	*C.V.(%)	7.0	3.5
15	range	0.52~0.63	0.239~0.304
	average	0.59	0.273
	C.V.(%)	6.6	7.8
25	range	0.62~0.71	0.241~0.321
	average	0.66	0.283
	C.V.(%)	4.7	9.1

*C.V.(%) means the coefficient of variation in percentage.

distinct groups (Figs 2 and 3). In conclusion, the observed intraspecific variations among the seven *Skeletonema costatum* clones from West Coast and Jinhae Bay, Korea again demonstrated the existence of non-uniformity among the clonal cultures within a single species (Conte et al., 1995; McQuoid and Hobson, 1995).

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