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Abundance of a unicellular, Chroococcoid picoplankton in the Nakdong River estuary, Korea

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낙동강 하구에서 초미소 자가 영양 플랑크톤에 관한 연구

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Picoplankton in the size range of 0.2-2.0 μm were enumerated in the Nakdong River estuary on 17 Octorber, 1991 by using an epifluorescence microscope. Numerous minute cells emitting yellow to orange fluorescence at various strengths were recognized and the cells were mostly spherical in shape. Picocyanobacteria seem to comprise most of the picoplankton observed. They also occurred in very polluted water. Cell densities of picoplankton were in the range of 683-3,878 cells·ml^{-1} at the surface water. In vertical profiles, the numbers were maximum at subsurface and minimum at surface layer. Percentage picoplankton biomass determined by chlorophyll ranged from undetectable levels to 5.9% of the total chlorophyll. The cell density and chlorophyll a concentrations of picoplankton were relatively low in the study area compared to those of other locations in world ocean, but the study of picoplankton may be important in understanding of microbial food web in the sea.

낙동강 하구에서 1991년 10월 17일 형광 현미경을 이용하여 0.2-2.0 μm 크기 범위의 초미소플랑크톤을 조사하였다. 여러 강도의 노란색부터 오렌지색 형광을 내는 많은 작은 세포가 관찰되었으며 모양은 대부분 둥근 형태이었다. 이들은 매우 오염된 해역에서도 출현하였다. 관찰된 초미소플랑크톤의 대부분은 picocyanobacteria 인듯 하였다. 표층에서 초미소플랑크톤의 세포수는 683-3,878 cells·ml^{-1}의 범위이었다. 수직분포를 보면 표층에서 최소이었고 다소 깊은 곳에서 최대이었다. 초미소플랑크톤의 엽록소 a 함량은 전체의 0-5.9% 범위이었다. 조사 해역에서 초미소플랑크톤의 세포수와 엽록소 a 양은 타 해역에 비하여 낮았으나 이러한 초미소플랑크톤의 연구는 해양에서 microbial food web의 이해에 중요할 것이다.

INTRODUTION

Data of standing stock and composition of phytoplankton are vital for the study of aquatic ecosystem and many studies on the phytoplankton biomass have been done with reporting the cell numbers. However, picoplankton in the size range of 0.2-2.0 μm (Sieburth et al., 1978) tends to be easily overlooked during the ordinary optical microscopic enumeration because the discrimination between detrital particles and agal cells smaller than 5 μm range is almost impossible(Takahashi and Hori, 1984).

The tiny picoplankton, which is photoautotrophic microorganisms, includes both prokaryotic cyanobacteria and eukaryotic algae, but the biomass of cyanobacteria is known to be more important than that of eukaryotic cells (Stockner and Antia, 1986). The cyanobacteria are characterized by containing high cellular levels of phycoerythrin

pigment, which is highly fluorescent in vivo. By using the fluorescent characteristics, many studies on the occurrence and biomass of picocyanobacteria have been done in various locations of the world ocean since Johnson and Sieburth(1979) and Waterbury et al.(1979).

The importance of picocyanobacteria were reported in both marine and freshwater ecosystem. Many studies have shown that picocyanobacteria are significant contributers to total primary production in open ocean (Platt et al.,1983; Takahashi and Hori, 1984; Glover et al., 1986, Prezelin et al., 1986; Prezelin et al., 1987), in coastal waters (Takahashi et al., 1985; Tracey et al., 1988; Hargraves et al., 1990) and in freshwater (Caron et al., 1985; Fahnenstiel et al. 1986; Fahnenstiel and Scavia, 1987; Weisse, 1988). In korean waters, studies on the picoplankton have not been done except the report of Shim et al.(1991), who presented the percentage chlorophyll a concentration and primary production of the total in the adjacent waters of Kori Nuclear Power Plant. However, direct microscopic observations of the picoplankton were not reported.

The purposes of this study were to report the occurrence of picoplankton including picocyanobacteria in the Nakdong River estuary and to enumerate the numbers by using the fluorescence microscope. The chlorophyll a concentrations of total and picoplankton were also measured. In addition, some parameters of water quality were measured to know whether the picoplankton occurrs in the polluted water because parts of the study area is known to become very polluted after the barrage construction.

MATERIALS AND METHODS

Field observation was performed in the Nakdong River estuary on October 17, 1991. The map and stations are shown in Fig.1. Water samples were collected by a bucket at the surface and by Van Dorn sampler from various depths at station 4 and 9.

Chlorophyll a concentrations were determined fluorometrically on 90% acetone extractions of cells retained on a 0.45 μm pore-sized membrane filter with a Turner Designs Model 10 Fluorometer equipped with appropriate filters (Yentsch and Menzel, 1963; Holm-Hansen et al., 1965). The extracted chlorophyll a measurements were made on a "unfractionated" (whole sample) and a second "picoplankton", defind as the fraction passing through a 3.0 μm membrane filter. Filteration pressures were always less than 15cmHg to minimize the cell loss from rupture of fragile picoplankton species. Stocknêř and Antia(1986) reported that low vacuum(10-20 cmHg) was preferable when filter was used for size separation. Membrane filters of pore size in the range of 3.0-0.2 μm have been used for separating and investigating the picoplankton(Takahash et al., 1985 ; Fahnenstial et al., 1986; Weisse, 1988).

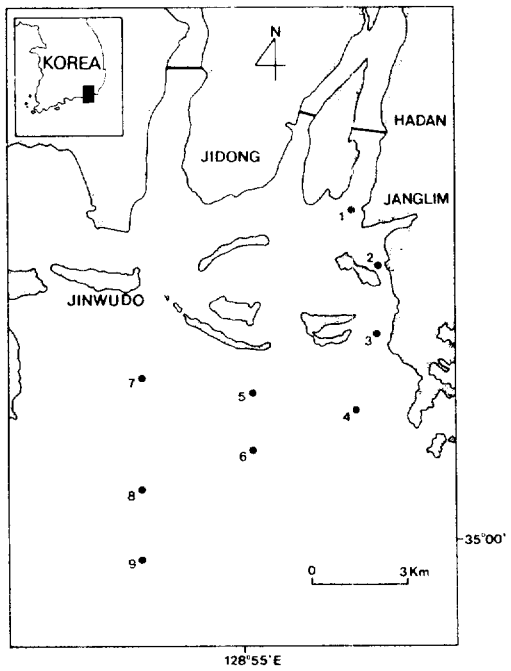

Fig. 1. Sampling stations in the Nakdong River estuary.

All samples were made in duplicate and the averages were taken for the results. The sample filters, which had been stored frozen, were soaked in glass tubes with 10 ml of 90% aceton and stored for 24 hrs in the dark refrigerator. After extraction was completed, the suspension was centrifuged for 5 minutes at 4000 rpm and the supernatant decan-

ted into a cuvette. The fluorescence before and 30 seconds after acidification (with several drops of 5% HCl) was measured with the fluorometer. Calculations were done after the blank at each sensitivity setting of the fluorometer was subtracted.

For the microscopic analysis of picoplankton, glutaraldehyde and paraformaldehyde were added to 100 m*l* of water sample at final concentrations of 1% and 0.03%, respectively. After the preserved samples were brought to the lab, 10 m*l* of each sample was filtered through a 3.0 μm pore-sized membrane filter. Then, slides were prepared by filtering onto 0.2 μm pore-sized Nuclepore filters which stained with 0.2% nigrosine previously and kept frozen immediately. These slides were counted within five days to minimize errors due to the fading of autofluorescence. The sample was observed at a magnification of 1000x, using an epifluorescence microscope (Vickers, M17). Excitation of the sample was made by the wavelength of 546 nm(green) using a mercury vapor lamps as light source. Cyanobacteria, which possesed phycobilins giving a light absorption between 520-600 nm, were identified by yellow to orange fluorescence excited at 546 nm. With the excitation wavelength at 546 nm, phycoerythrin, one of the phycobilins is known to give a characteristic fluorescence at about 580 nm (as yellow to orange).

Among phycoerythrin containing algae (red algae, cryptomonads, and cyanobacteria), red algae can be excluded easily by their larger size from picoplankton. Cryptomanads which have also phycoerythrin pigment probably interfere the cell counting of picocyanobacteria. However, cryptomonads have non symmetrical shape and in general larger size compared to picocyanobacteria. These features of cryptomonads may give reasonable differentiation from cyanobacteria except a few cases(especially for cryptomonads<2.0 μm). In addition, a few eukaryotic cells (<3 μm) with red fluorescence were observed, but excluded in counting. However, the fluoresceing cells are reported as picoplankton not as picocyanobacteria in this paper due to possible some fraction of cryptomonads. Picocyanobacteria, in fact, seems to comprise most of the picoplankton observed. Cell numbers were determined by direct microscopic observations from square (each length and width 100 μm) carved on the lenses. 25 to 45 times counting were made for determination of cell numbers inside the 100 m square for total area of filtered sample. The entire procedure for microscopic observations of the picoplankton samples followed Tsuji and Yanagita(1981) and Takahashi et al.(1985).

Water samples for nutrient analysis were filtered on shipboard through Gelman type A-E glass fiber filters, which were stored in polyethylene bottles and kept frozen until analysis were performed. All nutrient concentrations were determined colorimetrically by the methods of Strickland and Parsons (1972). Water tamperature and salinity were determined by using a T-S bridge and transparency were measured with white Secchi disk (30 cm diameter).

RESULTS

Surface water temperature and salinity were in the range of 19.2-20.5℃ and 10.1-32.4‰, respectively (Table 1). The lowest salinity occurred at station 2, located off Janglim stream, while the highest salinity at station 9. The temperature in more saline water was slightly higher than that in less saline water, which is in agreement with the report of Moon and Choi (1991) who showed lower temperature in the upper region of the estuary barrage than that in the lower region during fall. Vertical profiles of salinity at station 4 and 9 (Fig. 2) show increasing patterns with depth and temperature also increased with depth. Especially, the salinity difference between surface and bottom was 16.1‰ at station 4 though the depth was only 2 m.

Transparency, which was defined as Secchi disk depth, was in the range of 0.8-6.8 m. The highest depth occurred at station 9 and the lowest at station 1, just lower region of estuary barrage (Table 1).

Nutrient concentrations were in the range of 0.31-1.86 μg-at·l^{-1} for phosphate, 0.53-85.80 μg-at·l^{-1} for nitrate, 0.27-5.46 μg-at·l^{-1} for nitrite, 1.39-442.20 g-at·l^{-1} for ammonia and 3.42-87.98 g-at·

Table 1. Surface water temperature, salinity, nutrient concentrations and Sechii disk depth in the Nakdong River estuary on 17 October, 1991

Station	Temp. (℃)	Salinity (‰)	Nutrient (μg-at·l^{-1})					Sechii Disk depth (m)
			PO_4	SiO_2	NO_3^-	NO_2^+	NH_4^+	
1	19.7	13.0	1.24	72.37	85.80	3.10	204.40	0.80
2	19.2	10.1	1.86	80.09	84.61	4.44	337.80	1.00
3	19.5	15.9	1.56	87.98	84.24	5.46	442.20	–
4	19.5	16.9	0.93	53.95	63.37	2.78	92.80	0.80
5	19.8	19.6	0.52	14.56	24.32	0.98	51.40	0.70
6	20.0	25.8	0.62	25.00	14.50	1.00	51.95	1.40
7	19.1	18.5	0.31	49.12	63.14	1.71	68.05	1.20
8	19.8	31.9	0.31	6.75	1.42	0.53	41.95	5.00
9	20.5	32.4	0.36	3.42	0.53	0.27	1.39	6.80

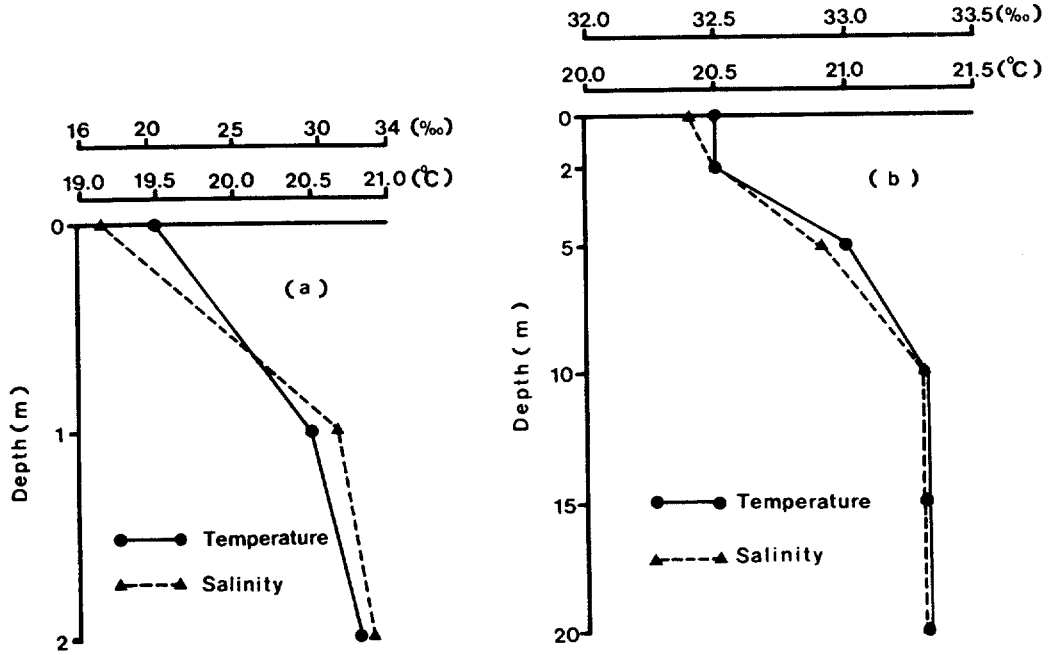

Fig. 2. Vertical profiles of temperature and salinity at station 4 (a) and station 9 (b).

l^{-1} for silicate (Table 1). Relatively high concentrations of all nutrient constituents occurred at station 1,2 and 3, the area where salinity was relatively low and many industrial complexed are located nearby Janglim stream. According to Moon and Choi (1991), the sources of nutrients in the Nakdong River estuary are Nakdong River discharge for silicate and nitrate and Janglim stream for phosphate and ammonia.

Total chlorophyll a concentrations in the study area was in the range of 0.90-4.86 μg·l^{-1} (Table 2). Relatively high concentrations occurred at station 1, 2 and 9. Picoplankton chlorophyll a concentrations ranged from undetectable levels to 0.10 μg·l^{-1} and at 5 among 9 stations, the concentrations were undetectable. Percentage picoplankton concentrations of chlorophyll a was 0 -5.9% (highest at station 3).

In the fluorescence microscopic observations, numerous minute cells emitting yellow to orange fluorescence at various strengths were recognized. The cells were mostly spherical in shape and their sizes ranged between 0.5 and 2.0 μm. Cell numbers of picoplankton counted in the study area were

Table 2. Chlorophyll a concentrations of total and picoplankton, percentages of chlorophyll a smaller than 3.0 m particle sizes and abundance of picoplankton (cells·ml^{-1}) at the surface waters of Nakdong River estuary on 17 October, 1991

Station	Chlorophyll a concentrations (μg/l)		% picoplankton of the total	Cell numbers of picoplankton
	Total	Picoplankton		
1	3.92	0.02	0.5	1138
2	4.86	ND	–	683
3	1.17	0.10	5.9	2379
4	1.11	ND	–	550
5	2.26	ND	–	911
6	1.08	0.02	1.9	3870
7	1.02	ND	–	832
8	0.90	0.02	2.2	1389
9	3.43	ND	–	804

*ND means "not detectable"

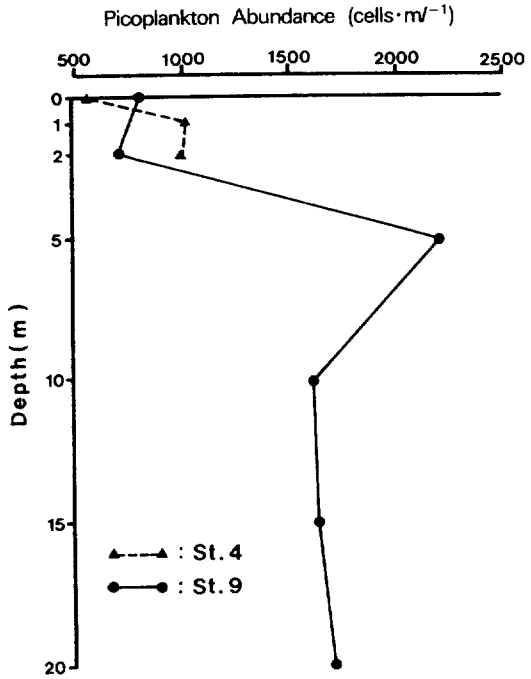

Fig. 3. Vertical profiles of picoplankton abundance at station 4 and 9.

in the range of 683-3,870 cells·ml^{-1} (average 1,395 cells·ml^{-1}) at the surface. The highest numbers occurred at station 6 and the lowest at station 4. Only when the number was more than 10^3 cells·ml^{-1}, the picoplankton chlorophyll a concentrations were detectable. In vertical profiles of picoplankton cell numbers (Fig. 3), the highest value occurred at subsurface and the lowest at surface layer. Maximum value at satation 4 and 9 occurred at 1m and 5m depth, respectively.

DISCUSSION

Picoplankton in this study area does not seem to be important in biomass of total phytoplnkton. Percentage of picoplankton biomass determined by chlorophyll by the size-separation technique ranged from undetectable levels to 5.9% of the total chlorophyll in the study area. These results were much less than those of previous studies carried out in coastal waters off Japan, where the percentage was reported as 20-100% (Takahashi et al., 1985) and those in adjacent water of Kori Nuclear Power Plant off Korea, where the percentage was between 4.6 and 66.0% (Shim et al., 1991). Picoplankton is known to be particularly important in the offshore or neritic waters in which total phytoplankton biomass is not large. However, in the Nakdong River estuary, there is a possibility that it becomes important in other season because the total chlorophyll concentration are very variable with season in the study area (Cho and Huh, 1988; Moon and Choi, 1991). Further investigation for seasonal variations of picoplankton biomass is needed.

Although the picoplankton biomass determined by chlorophyll was low, it was obvious that picoplankton smaller than 2 μm exist in the Nakdong River estuary. In the present study, picoplankton passing through 3 μm membrane filter were eva-

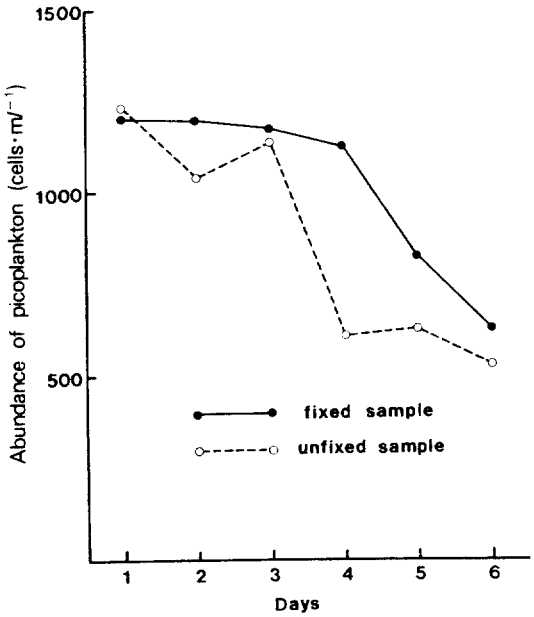

Fig. 4. Abundance of picoplankton with time after sampling were done, showing fading of autofluorescence.

luated and counted under an epifluorescence microscope, and they were easily distinguishable by their characteristic fluorescence. The cell numbers of picoplankton were between 683-3,870 cells·ml^{-1} and the numbers was not consistent with total chlorophyll concentrations (Table 2). The density was still low with relatively low concentrations of chlorophyll, compared to the density reported in the Narragansett Bay, where they occurred at typical concentrations of 5×10^6 cells·ml^{-1} (Tracey et al., 1988).

The reason of low cell density and chlorophyll concentration in the study area is not clear. It is not probable that the low biomass of picoplankton is caused by fading of fluorescence. As shown in Fig.4, the fluorescence in fixed samples was not much fading until four days after sampling. Most of our samples(14 among 16) were counted within 4 days and only 2 were counted at 5th days. Most of study on the biomass of picoplankton have been done in open ocean or lake and nothing has been done in brackish water such as Nakdong River estuary, where salinity varied between 10.1-32.4% during study period (Table 1). Based on the fact that the abundances of picoplankton were not consistent with salinity (Table 2), it is not probable that freshwater picocyanobacteria underwent osmotic stress when they met more saline water, resulting in low biomass. The picoplankton biomass and its importance in the brakish water need to be investigated.

As shown in Fig.3, picoplankton density was high at subsurface and these phenomena are in good agreement with the field investigation of Glover et al.(1985) who reported that picoplankton required lower light intensities to saturate photosynthesis and they utilized blue and in particular green light more efficiently for photosynthesis. According to Barlow and Alverte(1985), marine Synechococcus spp. exhibited dramatic photoinhibition of photosynthesis and reduction in growth rate at high photon flux densities. Subsurface maximum of picocyanobacteria was also reported in the western North Pacific Ocean and South China Sea (Takahashi and Hori, 1984), coastal water off Japan (Takahashi et al., 1985), Lake Ontario (Caron et al, 1985) and Lake Constance (Weisse, 1988). In this study of Nakdong River estuary, maximum picoplankton biomass occurred at 5m depth at station 9, while it occurred at 1m depth at station 4 (Fig.3). This is probably due to the fact that the transparency at station 9 was 6.8m while it was only 0.8 m at station 4 (Table 1).

It has not been studied before on the occurrence of picocyanobacteria in very polluted water. The station 1, 2 and 3 where many industrial complexes are located nearby Janglim stream are known to become very polluted after barrage construction. According to Moon and Choi(1991), concentrations of ammonia and phosphate in the just lower region of the barrage off Janglim is very high, dissolved oxygen is very low and there are many indicative phytoplankton species of water pollution there. The pollution was attributed to waste discharge from the Janglim stream and stagnation of water after barrage construction. In this study, the concentrations of phosphate and ammonia were more than 1.2 μg-at·l^{-1} and 200 μg-at·l^{-1}, respectively. However, picoplankton density was between 683-2,379 cells·ml^{-1} (average 1,400 cells·

ml^{-1}), which was not much difference with the average(1.395 cells·ml^{-1}) of total.

Although the picoplankton biomass in the Nakdong River estuary was low, the existence of picoplankton may be contributable to the microbial food web in the study area. According to Wright et al. (1982), picoplankton contribute significantly to the nutritional requirements of the animal when larger particles are not available. Fahnenstiel and Carrick (1991) reported that grazing was the major loss for picocyanobacteria population and most of grazing loss was attributed to small, heterotrophic flagellates. According to Tracey et al. (1988), the species composition of picoplankton affects the nutrition and growth of bivalve molluscs. The study of picoplankton in the Nakdong River estuary is probably important in understanding the microbial food web.

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REFERENCES

Barlow, R.G. and R.S. Alberte, 1985. Photosynthetic characteristics of phycoerthrin-containing marine *Synechococcus* spp. I. response to growth photon flux density. *Mar. Biol.*, **86**: 63-74.

Caron, D.A., F.R. Pick and D.R.S. Lean, 1985. Chroococcoid cyanobacteria in Lake Ontario: Vertical and seasonal distributions during 1982. *J. Phycol.*, **21**: 171-175.

Cho, C.H. and S.H. Huh, 1988. Community structure and distribution of phytoplankton in the Nakdong River estuary. *Ocean Research*, **10**: 39-45 (in Korean).

Fahnenstiel, G.L. and H.J. Carrik, 1991. Physiological characteristics and food-web dynamics of *Synechococcus* in Lake Huron and Michigan. *Limnol. Oceanogr.*, **36**: 219-234.

Fahnenstiel, G.L. and D. Scavia, 1987. Dyanamics of Lake Michigan phytoplankton: Primary production and growth. *Can. J. Fish. Aguat. Sci.* **44**: 499-588.

Fahnenstiel, G.L., L. Sicko-Goad, D. Scavia and E.F. Stoermer, 1986. Importance of picoplankton in Lake Superior. *Can. J. Fish. Aguat. Sci.*, **43**: 235-240.

Glover, H.E., D.A. Phinney and C.S. Yentsch, 1985. Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations, in various water masses in the Gulf of Maine. *Biol. Oceanogr.*, **3**: 223-248.

Glover, H.E., L. Campbell and B.B. Prezelin, 1986. Contribution of Synechococcus spp. to size-fractioned primary productivity in three water masses in the Northwest Atlantic Ocean. *Mar. Biol.*, **91**: 193-203.

Hargraves, P.E., R.D. Vaillancourt and G.A. Jolly, 1990. Autotrophic picoplankton in Narragansett Bay and their interaction with microplnkton.: In Coastal and Estuarine Studies. edited by E.M. Cosper, V.M. Bricelj and E.J. Carpenter, Springer-Verlag, 23-38.

Holm-Hansen, O., C.J. Lorenzen, R.W. Holms and J.D.H. Strickland, 1965. Fluorometric determination of chlorophyll. *J. Cons. Perm. int. Explor. Mer.*, **30**: 3-15.

Johnson, P.W. and J. McN. Sieburth, 1979. Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. *Limnol. Oceanogr.*, **24**: 928-935.

Moon, C.H. and H.J. Choi, 1991. Studies on the environmental characteristics and phytoplankton community in the Nakdong River estuary. *J. Oceanol. Soc. Korea*, **26**: 144-154.

Prezelin, B.B., H.E. Glover and L. Campbell, 1987. Effect of light intensity and nutrient availability on diel patterns of cell metabolism and growth in population of *Synechococcus* spp. *Mar. Biol.*, **95**: 496-480.

Prezelin, B.B., M. Putt and H.E. Glover, 1986. Diurnal patterns in photosynthetic capacity and depth-dependent photosynthesis-irradiance in *Synechococcus* spp. and larger phytoplankton in three water masses in the Northwest Atlantic Ocean. *Mar. Biol.*, **91**: 205-217.

Platt, T., D.V. Subba Rao and B. Irwin, 1983. Photosynthesis of picoplankton in the oligotrophic ocean. *Nature* (Lond.), **301**: 702-704.

Sieburth, J. McN., V. Smetacek and J. Lenz, 1978. Pelagic ecosystem structure; heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limol. Oceanogr.*, **23**: 1256-1263.

Shim, J.H., H.G. Yeo and Y.K. Shin, 1991. Ecological effect of thermal effluent in the korean coastal waters: . Signigicance of autotrophic nano and picoplankton in the adjacent waters of Kori Nuclear Power Plant. *J. Oceanol. Soc. Korea*, **26**: 77-82.

Stockner, J.G. and N.J. Antia, 1986. Agal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. *Can. J. Fish. Aguat. Sci.*, **43**: 2472-2503.

Strickland, J.D.H. and T.R. Parsons, 1972. A practical handbook of sea water analysis, 2nd ed. *Bull. Fish. Res. Bd. Can.* 167, 310 pp.

Takahashi, M., K. Kikuchi and Y. Hara, 1985. Importance of picocyanobacteria biomass(unicellular, blue-green algae) in the phytoplankton population of the

coastal waters off Japan. *Mar. Biol.*, **89**: 63-69.

Takahashi, M. and T. Hori, 1984. Abundance of picophytoplankton in the subsurface chlorophyll maximum layer in subtropical and tropical waters. *Mar. Biol.*, **79**: 177-186.

Tracey, G. A., P. W. Johnson, R. W. Steele, P. E. Hargraves and J. McN. Sieburth, 1988. A shift in photosynthetic picoplankton composition and its effect on bivalve mollusc nutrition: The 1985 "Brown Tide" in Narragansett Bay, Rhode Island. *Journal of Shellfish Research*, 7: 671-675.

Tsuji, T. and T. Yanagita, 1981. Improved fluorescent microscopy for measuring the standing stock of phytoplankton including fragile components. *Mar. Biol.*, **64**: 207-211.

Waterbury, J. B., S. W. Watson, R. R. L. Guillard and L. E. Brand, 1979. Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. *Nature* (Lond.), **277**: 293-294.

Weisse, T., 1988. Dynamics of autotrophic picoplankton in Lake Constance. *J. Plankton Res.*, **10**: 1179-1188.

Wright, R. T., R. B. Coffin, C. P. Ersing and D. Pearson, 1982. Field and laboratory measurements of bivalve filteration of natural bacterioplankton. *Limnol. Oceanogr.*, **27**: 91-98.

Yentsch, C. S. and D. W. Menzel, 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-sea Res.*, **10**: 221-231.

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