GROWTH OF PHYTOPLANKTON SPECIES POPULATIONS AND ECOLOGICAL REGULATION OF COMMUNITY STRUCTURE

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General Introduction

Various characteristic phytoplankton communities occur successively in aquatic environments with the progress of eutrophication of waters. Phytoplankton communities also exhibit the periodical annual changes in the same body of water with some degree of predictability. This progressive change of phytoplankton communities, which is termed to be succession, is a major characteristic behavior of phytoplankton and it is central for the consideration of phytoplankton ecology. Another fascinating research field in community ecology is the species diversity of phytoplankton community. How can so many different algae exist in the same body of water in very similar ways? What are the ecological variables that control a process of succession or diversity in a given environment?

These subjects have attracted the interest of aquatic ecologists, and then many investigations have been done intensively. However, most work has been confined to static description of changes in species composition or numerical calculation of diversity index. Since any successional changes in community are expected to be controlled strongly by surrounding environmental conditions prevailing at any given time, investigators

have attempted traditionally to predict the correlation between a change in relative abundance of species in a community, namely species diversity, and various environmental variables. However, such static descriptive researches are insufficient in the understanding of the mechanisms by which different phytoplankton communities are produced.

The character of phytoplankton community structure in natural water will result from differences in the growth of each component species. Such differences are believed to be due to the balance of the potential growth rate of the phytoplankton populations under a given environment and the removal rates of phytoplankton biomass such as zooplankton grazing, sinking, mortality and so on concurrently occurring in the water.

In the present study the potential growth of individual phytoplankton population was particularly concerned. Two major environmental parameters, light intensity and limiting nutrients, for phytoplankton growth were under consideration. Growth responses of various natural phytoplankton population were investigated under different light intensities and nutrient regimes. The growth responses of phytoplankton were further analyzed on the basis of photosynthetic responses. The study was conducted under field

conditions employing various lakes and ponds at different trophic status. Laboratory experiments under defined environmental conditions were also applied on necessity. Based upon the results on phytoplankton growth responses to light and nutrients, main controlling mechanisms of phytoplankton species succession and diversity in the field were evaluated by using growth models.

Chapter 1

Phytoplankton diversity in inland waters of different trophic status

1.1 Introduction

Diversity is an important attribute of a natural community and it has been widely used to characterize community structure. From a ecological viewpoint, it has been studied in relation to ecosystem characteristics such as development (ODUM 1969; MARGALEF 1968), stability (SANDERS 1968), primary production (CONNELL and ORIAS 1964) and heterogeneity (MACARTHUR and MACARTHUR 1961, ABEL 1974). In aquatic environments, the interest of the researchers has been focussed especially on the relationship between the phytoplankton diversity and the eutrophication of the water body. MARGALEF (1964, 1968) applied the information theory to the analysis of species diversity in phytoplankton assemblages and postulated that species diversity will became low in eutrophic waters and high in oligotrophic waters. MOSS (1973) investigated the phytoplankton diversity in three different trophic waters and confirmed the proposition presented by MARGALEF (1968).

The data are, however, still insufficient to generalize about MARGALEF's theory, and more extensive investigations are necessary to verify the relationship between the phytoplankton diversity and the trophic status of water bodies.

The purpose of the present study was to estimate phytoplankton diversity in different water bodies located in various regions of Japan and to examine the relationship between phytoplankton diversity and the trophic status of these water bodies.

1.2 Materials and Methods

Field surveys were made in 24 waters during the summer in 1975 and 1976. Water samples were collected from the surface layers with a 3 liter Van Dorn sampler. Water samples for the determination of chlorophyll *a* as an index of phytoplankton biomass were filtered through glass fiber filters (Whatman GF/C) and the concentration of chlorophyll a was measured according to the SCOR-UNESCO method(1966). The phytoplankton cells in the water samples were preserved in Lugol's solution, counted, and identified. In samples in which phytoplankton density was low, cells were counted after concentrating them 10 times by centrifugation for 15 minutes at 3,000 r.p.m.

The phytoplankton diversity was calculated by the SHANNON-WEAVER function (1963),

$$H = - \sum \frac{N_i}{N} \log_2 \frac{N_i}{N}$$

where H is the diversity as bits per cell, N_i and N are the cell number of i-th genus and the total cell number in the water sample, respectively. The diversity has generally been expressed on species level but in the present study it was expressed on genus level because of the difficulty in identifying all algal species presented in the sample. The diversity was dependent to some extent on the size of the sample. Fig.1 shows a plot of diversity for different sample size at different population densities. In each water sample, the phytoplankton diversity increased with the increase in the number of cells counted and reached stable values at certain levels. The stable values of the diversity index were obtained by counting about 20 cells in oligotrophic water, 70 cells in slightly eutrophic water and 400 cells in hypereutrophic water. Based upon these relationships, the number of identified cells (N) was about 70, 100 and 1000 cells for the samples of oligotrophic, eutrophic and hypereutrophic waters, respectively.

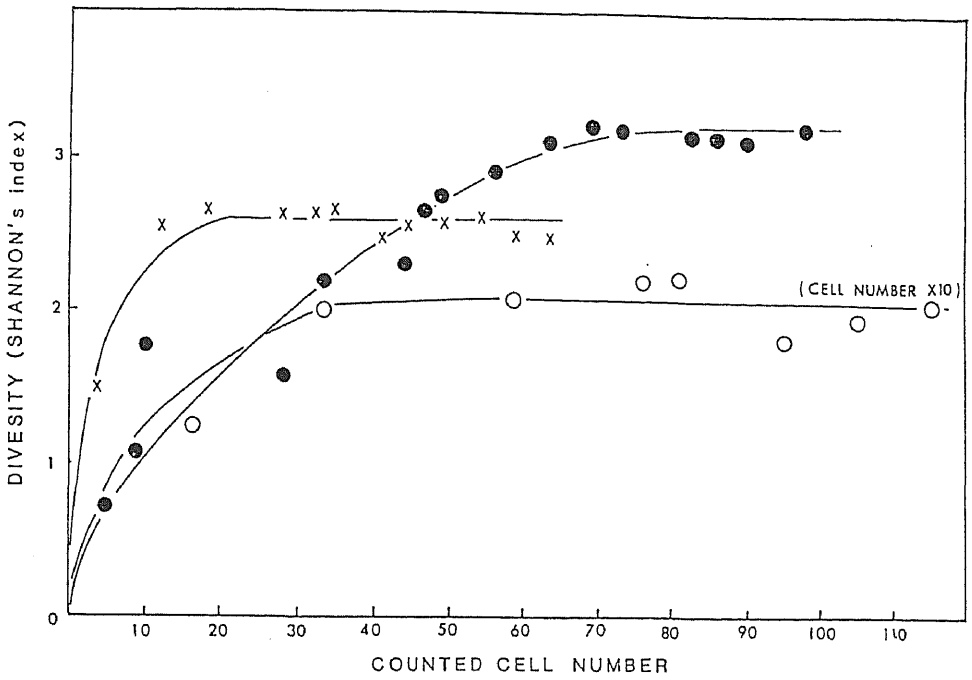

Fig. 1. Relationship between the SHANNON's index and sample size in oligotrophic water (x), slightly eutrophic water (●) and hypereutrophic water sample (○).

1.3 Results

(1) Phytoplankton diversity in relation to the trophic status of the water body

Fig.2 shows the relationship between the phytoplankton diversity and the trophic status of the water. Classification of the study sites was made by referring to the trophic lake types proposed by YOSHIMURA (1937), and the concentration of chlorophyll a was used as a measure of the trophic status.

Phytoplankton diversity was low in very oligotrophic waters; for example, it was less than 1 bit per cell in Lake Shikotsu, an extremely oligotrophic lake. It increased as trophic state moved to a mesotrophic condition, and high values, more than 3 bits per cell, were obtained in both mesotrophic and slightly eutrophic waters. However, the diversity was reduced in eutrophic waters, and the lowest values of 0.16 to 0.31 bits per cell were obtained in eutrophic and hypereutrophic waters.

The relation between the phytoplankton diversity and the trophic status is indicated by $Y = -0.39(\log X)^2 + 0.19(\log X) + 2.44$, where X is the concentration of

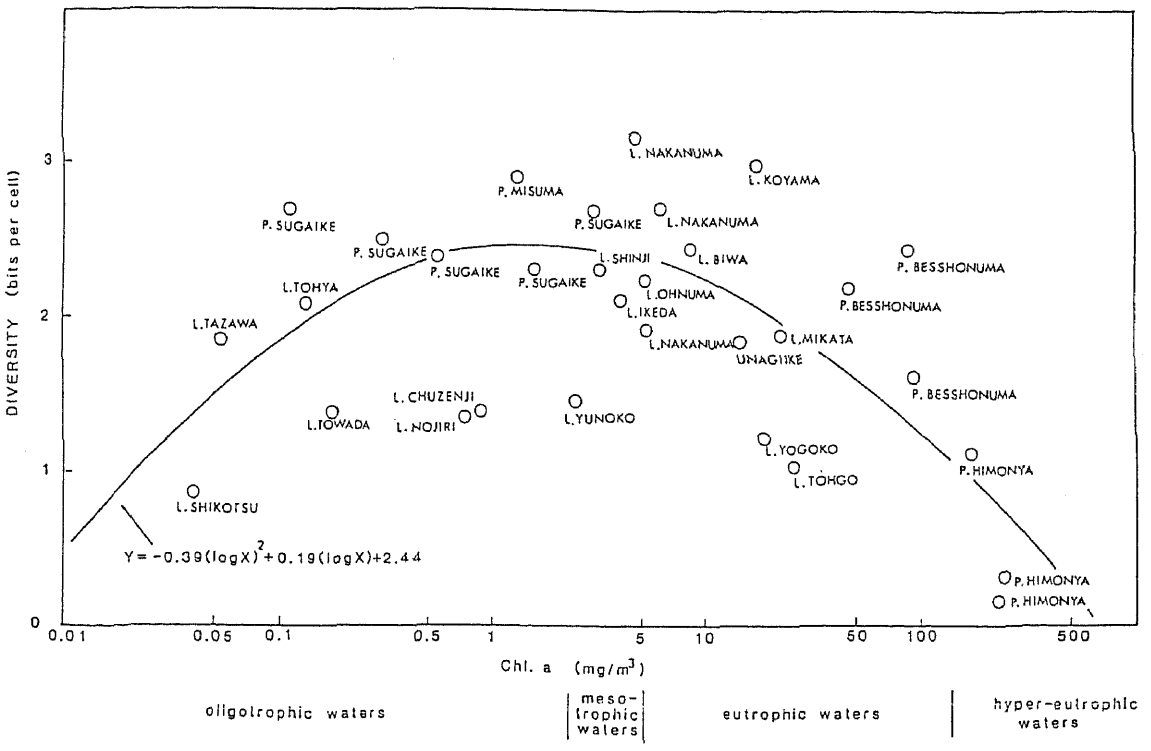

Fig. 2. Relationship between phytoplankton diversity and trophic status of waters.

chlorophyll a, Y is the phytoplankton diversity. The regression equation was determined by the least square method.

(2) Species composition affecting the phytoplankton diversity

The phytoplankton diversity is affected by the number of component phytoplankton taxa (richness) and the phytoplankton composition (equitability). Fig.3 shows the number of identified genera in each sample water. The richness was low (4 - 7 genera) in oligotrophic waters and high in eutrophic waters, but it decreased significantly in hypereutrophic waters. Although richness increased in highly eutrophic waters, the phytoplankton diversity decreased. The reduction of diversity may be caused by a decrease in evenness of equitability in water samples.

Fig.4 shows the number of cells of representative phytoplankters and their share in the total number of cells in the water samples.

Oligotrophic waters. The predominant phytoplankters were diatoms. Cyclotella ranged from 10 cells/ml to 10^2 cells/ml and accounted for about 50% of total cell number. It accounted for 80% in Lake Shikotsu and Lake

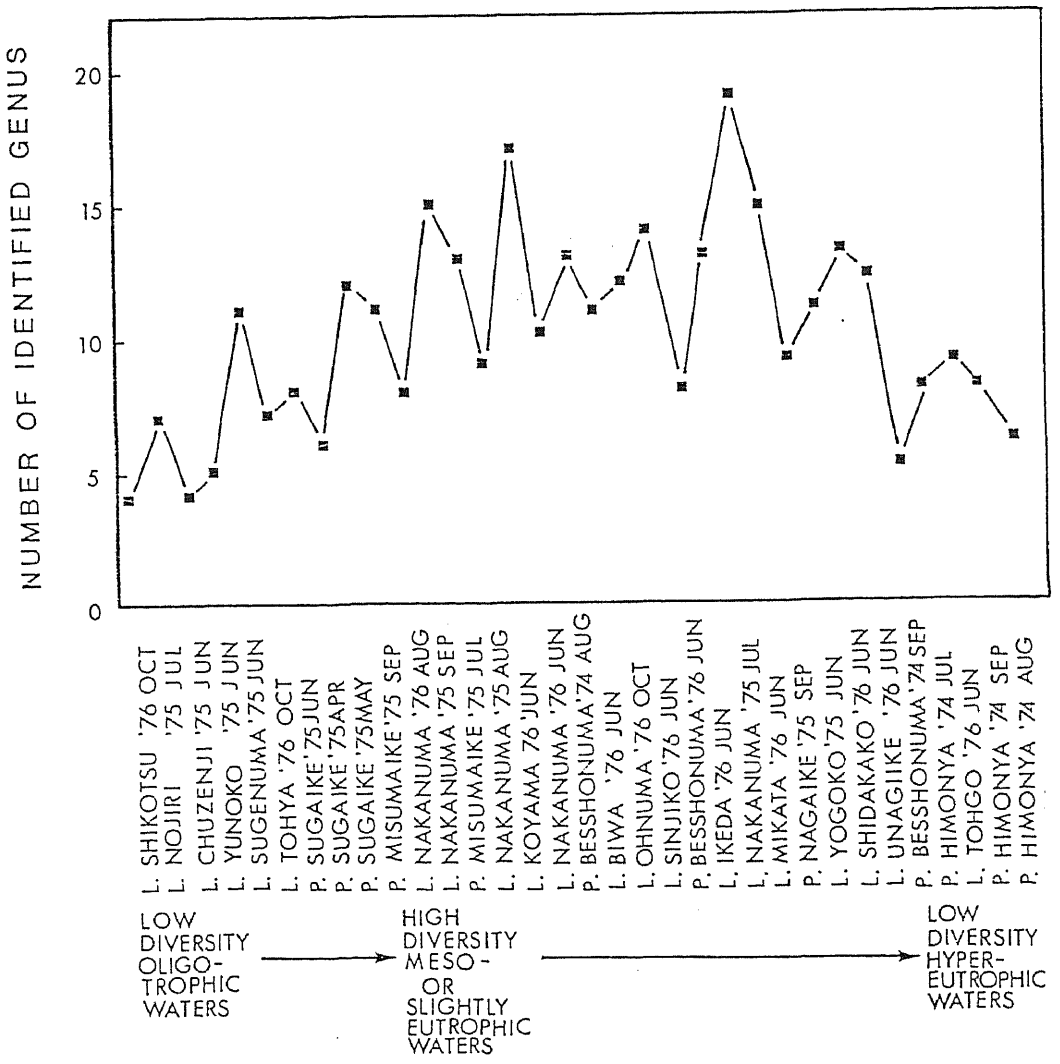

Fig. 3. The number of identified genus in each sample of water.

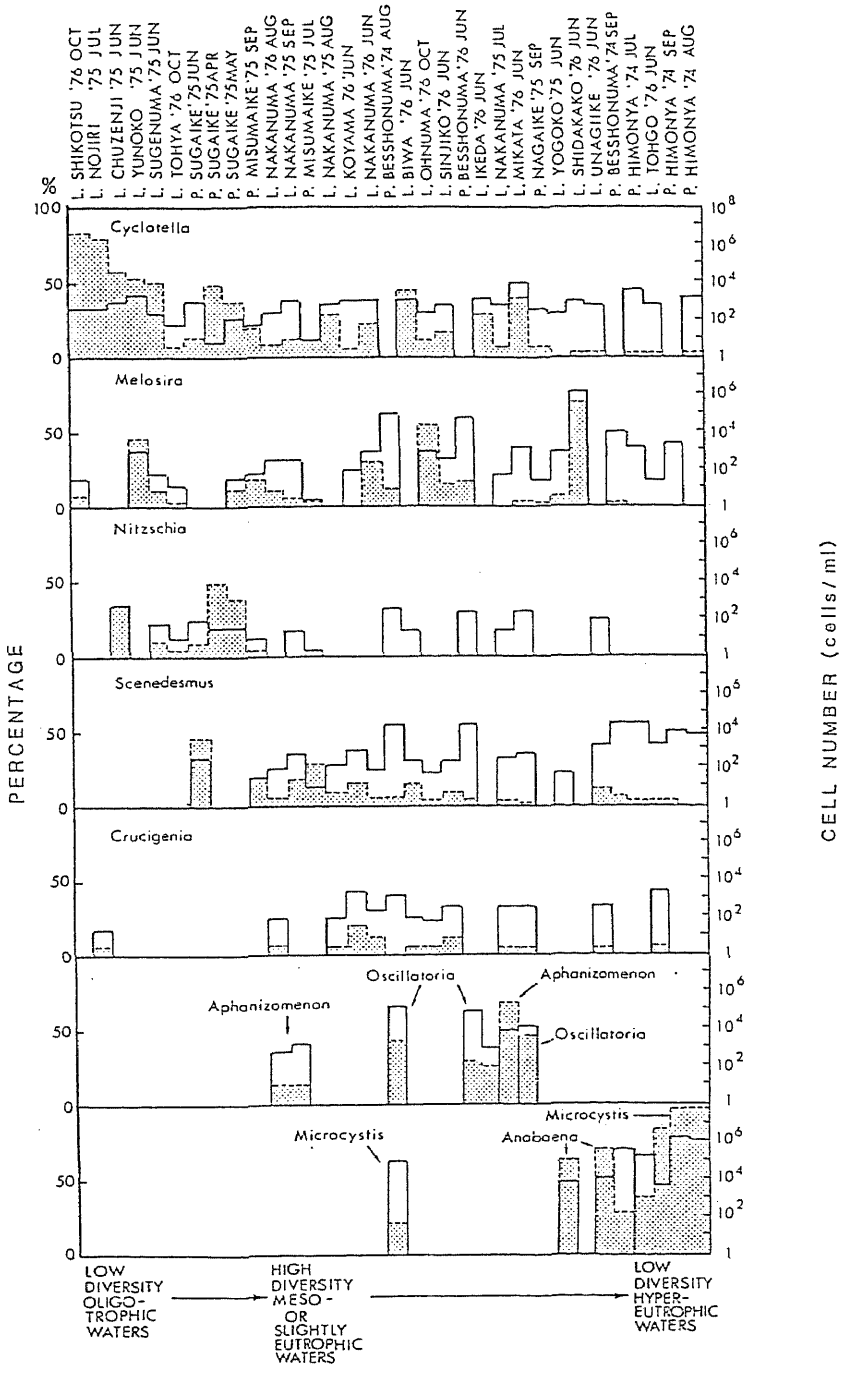

Fig. 4. The number of repesentative phyto-plankters(solid line) and their share in total number of cells(shaded place) in each sample of waters.

Nojiri. Nitzcshia and Melosira also prevailed in some oligotrophic waters. Their cell density was 10 to 10^3 cells/ml and they comprised 5 to 50% of the total cell number.

Meso- and slightly eutrophic waters. No one phytoplankton species formed a large population. The population of diatoms was almost the same size as that in the oligotrophic waters but the share of each diatom species in the phytoplankton assemblages was low, because a large amount of other phytoplankters such as planktonic green algae and blue-green algae always accompanied them. Scenedesmsus and Crucigenia ranged from 10^2 to 10^3 cells/ml and they comprised 5 to 20% of the total cell number. The blue-green alga, Aphanizomenon, was abundant sometimes and occuppied about 10% of the total cell number.

Eutrophic and hypereutrophic waters. Blue-green algae were most abundant in these waters. In particular, Microcystis formed a dense population and its share was more than 95% of the total cell number. Anabaena, Aphanizomenon and Oscillatoria also appeared frequently in some waters and their cell number ranged from 10^4 to 10^5 cells/ml and the share was 20 to 70%. Diatoms such as Cyclotella, Melosira and green algae such as Scenedesmus were insiginificant in eutrophic and

hypereutrophic waters but it should be noted that both diatoms and green algae were not excluded by blue-green algae. The cell numbers of diatoms and green algae were comparable to or in some cases larger than those in oligotrophic or mesotrophic waters.

The diversity and its relation to trophic status of waters can be summarized as follows. In oligotrophic waters, the phytoplankton assemblage consisted mostly of Cyclotella and this resulted in the low diversity that was observed. The absence of predominant species is the cause of the high diversity in meso- and slightly eutrophic waters. In eutrophic and hypereutrophic waters, the phytoplankton assemblage consisted of primarily blue-green algae such as Microcystis which lead to a low diversity.

1.4 Discussion

The results obtained in the present study coincided fairly well with MARGALEF's proposition with regard to the decrease of phytoplankton diversity in eutrophic waters, but differed somewhat in oligotrophic waters where the diversity was rather low compared with meso-trophic and slightly eutrohic waters.

In regard to the causal relation between the

phytoplankton diversity and the trophic status of waters, several explanations have been presented. MARGALEF (1968) emphasized that the high diversity in oligotrophic lakes and the low diversity in eutrophic lakes were due to the difference in the amount of information accumulated in the lakes. MOSS (1973) explained the relation between the phytoplankton diversity and the trophic status of waters by the chance of overlapping of each successional population in the water body. However, the low diversity obtained in the present study can not be elucidated by these hypotheses. These findings may be explained by the combination of nutrient conditions and the specific ability of phytoplankton species to take up the limiting nutrient. It is suggested that there are only a few phytoplankters which can adapt to a nutrient poor environment through their low half-saturation constants for nutrient uptake. This may explain the decrease in phytoplankton diversity in oligotrophic waters. No phytoplankters formed a remarkably large population in meso- and slightly eutrophic waters. Probably, the nutrients in such waters are not high enough to produce a phytoplankton community that consists of a few phytoplankton species. As stated by TILMAN (1977), characteristic nutrient utilization of different species, each of which is limited by a

different nutrient, may also reduce competition between species, and therefore many species may coexist. Thus the increase in richness and the evenness in equitability should lead to high diversity in meso- and slightly eutrophic lakes. In eutrophic and hypereutrophic waters, where high nutrients are being supplied continuously, the high level of nutrient and high productivity would support the coexistence of many phytoplankton species and result in high diversity. It, however, happened in nature that there were comparatively few species and the diversity was very low in highly eutrophic waters. This is probably due to a few species of phytoplankters with physiological characteristics such as high nutrient uptake ability, photosynthesis or buoyancy which allow them to form dominant populations.

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Chapter 2

Application of the grain density autoradiography to the study of phytoplankton production

2.1 Introduction

As a useful tool for the determination of photosynthetic activity of individual species in phytolankton assemblages, the grain density autoradiography has been employed by several investigators (WATT 1971, STULL et al. 1973, GUTELMACHER 1975), although BROCK and BROCK (1968) argued that this technique was not suitable for the quantitative study. KNOECHEL and KALFF (1976a, b) emphasized the serious errors that arised from each step in the processes of grain density autoradiography, and recommended an alternative technique, track autoradiography, for the determination of individual species productivity. They pursued the population dynamics of freshwater phytoplankton diatom species by the track autoradiography (KNOECHEL and KALFF 1978). PEAL and STULL (1979) found a higher degree of correlation between the techniques of grain density and track autoradiograhy in the study of a natural phytoplankton

community and claimed the validity of the grain density autoradiography.

As has been criticized by KNOECHEL and KALFF (1976a), the grain density autoradiography is simple technique but its reliability has not been proved sufficiently. The purpose of the present study is to check potential sources of error cited by KNOECHEL and KALFF (1976) in the grain density autoradiography and scrutinize the utility of this technique for the study of species dynamics of phytoplankton community.

2.2 Materials and Methods

Unialgal cultures of Cyclotella, Scenedesmus and Selenastrum were used for the present study. Each 100ml of algal suspension was taken into a 200ml Ehlenmeyer flask and 0.3 ml $NaH^{14}CO_3$ solution having an activity of 10 μCi/ml was injected into each flask. Flasks were then incubated in a water bath under illumination of 30 klux by white light fluorescent lamps at 20°C. Dark flasks were also prepared as controls. A series of samples were removed from flasks with pipettes at various time intervals over 4 hrs. Each 5 ml of samples was filtered through a 24-mm HA Millipore filter by gentle suction, and then the filter was washed with

small volume of distilled water and fumed in HCl vapor. Total radioactivity of ^{14}C incorporated into algal cells was measured by a liquid scintillation counter (Beckman LS 8100). Cell numbers counted with a Thoma's haemacytometer before and after the incubation were averaged and the mean cell number was used. Mean radioactivity per cell was calculated by dividing total radioactivity by cell number in the sample. For grain density autoradiographs each 2 ml of remaining samples was immediately filtered through a 24-mm HA Millipore filter by gentle suction. In the present experiment no fixative was applied to avoid possible chemography and destruction of cells. Each filter paper was attached to a microscope slide with 1% gelatin solution. The slide was then dried at 35°C for 24 hours in an oven and cleared with acetone vapor. SAKURA NR-M2 nuclear emulsion melted at 37°C in a water bath in the dark was poured into a small container for slide dipping. Under the safety light, each slide was dipped in the emulsion for a few seconds, withdrawn and dried in a vertical setting for one hour under a small fan. After drying, the slides were placed in a black plastic slide box containing dried silica gel. The boxes were stored at 4°C in a refrigerator during exposure of 2-10 days. Exposed slides were developed with developer (Konidol X

Super) in a staining vessel with a standard vertical holder made of stainless steel. Development time varied from 3 to 24 minutes. Following development, the slides were transferred to acetic acid stop bath for one minute, fixed in acid hardening fixer (Konifix) for 10 minutes, and washed gently in running tap water for 30 minutes. During the process samples were treated in a water-bath incubator at 20° C. Autoradiographs prepared in this way were photographed at 780 magnification by using a microscope (Nikon Optiphoto), and then grains on the photographs were counted. Background counts were estimated from slides prepared for dark bottle samples.

2.3 Results

(1) Determination of grain counting area

KNOECHEL and KALFF (1976a) argued that the number of produced grains and their spatial distribution are remarkably affected by cell size and shape, even if the absolute radioactivity is identical. They proposed to count all grains within a certain distance from a cell, the half-distance, in which more than a half of produced silver grains were located (SALPETER et al. 1969), and recommended distance of 10 μm for getting

enough counts.

At the first step of this study, therefore, the distribution of grains around a cell was examined to delineate the area to be counted. Slides used for this experiment were exposed for 2 days and developed for 6 minutes. Radioactivity incorporated into cells was 9.23×10^{-2} dpm/cell in a Cyclotella sample and 8.05×10^{-2} dpm/cell in a Scenedesmus sample. Grain were counted on microscopic photographs. As shown in Fig. 1, grains counted were mostly distributed within 10 μm from the cell margin. The number of grains within 10 μm from the margin was 82.3 in the Cyclotella sample and 68.3 in the Scenedesmus sample. The grain density, in which grains counted were normalized by a unit area and expressed as counts/100 μm^2, showed the highest value over the cell and decreased with increase of distance from the cell. The grain density became reduced to background level of 1.0 grain/100 μm^2 between 30-40 μm from the cell in Cyclotella and 1.5 grains/100 μm^2 between 10-20 um in Scenedesmus. This distribution pattern was also the same in the other samples exposed for longer time than 2 days.

It is generally noticed that the maximum path-length of a beta particle which originates from a point source in nuclear emulsion is about 100 μm for ^{14}C. However,

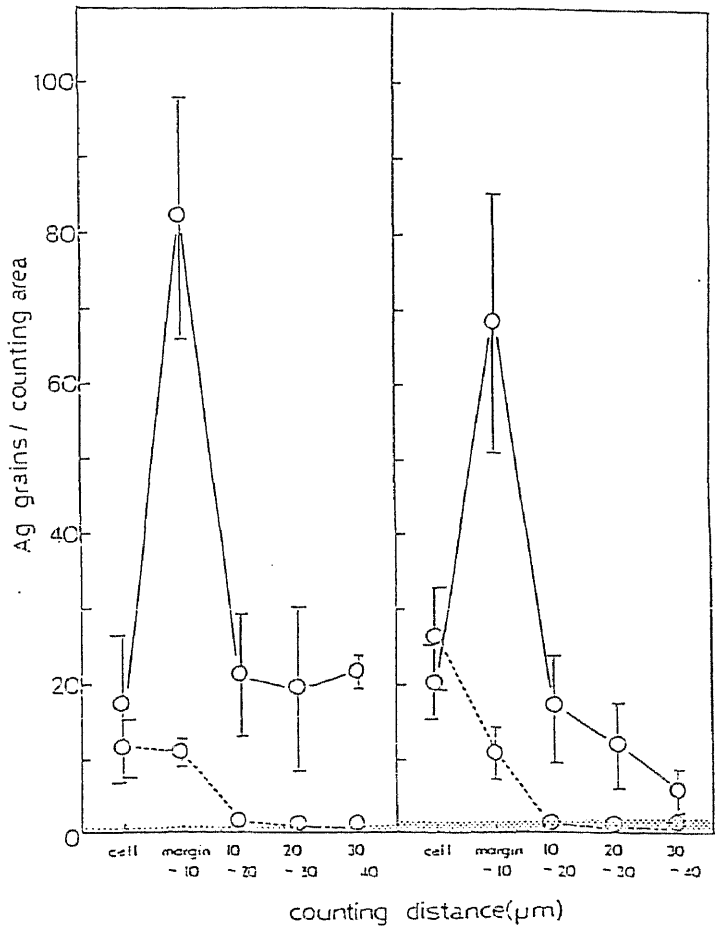

Fig. 1. The distribution of grains over the cell and surrounding area in Cyclotella (left) and Scenedesmus (right). Solid line, grain counts in each band area; dashed line, grain counts/100 μm^2. Shaded place represents background (background grain counts/100 μm^2). Vertical line indicates 95% confidence limits.

the grains outside the distance of 40 μm from the cell were so small that they were negligible in the present counting. Therfore, grains lying over the cell and within of 40 μm from the cell were all counted in the present study.

(2) Determination of exposure time

A long exposure time was required to develop all latent images, but excess background count due to the prolonged development has led to the difficulty of counting the grains. Fig. 2 shows the relationship between the development time and the grain count for two samples of Cyclotella. The radioactivity was 19.8 x 10^{-2} dpm/cell in one sample and 32.7 x 10^{-2}dpm/cell in another sample. Grain counts increased with development time in both samples and leveled off after 6 minutes. Grain counts at the plateau were 383.8 grains/cell for the high radioactive sample and 264.3 grains/cell for the low radioactive sample. After 12 minutes grain counts increased and reached the higher level of 693.2 grains/cell for the former sample and 477.4 grains/cell for the latter sample. Background count remained at very low level (6.4 count/cell) during the first 6 minutes, and thereafter it increased rapidly. From

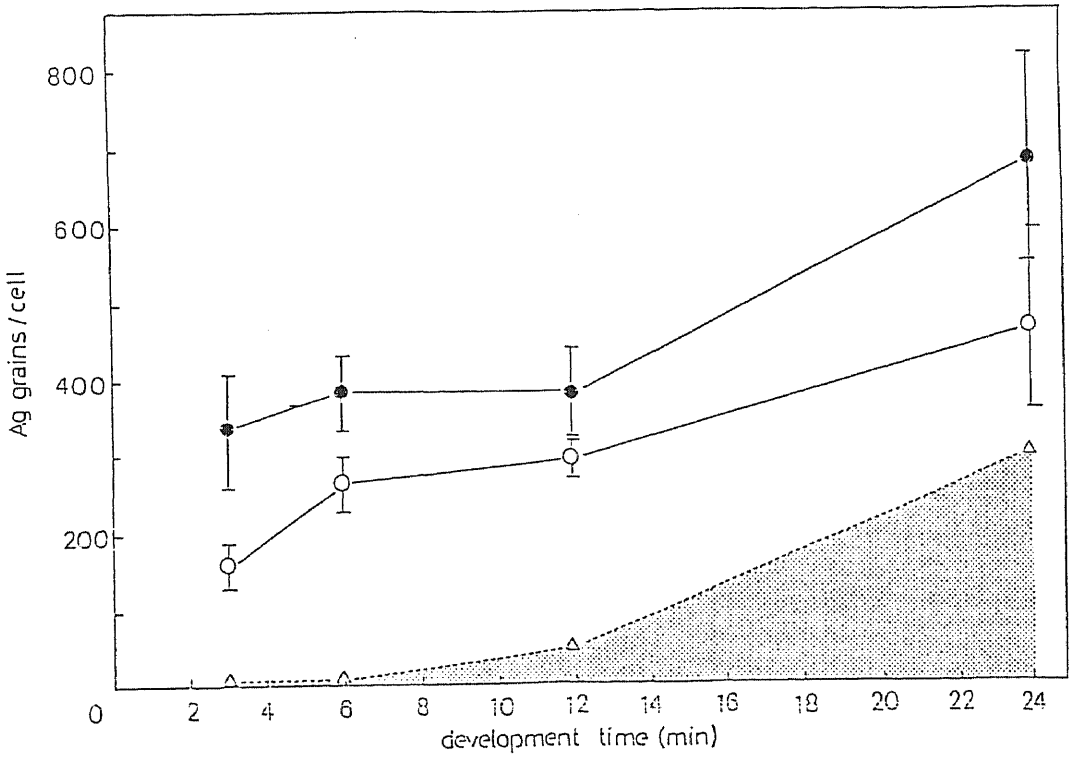

Fig. 2. Relationship between development time (min) and grain counts per cell in Cyclotella; 19.8×10^{-2} dpm /cell (○), 32.7×10^{-2} dpm/cell (●). Shaded area represents background counts. Vertical line indicates 95% confidence limits.

these results, it is inferred that the development time should not exceed 12 minutes. We adopted 6 minutes as development time because of the following two reasons; grain count reaches plateau by this time, and background count is negligible.

In micro-autoradiography, silver grains had been expected to increase linearly with exposure time. However, prolonged exposure has resulted in the underestimation of grains due to possible latent image erasure of interference of excessively produced grains (ROGERS 1967). Fig. 3 shows the relationship between the exposure time and the grain counts in a Cyclotella sample with radioactivity of 9.23×10^{-2} dpm/cell. Grain count increased linearly with exposure time during the first 7 days, and subsequently it decreased gradually. Grain density larger than 450 counts/cell made impossible to estimate grain numbers precisely, because of overlapping of developed grains. Thus less than 7 days were adopted for the exposure time in the present study, and photographs having grains less than 450 counts/cell were only applied for the estimation.

(3) Conversion of grain count to photosynthetic rate

Photosynthetic activity of individual cells in the

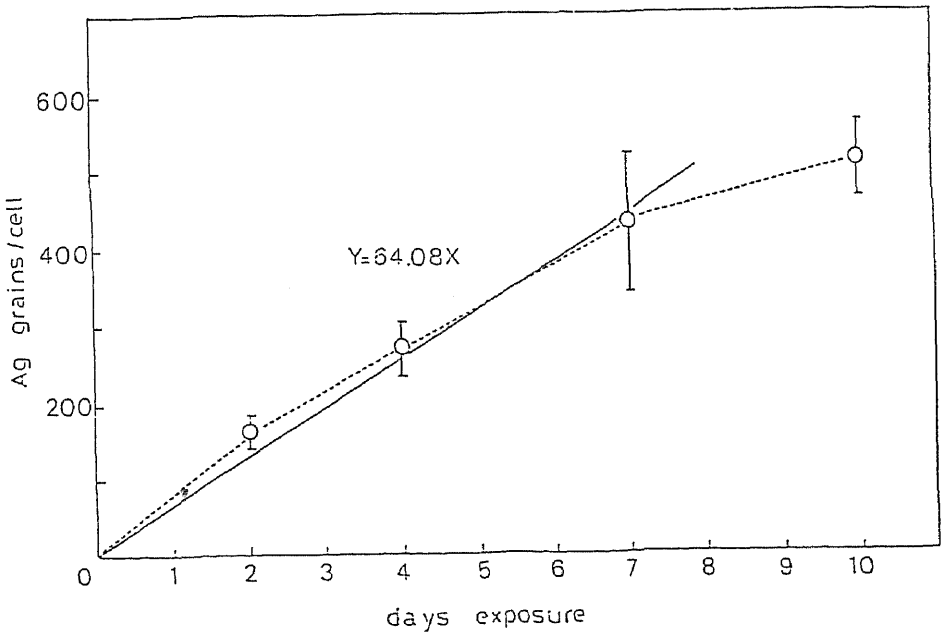

Fig. 3. Relationship between exposure time (days) and grain counts per cell in Cyclotella (9.23 x 10^{-2} dpm/cell). Vertical line indicates 95% confidence limits.

grain density autoradiography has been calculated from the apportionment of the total radioactivity to grains produced by each cell (WATT 1971, STULL et al. 1973, GUTELMACHER 1975). When phytoplankton assemblages consist of delicate species and nanoplankton and they are lost during the processes, this approach leads to an overestimation of the photosynthetic activity of the remaining species (KNOECHEL and KALFF 1976a). Thus, it is necessary to convert grain count directly to absolute disintegration rate (dpm). KNOECHEL and KALFF (1976a) stated that it would be desirable to convert grain count into an absolute disintegration rate through an internal standardization procedure. This conversion is made by the addition of specimens with a known activity to the sample. However, another alternative method was examined in the present study because we had no suitable reference source. Fig. 4 shows the interrelationship between radioactivity/cell and grain counts/cell. A good correlation was found between both parameters ($Y=587.06X+0.78$, $r=0.95$), and thereby the produced grains will be strictly corresponded to radioactivity incorporated into the cell. It would be possible to convert the grain counts to absolute disintegration rate from the above regression equation.

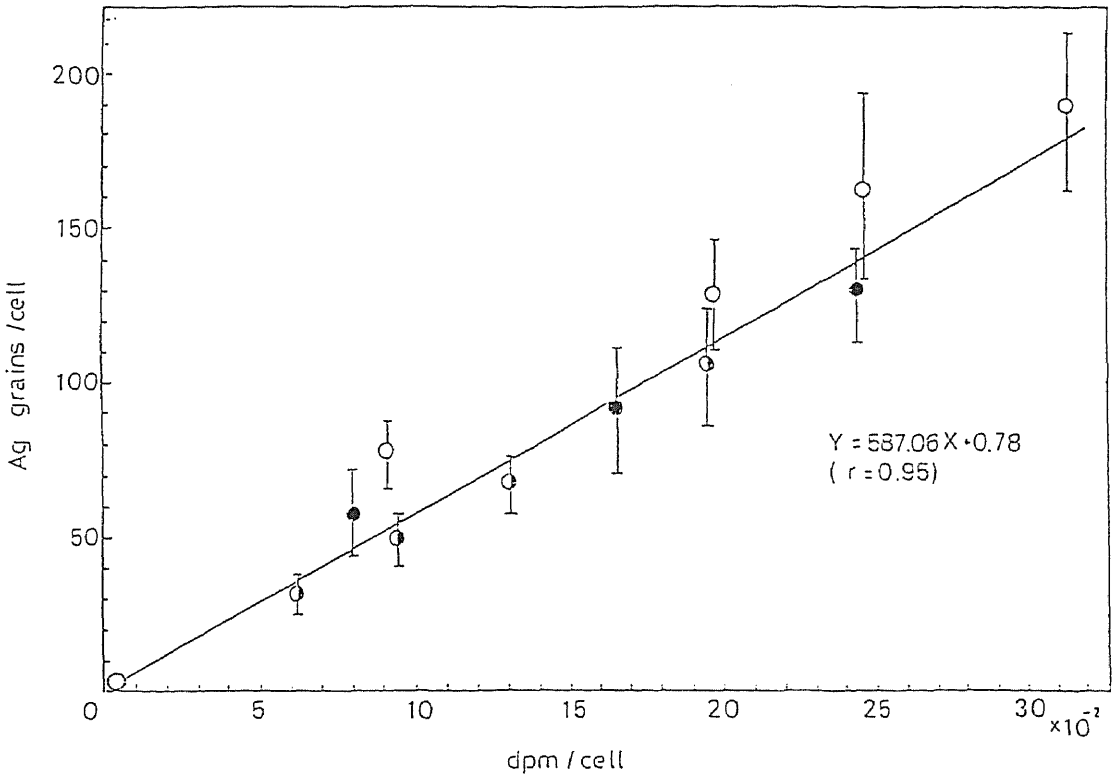

Fig. 4. Relationship between diel radioactivity (dpm/cell) and diel grain counts/cell in three different algal species; Cyclotella (○), Scenedesmus (●) and Selenastrum (◑). Vertical line indicates 95% confidence limits.

2.4 Discussion

KNOECHEL and KALFF (1976a, 1979) criticized the grain density autoradiography and emphasized inherent sources of error in this method. However, most of sources of error may be cancelled by suitable processes. Careful preparation of samples and proper selection of fixatives are necessary in the grain density autoradiography as well as track autoradiography. Latent image erasure is also a serious problem as cited by KNOECHEL and KALFF (1976a). Considerable attention must be payed on the chemography caused by fixatives and the effect of cell size and shape. Chemography was simply corrected by using dark bottle control, and the intensive interference of the grain count by chemography is reduced by the present procedure. If the samples are carefully treated, the effect of chemography is probably eliminated. This is evident from a low level of background counts in Fig. 1. The effect of cell size and shape can be cancelled by counting all grains within 40 μm from the cell.

It is difficult to convert the grain counts to absolute radioactivity. We calculated the absolute radioactivity from the linear relationship between radioactivity incorporated into the cell and the grains

produced. ROGERS (1967) has shown that this approach is suitable only for approximate estimation of absolute radioactivity. In this case, the inaccuracy may arise from the cross-fire effect of sources packed closely in tissue section. Since an algal cell is considered to be a single source, the cross-fire effect is probably excluded in phytoplankton samples. A regression equation presented in the present study would be useful for the conversion of grain counts to absolute radioactivity of a cell. The grain density autoradiography can be a useful tool for the measurement of photosynthetic activity of individual phytoplankton species if we treat samples carefully.

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Chapter 3

Photosynthesis characteristics and productivity of individual species in natural phytoplankton community

3.1 Introduction

Since phytoplankton growth is maintained fundamentally by photosynthetic organic matter production, it is the cornerstone in studies of phytoplankton population dynamics to make clear the photosynthetic characteristics of individual algal species. Photosynthesis measurements in aquatic environment were given a large impetus with the introduction of ^{14}C method by STEEMANN NIELSEN (1952). At present, there have been many researches on the community photosynthesis but little information is available for understanding of the photosynthetic character of individual species in natural phytoplankton community. This is mainly due to the lack of adequate technique for measurement of photosynthesis of individual species. Grain density autoradiography has been employed by several investigators to measure the photosynthesis of individual algal speceis in phytoplankton community.

However, this technique was criticized severely by BROCK and BROCK (1968) and KNOECHEL and KALFF (1976 a) because of several inherent sources of errors in the autoradiographic processes. In Chapter 2, we examined the sources of errors which were cited by KNOCHEL and KALFF(1976b). Checks were made on the following major points by use of three different unialgal cultures; development time, chemography, latent image erasure and geometrical effect, and it was concluded that major errors could be eliminated if the autoradiographic treatments were made carefully. In addition, a new method was presented for converting the grain count to absolute disintegration rate.

The present study was designed to test the possibility of using an impoved grain density autoradiography for measurements of photosynthesis of the component algal species in natural phytoplankton populations and to clarify the photosynthetic character of individual phytoplankton species growing under natural conditions.

3.2 Materials and Methods

Lake Nakanuma, a small eutrophic lake, is located in Ibaraki Prefecture 50 km northeast of Tokyo. The lake has a surface area of 1.2 ha with a maximum depth of

13.5 m and is protected from wind by low hills around it. The water is strongly stratified during the summer stagnation period from July to September, and a well developed thermocline is found between the depths of 6 and 8 m. A characteristic phytoplankton community dominated by blue-green algae has been observed at the depth near or below the thermocline. Such an almost monospecific population is considered to be usable as material for the verification of the applicability of an improved technique to the complicated natural phytoplankton community.

Water sample was collected with a pump system from various depths. The sample was transferred to 5 liter polyethylene bottles and immediately brought back to the RI experimental laboratory of the University of Tsukuba located at 30 km from the lake. Each 48 ml of sample water was taken into 50 ml BOD bottle, and 0.2 ml of $NaH^{14}CO_3$ having an activity of 10 μCi/ml was injected into the sample water. The samples were incubated in a water bath under various light intensities at in situ temperature. Dark bottles were also prepared as controls. After a 2 hr incubation, each 2 ml of sample was filtered gently through a 24-mm HA Millipore filter and the filter was used for grain density autoradiography. The samples for autoradiography was

prepared by the method described in Chapter 2. The slides for autoradiogram were exposed for 7 days.

Photosynthetic activity of each cell was calculated by the following equation,

$$P = \Sigma CO_2 \times \frac{\mu Ci}{\mu Ci^* \times t}$$

where P is the photosynthetic rate per cell of the given species (gC/cell/h); ΣCO_2, the available total inorganic carbon in the sample; μCi^*, the added radioactivity; μCi, the mean radioactivity of ^{14}C incorporated into individual cells of the given species; and t, the incubation time. The amount ofΣCO_2 was measured with an infrared gas analyzer(Maihak,UNOR 2).

Photosynthetic rate of individual cells is usually calculated from the apportionment of total radioactivity to grains produced in each cell. However, this approach leads to the overestimation of activity when phytoplankton community consists mainly of delicate phytoplankters or small ones. In the present study, the grain counts per cell (Y) were converted to absolute radioactivity (X, dpm/cell) by using the regression equation, $Y = 587.06X + 0.78$, which was given in Chapter 2. Total photosynthesis in the sample was deduced by summing up the amount of photosynthesis in each of the

given species population (PxN), where N is the number of total cells of the given species in the sample. Total radioactivity was also measured by a liquid scintillation counter (Beckhman LS 8100) after filtering the remaining sample in the bottle through 24-mm HA Millipore filters.

3.3 Results

(1) Physico-chemical conditions and phytoplankton community in Lake Nakanuma.

Thermocline developed from 4 to 8 m and light penetrated to the bottom of the metalimnetic layer. The concentration of NH_4-N in the epilimnion ranged from 0.2 to 1.4 µg-at N/l, and rapidly increased with depth and reached 54.2 µg-at N/l at 11 m. The concentration of PO_4-P was 0.06 µg-at P/l throughout whole water column. Cyclotella sp. and Scenedesmus quadricauda predominated in the epilimnion, whereas Oscillatoria mougeotii prevailed in the metalimnion.

The algal species found in the sample taken from 8 m on September 7 are listed in Table 1. Phytoplankton community was represented by blue-green algae, mainly Oscillatoria mougeotii, the cell number of which, 10750

Table 1. Cell numbers and percentages of cell number of algal species of a phytoplankton community collected from the depth of 8 m in Lake Nakanuma on September 7, 1979.

	cell number (/ml)	percentage of cell number (%)
Green algae		
Ulothrix sp.	470	2.0
Pediastrum duplex	70	0.3
Pediastrum tetras	40	0.2
Ankistrodesmus falcatus	450	1.9
Scenedesmus quadricauda	1450	6.1
Scenedesmus bijuga	610	2.5
Tetraedron minium	840	3.5
Crucigenia quadrata	70	0.3
Crucigenia rectangularis	1450	6.1
Crucigenia tetrapedia	190	0.8
Tetrastrum heterocanthum	190	0.8
Staurastrum sp.	50	0.2
Cosmarium sp.	30	0.2
Diatoms		
Cyclotella kützingiana	20	0.1
Cyclotella sp.	120	0.5
Synedra acus	50	0.2
Blue-green algae		
Chroococcus sp.	400	1.7
Coelosphaerium naegelianum	740	3.1
Oscillatoria mougeotii	10750	44.9
Aphanizomenon flos-aquae	150	0.6
Flagellates		
Phacus sp.	10	0.04
Cryptomonas sp.	1380	5.8
Gonyostomum sp.	120	0.5
Peridinium sp.	10	0.04
Trachelomonas sp.	5	0.02
Unidentified		
small flagellates	3840	16.1
small spherical algal colony	420	1.8

cells/ml, accounted for 50% of the total phytoplankton cells in the sample. Three other blue-green algae, Coelosphaerium naegelianum, Chroococcus sp. and Aphanizomenon flos-aquae, were also present, but their contribution was 3.1, 1.7 and 0.6% of the total phytoplankton cells, respectively. Subdominant algal groups were the flagellate Cryptomonas sp. and unidentified small flagellates. The former occupied 5.8% of the total phytoplankton cells and the latter 16%. More than 10 species of green algae were found, and in particular, two species, Scenedesmus quadricauda and Crucigenia rectangularis, showed a relatively high density of 1450 cells/ml. Three species of diatoms were present but their cell density was less than 0.5% of the total phytoplankton cells.

(2) Photosynthesis-light curve of dominant phytoplankton

As a useful indicator of photosynthetic characteristics the so-called P vs I curve has frequently been used by aquatic ecologists (STEEMANN NIELSEN and HANSEN 1959; TALLING 1957; ICHIMURA 1960) and the shape of the curve is also a convenient indicator for physiological state of phytoplankton. The P vs I curve is characterized by four parameters,

the initial slope (α) of the curve, the maximum photosynthetic rate (Pmax), the light intensity (Ik) at which the initial slope and Pmax intersect and the light intensity (Iopt) at which saturation occurs (TALLING 1957). In the present study, photosynthetic nature of individual species was visualized by use of the P vs I curve. The P vs I curves for dominant species taken from the surface layer are shown in Figs. 1, 2 and 3, and parameters α, Pmax, Ik and Iopt are summarized in Table 2.

During the summer stagnation period, the P vs I curves of Melosira varians showed a sun-type with a light saturation at 0.048 - 0.090 ly·min^{-1}. The initial slope of Melosira varians was gentle (2.3 X 10^{-14} - 4.3 X 10^{-14} gC/μm^3/h/ly·min^{-1}). During mid summer, Cyclotella sp. also exhibited a sun-type photosynthesis. Light saturation occurred at 0.028-0.080 ly·min^{-1} and Ik value ranged from 0.030 - 0.038 ly·min^{-1}. The initial slope of Cyclotella sp. was moderate (3.9 x 10^{-14} - 5.8 x 10^{-14} gC/μm^3/h/ly·min^{-1}).

Similar photosynthetic responses to light intensity were observed in the curves of Synedra acus and Tetraedron minimum. The Pmax of Tetraedron minimum was remarkably high. Crucigenia rectangularis have low light saturation, a low Ik value and a steep initial

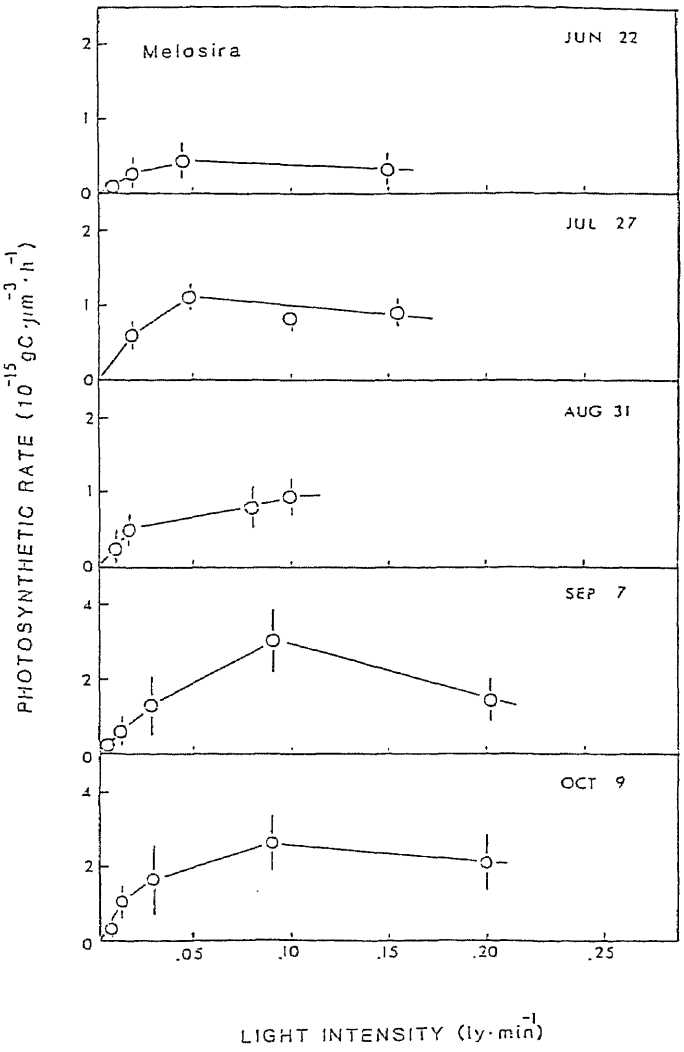

Fig.1. Photosynthesis-light curves of Melosira varians taken from the surface layer during the summer stagnation period.

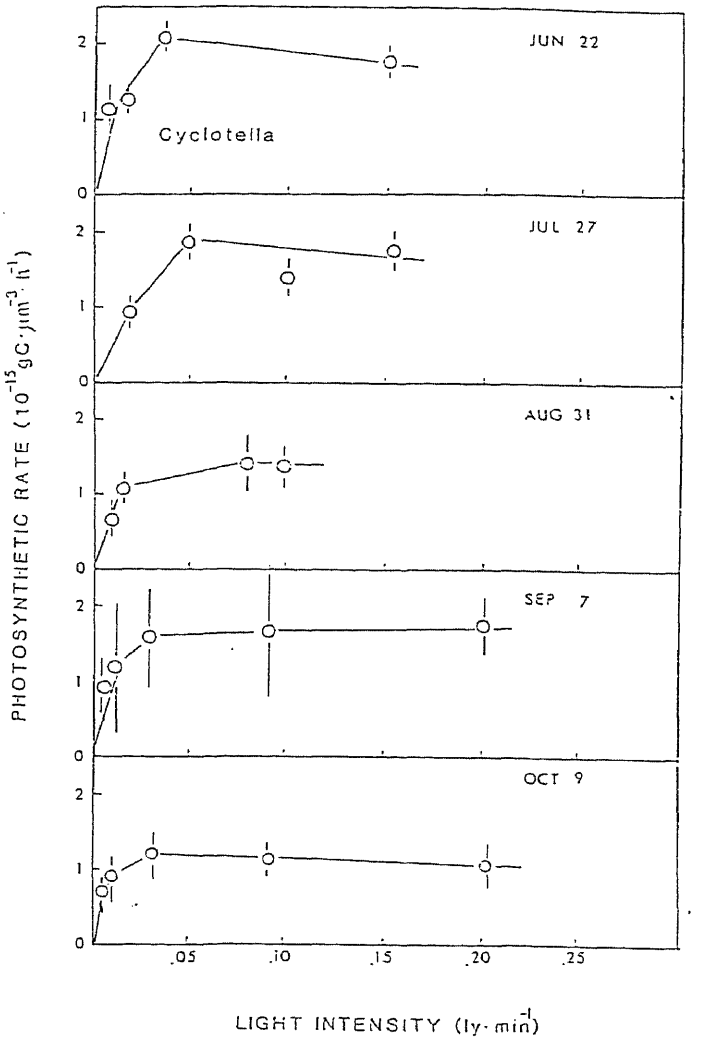

Fig. 2. Photosynthesis-light curves of Cyclotella sp. taken from the surface layer during the summer stagnation period.

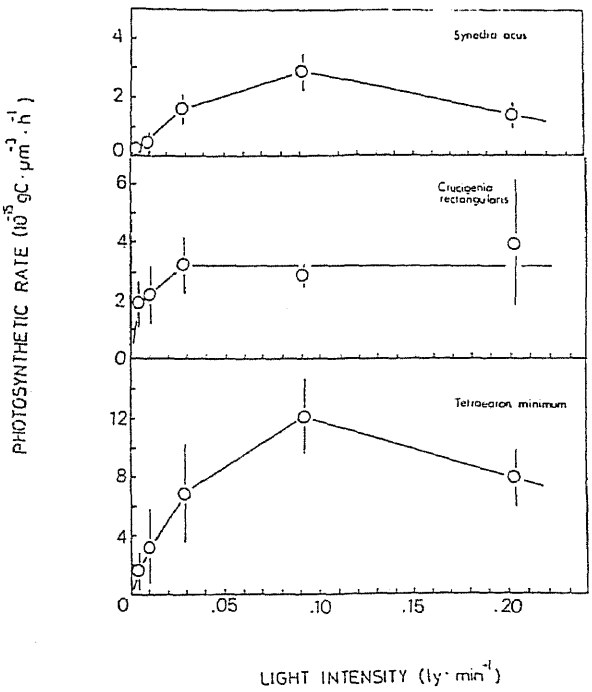

Fig. 3. Photosynthesis-light curves of three phytoplankton species taken from the surface layer during the summer stagnation period.

Table 2. Photosynthetic parameters of five freshwater phytoplankton species.

	light saturation ($ly \cdot min^{-1}$)	Ik ($ly \cdot min^{-1}$)	initial slope(α) (10^{-14} gC/um /h /$ly \cdot min^{-1}$)	Pmax (10^{-15} gC/ /μm^3/h)
Melosira varians				
JUL 27	0.048	0.034	2.3	1.1
AUG 31	0.090	0.036	2.8	0.9
SEP 7	0.090	0.072	4.3	3.1
Cyclotella sp.				
JUL 27	0.048	0.038	3.9	1.9
AUG 31	0.080	0.030	5.8	1.5
SEP 7	0.028	0.020	5.7	1.6
Synedra acus				
SEP 7	0.090	0.048	5.5	2.5
Tetraedron minimum				
SEP 7	0.090	0.046	23.5	12.3
Crucigenia rectangularis				
SEP 7	0.028	0.019	11.0	3.3

slope, but this species did not exhibit photo-inhibition even at 0.20 $ly \cdot min^{-1}$.

Fig. 4 shows the photosynthesis-light curve of Oscillatoria mougeotii taken from the metalimnetic layer. Curves of all samples showed a shade-type with light saturation at 0.017-0.035 $ly \cdot min^{-1}$. Light inhibition of photosynthesis occurred even at a light intensity as low as 0.025 $ly \cdot min^{-1}$. Initial slopes of the curves are summarized in Table 3. The average slope was 8.2×10^{-14} $gC/\mu m^3/h/ly \cdot min^{-1}$ for the sample in early July, and it became steeper during summer. The steepest inclination was 18×10^{-14} $gC/\mu m^3/h/ly \cdot min^{-1}$ for the sample taken from September 7 and the slope subsequently became gentle reaching 4.5×10^{-14} $gC/\mu m^3/h/ly \cdot min^{-1}$ for the 8 m sample on October 9. Photosynthetic rates of Oscillatoria mougeotii showed almost the same rate of 1.5×10^{-15} $gC/\mu m^3/h$ throughout the research period, except for the high rate of 2.2×10^{-15} $gC/\mu m^3/h$ on the September 7 and the lowest rate of 1.2×10^{-15} $gC/\mu m^3/h$ on October 9. These were remarkably low, compared with those in surface species.

(3) Photosynthetic activity of phytoplankton species

The radioactivity incorporated into the cells at light

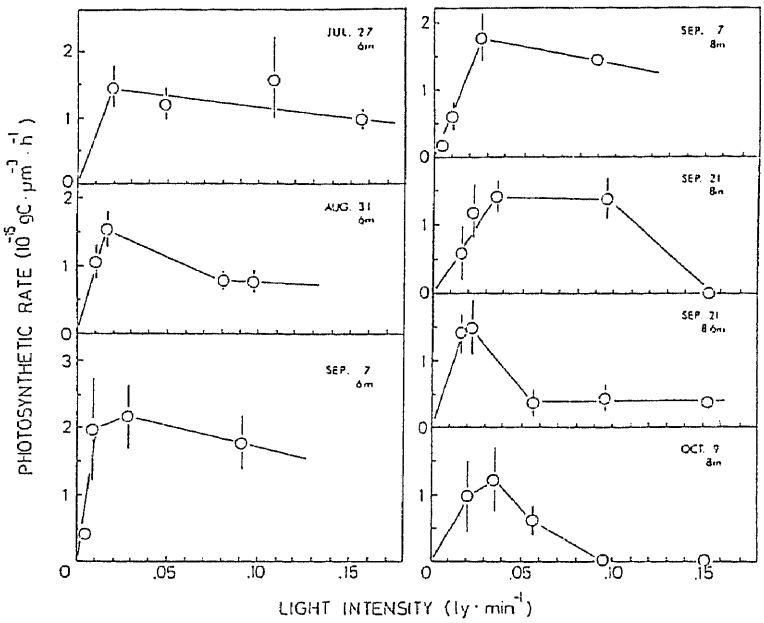

Fig. 4. Photosynthesis-light curves of Oscillatoria mougeotii taken from the metalimnetic layer during the summer stagnation period.

Table 3. Photosynthetic parameters of Oscillatoria mougeotii.

	light saturation ($ly \cdot min^{-1}$)	Ik ($ly \cdot min^{-1}$)	initial slope (10^{-14} $gC/\mu m^3/h$ /$ly \cdot min^{-1}$)	Pmax (10^{-15} $gC/\mu m^3/h$)
Oscillatoria mougeotii				
JUL 27(6m)	0.031	0.031	7.5	1.4
AUG 31(6m)	0.017	0.016	9.0	1.5
SEP 7(6m)	0.028	0.015	18.0	2.2
(8m)	0.028	0.028	6.6	1.8
SEP 21(8m)	0.036	0.036	3.8	1.4
(8.6)	0.022	0.022	8.3	1.4
OCT 9(8m)	0.036	0.036	4.5	1.2

saturation varied widely with species(Table 4). The high values were obtained, and they ranged from 7.2 x 10^{-3} dpm/cell/h for Cryptomonas to 62.28 x 10^{-3} dpm/cell/h for Peridinium sp., and the photosynthetic rates calculated from the radioactivities were 9.3 x 10^{-13} gC/cell/h to 80.6 x 10^{-13} gC/cell/h. Relatively high values were also measured in algae with a large cell volume such as the green algae Staurastrum sp. and Cosmarium sp., the diatoms Cyclotella kützingiana and Synedra acus. The ranges of their radioactivities were from 8.75 x 10^{-3} to 18.34 x 10^{-3} dpm/cell /h and photosynthetic rates were from 11.3 x 10^{-13} to 22.7 x 10^{-13} gC/cell/h. Radioactivity incorporated into Oscillatoria mougeotii showed a remarkably low value of 0.5 x 10^{-3} dpm/cell/h and it corresponded to 0.6 x 10^{-13} gC/cell/h. Lower photosynthetic rates of 1.2 X 10^{-13}, 1.2 x 10^{-13} and 3.0 x 10^{-13} gC/cell/h were also measured in other blue-green algae Aphanizomenon flosaquae, Coelosphaerium naegelianum and Chroococcus sp., respectively.

The photosynthetic rates per unit volume of the cell were also determined for several species. Cell volume was calculated from the length and width of the cell. The highest rate of 9.6 x 10^{-13} gC/μm^3/h was obtained in the diatom Cyclotella sp.and the lowest rate of 2.0 x 10^{-15}

Table 4. Photosynthetic properties of component of phytoplankton at light saturation. The sample was collected from the depth of 8m in Lake Nakanuma on September 7, 1979.

	radio-activity incorpo-rated (10^{-3} dpm /cell)	photo-synthetic rate per cell (10^{-13} gC /cell/h)	photo-synthetic rate per unit vol. (10^{-15} gC /μ^3/h)	produc-tion of popula-tion (10^{-10} gC /ml/h)	percent age of total produc-tion (%)
Green algae					
Ulothrix sp.	6.7± 2.2	8.7± 2.9		4.1±1.4	5.9
Pediastrum duplex	2.9	3.7		0.3	0.4
Pediastrum tetras	0.9± 0.1	1.1± 0.1		0.1±0.003	0.1
Ankistrodesmus falcatus	1.8± 0.3	2.4± 0.4		1.0±0.2	1.4
Scenedesmus quadricauda	2.1± 1.1	2.7± 2.1	6.2±4.9	3.9±3.0	5.6
Scenedesmus bijuga	3.5± 2.9	4.5± 3.7		2.5±2.3	3.9
Tetraedron minium	4.2± 0.5	5.5± 0.7	8.7±1.1	4.6±0.6	6.6
Crucigenia quadrata	1.3± 0.5	1.6± 0.6		0.1±0.04	0.2
Crucigenia rectangularis	1.4± 0.9	1.8± 1.1	4.0±2.4	2.5±1.5	3.6
Crucigenia tetrapedia	1.9± 0.8	2.5± 1.1		0.5±0.2	0.7
Tetrastrum heterocanthum	1.0± 1.0	1.3± 1.4		0.3±0.3	0.4
Staurastrum sp.	17.6	22.9		1.1	1.6
Cosmarium sp.	8.81	11.3		0.3	0.5
Diatoms					
Cyclotella kützingiana	18.3± 9.9	22.7±12.8	7.2±3.9	0.5±0.3	0.7
Cyclotella sp.	6.7± 3.4	8.7± 4.4	9.6±4.8	1.0±0.5	1.4
Synedra acus	17.5± 4.6	22.2± 6.0	2.3±0.6	1.1±0.3	1.6
Blue-green algae					
Chroococcus sp.	2.3± 0.7	2.9± 0.8		1.2±0.3	1.7
Coelosphaerium naegelianum	0.9± 0.6	1.2± 0.8		0.9±0.6	1.2
Oscillatoria mougeotii	0.5± 0.2	0.6± 0.2	2.0±0.2	6.8±2.5	9.8
Aphanizomenon flos-aquae	0.9	1.2		0.2	0.2
Flagellates					
Phacus sp.	15.3± 4.7	19.8± 6.1		0.2±1.1	0.3
Cryptomonas sp.	7.2± 0.5	9.3± 0.7		12.9±0.1	18.4
Gonyostomum sp.	27.7±17.3	35.8±22.4		4.4±2.7	6.3
Peridinium sp.	62.8	80.6		0.1	1.1
Trachelomonas sp.	23.0	29.9		0.1	0.1
Unidentified small flagellates	2.4± 0.4	3.2± 0.6		12.2±219	17.5
small spherical algal colony	11.5± 5.4	14.8± 7.0		6.2±2.9	8.9
the total production measured by grain density autoradiography				67.7	
the total production measured by scintillatin counter				112.7	

gC/μm^3/h was in Oscillatoria mougeotii. The green algae Tetraedron minimum and Scenedesmus quadricauda showed considerably high values of 8.7×10^{-15} and 6.2×10^{-15} gC/μm^3/h, respectively.

(4) Contribution of individual species for community production

The total photosynthetic production of phytoplankton community was determined by the two approaches. One is to sum up the production of each species population measured by grain density autoradiography and the other is to measure directly with a liquid scintillation counter. The results are shown in Table 4. The total production estimated by the former method was 67.7×10^{-10} gC/ml/h. The contribution of each algal group to the estimated total community production was 30.9% in the green algal populations, 17.5% in the small flagellate populations and 12.9% in the blue-green algal populations. Among the individual species populations, Cryptomonas sp. accounted for 18.4% of the total production and Oscillatoria mougeotii was the next and accounted for 9.8%. The green algae Tetraedron minimum, Ulothrix sp., Scenedesmus quadricauda and Crucigenia rectangularis contributed 6.6, 5.9, 5.6 and 3.6% of the

total production, respectively. The total production measured with a liquid scintillation counter was 112.74 x 10^{-10}gC/ml/h.

3.4 Discussion

Photosynthesis-light curves (P vs I curves) are important to evaluate the physiological states of phytoplankton species and are necessary to investigate phytoplankton species dynamics in relation to light environment. The phytoplankton species structure, its states and productivities are determined by various environmental conditions. P vs I curves for unialgal culture obtained in laboratory experiments provide only limited information about the responses of phytoplankton to light in the field. Therefore, studies of P vs I curves under field conditions may be essential in phytoplankton production ecology. There are, however, few published data for the P vs I curves of individual species in natural phytoplankton community. The depth-differentiation of P vs I curves have well been known in a well stratified water column. The curve is generally the sun-type for phytoplankton community in surface layers and the shade-type for that in deep layers (RYTHER 1956, ICHIMURA 1962). This was also

recognized in the present study. However, the ecological interpretation of such curves was impossible without consideration of features relating photosynthesis to light in individual species in mixed species assemblages. RYTHER(1956) investigated photosynthetic responses of marine phytoplankton to light by using unialgal cultures and showed that there exists a difference between three taxonomic groups (dinoflagellates, green algae and diatoms) with respect to their P vs I curves. The P vs I was a sun-type for the dinoflagellates, a shade-type for the green algae and an intermediate type for the diatoms. This difference may be due to the previous life history of phytoplankton species.

The quantitative determination of photosynthetic rate of individual phytoplankton species in natural mixed population has been made by a few investigators. KNOECHEL and KALFF (1975, 1976 a) determined the carbon fixation rate as 4.0×10^{-13} gC/cell/h for Tabellaria fenestrata and 6.0×10^{-13} gC/cell/h for Anabaena planktonica through track autoradiography. These rates coincide fairly well with those of the green algae but are little lower than those in flagellates obtained in the present study. Using track autoradiography, KNOECHEL and KALFF(1978) measured the in situ carbon fixation

rates for five freshwater phytoplankton diatoms Asterionella formosa, Fragilaria crotonensis, Melosira italica, Synedra radians and Tabellaria fenestrata, and showed the photosynthetic rates of 2.0×10^{-15}, 1.6×10^{-15}, 1.0×10^{-15}, 0.8×10^{-15} and 1.2×10^{-15} gC/μm^3/h for the respective species. These rates are somewhat lower than those obtained in the present study.

There are considerable differences between the community production determined by the two methods, grain density autoradiography and the scintillation counter method. The production measured by grain density autoradiography accounted for about 62.5% of that measured by the liquid scintillation counter method. If the production measured by the scintillation counter method is assumed to be the actual production, 38% of total production may be missed in the grain density autoradiography. This is probably due to the following reasons. The dense grains produced by prolonged exposure interfere with the counting of grains, and thereby the enumerated data became lower than the actual grain number. The 7-days exposure used in the present experiment is designed for blue-green algae in order to facilitate grain-counting, but it may be longer for other phytoplankton species. Thus, it is desirable in natural phytoplankton community to prepare several

autoradiograms exposed with different times and the maximum grain counts should be determined for each species. The ratio of the cell surface in contacted with emulsion to cell volume is smaller in large phytoplankters than in small ones, and then the efficiency of grain production may be lowered in the former than in the latter. As the result of this low efficiency, the ^{14}C radioactivity incorporated into the cell is supposed to be underestimated for larger phytoplankters. Since the conversion equation used in the present study is applicable only to nanoplankton species, the relation between incorporated ^{14}C radioactivity and produced grains should be further certified for larger phytoplankton species, through which the conversion equation will be more valid. In natural phytoplankton communities, it is not easy to count completely the total cells in the sample. This may also be responsible for underestimation of total production in grain density autoradiography. However, autoradiography is a technique improved for measuring production of individual species and not for assessing the total production. If several critical points mentioned above are examined carefully, grain density autoradiography can be used to measure the photosynthetic behavior of the component species of

phytoplankton community quantitatively.

3.5 References

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Chapter 4

Growth of individual phytoplankton speceis in mixed populations and its relation to light intensity in water body

4.1 Introduction

Light that penetrates into a water body decreases exponentially with the depth in a water body and different phytoplankton species may respond differently to light at their location within the euphotic zone. Photosynthesis-light intensity relationship established in Chapter 3 for different algal groups showed that green algae exhibit maximum photosynthesis rates at higher light intensities, blue-green algae at lower light intensities, and the diatoms at intermediate light intensities. STEEMANN NIELSEN (1939) classified oceanic phytoplankton into three groups based on field observations; oligophotic species which occur mainly below 50 m, mesophotic species which occur in the upper 100 m and euphotic species which occur in the upper 50 m. Such species occurrence patterns may be attributed to species-specific photosynthetic response to light gradient in aquatic environments and implicated

light as a factor regulating vertical distribution of species and community structure.

It has been widely known that Oscillatoria develope their biomass maximum at the depth near or below the thermocline in well stratified mesotrophic lakes during summer. This characteristic vertical distribution of the blue-green algae has drawn the attention of many investigators, and the physiological nature of these algae has been studied intensively. Some biological processes for formation of metalimnetic maxima have been shown by several investigators, but the formation mechanisms have not yet been fully elucidated (BAKER et al., 1969; WALSBY and KLEMER, 1974; KLEMER 1976). Such an almost monospecies population is also considered to be usable as material for the growth analysis of natural phytoplankton populations in relation to light conditions.

For an understanding of the dynamics of Oscillatoria population, information on their photosynthesis is essential. In recent years, new autoradiographic techniques have been developed to measure the photosynthesis of individual species in the phytoplankton community. KNOECHEL and KALFF (1978) measured individual photosynthesis of five different species of planktonic diatoms in a freshwater lake by

track autoradiography and explained their population dynamics. Using grain density autoradiography, OGAWA and ICHIMURA (1981) measured photosynthetic rates of individual phytoplankton species in a metalimnetic phytoplankton community and found that the rates for blue-green algae were considerably low, compared with those of the other species. This finding raises the question as to whether the deep Oscillatoria population is formed by biological processes or purely physical processes of accumulation of senescent cells which have sunk from the euphotic zone into the metalimnetic layer.

The purpose of the present study is to present a new approach to the studies of the growth of phytoplankton populations under natural conditions based on species specific photosynthetic responses to available light and to analyze the population dynamics based on organic matter production.

4.2 Materials and Methods

Samplings were made with a pump system at intervals of 1 m in the center of Lake Nakanuma. Water temperature was measured by a thermistor thermometer and the light attenuation by a selenium underwater photometer fitted with a neutral density filter. Water samples were

transferred into 5-liter polyethylene bottles and immediately brought back to the RI laboratory of the University of Tsukuba which is located 30 km away from the lake. Phytoplankton cells were counted under a microscope (Nikon Optiphoto) after concentrating the cells from 50 ml water sample 10 times by centrifugation for 15 minutes at 3000 r.p.m. Cell volume was determined from the length and width of a cell on microscopic photographs. Photosynthesis was measured by the ^{14}C method. Samples were poured into Winkler bottles of 50 ml, and 0.2 ml of $NaH^{14}CO_3$ solution having an activity of 10 μCi /ml was injected into the samples. These bottles were incubated in a water bath at the in situ temperature under different light intensities given by white fluorescent lamps. Light intensity was controlled by neutral density screens and measured by a LAMBDA LI-185 radiometer. Dark bottles were also prepared as control. After a 2-hr incubation, each 2 ml of a sample was filtered through a 24-mm HA millipore filter by gentle suction and the filter was washed with a small volume of distilled water, and then fumed in HCl vapor. These were used for grain density autoradiography (OGAWA and ICHIMURA 1980, 1981). Each filter was attached to a microscope slide, dried at 35°C for 24 hrs, and then made clear with acetone vapor. Under a safety light,

each slide was dipped in SAKURA NR-M2 emulsion and left in a vertical setting for 1 hr. The slides were placed in a black plastic slide box containing dried silica gel and stored at 4°C for 7 days. Exposed slides were developed by KONIDOL X SUPER and fixed by acid hardening fixer (KONIFIX). The resultant autoradiographs were photographed at 780 magnification by a Nikon Optiphoto microscope, and grains on the photographs were counted. The grain count per cell (Y) was converted to absoluted radioactivity (X) by the regression equation, $Y = 587.06X + 0.78$. Photosynthetic activity per unit volume of a given cell was calculated by the following equation,

$$P = C \times \frac{G'}{G \times t \times v} \qquad (1)$$

where P is the photosynthetic activity per unit cell volume of a given species ($gC/\mu m^3/h$); C , the available total inorganic carbon in the sample (gC/l); G', the radioactivity incorporated into a given cell (μCi); G the added radioactivity (μCi); t, the incubation time (hr) and v, the volume of a given cell ($\mu m^3/l$).

4.3 Results

(1) *In situ* growth of *Oscillatoria mougeotii* population

Figure 1 shows the changes in the vertical distribution of *Oscillatoria mougeotii* in Lake Nakanuma during summer in 1979. When the lake stratified slightly in mid July, a small population of *Oscillatoria mougeotii* appeared in the bottom layer of the euphotic zone (6 m). The thermal stratification was well established by the end of August, and a dense population developed in the thermocline showing a peak distribution at the depth of 6 m, where the light intensity was 2.4% of that of the surface. Thereafter the increase of population was reduced and the biomass peak shifted progressively towards the deeper layer within the thermocline until the middle of September. Then, *Oscillatoria mougeotii* decreased their cell number and only a small biomass remained in the hypolimnion by late October.

Growth of the *Oscillatoria mougeotii* population indicated by the total cell number in a water column can be expressed approximately by a logistic curve, as shown in Fig.2. Cell number reached a maximum of 19.5×10^5 cells/cm^2 in late September and subsequently decreased. Mean growth rate of population can be calculated by

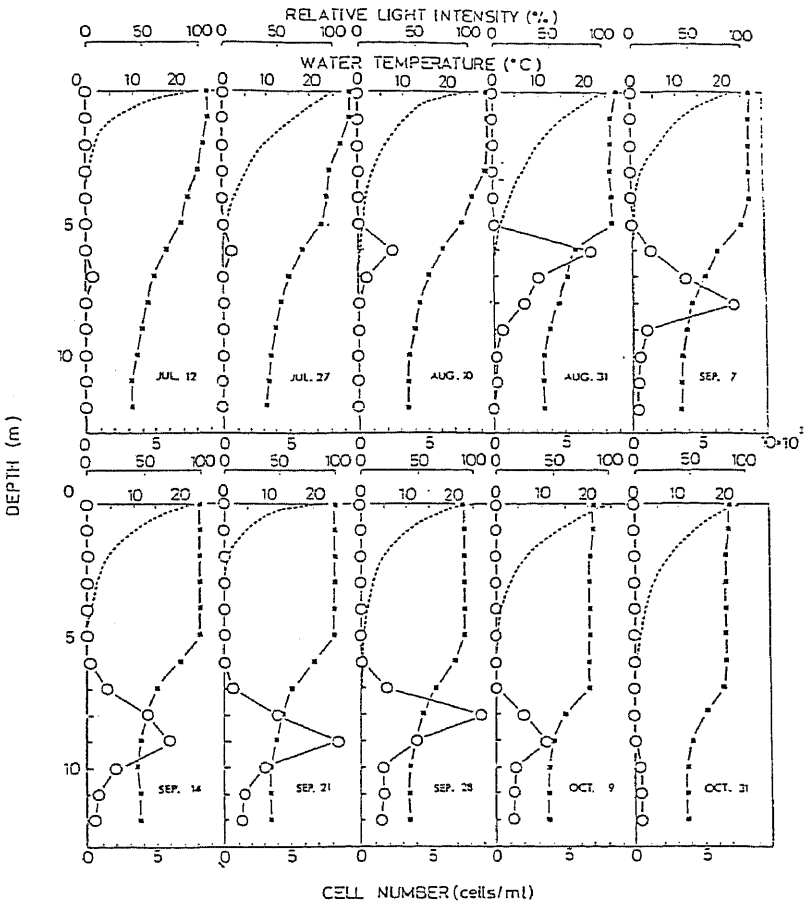

Fig. 1. Seasonal changes in the vertical distribution of Oscillatoria mougeotii (open circles), water temperature (filled squares) and relative light intensity (dashed lines) in Lake Nakanuma.

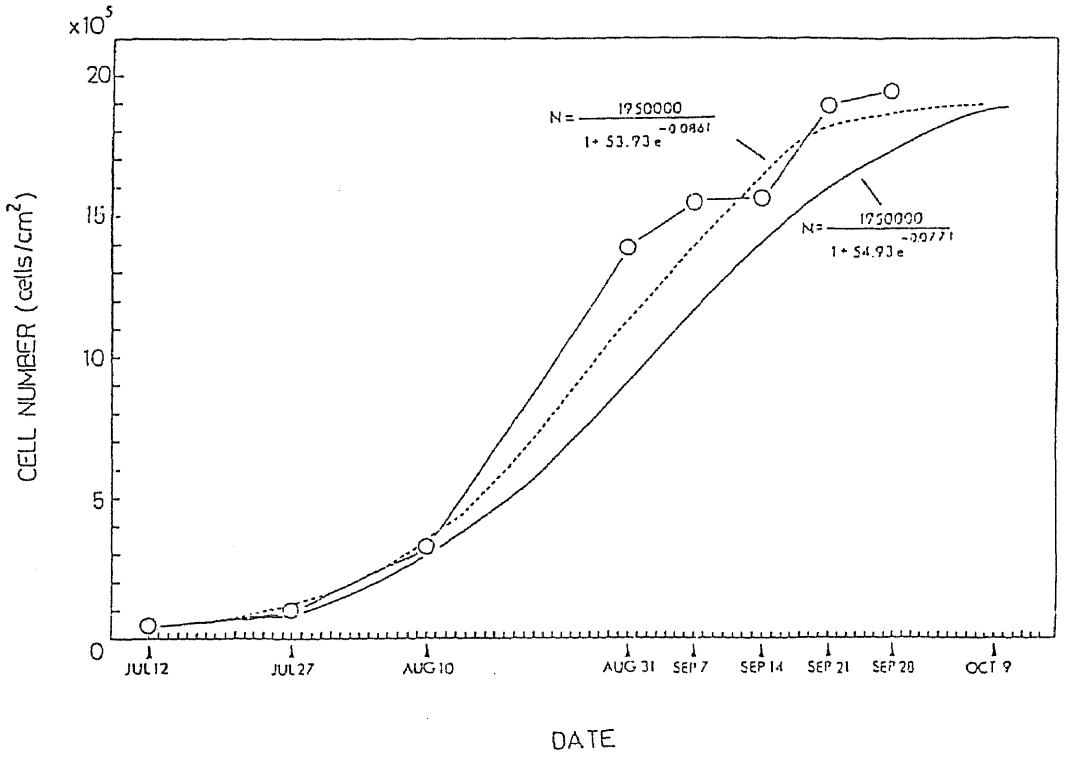

Fig. 2. Observed growth curve (-O-), and computed growth curves based on observed increasing rate μ (-----) and calculated intrinsic growth rate r (——) for Oscillatoria mougeotii population.

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \qquad (2)$$

where μ is the mean increase rate (day^{-1}); N_1 and N_2, the cell number of Oscillatoria mougeotii in water column per unit surface ($cells/cm^2$) at time t_1 and t_2 (day), respectively. The results are summarized in Table 1. The observed rate of increase from July 12 to July 27 was 0.064 (day^{-1}), and the maximum of 0.077 (day^{-1}) was obtained from July 27 to Augst 31. The Oscillatoria mougeotii population reached a maximum in early September at which the observed growth rate was 0.015 (day^{-1}) and subsequently dropped abruptly with decrease of the biomass.

(2) Growth analysis of Oscillatoria mougeotii population based on organic matter production

The observed population increase of Oscillatoria mougeotii in the entire water column can be approximated by the following equation;

$$N_t = \frac{K}{1 + k \cdot e^{-rt}} \qquad (3)$$

where N_t is the cell number (cells/cm) at time t (day); k, the constant of integration; r, the intrinsic rate of

Table 1. Observed growth rates (μ) of Oscillatoria mougeotii population determined from the changes in the cell numbers and the intrinsic growth rates (r) calculated from the net production.

Date	observed growth rate (μ)	intrinsic growth rate (r)
Jul. 12 - Jul. 27	0.064	
Jul. 27		0.043
Jul. 27 - Aug. 31	0.077	
Aug. 31		0.071
Aug. 31 - Sep. 7	0.015	
Sep. 7		0.077
Sep. 7 - Sep. 21	0.014	
Sep. 21		-0.006
Sep. 21 - Oct. 9		
Oct. 9		-0.003

increase (day^{-1}); and K, the ultimate maximum cell number for the population ($cells/cm^2$). The intrinsic rate of increase (r) corresponds to the increase rate of population (μ) in equation 2. The population growth of Oscillatoria mougeotii was calculated from the equation 3 by using observed values (K=1950000, r=0.086, k=53.93, starting population density N_o=35500) and the result is presented as the curve in Fig. 2.

Daily net production and total cellular carbon of Oscillatoria mougeotii are required for the actual calculation of the intrinsic rate based on production. The daily net production can be practically estimated either from direct in situ measurements or the photosynthesis-light curves combined with light profiles in the water column. In the present study, the latter approach was applied to the estimation of daily net production. The basic principles of this approach have been described by RYTHER (1956), and PARSONS and TAKAHASHI (1977). In the first step, the hourly net production of Oscillatoria mougeotii per unit volume of a cell at a given depth was calculated at different times of the day. For this purpose, the surface light intensity at a given time (I_t) was estimated by the following equation,

$$I_t = Imax \cdot \sin^3(\pi/D)t$$

where I_{max} is the surface light intensity at solar noon and D is the day length (hr). Light intensity at a given depth was estimated from the relative light attenuation which was measured directly in the lake and the surface light intensity(I_t). The photosynthesis-light curves presented in Fig. 3 were applied to the calculation. The daily net production per unit cell volume was then obtained by integrating the hourly net production over a period from sunrise to sunset. The diel net production (24 hrs) was determined by subtracting night respiration from the daily net production. In this case, respiration was assumed to be 1/20 of the photosyntetic rate at light saturation. The diel net production in a unit water volume at a given depth was obtained by multiplying the production per cell volume with total cell volume in a unit water volume at that depth. Finally, the diel net production of Oscillatoria mougeotii in a unit water column was calculated by summing up the diel net production at each respective depth. Carbon content per cell is required to convert the net production to the number of Oscillatoria mougeotii cells. This was deduced by using the STRATHMANN's regression of carbon vs volume for algae (STRATHMANN, 1967). Mean cell volume of Oscillatoria mougeotii was 31.68 μm^3, so that cell

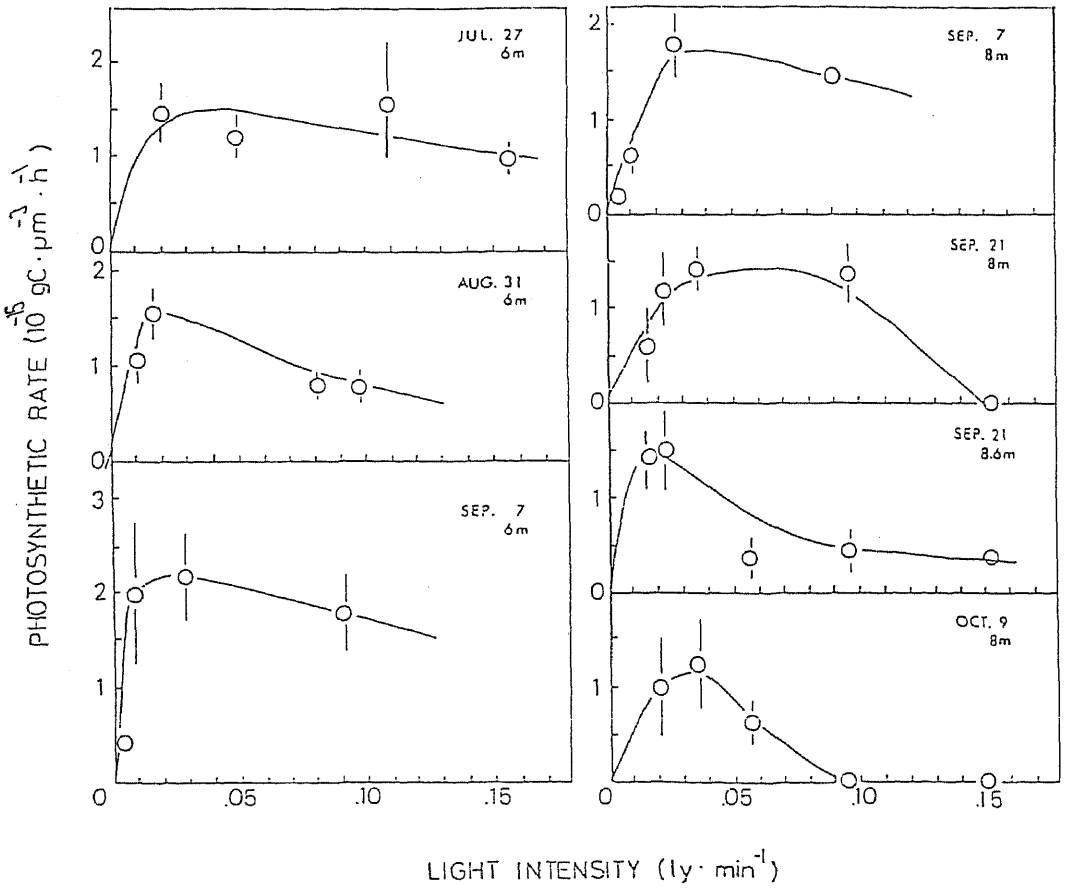

Fig. 3. Photosynthesis-light curves of Oscillatoria mougeotii used for the calculation of diel net production.

carbon was taken to be 6.90×10^{-12} gC/cell. Since carbon contents of algal cells under nutrient-limited natural conditions have been reported as a half of those of the cultured cells which are not nutrient limited (ANTIA et al., 1963), 3.45×10^{-12} gC/cell was assumed to be cell carbon for Oscillatoria mougeotii. Substituting the cell number, which was estimated by dividing the diel total net production by cell carbon, into equation 2, the intrinsic rate ($r = \mu$) was obtained. It was 0.043 (day^{-1}) in the early stage of growth on July 27. This was fairly low, compared to the observed growth rate (μ) of 0.064 (day^{-1}) in Table 1. Both rates of 0.071 (day^{-1}) on August 31 and 0.077 (day^{-1}) on September 7 were similar to the observed rates for the period from July 27 to August 31 but higher than 0.015 (day^{-1}) for the period from August 31 to September 7. Negative rates such as -0.006 (day^{-1}) and -0.003 (day^{-1}) were obtained in senescent population during late autumn.

On the assumption that the mean intrinsic rate of increase (μ) is 0.077 (day^{-1}), the ultimate maximum cell number (K) is 1950000 cells/cm^2, the constant of integration is 54.93 and the starting population density N_o is 35500. The calculations were made for in situ growth of the Oscillatoria mougeotii population by

using equation 3, and the results are given in Fig.2. The loss of standing algal crop resulting from impair processes such as mortality, sinking, grazing, and horizontal advection might contribute significantly to population dynamics but this was not considered in the present calculation. The predicted growth curve resembled the observed one fairly well in shape and magnitiude.

(3) Growth analysis of model phytoplankton community that consists of three species populatios in hypothetical water body

Calculation procedure was nearly the same as that used for a population of Oscillatoria mougeotii. Diel net photosynthesis (Pn) of individual cells at a given depth in the water body was calculated by the following equation (TAKAHASHI, personal communication),

$$Pn = Pmax \cdot D \cdot \left(1 - \frac{1}{1 + a \cdot Idmax} \right) - 24\ R \qquad (4)$$

where Pmax is the maximum photosynthetic rate(gC/cell volume/hr) on P vs I curve, D is the day length (hr) Id·max is the light intensity at a given depth (d), a is the constant for P vs I curve and R is the respiration

(gC/cell volume/hr). The light intensity Id·max was calculated by

$$Idmax = Imax \cdot \sin^2(\pi / D) \cdot e^{-kd} \qquad (5)$$

where I max is the surface light intensity at solar noon, k is the extinction coefficient. The respiration (R) was estimated to be 1/20 of Pmax. Diel increase of biomass (cell number ΔN) per one cell was calculated by dividing the diel net photosynthetic product per cell by cellular carbon of each species,

$$\Delta N = \text{photosynthetic product} / \text{cellular carbon} \qquad (6)$$

where cellular carbon was estimated from cell volume by the STRATHMANN's equation. The growth rate for each species was calculated as follows,

$$\mu = \ln(1 + \Delta N) - \ln 1 \qquad (7)$$

The growth of individual species populations was predicted by substituting μ into the equation $N = N_0 e^{\mu t}$, where N_0 and N are the cell number of initial and t days later.

Diel photosynthetic products at depths 1, 4 and 7 m

were calculated by equation 4 for three species; Melosira varians, Cyclotella sp. and Oscillatoria mougeotii. The values of a in equation 4 were determined to be 26.1 for Melosira varians, 88.1 for Cyclotella sp.and 310.2 for Oscillatoria mougeotii and Pmax were 1.5 x 10^{-15}, 1.68 x 10^{-15} and 1.68 x 10^{-15}gC/μm^3/h respectively, from P vs I curves presented in Fig. 4. The value I max was assumed to be 0.6 ly·$min^{-1}$and the mean extinction coefficient k of 0.75 was chosen on the basis of the field surveys made in Lake Nakanuma. Results are summarized in Table 2. Photosynthetic rates of Melosira varians and Cyclotella sp. are 9.85 x 10^{-15}gC / μm^3/day and 15.36 x 10^{-15} gC/μm^3/day, respectively at 1 m depth, and decreased with towards deeper layers. On the contrary, the photosynthetic rates of Oscillatoria mougeotii were higher at depths 4 and 7 m than at 1 m depth. The diel increase of cell number and the growth rate were high at 1 m depth for both Melosira varians and Cyclotella sp., while those of Oscillatoria mougeotii were higher at deeper layers.

The diel net production at a given depth was calculated for three species populations and their successive growth was predicted by summing up the diel net production. The starting population density is assumed to be 1000 cells/ml. The results are shown in

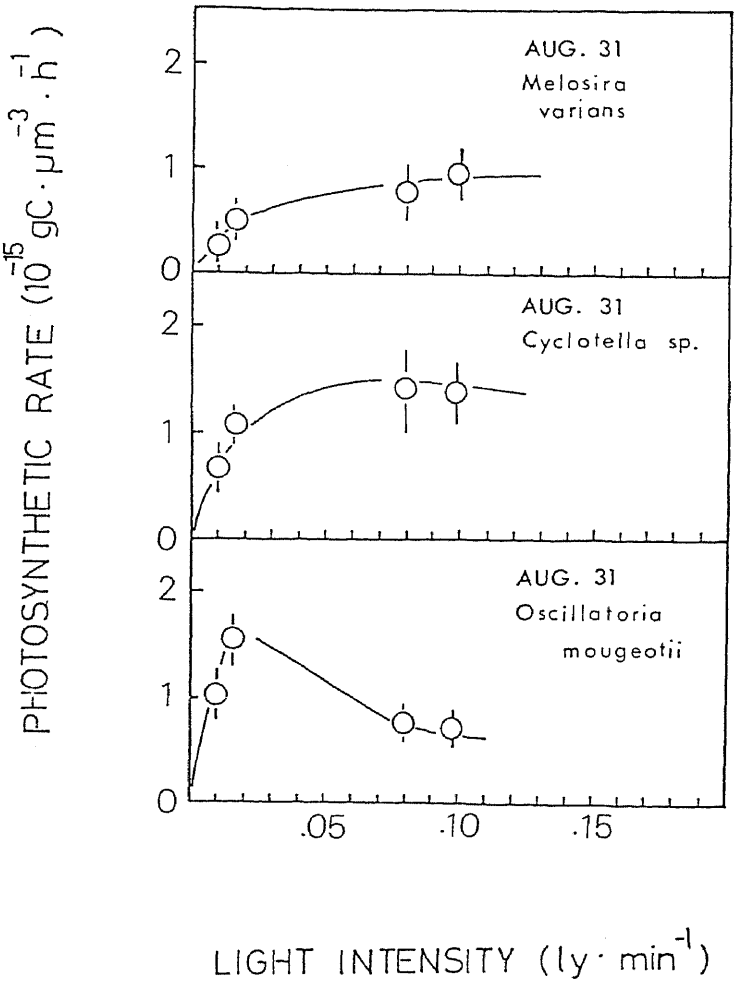

Fig. 4. Photosynthesis-light curves of Melosira varians, Cyclotella sp. and Oscillatoria mougeotii used for the calculation of diel net production of each species.

Table.2 Diel net production, diel cell increase and specific growth rate (μ) of three phytoplankton species.

relative light intensity	diel net production (10^{-15} gC/ μm^3/day)	diel net production (10^{-15} gC/ cell/day)	cell increase	μ
Melosira varians				
61.4% (1m)	9.85	1654.8	0.08	0.09
26.8% (4m)	8.39	1409.5	0.07	0.07
2.9% (7m)	2.95	495.6	0.02	0.03
Cyclotella sp.				
61.4% (1m)	15.36	1396.5	0.13	0.12
26.8 (4m)	13.98	1271.0	0.11	0.10
2.9% (7)	6.20	563.7	0.05	0.05
Oscillatoria mougeotii				
61.4% (1m)	7.6	237.7	0.07	0.07
26.8% (4m)	10.2	320.8	0.09	0.09
2.9% (7m)	8.8	277.3	0.08	0.08

Fig. 5. After ten days, both sun-type populations of Cyclotella sp. and Melosira varians predominate at the euphotic layer (1 m, 4 m) and the shade type Oscillatoria population prevails at the bottom of the euphotic layer where the light intensity is reduced to 5% of the surface. The calculated vertical distribution patterns of three species populations which are common in Lake Nakanuma coincided fairly well with the observed data.

4.4 Discussion

The coincidence of predicted and observed growth curves of Oscillatoria mougeotii populations suggests that the loss of standing crop would be negligible in the deep Oscillatoria mougeotii populations for the substantial period. The increment of Oscillatoria mougeotii population in the meta- or hypolimnion may be due to in situ photosynthetic growth rather than the accumulation of senescent algae sinking from the euphotic layer. In this connection, it is interesting to note that this species could not be detected in the epilimnion throughout the study period.

As for the occurrence of the discrete metalimnetic maximum of Oscillatoria mougeotii, more data are

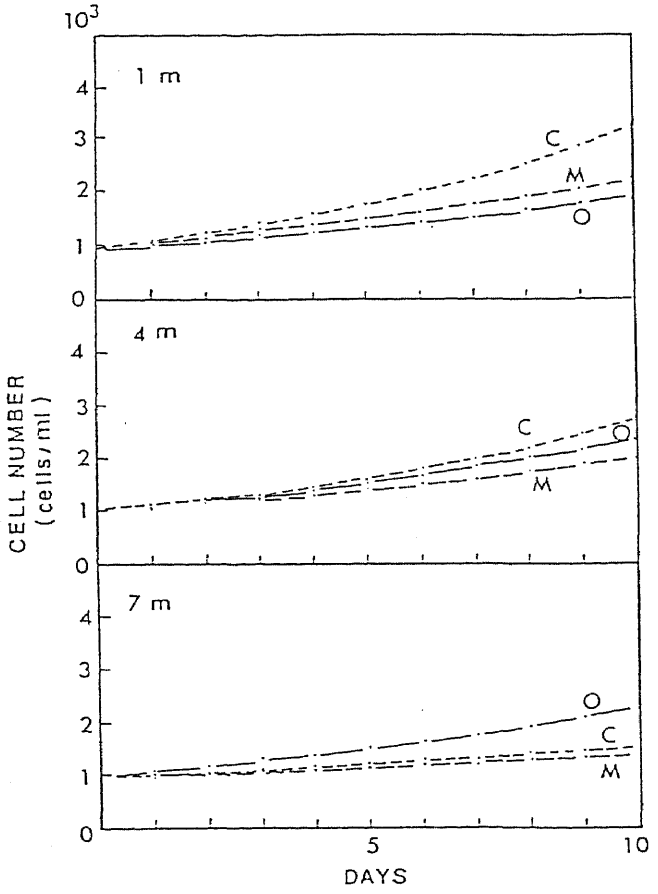

Fig. 5. The population growth of three phytoplankton species at different depths; Melosira varians (M), Cyclotella sp. (C) and Oscillatoria mougeotii (O). The growth of each species was estimated from the diel net production of respective species.

necessary to analyze its formation mechanisms. Surface concentrations of nitrogen and phosphorus in Lake Nakanuma were reduced to undetectable levels during summer but they were high in discontinuity layers. Under such conditions of stable stratification, blue-green algal species such as Anabaena, Microcystis, Aphanizomenon are able to control their vertical position by the regulation of the buoyancy (REYNOLDS, 1973, 1976). It is therefore inferred that metalimnetic maxima of Oscillatoria mougeotii are probably formed by the interaction of their photosyntetic activity and buoyancy in relation to nutrient availability, as documented by REYNOLDS (1973) and KLEMER (1976).

The simulation for Oscillatoria mougeotii has dealt with the community of a single algal species that has typical shade-type photosynthesis properties. When this extreme simplification is taken into consideration, the agreement with observed data is encouraging. The results can certify the predictive ability of such mechanistic approach to phytoplankton ecology.

The growth dynamics of three species populations was predicted by the mechanistic models, and it well interpreted natural phenomena. The field observations on the vertical distribution of three species in Lake Nakanuma suggest that because of comparatively slow

growth rates prevailing in deep layers, it is unable to compete for the niche with the faster growing diatoms found in the surface layer. Oscillatollia mougeotii with a shade-type P vs I curve should be dominant at intermediate depths, because of its photosynthetic ability at the light intensity lower than that required for the survival of the other two diatom species. Vertical differentiation of species distribution will mainly be regulated by the species specific photosynthetic behavior to light gradient in a water body. The results reported here used only one niche dimension, but the mechanistic approach could easily be expanded to include more dimensions such as nutrients.

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Chapter 5

The relationship between phytoplankton diversity and trophic status of inland waters

5.1 Introduction

Species diversity, one of the important characteristics of a natural community, has long attracted the interest of researchers. For aquatic communities, the relationship between phytoplankton diversity and the trophic status of water has been discussed by several investigators. MARGALEF (1968) applied the information theory to a study of phytoplankton species diversity, and proposed that diversity was low in eutrophic waters and high in oligotrophic waters. MOSS (1973) studied phytoplankton diversity in waters having three different trophic levels and agreed with MARGALEF's hypothesis. Our studies on 24 bodies of water with different trophic status have produced results somewhat different from those of previous studies. We found that phytoplankton diversity was low in oligo- and hypereutrophic waters and high in meso- and subeutrophic waters.

MARGALEF (1968) interpreted the changes in phytoplankton diversity to be changes in the amount of information

accumulated in each water mass. MOSS (1973) explained that phytoplankton diversity varied according to the degree of overlapping among successional species populations. We believe that both high and low nutrient waters create a low diversity, while a multispecies, high diversity phytoplankton community prevails in moderately nutrient-rich waters.

The purpose of this study is to define the relationship between species diversity and nutrient level, and to propose a plausible mechanism for controlling species diversity of the phytoplankton community.

5.2 Materials and Methods

Field studies were carried out in Himonya Pond, Tokyo, a 1 ha hypereutrophic pond of 2 m maximum depth, and in Lake Nakanuma, Ibaraki Prefecture, a 1.2 ha eutrophic lake of 13.5 m maximum depth. Water samples were collected monthly using a 1.5 cm inner diameter polyethylene tube which was held by hand and lowered by a string to a depth just above the thermocline, and then pulled up by the string.

All the phytoplankters in the water column of the mixed layer were collected by this method. Samples were filtered through glass fiber filters (Whatman GF/C).

Phosphate, ammonium, nitrate and nitrite concentrations in the filtrate samples were determined according to the methods described by STRICKLAND and PARSONS (1972). The amount of chlorophyll a on the filters was measured spectrophotometrically (SCOR-UNESCO 1966).

Diversity of the phytoplankton community was expressed on the genus level because of the taxonomic difficulties of identifying all the algae to the species. A 50 ml sample was fixed by Lugol's solution, and phytoplankters were identified in random fields of a counting chamber. The identified cell numbers were about 1000 cells and 100 cells for the samples of Himonya Pond and Lake Nakanuma, respectively. Phytoplankton diversity (H) was calculated by the SHANNON-WEAVER (1963) function,

$$H = - \sum \frac{N_i}{N} \log_2 \frac{N_i}{N}$$

where H is expressed in bits per cell, N_i is the cell number of i-th genus and N is the total of counted cells.

5.3 Results

(1) Phytoplankton component in the two bodies of water

The amounts of chlorophyll a in Himonya Pond varied from 20 mg/m^3 in Dcecmber to 246 mg/m^3 in September(Fig.

1-A). These large amounts of chlorophyll *a* indicate that Himonya Pond is hypereutrophic. A blue-green alga, *Microcystis aerginosa*, dominated throughout the survey period, and small numbers of the blue-green algae, *Oscillatoria* and *Anabaena*, and the diatom *Fragilaria*, were also present. *Microcystis* density started to increase in April and showed two maxima of 9.4×10^5 cells/ml in late June and 15×10^5 cells/ml in late September. It then decreased to 100 cells/ml in late November. *Oscillatoria* appeared in measurable numbers in April and increased to high concentration of 9.3×10^5 cells/ml in late May, and then dropped abruptly to a small number of 10 cells/ml in August. A small peak of *Aphanothece* occurred in November. *Anabaena* and *Fragilaria* were in relatively low numbers throughout the year.

Chlorophyll concentrations in Lake Nakanuma ranged from 3 mg/m^3 in August 1976 to 17 mg/m^3 in May 1975 (Fig. 2-A). Such moderate amounts of chlorophyll *a* show that Lake Nakanuma is eutrophic. The phytoplankters were the diatoms, *Melosira varians*, *Synedra ulna* and *Cyclotella*, and the green alga *Scenedesmus*. *Melosira varians* dominated from winter to spring with a maximum of 3.1×10^3 cells/ml in April 1975, and was relatively low in number during the summer. *Synedra*

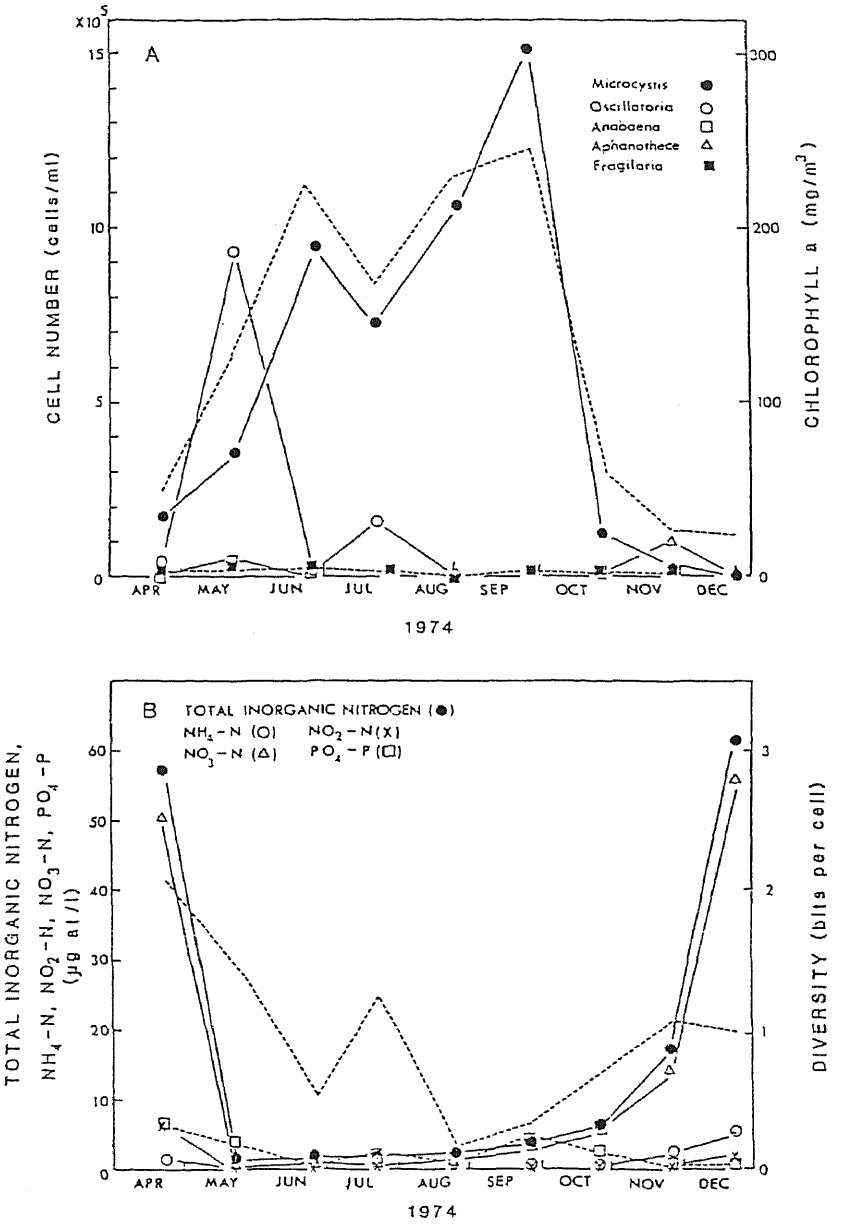

Fig. 1. A: Seasonal changes in chlorophyll a (dashed line) and dominant phytoplankton in Himonya Pond. B: Seasonal changes in phytoplankton diversity (dashed line) and inorganic nutrients in Himonya Pond.

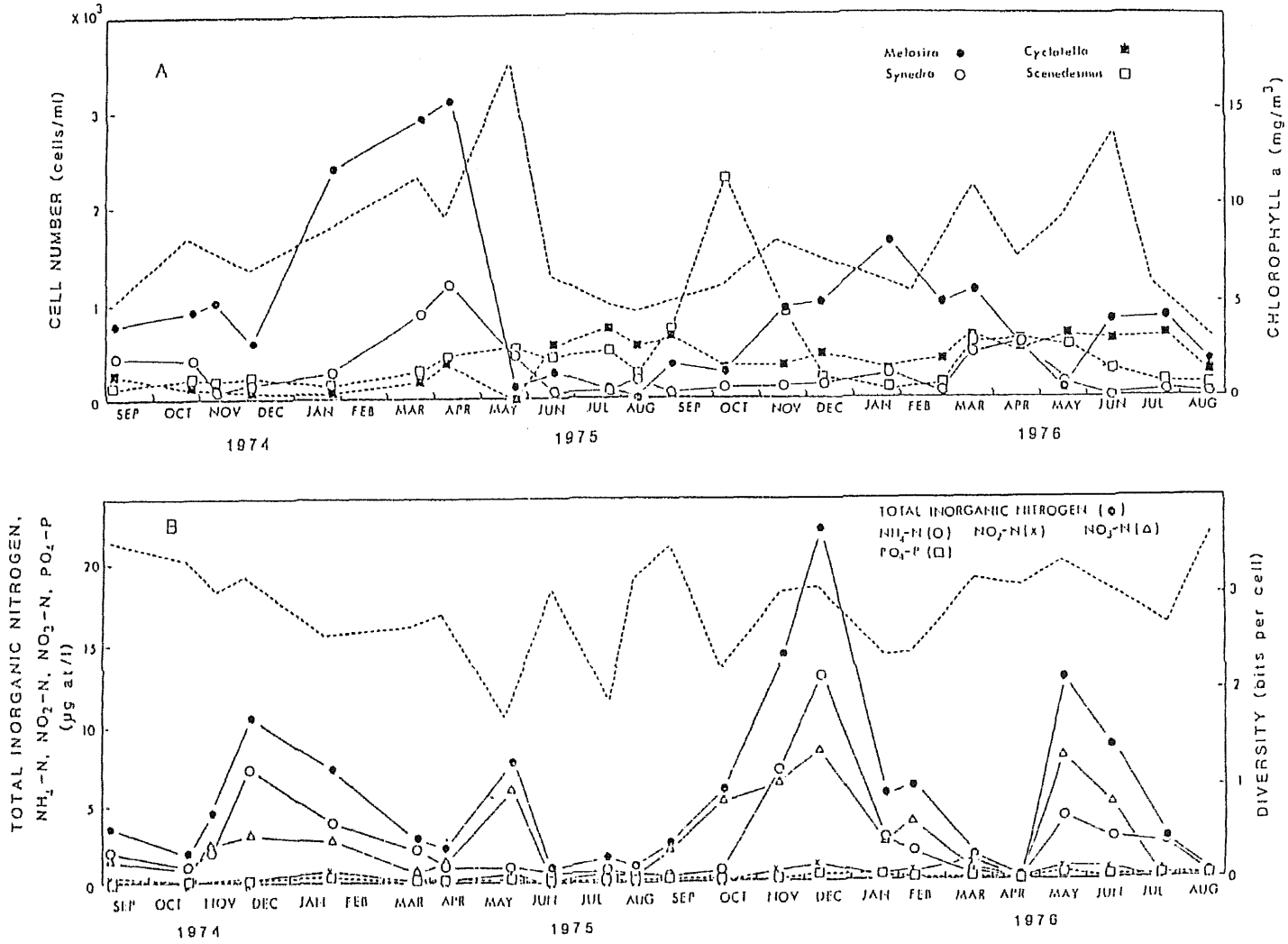

Fig. 2. A: Seasonal changes in chlorophyll a (dashed line) and dominant phytoplankton in Lake Nakanuma. B: Seasonal changes in phytoplankton diversity (dashed line) and inorganic nutrients in Lake Nakanuma.

ulna was relatively abundant in April but low in the other seasons. Scenedesmus showed a peak of 2.3×10^3 cells/ml in October 1975 and remained in substantial numbers throughout the year.

(2) Phytoplankton diversity and nutrient concentrations in the two bodies of water

Seasonal changes in the diversity in Himonya Pond approximated those of NO_3-N and the total inorganic nitrogen, although the diversity decreased more slowly than the nitrogen (Fig.1-B). High values of 2 bits/cell for the diversity index, 50 µg-at N/l for NO_3-N and 56.7 µg-at N/l for the total inorganic nitrogen appeared in the spring. As the NO_3-N and total nitrogen were depleted in the summer, the diversity decreased reaching the lowest at 0.31 bits/cell in late August when NO_3-N and the total inorganic nitrogen were 1.2 µg-at N/l and 1.5 µg-at N/l, respectively. All three factors began to increase in late autumn and reached fairly high levels of 1 bit per cell, 55.2 µg-at N/l and 61.0 µg-at N/l, respectively, by the end of the year.

The diversity within the phytoplankton community of Lake Nakanuma fluctuated between 2.0 to 3.5 bits/cell,

although the concentrations of NH_4-N, NO_3-N and the total inorganic nitrogen showed large seasonal changes with two peaks in May and December (Fig.2-B). The concentrations of NH_4-N and NO_3-N began to increase in early autumn, and NH_4-N reached a high level of 12.5 μg-at N/l and NO_3-N a high level of 8.0 μg-at N/l in December 1975 (Fig.2-B). These nutrients decreased to low levels less than 2.0 μg-at N/l by April 1975, but increased again in late spring. On the other hand, the diversity decreased slightly from autumn 1974 to spring 1975. Sharp reduction of NH_4-N and NO_3-N occurred in early summer of both years, and low levels developed by the end of August, although the diversity oscillated at a higher level. Thus, the correlation between diversity and nutrient concentration was not as obvious in Lake Nakanuma as in Himonya Pond.

(3) Model analysis of nutrient effects on the growth of phytoplankton populations

Field observations demonstrate that extremely high concentrations of nutrients induce development of a phytoplankton community containing only a few populations, whereas moderate nutrient concentrations promote formation of a multispecies phytoplankton

community. The changes in phytoplankton diversity as a function of the nutrient condition of the waters can be expressed in the following simple mathematical model which was designed to simulate the growth of phytoplankton under various nutrient conditions.

During the exponential phase, the growth of a population in a phytoplankton assemblage can be expressed by

$$n_{it} = (1 - D) \cdot n_{io} \cdot e^{r_i t} \qquad (1)$$

in which n_{io} and n_{it} are the cell number or biomass of i-th population at time zero and after a period of time t (day), e is the base of natural logarithm, r_i is the specific growth constant, and D is the mortality (day^{-1}). The dependence of phytoplankton growth on nutrients can be represented by the Monod equation,

$$\mu_i = \mu_{mi} \cdot \left(\frac{S}{k_{si} + S} \right) \qquad (2)$$

where μ_i and μ_{mi} are the specific growth rate and the maximum specific growth rate of i-th species populations in unit of time (day^{-1}), s is the concentration of nutrient in waters (μg-at/l), and k_{si} is the half saturation constant for i-th species population . From eq. 2, the specific growth constant for i-th species

population will be

$$r_i = \ln 2 \cdot \mu_i \qquad (3)$$

Substituting eq.3 into eq.1, the relation between the growth of species population and the external concentration of nutrient can be written by

$$n_{it} = (1 - D) \cdot n_{io} \cdot e^{\ln 2 \cdot \mu_i} \qquad (4)$$

As inorganic nutrients are incorporated into the phytoplankton cell during the growth, the concentration of nutrients in the water decreases as the biomass of the phytoplankton assemblage increases. This situation can be expressed by

$$s_t = s_o - s_c \Sigma (n_{it} - n_{io}) \qquad (5)$$

where s_o and s_t are nutrient concentrations at time zero and after a period of time t (day), $(n_{it} - n_{io})$ is the inrease in cell number of i-th species population during the period from t_o to t, and s_c is the nutrient content in each phytoplankton cell. The successive growth of each different poulation can be obtained by repeated use of eqs. 2, 3, 4 and 5. Thereby, the cell number of each population and the total cell number of the phytoplankton assemblage can be estimated in a time sequence. The successive changes in species diversity can also be computed by the SHANNON-WEAVER function.

We applied this theoretical growth model to a phytoplankton assemblage containing three species (sp. 1, 2 and 3) all with different k_s and μ values. Inorganic nitrogen was considered to be a limiting nutrient which regulates phytoplankton growth. One hundred, 10 and 1 µg-at N/l were used as the three initial nitrogen concentrations (s). These amounts roughly correspond to those of hypereutrophic, meso- or eutrophic and oligotrophic waters, respectively. Other constants used in the calculation are presented in Table 1. The k_s values were adopted from the data for nitrate uptake of diatoms cited in the table of PARSONS and TAKAHASHI (1977). The initial cell number (n_{io}) was arbitrarily assumed to be 14 cells/ml and the nitrogen content 5 pg/cell for each species.

Under a high nutrient level, the sp.1 population increased rapidly with an abrupt exhaustion of nutrients (Fig.3-A). The number of cells reached a maximum of 25 x 10^4 cells/ml after 12 days, and subsequently maintaind a steady state with only a small fluctuation. The nitrogen concentration was depleted within two weeks, and after that it maintains a steady state with a small oscillation from near-zero to 10 µg-at N/l. This fluctuation may result from a balance between the release of nutrients through mineralization of dead cells

Table 1. Constants used for the growth model.

	k_s (μg-at N/l)	μ (doublings/day)	initial cell number(/ml)	mortality (/day)	N content per cell (pg/cell)
species 1	6.0	1.5	14	0.1	5
species 2	2.5	1.0	14	0.1	5
species 3	0.5	0.5	14	0.1	5

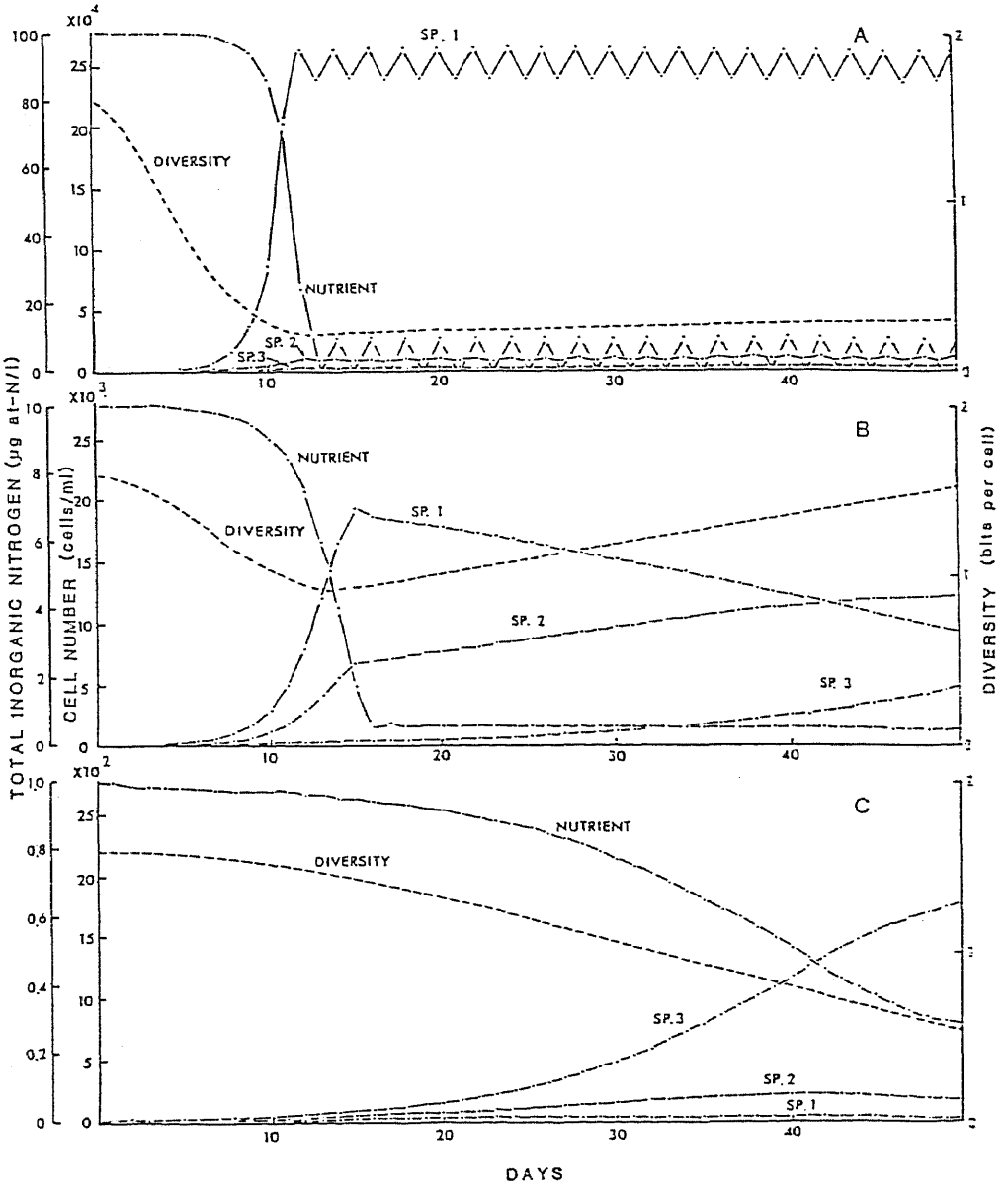

Fig. 3. Model estimations of growth of each species population in a phytoplankton assemblage consisting of three different species. Data were calculated by the growth model under different concentrations of inorganic nitrogen. Initial nitrogen concentrations were 100 µg-at N/l, 10 µg-at N/l and 1 µg-at/l.

(mortality is assumed to be 0.1 per day in the calculation) and the rate of nutrient uptake by living cells. The growths of sp.2 and 3 are considerably slower. Consequently, the sp.1 population predominates over the other species under high nutrient levels, and species diversity declines.

When the amount of the nutrient was not so abundant as in the hypereutrophic waters, sp. 1 grows faster during the initial stages with a maximum population of 19 x 10^3cells/ml, and subsequently, gradually declines due to a decrease in nutrient (Fig.3-B). The growth of sp.2, and especially of 3, is slower than sp. 1 but steady, and their cell numbers exceed the number of sp. 1 after 40 days in the former and two months in the latter. Therefore, no species predominates and the phytoplankton diversity is maintained at a high level.

In the oligotrophic environment (Fig.3-C), sp.3 grows slowly but continuously, while sp. 1 and 2 are extremely difficult to grow because of the lack of nutrients. As a result, sp. 3 predominates in the community and species diversity is reduced fairly well.

The calculated diversity at a steady state is 1.25 in the eutrophic environment and 0.25 - 0.50 in the hyper-eutrophic or oligotrophic waters. Compared with the calculated values, diversity varied seasonally from

2.0 to 3.5 in the eutrophic Lake Nakanuma and from 0.3 to 1 in the hypereutrophic Himonya Pond. The calculated and the observed values of diversity coincide fairly well.

5.4 Discussion

Several explanations have been proposed to explain the relationship between phytoplankton diversity and the trophic status of waters. Based on information theory, MARGALEF(1968) proposed that eutrophic waters are immature ecosystems affected by many disturbances such as the inflow of urban drains and fertilizers, and they contain a small amount of information (low diversity), while oligotrophic waters are mature ecosystems containing a large amount of information (high diversity). However, this approach to phytoplankton diversity does not clarify how the nutrient condition of the water affects phytoplankton communities, or how diversity responds to the trophic status of waters.

We have illustrated the mechanism which regulates phytoplankton diversity by considering the amounts of nutrients in two bodies of waters and their effect on phytoplankton growth. The results obtained demonstrate the relation of species diversity of phytoplankton

community to the trophic status of water. Namely, the diversity is low in hypereutrophic or oligotrophic waters, and high in meso- or slightly eutrophic waters. The calculations coincieded well with the patterns obtained in field surveys.

Another explanation in which nutrient concentrations in the environment were taken into account was proposed by PETERSEN(1975). He constructed a model in which two nutrients limited phytoplankton growth. In his model, the ratio of nutrient 1 to 2 regulated phytoplankton diversity. On the other hand, our model indicates that absolute amounts of nutrients can regulate the phytoplankton diversity.

Various hypotheses have been proposed to explain the mechanism which causes the differences in phytoplankton diversity. Some of these were based on the continuous variation in environmental conditions (HUTCHINSON 1961; GRNNEY et al. 1973), a patchy distribution of the phytoplankton (RICHERSON et al. 1970), or the chance for overlapping of the succeeding phytoplankton populations (MOSS 1973). Our proposal is compatible with these hypotheses. The phytoplankton diversity in natural communities would be better explained by combining our hypothsis with those previously proposed.

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Chapter 6

A mechanistic approach to competition among species in phytoplankton community for limited nutrients

6.1 Introduction

The species composition and succession of phytoplankton communities in lakes are associated with the trophic status of waters. This phenomenon will be attributed to the interspecific competition and the difference in growth responses of individual phytoplankton species to successive changes in environmental factors such as light, temperature and nutrient. Especially, nutrient regulation seems to be important at low concentrations of nutrients. Therefore,it is expected that the most fundamental approach to the understanding of mechamisms for species succession of phytoplankton communities is to predict the species-specific nutrient utilization and its relation to phytoplankton growth. CAPERON (1967) and DUGDALE (1967) showed that the rate of nutrient uptake by phytoplankton is dependent on external nutrient concentrations at low concentrations and this relationship can be described by the MICHAELIS-MENTEN kinetics. EPPLEY and THOMAS (1969) demonstrated

experimentally that the nutrient effect on phytoplankton growth can be approximately expressed by a hyperbolic function, the Monod equation, in which the essential feature can be explained by the two parameters, half-saturation constant k_s and the maximum attainable growth rate μ_m. Many studies have already revealed that phytoplankton inhabiting in eutrophic waters have higher k_s and μ_m values than those in oligotrophic waters (EPPLEY et al.,1969; EPPLEY and THOMAS, 1969; CARPENTER and GIULLARD, 1979). The Monod equation has been used recently by TILMAN and KILHAM(1976) and TILMAN (1977) to illustrate interspecfic competition of phytoplankton species for nutrients and they stressed that differences in k_s and μ_m are essential when different phytoplankton species compete their growth in natural waters of various trophic status.

In the present study all these past findings were reexamined in natural lakes of diffenent trophic conditions. Furthermore, differences in species diversity observed in natural waters were analyzed in respect of the differences in phytoplankton growth from the viewpoint of nutrient availability. Simple growth models were employed to account for the dynamics of phytoplankton community structure.

6.2 Materials and Methods

Field surveys were carried out in the water bodies of different trophic conditions. The majority of which belong to hypereutrophic or eutrophic types. Water samples were collected with an integrating sampler. A 1.5 cm inner diameter polyethylene tube connected to a suction-pump was lowered to the desired depth, and sample water was pumped up. This sampler is advantageous because it can supply a homogeneous collection of phytoplankton and collect all the phytoplankton in a complete column of water down to a desired depth (LUND and TALLING, 1957). Collected water was filtered through glass fiber filters (Whatman GF/C). Nutrient (phosphate, ammonium, nitrate and nitrite) concentrations in the filtrate samples were determined according to the methods described by STRICKLAND and PARSONS (1972).

The growth of a species population was approximated by the logistic equation,

$$n = \frac{K}{1 + k \cdot e^{-rt}} \qquad (1)$$

where n is the cell number at the time t, K is the attainable maximum cell number, r is the intrinsic

growth rate and k is the integration constant. The values of r and k were calculated by using the observed n and K. Specific growth rate (μ) was calculated by the following equation based upon the cell number increase estimated from the logistic equation,

$$\mu = \frac{1}{\ln 2} \cdot \frac{\ln n_2 - \ln n_1}{t_2 - t_1} \qquad (2)$$

where μ is the specific growth rate (doublings/day), and n_1 and n_2 are the calculated cell number(cells/ml) at t_1 and t_2 , respectively. Diversity of the phytoplankton was expressed on the genus level and it was calculated by the SHANNON-WEAVER function (1963) as described in Chapter 1 and 5.

6.3 Results

(1) Growth of some species populations in natural phytoplankton communities

Growth patterns of typical species populations are shown in Fig. 1. Growth of individual populations was expressed approximately by the logistic curve. The maximum cell number(K), the integration constant (k) and the intrinsic growth rate(r) for each species are

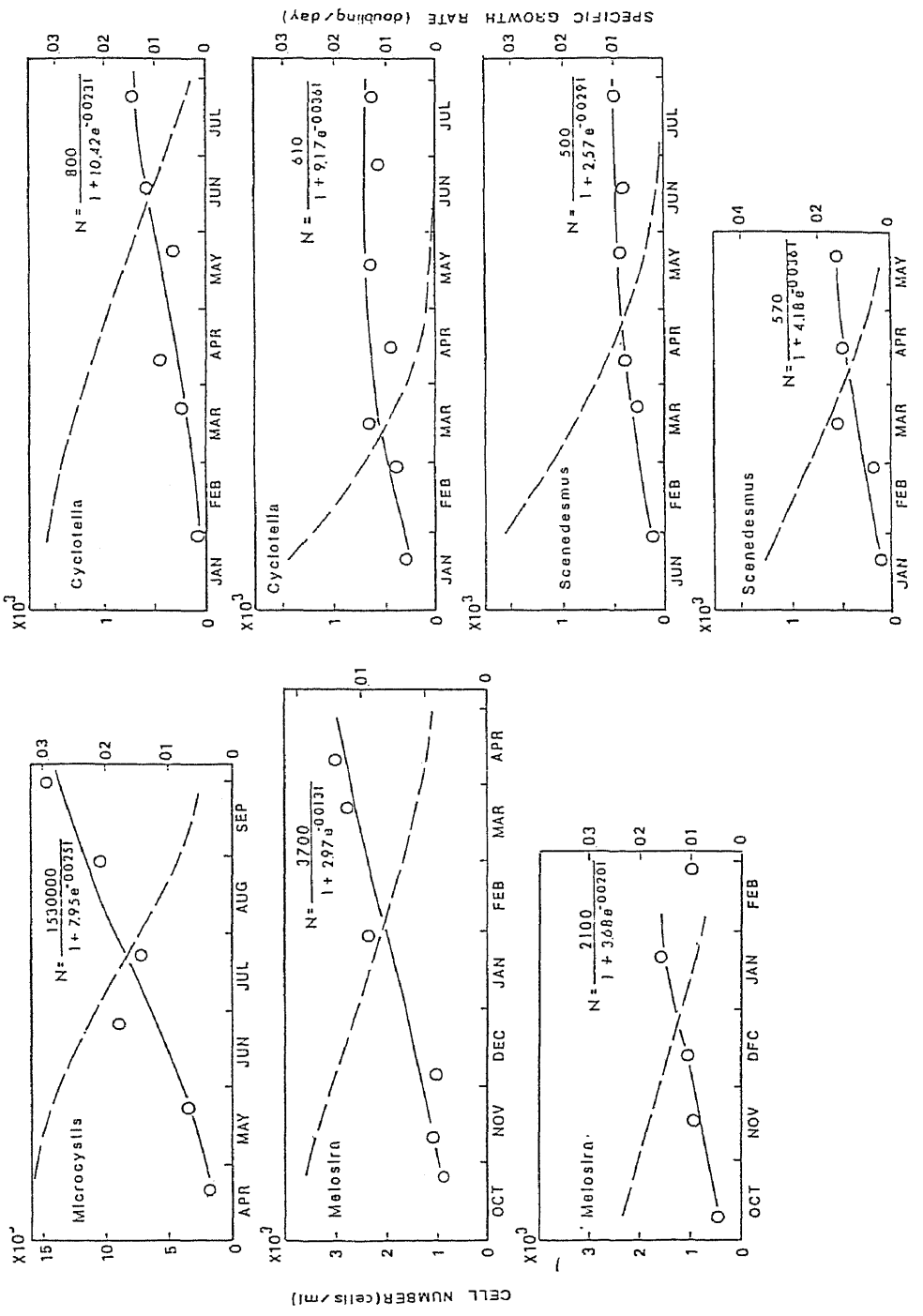

Fig. 1. Seasonal changes of cell number(○) and specific growth rate (dashed line) of representative phytoplankton. The growth curves (solid line) were calculated by use of the logistic equation.

summarized in Table 1. Microcystis was the most dominant phytoplankton in hypereutrophic waters during the summer. The values of K, k and r were 15 x 10^5 cells/ml, 7.95 and 0.025(day^{-1}), respectively, during the active growing season. Melosira predominated in eutrophic waters during the period from autumn to spring. The cardinal values for the growth curve of Melosira were K = 2.1 x 10^3 - 3.7 x 10^3, k= 2.97 - 3.68 and r = = 0.013 - 0.02. Cyclotella prevailed in eutrophic waters during summer and the cardinal values were K= 6.1 x 10^2- 8.0 x 10^2, k = 9.17 - 10.42 and r = 0.029 - 0.035. Scenedesmus also appeared in eutrophic waters during summer and the cardinal values were K = 5.0 x 10^2 - 5.7 x 10^2 , k = 2.57 - 4.18, r = 0.029 - 0.036. The growth curves which were calculated from eq. 1 by using these measured values are shown in Fig.1. The specific growth rate (μ) of each species population was calculated on the growth curve by using eq.2. The results are also shown in Fig. 1. The specific growth rate was high at the initial growth stage, and it gradually decreased with the progress of season. The lower values were predicted at the later growth stage.

(2) Relationship between specific growth rate and

Table 1. The maximum cell number (K), the integration constant (k) and the intrinsic growth rate for four freshwater phytoplankton.

	K (cells/ml)	k	r
Microcystis	1530000	7.95	0.025
Melosira	2100 - 3700	2.97 - 3.68	0.013 - 0.200
Cyclotella	610 - 800	9.17 - 10.42	0.023 - 0.036
Scenedesmus	500 - 570	2.57 - 4.18	0.029 - 0.036

nutrient concentration

The specific growth rates of each species were plotted against the given concentrations of nitrogen and phosphate encountered at the sampling time. As shown in Fig. 2, the relationship between specific growth rate and concentration of inorganic nitrogen and phosphate was approximated by the MONOD equation;

$$\mu = \mu_m \frac{S}{k_s + S} \qquad (3)$$

where μ and μ_m are the specific growth rate and maximum specific growth rate (day), respectively, s is the concentration of nutrient in water (μg-at/l) and k_s is the half-saturation constant. In each species population, the values of μ_m and k for total inorganic nitrogen and phosphate were determined by using the Monod equation, and the results are summarized in Table 2. The responses of specific growth rate to nutrient concentrations differed significantly between species. The values of μ_m ranged from 0.044 in Scenedesmus to 0.027 in Melosira for nitrogen, and from 0.046 in Microcystis to 0.024 in Melosira for phosphate phosphorus. Interspecific difference of the half-saturation constant was also noticeable. The measured

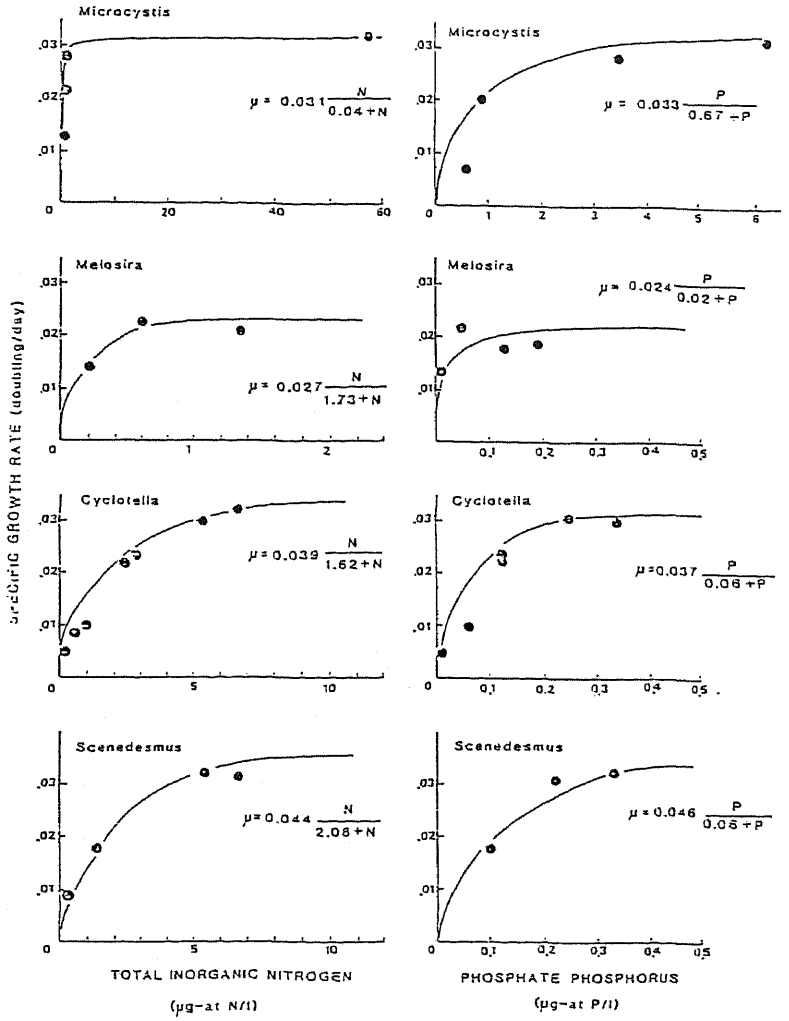

Fig. 2 Growth rates of Microcystis, Melosira, Cyclotella and Scenedesmus as a function of total inorganic nitrogen and phosphate phosphorus.

k_s in Microcystis was 0.68 µg-at P/1 for phosphate and 0.04 µg-at N/1 for total inorganic nitrogen. On the contrary, the k_s in Melosira, Cyclotella and Scenedesmus ranged from 0.02 to 0.7 µg-at P/1 for phosphate, and from 1.62 to 2.08 µg-at N/1 for total inorganic nitrogen.

(3) Growth analysis of species populations in a simulated model phytoplankton assemblage

The mathematical growth model employed in the present study was the same in principle as that used in Chapter 5. A phytoplankton assemblage was assumed to be composed of three species of Microcystis, Cyclotella and Scenedesmus. These species are widely distributed in hypereutrophic and eutrophic lakes. Procedures of growth analysis will be described briefly. At the exponential growth stage, the growth of a population of each component species in a phytoplankton assemblage can be difined as follows;

$$n_t = (1 - D) \cdot n_0 \cdot e^{-\mu t} \qquad (4)$$

where n_0 and n_t are the cell numbers of a population at time zero and after a period of time t, e is the base

of natural logarithm, r is the specific growth constant, and D is the mortality(day^{-1}).

The relationship between phytoplankton growth rate and limiting nutrients can be described by the following external nutrient control model;

$$\mu = \mu_m \cdot \frac{s}{k_s + s} \cdot \frac{s'}{k_s' + s'} \qquad (5)$$

where s and s' are the concentration of nutrients (s and s' are total nitrogen and phosphate in the present study), and k_s and k_s' are the half-saturation constants of the nutrient-response (k_s and k_s' correspond to the constants for total nitrogen and phosphate in this study, respectively).

The specific growth constant r in eq. 4 will be

$$r = \ln 2 \cdot \mu_m \cdot \frac{s}{k_s + s} \cdot \frac{s'}{k_s' + s'} \qquad (6)$$

From eqs.4 and 6, the dependency of species population growth on external concentrations of nutrient can be written by

$$n_t = (1 - D) \cdot n_o \cdot e^{(\ln 2 \cdot \mu_m \cdot \frac{S}{k_s + S} \cdot \frac{S'}{k_s' + S'}) \cdot t} \qquad (7)$$

Then the decrease of external nutrients (nitrogen

and phosphate) caused by the growth can be expressed as follows;

$$s_t = s_o - s_c \quad (n_t - n_o)$$
$$s_t' = s_o' - s_c' \quad (n_t - n_o) \qquad (8)$$

where s_o and s_t are the concentration of external total inorganic nitrogen at time zero and t; s_o' and s_t' are the concentration of external phosphate at time zero and t; s and s' are the content of nitrogen and phosphate in a phytoplankton cell, respectively. At the first step for the estimation of parameter s and s' in the three species Microcystis, Cyclotella and Scenedesmus, the carbon content in a cell was calculated from the cell volume of each species by using the STRATHMAN equation (1967). Then nitrogen content(s_c) was determined from the carbon content by use of a C:N ratio of 3 (ANTIA et al.,1963). The content of phosphate (s_c') per cell was estimated from the content of carbon per cell by using a N:P ratio of 25 for Microcystis, 15 for both Cyclotella and Scenedesmus. The parameters μ_m, k_s and k_s' for the three species were taken from Table 2. The concentration of nitrogen and phosphate in a postulated water was set to be 50 μg-at

Table 2. The growth parameters and nutrient contents for four freshwater phytoplankton.

	k_s (μg-at N /l)	k'_s (μg-at P /l)	μ_m (doublings /day	N content (μg-at N /cell)	P content (μg-at P /cell)
Microcystis	0.04	0.67	0.031 - 0.033	3.75×10^{-7}	1.43×10^{-8}
Melosira	1.73	0.02	0.024 - 0.027	1.07×10^{-6}	7.13×10^{-7}
Cyclotella	1.62	0.06	0.037 - 0.037	1.10×10^{-6}	7.33×10^{-7}
Scenedesmus	2.08	0.06	0.044 - 0.046	1.39×10^{-6}	9.26×10^{-7}

N/l and 10 µg-at P/l for a hypereutrophic water, and 10 µg-at N/l and 1 µg-at P/l for a slightly eutrophic water. These nutrient concentrations are frequently encountered in natural surface waters of lakes. By using these parameters, the successive growth of each different population under given conditions was calculated with a computer by repeated use of eqs. 5, 6, 7 and 8. The results are indicated in Figs. 3A and B. Under the hypereutrophic status three species populations increased equally in the first 20 days, depending on abundant nutrients. During this period total inorganic nitrogen decreased rapidly and reached a low level of 0.001 µg-at N/l. On the contrary, phosphate still remained at a high level of 7.9 µg-at P/l. After 20 days both species Cyclotella and Scenedesmus did not show any increase and maintained a steady state. Microcystis increased exponentially and reached a maximum of about 25 x 10^4 cells/ml after 180 days. At the steady state Microcystis population comprised 70% of total cell number in a postulated phytoplankton assemblage. Diversity of this phytoplankton assemblage dropped to the low level of 0.90 at the steady state. Both Cyclotella and Scenedesmus have a high k_s for nitrogen, and therefore they are not able to increase in nitrogen-depleted waters even though

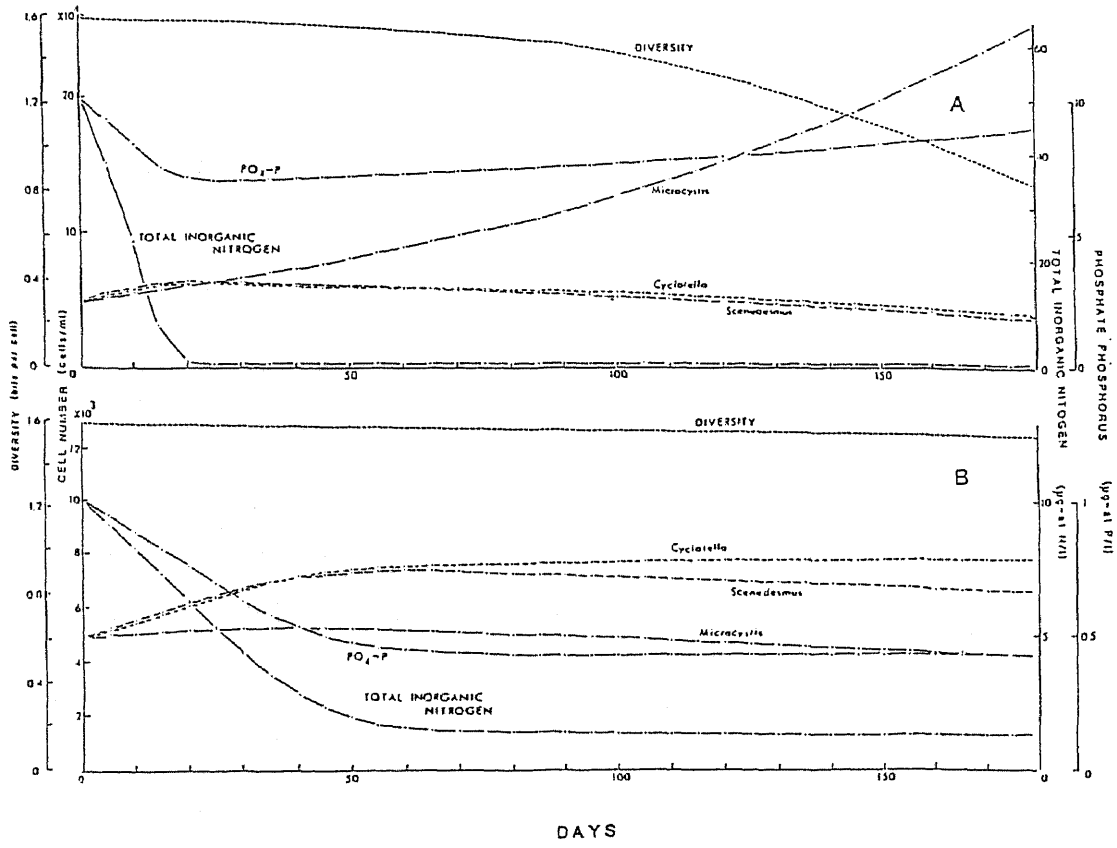

Fig. 3 Model estimations of growth of Microcystis, Cyclotella and Scenedesmus under different concentrations of inorganic nutrients. Initial concentrations of total inorganic nitrogen and phosphate phosphorus were 50 µg-at N/1 and 10 µg-at P/1 for (A), and 10 µg-at N/1 and 1 µg-at P/1 for (B).

phosphate existed at a high level. In the medium of slightly eutrophic status, the populations of Cyclotella and Scenedesmus increased slowly at the initial stage and reached a plateau after 2 months in the former and 180 days in the latter, with a cell number of 7.9×10^3 and 7.7×10^3 cells/ml, respectively. The decrease in nutrients occurred gradually and reached the steady state after one month. The populatons of Scenedesmus and Microcystis decreased slightly at the latter stage. Under such slightly eutrophic conditions any phytoplankton species cannot develop into a large population and the phytoplankton diversity is maintained continuously at a high level of 1.58.

6.4. Discussion

As shown in Chapter 1, the species composition of phytoplankton communities and the relative dominance of the different species, namely species diversity, correlated strongly with the trophic status of waters. The ecological significance of nutrients to this natural phenomenon may be interpreted through investigation of nutrient kinetics of individual phytoplankton species, and the investigation may elucidate the mechanisms of population dynamics. A positive correlation was

verified between phytoplankton growth rates and limiting nutrient concentrations in the present study as well as the study of EPPLEY and THOMAS(1969). This relation was described by the MONOD equation, and the parameter k_s was considered to be species specific because the parameters characterize the relative ability of individual species to use low levels of nutrients. Theoretically, interspecific competition for nutrients determines community species structure; phytoplankters characterized by lower k_s values predominate in waters with lower nutrient concentrations, while species charracterized by higher k_s values dominate in waters with richer nutrients. This suggests that the progressive changes in available nutrients result in continuous selection of species which adapts to nutrient changes. Thus, there have been numerous measurements of k_s for many species of marine phytoplankton. However, most of them have been made on nutrient uptake rather than the growth , and can provide less physiological information. In recent years, there have been reported studies on the k_s for growth of natural phytoplankton. CARPENTER and GUILLARD (1971) have shown that the k_s values for NO_3-N-limited growth of planktonic algae isolated from coastal water varied from 1.6 to 6.8 µg-at N/l. Similar level of the k_s for NO_3-N has been reported in NO_3-N-

limited natural phytoplankton population by EPPLEY et al. (1969). In the present study, the k_s values for nitrogen-limited growth ranged from 1.62 μg-at N/l for Cyclotella to 2.08 μg-at N/l for Scenedesmus, except for the low value in Microcystis. These values coincided well with those of marine species isolated from eutrophic regions. It should be noted that Microcystis has a low k_s value for nitrogen-limited growth, and that the k_s value was similar to the nutrient concentrations encounterd in summer under natural conditions. Microcystis frequently formed the remarkable dense population and comprised more than 90% of total cell number even when the inorganic nitrogen was reduced to a undetectable level.

There is considerably less information available on the k_s values for PO_4-P-limited growth. BENDORF (1973) reported that the k_s for PO_4-P-limited growth of Asterionella formosa was 0.02 μg-at P/l. TILMAN and KILHAM(1976) have shown that the k_s of Asterionella formosa was significantly lower than that of Cyclotella meneghiniana. The k_s value was 0.02 μg-at P/l for the former and 0.25 μg-at P/l for the latter. In the phosphate uptake experiment made by RHEE(1973) for Scenedesmus sp., the k_s value of 0.6 μg-at P/l was reported. The results obtained in the present study

were 0.06 µg-at P/l for Cyclotella and 0.10 µg-at P/l for Scenedesmus.

The interspecific competition for nutrients is often believed to be a major determinant of the species composition and succession of phytoplankton communities. As examined experimentally by TILMAN(1977) and explored theoretically by HSU et al. (1977), the species with the lowest k_s may overcome all other species. The dominance of this species could be a cause of decrease in the species diversity in phytoplankton communities. For predicting the dynamics of phytoplankton communities, the MONOD nutrient kinetics has been used by many investigators but several important factors have been frequently ignored in application. First, it has been assumed that the uptake and growth of phytoplankton do not affect the concentration of the nutrients. However, nutrient concentration is the property of the environment that responds to changes of phyptolankton, and growth is not independent of changes in nutrient levels. Second, the MONOD nutrient kinetics has usually been used under the simple assumption of an average phytoplankton population, although knowledge of complex multispecies communities is necessary for treatment of species competition and succession. The errors associated with the calculation in which an average k_s value is used

instead of many k_s values for individual species may not be negligible in diverse communities that consist of many species. The kinetics of different species is not similar. For examination of this subject, the dynamics of the three species populations and two nutrients, inorganic nitrogen and phosphate phosphorus, were predicted in the present study by use of the MONOD model and the logistic population growth model. Both models were numerically solved by using the the parameters k , μ_m and concentrations of nutrients (s) observed in nature. The population dynamics predicted by the models for the three species populations are consistent with the results in Lake Nakanuma and Himonya Pond. Thus, this consistency supports the applicability of the nutrient competition theory proposed to explain succession of phytoplankton communities.

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Summary and General Conclusion

During the last two decades, the investigations of primary production of aquatic ecosystem have progressed markedly in phytoplankton ecology. In these studies, phytoplankton communities that consists of many different species have been mostly treated as a whole and little attention has been paid to the metabolic activities of individual component species. On the other hand, intensive studies have been done on the physiological characteristics of individual phytoplankton species by using chemostat cultre or batch culture. The nutrient uptake kinetics and its relation to phyto-plankton growth has been also studied by marine ecologists for understanding of species competition for nutrients which are considered to be important in determination of succession of phytoplankton communities. Furthermore, conbined effects of environmental variables such as light, temperature and nutrients have also been investigated in unialgal culture. The behavior of each phytoplankton species in nature can now be explained to some extent on the basis of physiological knowledge obtained by these studies. However, there have been only a few studies concerned with phytoplankton communities from the viewpoint of

population dynamics.

The present study was carried out to explain the mechanisms regulating interspecies competition and succession of phytoplankton community in lakes. Especially, photosynthetic characteristics and nutrient kinetics of individual species were taken into account, because the two factors (light and nutrients) play a most important role in regulation of phytoplankton dynamics.

This dissertation is divided into six parts, first of which deals with some basic subjects concerning community diversity and trophic status of waters. Phytoplankton diversity in 24 water bodies of different trophic status was evaluated by the SANNON index. Higher diversity was obtained in meso- and slightly eutrophic waters and a low diversity was observed in oligotrophic- or highly eutrophic waters. This finding differed somewhat from that reported by MARGALEF(1964). He showed that species diversity of phytoplankton community was high in oligotrophic lakes and low in eutrophic lakes. This discordance may be partly due to the difference in definition of lake types. With the progress of eutrophication, species structures of phytoplankton communities would change successively from a simple species structure in oligotrophic waters to a

complex multispecies structure in mesotrophic waters and then again to a simple structure in hyper eutrophic waters.

The second part presents the new technique for measurement of photosynthetic activity of individual species in mixed phytoplankton populations. Particulary, the applicability of grain density autoradiogaphy to the quantitative determination of phytoplanktonic species photosynthesis was tested. Sources of errors which have been inherent in this method were checked by using cultured algae. The effects of development time, chemography and latent image erasure were completely excluded by the newly devised technique. Grain density autoradiography could now be applicable to the quantitative determination of the photosynthesis of a phytoplankton cell, if the treatments are made carefully. This new method is considered to be a useful tool in the field of phytoplankton ecology.

The third part deals with phytoplankton species production in natural phytoplankton communities in the waters of different trophic status. Phytoplankton species production varied widely with species and their physiological states. The data also gave information on the contribution of the component species production

to total community production.

In the fourth part, photosynthetic characteristics and *in situ* growth of individual dominant species were studied in a samll eutrophic lake. Photosynthetic nature of individual species expressed by the photosynthesis-light curve was a shade type in samples taken from deep metalimnetic layers and a sun type in samples from shallow euphotic layers. By using the observed photosynthesis-light curve, the successive growth of phytoplankton species under a given light condition was calculated by a simple growth model. The growth patterns predicted by the model agreed with the observed ones. Thus, it is concluded that the development of phytoplankton populations would partly be regulated by species-specific photosynthetic responses to the light conditions. These results can be considered to be evidence for the predictive ability of a mechanistic approach to vertical successional changes of phytoplankton communities in lakes.

The fifth part is designed to confirm the correlation between species richness of phytoplankton communities and nutrient levels of waters, and to propose a plausible mechanism for regulation of species diversity. Species diversity in both eutrophic and hypereutrophic waters showed seasonal changes, and phytoplankton

communities were more diverse in the eutrophic waters than the hypereutrophic waters. The dependence of phytoplankton growth on nutrient is expressed by the MONOD equation. By using this simple growth model, the relationship between phytoplankton diversity and the nutrient of waters was evaluated under plausible conditions. A low diversity phytoplankton community developed in hyper- or oligotrophic conditions and a plankton community with high diversity occurred in meso- or eutrophic waters. This result suggested that species respond in special ways to different nutrient levels.

The final part is concerned with the relationship between growth rates of phytoplankton species and external nutrient concentrations in natural waters, and is designed to test whether theoretical approaches presented in the Chapter 5 provide valid information on the interspecific nutrient competition for phytoplankton dynamics. In the present study, the dependence of growth rate of phytoplankton species on the two major nutrients, nitrogen and phosphate, was described by the MONOD equation. The half-saturation constant k_S of natural phytoplankton species was 0.04 to 1.62 µg-at/l for NO_3-N-limited growth and 0.06 to 0.1 µg-at P/l for PO_4-P-limited growth. The dynamics of the mixed

populations of three species and nutrients was predicted under given conditions by use of the MONOD model of nutrient competition. The calculated results showed that the species with lower k_s were able to grow at lower nutrient concentrations much better than those with higher k_s. The predicted population dynamics were in good agreement with the observed dynamics. Thus, the nutrient competition theory may serve as a mechanistic basis for determination of species composition and succession of phytoplankton communities.

Reference

MARGALEF, R. (1964) Correspondence between the classic types of lakes and the structural and dynamic properties of their populations. Verh. Int. Ver. Limnol. 15: 169 - 175.