Note

The Use of Dialysis Culture Chamber to Measure N/C and P/C Ratios of Individual Phytoplankton Species

Yasunori WATANABE

Abstract

A dialysis culture chamber was constructed and used to determine cellular N/C and P/C ratios and *in situ* growth rate of individual phytoplankton species. The chamber was made of acrylic tubing and membrane filters of 47 mm in diameter for the diffusion boundary. A much higher permeation rate of dissolved ion into the chamber was obtained when the Nuclepore filters (0.2 and 3.0 μm porosity) were used as the boundary, compared to the case when the cellophane membrane was used. Six species of planktonic algae were separately incubated in the chamber equipped with 1.0 μm porosity Nuclepore filters and suspended in a eutrophic pond for four days. The species specific growth rate and the cellular N/C and P/C ratios of these algal populations were obtained. The use of the dialysis culture chamber was thought to be effective in studying chemical composition of the species population in the fields. A few comments on the field use of dialysis culture chambers were made.

Key words : Dialysis culture chamber, Algal N/C and P/C ratios, Individual phytoplankton species, nutrient deficiency

Relative content of nitrogen and phosphorus to carbon (N/C and P/C ratios) in phytoplankton have been shown as good indices of the nutrient status of the community and the water bodies (HEALEY and HENDZEL, 1980 ; TEZUKA, 1985). Although it is difficult to obtain the values of N/C and P/C ratios for an individual phytoplankton species which is usually present in mixed species populations under natural conditions, the species characteristics of these ratios must be of considerable interest for analyzing the nutritional status of each population and interspecies competition for nutrient as well as for assessing the food relation between phyto- and zooplankton.

In the present study, we constructed a dialysis chamber in which an individual species of planktonic algae was separately incubated and then suspended in a eutrophic pond. The N/C and P/C ratios could be determined for each species incubated under the same environmental conditions.

The dialysis culture chamber was composed of 3 mm thick transparent acrylic tubing, acrylic plates and membrane filters (Fig. 1). The volume of the culture space was 28 m ℓ (25 mm in thickness and 38 mm in diameter). Membrane filters of 47 mm in

diameter were used as a permeable boundary between the culture space and outer environment. Glass fiber filters (GF/C, 47 mm in diameter) of which centers were cut out were used as "O-rings" to ensure the sealing of the spaces between the membrane filters and the acrylic plates on both sides. The chamber was assembled with six sets of stainless-steel bolts and nut. Two sampling ports were made by tapping 10 mm holes through the central section of the chamber and fitting them with

Fig. 1. The dialysis culture chamber. MF : membrane filters of 47 mm in diameter, O : o-rings made of glass fiber filter, Sp : sampling port, P : silicon rubber plug, B and N : stainless steel bolt and nut.

silicon rubber plugs after algal culture was filled in.

The exchange rate of water (or solutes) through the filters is an important characteristics for the experiments with the chamber system. The rates of diffusion and permeation of an inorganic ion from the outer environment to the inner space of the chamber were determined for the chambers with polyethylene sheet, cellophane membrane, or Nuclepore filters as a dialysis boundary (Fig. 2). The chambers filled with distilled water at the beginning were placed in a tank with 10 ℓ of NaNO_2 solution ($3.5 \text{ mg N } \ell^{-1}$). The solution was kept at about 22°C and gently stirred during the experimental period. The water in the chambers was subsampled at intervals and the NO_2^- concentration was

Fig. 2. Permeation of nitrite ion into the dialysis culture chamber equipped with polyethylene sheet, cellophane membrane, or Nuclepore filters. The broken line shows the ion concentration in the surrounding water.

monitored. Polyethylene sheet did not allow to permeate the ion completely into the chamber, showing no leakage of the chamber assemblage. Cellophane membrane sheet used for the usual dialysis bag (Seamless Cellophane Tubing, Union Carbide Co.) allowed permeation of the ion, but more slowly than Nuclepore filters with a pore size of 0.2 and $3.0 \mu\text{m}$. The mode of the ion permeation through the Nuclepore filters was almost exponential; the half exchange period was about 1.7 and 0.9 hr for 0.2 and $3.0 \mu\text{m}$ pore filters, respectively. Although the exchange rate would vary with such environmental parameters as water temperature and water turbulence, the exchange rate of the Nuclepore-chamber appears to be sufficient to sustain the nutrient uptake for the growth of phytoplankton cells in the chamber unless very dense algal suspension is contained. Because doubling time of phytoplankton cells is usually more than several hours.

Six algal species, that is, *Microcystis aeruginosa*, *Pediastrum boryanum*, *Scenedesmus quadricauda*, and *Fragilaria* sp. isolated from Himonya Pond, *Anabaena macrospora* from Lake Kizaki, and *Chlorocella ellipsoidea* from a culture collection were precultured in the laboratory and separately incubated in the chambers equipped with $1.0 \mu\text{m}$ Nuclepore filters. And then they were suspended at 20 cm depth (relative light intensity of about 30%) in hypereutrophic Himonya Pond for four days, when the water temperature was 24°C . The algal population density, cellular N/C and P/C ratios were measured before and after the incubation (Table 1). The cellular C and N were determined with a CHN analyzer (Yanagimoto, MT-3), and cellular P was

Table 1. Changes in cell density, growth rate, N/C and P/C (weight ratio) of phytoplankton species incubated in the dialysis culture chambers which were suspended in Himonya Pond. N/C and P/C are the mean of two replicates.

Species	Cell density (No. $\text{m}\ell^{-1}$)		Growth rate (μ : day^{-1})	N/C		P/C ($\times 100$)	
	0 day	4 day		0 day	4 day	0 day	4 day
<i>Microcystis aeruginosa</i>	7.57×10^5	1.15×10^6	0.105	0.174	0.162	7.30	1.60
<i>Anabaena macrospora</i>	1.02×10^3	$7.00 \times 10^{1*}$	-0.669	0.176	0.132	2.34	7.50***
<i>Scenedesmus quadricauda</i>	3.36×10^4	1.76×10^5 **	0.414	0.148	0.134	5.79	1.67
<i>Chlorocella ellipsoidea</i>	1.06×10^7	1.00×10^7	-0.015	0.162	0.154	4.76	2.56
<i>Pediastrum boryanum</i>	5.82×10^3	7.08×10^3 ***	0.049	0.165	0.143	2.70	1.24
<i>Fragilaria</i> sp.	6.75×10^4	1.40×10^5	0.182	0.150	0.138	2.91	2.59
pond seston				0.161	0.160	1.32	1.37

* trichome length(mm), ** number of coenobium, *** doubtful datum in single analysis

determined colorimetrically after oxidizing the algal samples with potassium persulfate in an autoclave. The chambers with no algal inoculation were used to correct the background C, N and P derived from seston in the pond water which would enter into the chamber through the filter. As the cellular size of all the algal species examined was more than $1\ \mu\text{m}$, loss of the cells through the filters could be neglected. The cell increment in the chamber corresponds to the net growth of the algal population during the incubation period. The growth rate in terms of population density (μ , day^{-1}) during the incubation differed among the phytoplankton species: *M. aeruginosa*, *S. quadricauda* and *Fragilaria* sp. showed a positive growth while no significant change was observed in *C. ellipsoidea* and *P. boryanum*, and a highly negative growth in *A. macrospora*. The best grown alga was *S. quadricauda*; its mean doubling time was 1.67 d which is fairly high value as a field population. The case of *A. macrospora* suggests that decomposition or autolysis of the cells exceeded the cell division rate. Many bleached cells of the alga were observed microscopically in the incubated sample.

The ratios (by weight) of N/C and P/C observed were species specific. Both the ratios in all the species were higher at the beginning than after the four-day incubation in the pond, probably due to the effect of precultures of the algae which were made with inorganic medium containing a high level of nitrogenous and phosphorus ions. After the incubation, the N/C ratio of *M. aeruginosa* was very similar to that of the pond seston which was dominated by *Microcystis* spp. and several desmid species. However, all other species showed a lower N/C ratio, irrespective of the presence or absence of positive growth. The relatively high N/C ratio (0.161) of the pond seston may partly be attributable to the small particles including bacterioplankton, because the seston entering the control chambers with no algal inoculation showed N/C and P/C ratios of 0.22 and 0.0156, respectively. The P/C ratio in all species except *P. boryanum* was higher than in the pond seston. Among them *C. ellipsoidea* and *Fragilaria* sp. showed the ratio about twice the ratio of the seston. This may be due to their ability of P storage in the cell.

Although these observations were preliminary and some further examinations on such as the effect of incubation period on the algal growth are needed, N/C and P/C ratios of individual phytoplankton

species obtained here have never been obtained by an analysis of total seston in water. The difference in these ratios between species which were incubated under the same environmental conditions suggests that 1) N and P requirement are different among species, or 2) the N and P contents observed in each species are the result of a competitive interaction between species.

SAKSHAUG and OLSEN (1986) have reported that the N/C ratio was highly species specific even under N saturated conditions; for example, the N/C ratio in the blue green *Anabaena flos-aquae* was 0.29 against 0.07–0.082 in the desmid *Staurastrum luetkemuellerei*. They also suggested the significance of the difference in the nutrient requirement in analysis of interspecific competition. The use of dialysis culture chamber will contribute to measure the chemical composition of phytoplankton species under a nearly natural condition and to assess the effect of nutrient limitation on their population growth in the fields.

The dialysis chamber, in its present form, is also available for determination of the growth rate (cell division rate) of individual species (CRUMPTON and WETZEL, 1982), or to examine survival and morphological change of microorganisms under nearly natural conditions (TRAINOR, 1965; MCFETERS and STUART, 1972). The cellophane dialysis bag may also be useful. However, in studies using such chamber or bag systems, one should be sure that the permeation rate can support the algal growth in the chamber or bag similar to the case under field conditions. Other problems should also be discussed in connection with the field use of the dialysis culture chamber.

Microbial growth on the membrane boundary has often been pointed out as the fouling affecting the algal growth in the chamber. We compared three kinds of boundary materials, cellophane, Nucleopore filter and a membrane filter of nitroacetate (Millipore, RA), by incubating the chambers equipped with these membranes in the hypereutrophic pond for two weeks. The fouling was most serious on the Millipore chambers and least serious on the Nucleopore ones. The texture of the Nucleopore filter was thought to be most suitable for the present purposes in these materials. The precipitation of the algal cells in the chamber during the incubation was still thought to be one of the drawbacks of the chamber experiment. Some steps should be taken to keep the algal cells in suspension in the chamber. One of the advantages of the dialysis chamber reported here

is that the membrane or filter boundary is easily changeable according to the purpose and organisms of the study. The use of the chamber equipped with the Nuclepore filters of suitable pore size can keep the algal cells under the conditions closer to the natural habitat, as compared with cells incubated in a cellophane bag which allow permeation only by low molecular weight substances.

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摘 要

プランクトン藻の種類別 C : N : P 比測定への 透析培養チェンバーの利用

プランクトン藻を種類別に野外条件下で培養し、増殖速度と細胞態の N/C と P/C 比を測定するために小型の透析培養チェンバーを製作した。チェンバーの基本構造は透明アクリルパイプの両端に直径 47 mm のヌクレポアフィルターを装着した容量 28 ml のものである。フィルターは研究の目的と対象生物に応じて種々の孔径のフィルターあるいは半透膜などと交換が可能である。チェンバー内外の水の交換性を調べたところ、ヌクレポアフィルター（孔径 0.2 および 3.0 μm ）はセロファン膜よりも交換速度が速かった。また、野外での培養時に障害となる膜面への微生物の付着もヌクレポアフィルターは比較的になく、透析培養の界面素材として適していることがわかった。

1.0 μm 孔径のヌクレポアフィルターを装着したチェンバーに、6 種類のプランクトン藻を個別に封入して富栄養池中で 4 日間培養した。その結果、種類別の増殖速度および細胞態の N/C, P/C 比が求められた。野

外における透析チェンバー利用についての有効性と問題点について考察した。

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(著者：渡辺泰徳，東京都立大学理学部生物学教室，〒158 東京都世田谷区深沢 2-1-1；Yasunori WATANABE, Department of Biology, Tokyo Metropolitan University, 2-1-1, Fukazawa, Setagaya-ku, Tokyo 158, Japan)

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