THE POPULATION DYNAMICS OF THE BACTERIOPLANKTON COMMUNITIES IN THE MESOTROPHIC BOG ENVIRONMENTS

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CONTENTS

RESULTS AND DISCUSSION

SUMMARY

The significance and contribution of bacterioplankton activities in the geochemical dynamics were studied with special reference to the eutrophication process in the mesotrophic bog ecosystems. The research was made on the bacterioplankton communities in Matsumi-Ike Bog and Doh-Hoh-Numa Bog in Tsukuba Science City. The microbial role in the dynamics of the bogs was analyzed statistically with special reference to the important environmental factors: i.e., the concentration of dissolved organic carbon (DOC), the population density of bacterioplankton and the water temperature.

In Matsumi-Ike Bog, every ecological factor showed the steady-state oscillation in a range near the eutrophic part of the mesotrophic system. In this bog, the population growth rate of bacterioplankton community was determined with the chemostat. The growth rate was regulated by the DOC concentration as the limiting factor. The growth rate was also affected profoundly by non-limiting factors, primarily by the population density of bacterioplankton and secondly by the water temperature. Through a series of natural feed-back loops as influenced by these environmental factors in the bog, the generation time of bacterioplankton within the 95% confidence interval was maintained in the range between 5 and 20 hours (growth rate between 0.03 to 0.14/hr).

In the conventionally natural environment of Doh-Hoh-Numa Bog, the population growth rate of bacterioplankton was also regulated primarily by the DOC concentration as the limiting factor in two different modes, as dividing the study year into two distinguished periods: one period while phytoplankton was the major DOC source, and the other period while dead littoral vegetation in the bog sediment was responsible for supplying the DOC into the watercolumn. The annual average of the growth rate of bacterioplankton was 0.08/hr (generation time of 87 hours), therewith the daily bacterial production rate was 63 μgC/l/day on the annual average.

A new type of water purification system, the "Bio-filter system", has been introduced into Doh-Hoh-Numa Bog in 1985. Rapid oligotrophication by the Bio-filter system has changed the ecosystem of Doh-Hoh-Numa Bog from a moderate mesotrophic system to the oligotrophic end of the mesotrophic system. Since the Bio-filter system started its operation, the population density of bacterioplankton and the concentration of inorganic nitrogen have decreased with statistical significance, whereas the concentrations of phosphate and DOC have increased significantly for some months. The DOC concentration was higher during the operation period than non-operation period, although environmental factors relevant to the autotrophic process were shifted to the direction of oligotrophication. The bacterioplankton in the bog were shown to modify their

physiological kinetics to maintain the growth rate at the optimal level, even when they were exposed to the rapid oligotrophication. The population growth rates thus showed no obvious increase or decrease with this rapid artificial oligotrophication within the mesotrophic system, and continued to be controlled primarily by the DOC concentration even after the Bio-filter system operation was discontinued.

The bacterioplankton activities are analyzed to response so well to any eutrophication process in the mesotrophic bog environments that a stable steady-state equilibrium has been attained between the biological processes of microorganisms and their physico-chemical environment.

INTRODUCTION

1. Bacterioplankton in Nature

The bacteria inhabit every place in the bioshpere: Their habitats range from the favourable conditions such as rumen or sewage, to less comfortable habitats such as Antarctic ice (Sullivan & Palmisano, 1984), where other multicellular organisms can rarely live (Kushner, 1978). They can live in the very vicinity of deep-sea hydrothermal vents (Karl, 1987; Jannasch, 1984), and survive even in an artificial Mars condition (Imshenetskii et al., 1984).

In the biosphere, every biological element passes through a continuous cycle. In this cycle, microorganisms have been shown to be greatly responsible for the decomposition of organic materials due to (e.g., Seki, 1982a):

1) their omnipresence throughout the biosphere,
2) their versatile activity to decompose and utilize almost all kinds of organic compounds,
3) their high rates in metabolism and growth.

For example, over 90% of the carbon dioxide production in the biosphere has been estimated to result from the heterotrophic processes of microorganisms (e.g., Stanier et al., 1963). As such an estimation has only been made approximately for the microbial community as a whole, each process of a certain group of microorganisms in a certain natural habitat has not been

determined systematically.

This particular study focuses on the growth rate of heterotrophic bacterioplankton in the aquatic environments. This is because the bacterioplankton may play major roles in the heterotrophic processes among all kinds of microorganisms, and their growth rates can represent the physiological activity and their contribution degree to the production in the detritus food chains (e.g., Seki, 1982a). The growth rate of microorganisms has been determined by various methods as reviewed by Brock (1971), whereas it is also shown that the bacterial growth reflects rapidly the changes of environmental conditions. Thus the bacterial growth in nature can be used as a promising indicator to assess the environmental changes. In this case, the methods for determining the growth have been well developed accordingly to the bacterial status such as free-living or attached (Brock, 1971).

Bacterial forms in the aquatic environments can be classified generally into two groups depending on the mode of life with special reference to the liquid-solid phases in natural waters: i.e., bacterioplankton in the free-living form, and epibacteria (attached bacteria) on the solid surface. Quantitative significance of each bacterial form has not been clarified both in the heterotrophic processes in the geochemical cycles and in trophodynamics of the food webs. Some works show the important contribution of bacterioplankton in these processes

(e.g., Fuhrman & Azam, 1980; Jordan & Likens, 1980; Wiebe & Pomeroy, 1972; Zimmermann et al., 1978), the others emphasize the contribution of epibacteria to the processes (e.g., Goulder, 1977: Harvey & Young, 1980; Kirchman & Mitchell, 1982; Pedros-Alio & Brock, 1982; Seki, 1972; Wilson & Stenvenson, 1980). Their conclusions are manifold partly because of the different aquatic environments for their studies and partly because of the lack of suitable techniques. The lack of suitable techniques causes the greatest problem in counting the epibacteria, although some devices have been developed; The slide-glass suspending methods have usually been most successfully applied to determine the growth rate of attached bacteria (e.g., Ierusalimsky, 1954). The capillary methods have been practically applied for individual cell (Perfil'ev & Gabe, 1969). Still the lack of suitable techniques becomes absolutely critical at the occasion of the quantitative estimation for epibacteria inside the aggregates in the natural waters. Some aggregates as large as the size of "marine snow" can rarely be examined under microscopic examination due to the problem of sampling size, although these large particles with numerous epibacteria must take part in definitely significant fraction of particles in natural waters (Hood, 1970). Hence the ecological contribution of epibacteria to the aquatic ecosystems should be underestimated, in spite of their possibly greatest responsibility for most of the biochemical changes that living

things bring about in the aquatic environments due to favorable effect of solid surface on the epibacterial metabolism (ZoBell, 1943).

On the other hand, the biomass and growth rates of bacterioplankton can be quantified more precisely and easily with the current microbiological techniques. At present the study of their population dynamics is thus of great use for their contribution to the aquatic ecosystems.

Here the word "bacteria" (singular, bacterium) is derived from Greek bakterion, which is diminutive of baktron meaning Walking-stick. This is akin to Latin bacillum, which is composed of bac- (baculum, = walking-stick) and -illum (diminutive suffix). Bacteria were regarded as animals from the first observation in 17th century by Antony van Leeuwenhock at the time of 1841, when F. Dujardin placed them into a kingdom Protista, midway between the animal and plant. However, in 1859, C. Davaine showed their alliance with algae. Later Louis Pasteur called the anaerobic bacteria "animalcules infusories" in his paper in 1861. The term "bacteria" started to be used commonly in the decade of 1870 by Ferdinand Julius Cohn and Robert Koch. Cohn classified bacteria in his papers "Untersuchungen uber Bakterien" (1872-1875) as ".... the smallest, and at the same time the simplest and lowest of all living forms, we call Bacteria".

The word "plankton" was derived from Greek planktos (=

drifting), composed of plank- (variant of plang- which is stem of plazesthai = to drift) and "-tos" (verbid suffix). Plankton are classified into various sub-groups according to species, sizes, and habitats as follows:

Organism	Depth
bacterioplankton	epiplankton
phytoplankton	mesoplankton
zooplankton	bathyplankton
	hypoplankton

Size	Light Intensity
megaloplankton	panteplankton
macroplankton	phaoplankton
mesoplankton	knephoplankton
microplankton	skotoplankton
nannoplankton	
picoplankton	
ultraplankton	

Habitat	Period
marine plankton	holoplankton
brackish plankton	meroplankton
freshwater plankton	tychoplankton
neritic plankton	
oceanic plankton	
limnoplankton	
eulimnoplankton	
heleoplankton	
potamoplankton	
(aeroplankton)	

2. Eutrophication

At different aquatic ecosystems with the common characteristics in relation to eutrophication, the bacteria therein play their role differently in recycling of biological elements as regulated not only by the limiting factor (Liebig, 1840) but also greatly by the different non-limiting factors (Kang & Seki, 1984; Liu & Seki, 1987, in press; Masaki & Seki, 1984; Ohle, 1965; Shiraishi et al., 1985; Tsuchida et al., 1984). As an extreme example, Ohle (1980, 1984) shows also that aquatic bacteria contribute to the Short Circuit Metabolism playing a critical role in the eutrophication process in lakes through active mineralization of organic compounds. Hence, the bacterial contribution to the biochemical processes in an aquatic ecosystem can become multiple by various combinations of environmental factors in the same trophic system.

The components and activities of bacterial populations seem to be kept in particular states for each type of aquatic ecosystem. This is assumed from the studies that compared natural aquatic ecosystems at various eutrophication levels (e.g., Bell et al., 1977, 1982; Fuhrman & Azam, 1980; Kirchman & Mitchell, 1982; Lovell & Konopka, 1985a, 1985b, 1985c; Murray & Hodson, 1985; Rheinheimer, 1977; Riemann & Sondergaard, 1984; Seki, 1982a, 1982b; Seki & Iwami, 1984), that explored experimentally the artificial eutrophication of inland waters (e.g., Brock, 1985; LeBrasseur et al., 1978; Parsons et al.,

1972a, 1972b, 1977; Schindler, 1977; Seki et al., 1980a, 1980b; Stockner, 1977; Stockner & Manzer, 1978; Stockner & Shortreed, 1979), and that investigated the recovery of aquatic ecosystems from eutrophication (e.g., Edmondson, 1970; Edmondson & Lehman, 1981; Fiala & Vasata, 1982; Haslauer et al., 1984; Schindler, 1974).

In Edmondson's study at Lake Washington, recovery was accomplished after a decade of sewage diversion; whereas the recovery in most experimental lakes (e.g., Schindler, 1974) took place within several years after experimental fertilization. Even these shorter periods of oligotrophication must have an extremely sluggish impact on the microbial population with the time scale of their generation time, because their generation times are usually less than a few days.

Here, the eutrophication is characterized as a natural or artificial process of enrichment in inland waters and marine environments (Seki, 1983; Seki & Iwami, 1984). The word "eutrophication" is derived from "eutrophy" or "eutrophie" which means "good nutrient". The word "eutrophy" and its antonym "oligotrophy" (poor nutrient) was originally a term for expressing the soil nutritional states. These words were first introduced to limnology by Einar Naumann (1981-1934); the terms were agreed by a "father of limnology", August Thienemann (1882-1960) in the early 20th century. Then the word became of popular use not only in limnological concern but also in a view of

aquatic ecosystems. The reverse process of eutrophication is named "oligotrophication", which is occasionally applied for natural conditions in such a case of artificial recovery of the polluted waters.

3. Bogs

Inland waters are divided commonly into running waters and still waters. Still waters are further categorized typically into lakes, ponds, marshes, and bogs. The availble termionology is quoted from Glossary of Environmental Terms (1968):

Lake ---- any standing body of inland water, generally of considerable size.

Pond ---- a relatively small body of water, usually surrounded on all sides by land.

Marsh --- in general, any area of continuously saturated or spongy ground having poor drainage, hence synonymous with swamp.

Bog ----- a quagmire or morass; an area of wet, peaty, spongy ground, usually lacking in mineral nutrients, often interspersed with pools of open water, where any dense body is likely to sink.

Natural waters having the same limiting factor with different non-limiting factors could be represented by bogs and ponds in the watershed of Lake Kasumigaura, the Kanto Plain, Japan. The bogs and ponds are numerous in the watershed with the

magnitude of ponds having areas less than a few hectare. The depth of their water column are various, usually less than a few metre. Many of these bogs and ponds have been enriched by agricultural drainages or domestic wastes. Some are still free from the direct influence of these human perturbations. Among these natural waters, Matsumi-Ike Bog and Doh-Hoh-Numa Bog were selected as the study areas.

MATERIALS AND METHODS

1. Sampling

1.1 Period, Frequency and Time of the Day

Selection of the study period and the sampling frequency is important in planning field researches. For a study on the seasonal variation of any limnological environment, at least the study duration of one year with the frequency of weekly interval has been assigned to be necessary among most limonologists.

Sampling hour of the day is another important problem because of diel variations of the environment. Morning sampling was applied in this study because the water temperature around 10:00 am is reported to represent the daily average (Iwata, 1986), and the autotrophic and heterotrophic processes in the aquatic system are in the approximately balanced condition.

Therefore, in this study, sampling was made weekly at 9:00 am during the following periods of the study year at both Matsumi-Ike Bog and Doh-Hoh-Numa Bog as:

Matsumi-Ike Bog: for the study of the natural condition
from 13 April 1983 to 10 April 1984

Doh-Hoh-Numa Bog: for the study of the natural condition
At the main basin;
from 13 April 1984 to 29 March 1985

Doh-Hoh-Numa Bog: effect of the Bio-filter system

At the main basin and the marginal basin;

Period I; During the system operation;

from 21 June 1985 to 23 October 1985

Period II: After the system discontinuance;

from 1 November 1985 to 14 June 1986

1.2. Sampling Size

Sampling sizes was decided according to purpose and objects of this study. For plankton studies, Haury (1986) reviewed the horizontal distribution of various zooplankton species at scales ranging from 1 meter to 100 kilometers. Also for chlorophyll-a, its spatial scales (length) have been reported to be 32 km on the innner continental shelf and 84km on the puter shelf (Yoder et al., 1987). As bogs and ponds having the area of several hectare and depth of one meter have neither marked stratified structure nor regular horizontal current, the limnological characteristics can be represented approximately with one or two stations and several-liters of water. Actually, areal survey of Doh-Hoh-Numa Bog with the mesh-size of 15m revealed the horizontal heterogenities (patchness) of inorganic nutrients and chlorophyll-a without the concentration gradients of the order of magnitude (Suzuki, 1988).

Hence, at both bogs in this study, the sampling was performed at one or two stations in each bog with the collection

of several litres of water.

1.3. Sampling Equipments

Sterilized Hyroht samplers (1,000 ml) for bacterioplankton and polythene bags (5 to 10 litre) for chemical analyses were used in this study (Seki, 1976). Hyroht sampler is most convenient for aseptical sampling of bacterioplankton in shallow waters. Polythene bags washed with 1N hydrochloric acid (HCl) are desirable for water sampling without metal-contamination.

1.4. In Situ Procedures

Surface water temperature was measured with alcohol thermometers (-20 to 100^{o}C). Dissolved oxygen in the water samples collected in glass bottles (ca. 100 ml) was determined by the Winkler method (Strickland & Parsons, 1972). Samples for the bacterioplankton population density were fixed with borax-neutralized formalin (sample:formalin = 20:1 in volume).

2. Chemical Analyses

2.1. Water Filtration

Each sample water was suction-filtered through glass-fiber filters (Whatman GF/C) which were pre-conbustioned at 450^{o}C to remove organic materials. The suction unit was composed of a vacuum pump, a suction bottle, a filter head and a funnel

(Figure 1). The GF/C filter can collect particles larger than 1 um; materials passing through the filter were regarded as "dissolved". Particles collected on the filters were analyzed for particulate organic carbon (POC) and chlorophyll-a. The filtrate was used for chemical analyses of dissolved organic carbon (DOC) and inorganic nutrients. Both the samples collected on the filters and the sample filtrate were kept frozen at $-20^{o}C$ for the chemical analyses.

2.2. Chemical Measurements

The concentrations of inorganic nutrients (nitrate-N, nitrite-N, ammonium-N, and phosphate-P) were colorimetrically determined by the methods of Golterman et al. (1978).

The POC concentration was determined by combustion of the sample filter using Yanagimoto CHN Analyzer MT-2 (Kyoto).

The DOC concentration was determined by the dry-combustion method with Beckman TOC Analyzer 915B (Fullerton); this method is gives higher DOC values than the wet-combustion method (Strickland & Parsons, 1972).

Chlorophyll-a concentration was spectrophotometrically determined by the method of Strickland & Parsons (1972). There have been many empirical equations for determining the chl-a concentration, and the one of the most popular equations is the SCOR/UNESCO equation (1966):

$$[\text{Chl-a}] = 11.64\ A_{663} - 2.16\ A_{645} + 0.10\ A_{630}$$

where [Chl-a] is μg of chlorophyll-a in 1 ml of 90% acetone and each A is the absorbance at the subscribed wavelength (nm). Each A value is blank-corrected with A_{750} values. However, the following equation of Jeffrey & Humphrey (1975) was applied in this study:

$$[\text{Chl-a}] = 11.85\ A_{664} - 1.54\ A_{647} - 0.08\ A_{630}$$

because it gives more accurate values for mixed phytoplankton populations in the freshwater environments.

3. Counting of the Bacterioplankton Cells

The population densities of bacterioplankton were determined by epifluorescent microscopy with the acridine orange staining as shown in Figure 2 [original methods in Hobbie et al. (1977) and Zimmermann et al. (1978)]: A known volume of each sample water was filtered through a Nuclepore filter (pore size of 0.2 μm) pre-stained with Sudan black (6 mg dissolved in 100 ml of 50% ethanol). The bacterioplankton cells collected on the filter were stained with acridine orange (0.01% in 6.6 mM phosphate buffer, pH 6.7). Then the stained bacterioplankton were counted under an epifluorescent microscope (Plates 1 and 2) with following components (Nikon, Tokyo):

Light source	High-pressure mercury lamp
Excitation filter	B (with a dicroic mirror)
Absorption filter	515W
Objectives	Plan (x40, x100)
Eyepieces	CFW (x10)

or

Light source	High-pressure mercury lamp
Filter casette	Excitation filter B2 (EX450-490) Dicroic mirror DM510 Absorption filter BA520 (x1.25)
Objectives	Plan (x40, x100)
Eyepieces	CFW (x8)

Details of the microscopy are described in Ploem & Tanke (1987).

In order to minimize the counting errors, cell counts per microscopic field were made with cell number between 40 and 100 (occasionally above 100). This range is within the limit of reasonable counts suggested by Kirchman et al. (1982).

4. Measurement of the Bacterioplankton Growth

The growth implies in two ways: 1) the increase in size and volume of individual cells and 2) the increase in the cell numbers. These two are proportional when bacteria are in the "balanced growth"; these two do not necessarily have direct relationship, especially when bacteria are in "starvation" (Morita, 1982). During the balanced growth, increase in protein, ribonucleic acid, deoxyribonucleic acid, lipid, or other macromolecules is coincident with an increase in cell mass and cell numbers (Drew, 1981). It is this growth state that in practice is the only reproducible state of bacterial cells (Ingraham et al., 1983). On the other hand, there have been three patterns observed for the starvation-survival of aquatic bacteria: 1) an initial increase in viable cells followed by a decrease until a constant number was reached, 2)

an increases in viable cells until a constant number was reached, and 3) a decrease in viable cells until a constant number was reached (Amy & Morita, 1983). These starvation-survival patterns frequently involve the decrease in size down to 0.2 or 0.3 μm (Novitsky & Morita, 1976).

In this study, "growth" refers only to increase in cell numbers (population growth), not including the increase in biomass.

4.1. Mathematical Basis of the Bacterial Growth

The equation of bacterial growth is expressed as:

$$dN/dt = \mu N \quad \text{---------- Equation 1}$$

The integrated form of Equation 1 is

$$N = N_0 e^{\mu t} \quad \text{---------- Equation 2}$$

where N is the cell number, N_0 is the initial cell number, t is time, and μ represents the specific growth rate or the intrinsic growth rate. In this thesis, μ is simply termed the growth rate. The growth rate μ can be fundamentally measured by increase in cell number, as known by transforming Equation 2 as:

$$\mu = (\ln(N/N_0))/t \quad \text{---------- Equation 3}$$

Bacterial growth by the binary fission can be expressed by another equation as:

$$N = N_0 \, 2^{t/q} \quad \text{---------- Equation 4}$$

where q is the generation time. From Equations 2 and 4, the relationship between the growth rate (μ) and the generation time

(q) is shown to be as:

$$q = (\ln 2)/\mu \quad \text{---------- Equation 5}$$

The numerator of ln2 (natural logarithm of 2, approximately 0.693) is thus proved to be due to the mode of bacterial growth, i.e. the binary fission.

4.2. Determination of the Bacterial Growth Rate

Among various methods established for determining the growth rates of microorganisms (Brock 1971), the chemostat culture method was used preferentially in this study, with the batch culture method occasionally when it is necessary.

Batch Culture

For determining the bacterioplankton growth rates, the batch culture method is the simplest application of Equations above; the growth rate μ can be determined by counting both the initial cell number and the cell number after incubation during a known period. This batch culture method has been successfully applied for marine species (e.g., Carlucci & Williams, 1978), and similar method was used for determining the growth of estuarine bacterioplankton at various experimental salinities (Naganuma & Seki, 1988). The batch culture method, however, can be used only with limited incubation time, because it is a closed culture system. This closed systems is only applicable to simulate the in situ conditions before dilute nutrients are consumed up.

Thereafter the physiological conditions of bacterioplankton change even into the level of starvation strategy with prolonged incubation (Amy & Morita, 1983; Morita, 1982; Novitsky & Morita, 1976; Torrella & Morita, 1981).

In this study, the batch culture method was used for determining the bacterioplankton growth rate at different temperatures in Matsumi-Ike Bog and Doh-Hoh-Numa Bog (Figures 3 and 4). Eight quintuplets of 10ml-subsamples were incubated at 5, 10, 15, 20, 25, 30, 35 and 40^{o}C, respectively. Of a quintuplet, subsamples were fixed with formalin one by one at 1, 2, 3, 4 and 5 hours after the start. The growth rate at a temperature was calculated from increase in cell number during the incubation. Unlike other measurements, this determination was made biweekly or triweekly.

Chemostat Culture

Changing in the nutrient concentration in the culture liquid never takes place by the chemostat culture (Figure 5). During the chemostat culture, the nutrient concentration available for bacterial cells is kept constant, with the appropriate rate of nutrient supply (Malek & Fencl, 1966). The chemostat can closely simulate the in situ nutritional condition by using in situ water as the culture medium.

4.3. Theory of the Chemostat Culture

The chemostat was independently designed by Monod (1950) and Novic & Szilard (1950). The name of "chemostat" was given by Novic & Szilard, while Monod gave theoretical bases and a less popular name of "bactogen".

The chemostat is a kind of continuous culture systems, and there are other systems such as: "turbidostat" maintaining the population density of microorganisms at a constatnt turbidity (Bryson & Szybalski, 1952); "phauxostat" employing the growth-dependent pH change to control the input rate of fresh medium.

For the growth rate in the chemostat culture, Equation 1 should be rewritten as:

$$dN/dt = (\mu - D)N \quad \text{---------- Equation 6}$$

where D is the dilution rate, i.e., the flow rate (F) divided by the culture volume (V); D = F/V. The chemostat system was originally for maintaining the population density of microorganisms constant, i.e., dN/dt = 0. The time-independence of density is termed the steady-state; the constant density is the steady-state density. In the steady-state, the growth rate must be equal to the dilution rate; $\mu - D = 0$. Hence, the <u>in situ</u> growth rate of bacterioplankton population must be determined before the chemostat culture reaches the steady-state. The dilution rate (D) must be within a certain range. D above the critical dilution rate (D_c) caused the mass washout of microorganisms (Herbert et al., 1956); D below the lower limit

cannot support the maintenance energy of microorganisms (Stouthamer & Bettenhaussen, 1973; Tempest & Neijssel, 1984).

On the theoretical basis above, Jannasch (1969) applied first the chemostat for determining the growth rate of marine bacterial isolates. The growth rate (u) can be calculated by integral and transformation of Equation 6 as:

$$\mu - D = \{\ln(N/N_0)\}/t \quad \text{------- Equation 7}$$

where μ-D is termed the washout rate (A); the growth rate (μ) can be obtained from A+D. The washout rate (A) can also be obtained by semi-log plotting of the population density versus time. That is, the washout rate (A) is the linear slope calculated from the plots by the least square method. Linearity of every slope must be shown to be significant by the statistical F-test.

Later, Jannasch (1974) pointed out that 1) the <u>in situ</u> environments are in the unsteady-state, 2) the <u>in situ</u> environments have no dilution rates, and and 3) mixed bacterioplankton populations are under selective pressure and only single species can be selected in the chemostat culture. However, natural environments are in the steady-state during a short period, while the growth rate can be determined as the steady-state before the population structure receives the dilution effect. Moreover, mixed populations of microorganisms react as a single species within a short time scale (e.g., Counotte & Prins, 1979; Hendricks, 1972; Meyer-Reil, 1977).

Therefore, the chemostat method at a certain condition can be used for the assessment of microbial process in nature.

4.4. Chemostat Culture in This Study

In order to simulate the *in situ* conditions, the *in situ* bog water was used as culture medium; the water was collected one day before the sampling, filtered through Whatman GF/F filters, and autoclaved. The chemostat culture was made immediately after the sampling at *in situ* temperature in the dark, with stirring at 200rpm. At least 4 subsamples were collected in course of the chemostat culture before the natural growth rate was modified in the chemostat. The washout rate (A) was negative in most measurements, i.e. $\mu - D < 0$. The dilution rate (D) was set between 0.14 to 0.16 /hr according Seki (1976): the flow rate (F) of 70 to 80ml/hr and the culture volume of 500ml. The chemostat apparatus used for this study composed of a Mini Jerfermenter M-100 (Tokyo-Rika-Kikai, Tokyo) and Micro Tube Pumps MP-3 or MP-32 (Tokyo-Rika-Kikai, Tokyo).

Within the first 5 to 12 hours of the chemostat culture, the population density of bacteriolplankton decreased linearly against the time (Figrue 6), thereafter it reached the steady-state (Figure 7). Another washout was, in another case, observed after 40 hours from the initiation of incubation (Figure 8). This washout recovery is probably due to: 1) the clump formation by bacterioplankton and 2) the attachment and growth on the wall of

culture vessel. This wall growth results from the "sticky" character of the culture cells and their innate tendency to attach to glass surfaces (Dykhuizen & Hartl, 1983). This wall growth is not serious within a short culture period. Practically, the wall growth is of less concern with the common population densities of bacterioplankton in the eutrophic and mesotrophic waters, or at low dilution rates of the chemostat. This is because only small fraction of the bacterial cells in the liquid attaches onto the chemostat wall during the experiment.

The growth rate was, thus, determined within the time of first "linear decrease", mostly within the first 4 hours of the culture. As an example (Figure 7), the growth rate was determined by regressing the plots between 1 and 4 hours. The 0-hour plot was excluded because the population density of bacterioplankton often increase within the first 0.5 and 1 hour (Figure 9), possibly due to the mechanical destruction of detritus and bacterial aggregates.

During the chemostat culture for less than 5 hours with samples from Matsumi-Ike Bog, the washout modes were found to be classified into four types (Figure 9). Type 1 shows convex modes, where the growth rates are high during the first half an hour and then reduce to constant levels. Type 2 shows concave modes, where the growth rates are below the dilution rate at first, but become equal to or above the dilution rate. Type 3 shows winding

modes, where the growth rates fluctuate as being high at the first stage, low next, then equal to or above the dilution rate. Type 4 shows sharp rising modes, where the growth rates rise sharply in the first half an hour, and sharp decrease follows during the next half an hour. This initial-high growth in Type 4 cannot be responsible for their actual growth, and it is caused by mechanical destruction of bacterial aggregates, due to vigorous stirring in the chemostat. Adding to the four types of washout modes, the environmental conditions of Matsumi-Ike Bog were classified into twelve categories (Figure 10), based on threshoulds of various parameters; i.e., the DOC concentrations of 3.9 and 5.1 mgC/l, the bacterioplankton population density of 5.9×10^9 cells/l, and water temperature of $6.5^{\circ}C$ at which the master biochemical reaction in their growth alternates. Between the washout types and the environmental categories, some relationships were found as follows:

washout type	environmental category
1	3, 6, 8, 10
2	1
3	4, 7, 11, 12
4	5

except Categories 2 and 9, which were not found in Matsumi-Ike Bog during the study year.

These relationships lead to some conclusions: The Type 2

washout reflects the low *in situ* growth rates which were caused by the lower DOC concentration, the lower population density and the lower temperature, as characterized Category 1. The Type 3 washout is responsible for higher species diversity with higher population density at the conditions as Categories 4, 7, 11 and 12. The Type 4 washout relates to the mechanical destruction of bacterial aggregates, and the aggregates are formed at the conditions with the intermediate DOC concentration, the lower population density and the lower temperature, as characterized Category 5. On the other hand, the Type 1 washout is not related to any environmental category. This is possibly because the growth is regulated equally by multiple environmental factors.

5. Measurement of the Bacterioplankton Production

The bacterial production (cells/l/day) can be estimated by multiplying the population density (cells/l) by the growth rate (/day = x24/hr). When the bacterial *biomass* production (e.g., carbon g/l/day) is required, the cell-to-biomass conversion factor (gC/cell) should be known. The conversion factor can be obtained from bacteria variables as: 1) specific biovolume (μm^3/cell); 2) buoyant density ($g/cm^3 = x10^{-12}/\mu m^3$); 3) ratio of dry/wet weight (%); and 4) ratio of carbon/dry weight (%).

The specific biovolume has been determined in various waters by various methods, ranging more than 100-fold between authors:

μm^3/cell	Sample	Reference
0.058 (winter) 0.076 (spring) 0.102 (summer) 0.133 (autumn)	Howe Sound	Albright & McCrae (1987)
0.13	stream sediments	Bott & Kaplan (1985)
0.061 (rods) 0.036 (cocci)	Newport River Estuary	Bowden (1977)
0.14-0.39	culture	Bratbak (1985)
0.100 (Jan-Feb) 0.160 (March-Dec)	Lake Bysjon	Conveney et al. (1977)
0.053-0.10	Frying Pan Shoals, NC.	Ferguson et al. (1984)
0.145 0.81	Scripps pier coastal California	Fuhrman (1981)
0.08	Bothnian Sea	Hagstrom (1984)
0.0024-0.79 (0.21)	Sea of Japan	Kameneva & Mishustina (1985)
0.009-0.09 (0.04)	Barents Sea	Mishustina & Kameneva (1981)
0.032-0.048	Lake Plussee	Krambeck (1984)
0.018-0.130 0.015-0.022	Lake Plussee 10 lakes in FRG	Krambeck et al. (1981)
0.023-0.073	Crane Neck, Long Is.	Lee & Fuhrman (1987)
0.66	Lake Valencia	Lewis et al. (1986)
0.12 0.12 0.08	Sapelo Island, Ga. Duplin River Estuary Skidway River Estuary	Newell & Christian (1981)
0.072-0.096 0.076-0.096	Newport River Estuary Gulf of Mexico	Palumbo et al. (1984)
0.159	Lake Mendota	Pedros-Alio & Brock (1982)

0.131	Atlantic	
0.120	forest stream	Rheinheimer (1985)
0.149	Elbe River	
0.357	sewage	
0.04-0.24	Finnish lakes	Salonen (1977)
0.19-0.27	Sargasso Sea (100m)	
0.16-0.22	Narragansett Bay	Sieracki et al. (1985)
0.08-0.11	Barber Pond, RI.	
0.028-0.104 (free-living)	Lake Constance	Simon (1987)
0.048-0.350 (attached)		
0.06	Kiel Fjord Kiel Bight	Zimmermann (1977)

In this study, the cell volume was determined to be 0.1 μm^3/cell for bacterioplankters in Matsumi-Ike Bog and Doh-Hoh-Numa Bog by epifluorescence microphotography. This biovolume of 0.1 μm^3 is reasonable when compared with the values above.

The buoyant density has been determined to be constant as follows:

g/cm^3	Reference
1.09	Bakken & Olsen (1983)
1.09-1.13	Bratbak & Dundas (1984)
1.025	Brock (1985)

As 1.0 to 1.1 g/cm^3 is accepted in general, the value of 1.0 g/cm^3 is used as the buoyant density of bacterioplankton in this study.

The ratio of dry/wet weight is obtained from the water content of bacterial cell, as:

dry weight (%) = 100 - water content (%)

with the proposed water content as listed below:

Water content (%)	Reference
80	Generally accepted (e.g., Luria, 1960)
70 (67-88)	Bakken & Olsen (1983)
43-69	Bratbak & Dundas (1984)
77	Ferguson & Rublee (1976)
85	Kuznetsov & Romanenko (1966)
74	Larsson & Hagstrom (1982)

Thus in this study, the value of 20% is used as the dry/wet weight of ratio the bacterioplankton cells.

The ratio of carbon/dry weight has been determined to be approximately 50% as the values recently measured:

Carbon content (%)	Reference
50	Generally accepted (e.g., Luria, 1960)
34.4	Ferguson & Rublee (1976)
9.79*	Pedros-Alio & Brock (1982)

* Carbon/wet weight

Here the value of 50% is used as the carbon/dry weight ratio of the bacterioplankton cells.

Thus the cell-to-biomass conversion factor is obtained from:

0.1	x	1.0	x	0.2	x	0.5	=	1.0
(μm^3/cell)		($x10^{-12}$ $g/\mu m^3$)						($x10^{-14}$ gC/cell)

Hence, $1x10^{-14}$ gC/cell and $1x10^{-13}$ gC/μm^3 are the appropriate conversion factor for bacterioplankters both in Matsumi-Ike Bog and Doh-Hoh-Numa Bog. These factors are reasonable when compared with other values of $0.94x10^{-13}$ gC/μm^3 (Pedros-Alio & Brock, 1982), $1.06x10^{-13}$ gC/μm^3 (Nagata, 1986), $1.43x10^{-13}$ gC/μm^3 (Larsson & Hagstrom, 1982). Using these conversion factors, the bacterioplankton biomass production can be estimated as follows:

bacterioplankton production	=	population density	x	growth rate	x	conversion factor
(gC/l/day)		(cells/l)		(x24/hr)		($x10^{-13}$ gC/cell)

6. Counting of the Zooplankton Individuals

Zooplankton in Matsumi-Ike Bog were collected by vertical haul from bottom to water surface with a plankton net (NGG 54; net diameter of 30 cm, mesh size of 54 mesh/inch, opening of 334 μm). Sample zooplankton were fixed with borax-neutralized formalin (final concentration of 5%) immediately after the collection, and were counted under a stereoscopic microscope (Olympus, Tokyo).

7. Statistical Analyses

The degrees of correlation were shown with r^2, where r is the correlation coefficient (Sokal & Rohlf, 1973).

The statistical significance of every correlation and regression was examined by the F-test (Sokal & Rohlf, 1973).

The statistical significance of difference between two groups was also examined by the F-test.

RESULTS AND DISCUSSION

1. Bacterioplankton Community in Matsumi-Ike Bog

Limnological chracteristics of Matsumi-Ike Bog

Matsumi-Ike Bog (Figure 11) is located in the campus of the University of Tsukuba, Tsukuba Science City, Japan (140^{o} 06.7'E, 36^{o} 05.3'N). Total area of the bog is 1.5 hectare; Kami-Ike has 0.7 hectare and Shimo-Ike 0.8 hectare. The maximum depth is less than one metre. Matsumi-Ike Bog has been artificially separated in 1976 into these two basins of Kami-Ike and Shimo-Ike. The bog water can flow from Kami-Ike to Shimo-Ike through earthend pipes, then flows out into neighbouring the River Hanamuro-gawa, which flows into Lake Kasumigaura at Tsuchiura-iri Inlet. Kami-Ike has been embanked and surrounded by university buildings and lawns since 1976; Shimo-Ike has been conserved as a natural bog. Matsumi-Ike Bog receives water inputs of rain-fall and rain drainage. No river or stream flows into the bog.

Sampling was made at two stations: Station 1 at the north-west shore of Kami-Ike and Station 2 at the head of Shimo-Ike (Figure 11). All ecological parameters were similar both at Stations 1 and 2 (Table 1), thereby the results from Stations 1 and 2 were dealt for the common statistical analyses. At both stations, it was shown that every ecological parameter in the surface water of Matsumi-Ike Bog showed the steady-state

oscillation in a range near the eutrophic part of the mesotrophic system (Figures 12 and 13).

Surface water temperature of the bog showed a typical fluctuation in the temperate zone (Figure 12). The highest and the lowest surface temperature at 9:00 am were 29.2^{o}C on 12 August 1983 and 3.0^{o}C on 27 January 1984, respectively; the average was 15.0^{o}C. The temperature fluctuation was regressed into an annual sine-curve with statistically high significance as:

$$t = 9.8\sin[(w-4)\pi/26] + 15.0 \quad \text{------ Equation 8}$$

$$(F = 259, F_{0.01} = 6.9)$$

where t is temperature (^{o}C) and w is the number of weeks elapsed since 10 April 1983. The bog water surface was frozen at each sampling time from 28 December 1983 until 10 February 1984.

The dissolved oxygen (DO) concentration at the surface of the bog was between 4 and 15 mgO_2/l throughout the year (Figure 13). The DO fluctuation in this range little affects on aquatic bacterial metabolisms in the bog, because the critical DO concentration for bacterial respiration was estimated to be as low as 0.43 mgO_2/l for natural bacterial community (Pamatmat, 1971; Seki et al., 1984) as well as for a pure strain of marine bacterium (ZoBell, 1940). It has been also suggested that the multiplication and respiration of lake bacteria are independent of DO throughout the range from 0.30 to 36 mgO_2/l (ZoBell, 1946). The average DO concentration was 10.1 mgO_2/l; 10.5 mgO_2/l at Station 1 and 9.6 mgO_2/l (Table 1). Annual fluctuation had a

pattern of summer-low and winter-high. The highest DO concentration was 15.7 mgO_2/l on 13 January 1984 at Station 1, and the lowest was 4.0 mgO_2/l on 5 August 1983 at Station 2.

The concentration of inorganic nitrogen (total of NO_3-N, NO_2-N and NH_4-N) increased its fluctuation range at the beginning of summer by reaching the annual maximun at Station 2 from 69.4 µg-at/l on 5 August 1983 to 4.9 µg-at/l on 26 August 1983 (Figure 13). The fluctuation became progressively smaller toward the beginning of spring. The average concentration was 17.6 µg-at/l; 15.1 µg-at/l at Station 1, and 20.0 µg-at/l at Station 2 (Table 1). NO_3-N and NH_4-N showed alternative dominance in the concentration; NO_3-N contribution was 53.7% of inorganic nitrogen concentration, and NH_4-N was 42.3%, on the annual average.

The concentration of inorganic phosphorus (phosphate-phosphorus) increased drastically at the beginning of August and maintained higher level until its concentration reasched the annual maximum of 7.6 µg-at/l on 18 November 1983 at Station 2 (Figure 12). Thereafter the concentration drastically decreased within half a month down to a moderate level. The moderate level was maintained throughout the winter until beginning of the spring bloom of phytoplankton. Through this fluctuation, the average concentration was 1.3 µg-at/l; 1.2 µg-at/l at Station 1, and 1.4 µg-at/l at Station 2 (Table 1).

The concentration of dissolved organic carbon (DOC) showed annual fluctuation with less obvious pattern of high

concentration in late spring and low in late winter (Figure 13). The highest concentration was 8.6 mgC/l on 10 June 1983 at Station 2, and the lowest was 0.5 mgC/l on 2 March 1984 at Station 2. The annual average was 4.6 mgC/l; 4.7 mgC/l at Station 1 and 4.5 mgC/l at Station 2 (Table 1).

The concentration of particulate organic carbon (POC) was the greatest during the spring bloom of phytoplankton, thereafter the POC concentration became lower progressively toward the end of winter (Figure 13). Thus the primary constituents of particles were possibly living phytoplankton and phytodetritus (Brown & Parsons, 1972). Actually, each POC pulse corresponded to a phytoplankton pulse that was formed during the natural selection of predominant phytoplankton species. The maximum POC pulse formed on 12 August 1983, for example, was identical with the phytoplankton pulse predominantly composed of Nitzschia species (Shiraishi et al., 1985). This maximum POC concentration was 7.6 mgC/l at Station 1. The annual average was 2.0 mgC/l; 2.1 mgC/l at Station 1 and 1.9 mgC/l at Station 2 (Table 1).

The concentration of chlorophyll-a showed several pulses throughout the year (Figure 13). On 12 August 1983 at Station 1, the maximum pulse that was the annual highest peak of 124.9 µg/l was observed. This maximum pulse was due to the phytoplankton bloom of diatom species Nitzschia as described above. Other pulses occurred in June, July and August, with the pulse height of 50 to 100 µg/l. On the other hand, low chlorophyll-a values

(below 10 µg/l) were observed in winter, and the lowest was 3.2 µg/l on 27 January 1984 at Station 1. The average chlorophyll-a concentration was 20.9 µg/l; 23.0 µg/l at Station 1 and 18.9 µg/l at Station 2 (Table 1).

Zooplankton in Matsumi-Ike Bog

Zooplankton collected by the net sampling were largely divided into two groups: copepods and cladocerans. They formed sharp pulses, accompanying pulses of the bacterioplankton population density and the chlorophyll-a concentration (Figure 14). Assuming that chlorophyll-a concentration indicates phytoplankton biomass, quantitative aspects of biomass flow in the bog food web can be reasonably explained throughout the year: The grazing food chain was major processes in the bog food web in spring and summer, when the prey-predator relationship was apparent among the abundance of phytoplankton and zooplankton. During this period, the predominant zooplankton were cladocerans; these larger herbivores prefer to eat larger particles as living phytoplankters in their selective choice of food items. On the other hand, the bog food webs in autumn and winter was composed mainly of the detritus food chains, where the prey-predator relationships were quantitatively apparent between fine detrital particles or bacterioplankters and small bodied copepods.

Bacterioplankton in Matsumi-Ike Bog

i) Population Density

The population density of bacterioplankton was fluctuated with a pattern of autumn-high and spring-low, ranging from the magnitude of 10^8 cells/l to 10^{10} cells/l (Figure 15). Drastic increase was observed on 14 October 1983, reaching each annual maximum of 1.7×10^{10} cells/l at Station 1 and 2.0×10^{10} cells/l at Station 2. On the other hand, the lowest values were found in spring; 2.9×10^9 cells/l on 15 April 1983 at Station 1, and 5.0×10^8 cells/l on 6 May 1983 at Station 2. The annual average density was 8.9×10^9 cells/l; 9.2×10^9 cells/l at Station 1 and 8.6×10^9 cells/l at Station 2.

ii) Population Growth Rate

The population growth rate showed two patterns of fluctuation: 1) the annual fluctuation showed a pattern as high in summer and low in winter, and 2) week-to-week fluctuation was larger in winter but smaller in other seasons (Figure 15). The highest growth rate was 0.33/hr (generation time of 2.1 hours) on 29 July 1983 at Station 2, while the lowest was 0.02/hr (350 hours) on 13 January 1984 at Station 1. The annual average was 0.12/hr (5.8 hours) at both stations.

iii) Production Rate

The fluctuation pattern of bacterioplankton production was

more related to rather that of the growth rate than that of the population density (Figure 15). The annual maximum was prominent as 1,055 μgC/l/day at Station 2 on 29 July 1983, when the annual maximum of growth rates and the high peak of population density were found. On the other hand, smaller production was found during winter and just at after the spring peak, with the annual minimum of 10 μgC/l/day on 6 May 1983 at Station 2. The annual average was 238 μgC/l/day; 251 μgC/l/day at Station 1, and 225 μgC/l/day at Station 2.

iv) DOC - Growth Relationship

The effect of nutrient concentrations on bacterial growth rates (F[D]) can be shown theoretically by modifying the kinetic models of Monod (1949), who proposed the following equation expressing a relationship between bacterial growth rates and substarate concentrations:

$$\mu = \frac{\mu_m [S]}{K_s + [S]}$$

where μ and μ_m represent the growth rates and the maximum growth rates, respectively. [S] is the concentration of a limiting substrate, and K_s is the half-saturation constant. This equation is dynamically identical to the Michaelis-Menten equation of enzyme reactions. When the effects of excessive concentration are taken account, the Monod equation is modified as follows:

$$\mu = A \mu_m [S] \exp(1 - A [S])$$

where A is a constant. Steele (1962), for example, applied this modified equation to the effects of inhibition in intense light in order to analyze the photosynthesis-light relation. The same modification was possible for the Monod model in relation to the DOC effect on the population growth rates of bacterioplankton, and the model in Matsumi-Ike Bog could be expressed as a highly significant regression (Figure 16):

$$F[D] = 0.17(D-3.9)\exp\{1 - 0.84(D-3.9)\} \quad \text{---- Equation 9}$$

$$(F = 144, F_{0.005} = 8.5)$$

where D is the DOC concentration (mgC/l) in the bog water. From this mathematical model, it was shown that major constituents of the natural bacterioplankton community can start their multiplication at the DOC concentration of 3.9 mgC/l in the bog environment. The inhibitory effect of higher DOC concentration was obviously shown by this model. Moreover, the optimum DOC concentration of 5.1 mgC/l was given by this model. The the shortest generation times of the bacterial population in the bog was thus shown theoretically to be several hours. This generation time is the threshold at the boundary between the mesotrophic and eutrophic systems.

v) Density - Growth Relationship

The bacterial growth is affected significantly by the bacterial population density, especially when the density is maintained at maximal levels in a certain aquatic ecosystem.

This effect in Matsumi-Ike Bog must be great because the bacterioplankton population density in the bog water was in highest levels of the mesotrophic system (levels in various systems, see Seki & Nakano, 1981). The effect of the population density on the growth rate can be analysed by the model of Allee's principle. According to Allee's principle, the degree of aggregation of metazoans varies not only with the lack of aggregation but also the excessive aggregation (Odum, 1971). As common characteristics among microorganisms, an aggregated group must have better growth and survival properties than individuals, while the size of the group is within a certain limit. The formation of microcolonies is an adaptation based on mutual protection or assistance within the population; even motile bacteria having capability of moving away from the colony remain as aggregates within a certain limit (Atlas, 1986). The effect of the bacterioplankton population density on their growth rate (F[P]) in Matsumi-Ike Bog could be shown as a highly significant regression (Figure 17):

$$F[P] = 0.02\ P \exp(1 - 0.17\ P) \quad \text{---------- Equation 10}$$

$$(F = 130,\ F_{0.005} = 8.5)$$

where P is the population density ($\times 10^9$ cells/l) in the bog water. From this mathematical model, it was shown that the interaction of bacterioplankton cells is the most favourable for their growth at the density of 5.9×10^9 cells/l. Below this optimum population density, dominant bacterioplankters were

mostly rods having an average cell size with 2 um in diameter and 5 um in length. On the other hand, above the optimum density, the inhibitory effect of higher population densities on their growth was evident; the cell size of the bacterioplankters became drastically smaller, and many changed from rods to cocci of approximately 0.5 um in diameter.

vi) Temperature - Growth Relationship

There is a certain range of temperature where the temperature-growth relation of a bacterium follows the Arrhenius equation (Johnson et al., 1954):

$$r = S \exp(-E/RT)$$

where r is the rate of reaction, S is a constant, E is the activation energy, R is the gas constant, and T is the absolute temperature. Taking the logarithm, another form can be obtained:

$$\ln[r] = (-E/R)/T + \text{constant}$$

If this type of plot (frequently termed an Arrhenius plot) is made for bacterial growth rate, a different response from chemical reactions can be seen. The Arrhenius plots for bacterial growth rates have limited linear range, above and below which the growth rates decline rapidly. Ratkowsky et al. (1982) found the linear relationship between the square root of growth rate and temperature (^{o}C). Ingraham et al. (1983) stated: "Although this relationship has no theoretical basis and fails by extrapolation to predict accurately the minimum temperature of growth, it

should be proved useful in predicting intermediate growth rates from limited data". The linear range could become wider for a natural community composed of different bacterial groups in their response to temperature. This is because the best master reaction of each thermally optimum group controls alternately an overall process of the community growth over a wider temperature range. This was evident for the population growth of natural bacterioplankton in Matsumi-Ike Bog (Figure 18), where the thermal effect on the growth rates (F[T]) was highly significant following the Arrhenius equation:

$$\log F[T] = -1970/K + 5.09 \quad \text{---------- Equation 11}$$

$$(F = 77, F_{0.005} = 8.5)$$

where K is absolute temperature of the bog water.

Microbiological Profile of Matsumi-Ike Bog

There have been various classifications for trophic states of lakes. The most popular classification is based on the concentrations of inorganic nutrients, as it is dealt at the section "Limnological Characteristics of Matsumi-Ike Bog". Another is biological, based on chlorophyll-a concentration (μg/l) as listed below. According to any classification below, Matsumi-Ike Bog having the average chlorophyll-a concentration of 20.9 μg/l can be identified as eutrophic throughout the year:

Oligo-	Meso-	Eutrophic	References
< 2	< 6	<	Rast & Lee (1978)
< 2.5	< 5	<	Sakamoto (1966)
< 2.5	< 6.5	<	Carlson (1977)
< 3	< 7	<	Forsberg & Ryding (1980)
< 4	< 10	<	Nat. Acad. Sci. (1972)
< 4.3	< 8.6	<	Dobson et al. (1974)
< 7	< 13	<	USEPA (1974)

However, these classification can be applied strictly for lakes, not for bogs, because there are two types of eutrophication process (Odum, 1971):

Depth	Nutrient concentration: Low	High
Shallow	Morphometric Eutrophy	→ Eutrophic
Deep	Oligotrophic	Morphometric Oligrotrophy

Here Matumi-Ike Bog is in the state of morphometric eutrophy, or in an essential definition, mesotrophy. Thus the mesotrophic range should be wider than those classifications above.

The population growth rates of natural bacterioplankton in the Matsumi-Ike Bog were measured with the chemostat, and the average growth rate was 0.12/hr, a generation time of 5.8 hours. The highest rate was 0.33/hr; such a high growth as the generation time of 2.1 hours was rarely measured in Matsumi-Ike Bog. This extraordinary high growth activity in the mesotrophic environment was due possibly to that eutrophic microenvironments

exist sporadically in the mesotrophic macroenvironment. The generation times of bacterioplankton within the 95% confidence interval were statisticaly determined in the range from 5 to 20 hours. The growth rates were thus maintained in this range by a series of natural negative feedback loops as influenced by a number of environmental factors in the bog (Seki, 1982a; Seki & Iwami, 1984).

The bacterioplankters must multiply at the expense of dissloved organic matters in Matsumi-Ike Bog. Then they should be eaten by detritivores through the detritus food chains (Seki, 1972). For example, the production of bacterioplankton in the bog was determined on 14 October 1983 by the chemostat to be as high as 415 μgC/l/day. This is equivalent to the primary production at the coastal area on the continental shelf (e.g., Whittaker, 1970). When referred to the following decrease of bacterioplankton population density, the bacterioplankton biomass was then eaten by detritivorous copepods with the grazing rate of 433 μgC/l/day. Such an efficient biological production through the detritus food chains was alomst comparable to significance of the grazing food chains during blooms of phytoplankton, and could be the common phenomenon in natural bogs in the watershed of Lake Kasumigaura.

The DOC concentration was shown to be the limiting factor regulating the population growth rates of bacterioplankton in

Matsumi-Ike Bog. However, only very few of bacterioplankton ultramicrocells were detected in the bog at any season of the study year, although some inefficint bacterial species in exploiting nutrients were reported (e.g., Jannasch, 1967). They fall into the starvation-survival phases due to the shortage of availability of dissolved organic materials (Morita, 1982). Many bacterioplankton in the bog reduced their cell sizes down to 0.5 um. This size is larger than the bacterial ultramicrocells at the real starvation-survival phases (Novitsky & Morita, 1976; Torrella & Morita, 1981).

Historically, mesophiles and psychrophiles have been earlier proposed physiologically to be distinguished by the temperature characteristic, i.e., E in the following Arrhenius equation, than their ecological characterization (Ingraham, 1958):

$$\ln [\text{growth rate}] = (-E/R)/K$$

where R is the gas constant and K is absolute temperature. This concept was later challenged by Hanus & Morita (1968), who found no significant correlatiion between the temperature characteristics of psychrophiles, psychrotrophs and mesophiles. Then the cardinal temperatures (minimal, optimal and maximal growth temperatures) have become to be used for distinguishing them. Thus, at present, obligate psychrophiles are defined as organisms having an optimal temperature for growth at about $4^{o}C$, a maximal temperature for growth at about $10^{o}C$, and a minimal

temperature for growth at $0^{o}C$ or below (Morita, 1975). Facultative psychrophiles (or psychrotrophs) are defined as organisms able to grow at $0^{o}C$ but also able to grow at temperatures of 25 to $30^{o}C$ (Brock et al., 1984). In the temperature range of this bog, dominant bacteria are obligate and facultative psychrophiles. The effect of temperature on the population growth rates in Matsumi-Ike Bog was expressed highly significantly throughout the year, following the Arrhenius equation. Moreover, there was obviously a sharp break in this over-all process at about $6.5^{o}C$. This could be attributed to the two major master reactions of the facultatively and obligately psychrophilic bacterioplankters inhabiting in Matsumi-Ike Bog.

2. Bacterioplankton Community in Main Basin of Doh-Hoh-Numa Bog -- Before the Introduction of the Bio-filter System

Limnological Characteristics of Main Basin of Doh-Hoh-Numa Bog

Doh-Hoh-Numa Bog is located in Tsukuba Science City of Japan (140^{o} 07.6'E, 36^{o} 03.5'N), and is one of the bogs in the watershed of Lake Kasumigaura (Figure 19). Doh-Hoh-Numa Bog was once connected to the River Ono-gawa which flows into Lake Kasumigaura at Edosaki-Iri Inlet. The bog with area of 3.4 hectare is consisted of two basins: a main basin (3.2 hectare) and a marginal basin (0.2 hectare). A sampling station (Station 1) was selected at the deepest site in the main basin.

Along the east coast of the main basin, there is a well-developed reed community consisted of Phragmites and Typha. Water shield (Brasenia chreberi) occupied the south-eastern half and southern coastal zone of the main basin in May. Instead, Potamogeton distinctus flourishes in summer.

Doh-Hoh-Numa Bog receives water-inputs of rain-fall, rain-drainage, pumped underground water, and lesser amount of the effluent from a swimming pool into the marginal basin. No river flows in and out Doh-Hoh-Numa Bog. The depth is less than one metre. In an extreme case, approximately 50% of the bog area was dried up during late summer and autumn of 1984 because of high temperature and little precipitation. However, the bog was never dried up in summer and autumn of 1985. The bog surface was

frozen at sampling times occasionally from 28 December 1984 until 15 February 1985, and from 10 December 1985 until 7 March 1986.

Sampling was made at Station 1 (Figure 19), where the bog was the deepest. Sampling and other measurements in the field were made on a boat, taking good care not to stir up the bottom mud into the water column.

Every trophic parameter in the surface water of Doh-Hoh-Numa Bog showed seasonal fluctuation near the oligotrophic part in a range of the mesotrophic system (Figures 20 and 21).

The annual fluctuation of water temperature of the bog was typical in the temperate zone, and was expressed as a highly significant regression curve as follows (Figure 20):

$$t = 12.8 \sin((w-5)\pi/26) + 14.9 \quad \text{---------- Equation 12}$$

$$(F = 141,\ F_{0.01} = 7.2)$$

where t is temperature (^{o}C) and w is the number of weeks elapsed since 13 April 1984. From this eqaution, the average temperature was found to be 14.9^{o}C (Table 2). The highest temperature at the sampling hour of the day was 32.0^{o}C on 17 August 1984; the lowest was 0.3^{o}C on 15 February 1985.

The DO concentration at the surface water annually fluctuated as low in summer and high in winter, ranging from 4.4 mgO_2/l on 21 September 1984 to 14.5 mgO_2/l on 28 December 1984 (Figure 20). This DO minium in this bog is still far above the minimum level required by bog fishes, and then the summer-kill in

fishes (Welch, 1952) has never taken place in Doh-Hoh-Numa Bog. The annual average DO concentration was 8.8 mgO_2/l (Table 2).

The concentration of inorganic nitrogen (NO_3-N, NO_2-N and NH_4-N) annually fluctuated as follows (Figure 20): It began to increase progressively from May by reaching its annual maximum of 34.2 μg-at/l on 23 November 1984. Then it was almost constant throughout the winter. At the beginning of spring in 1985, it was almost at the same level as that at the beginning of spring in 1984. Thus its annual cycle was completed without obvious changes. The annual average was 6.9 μg-at/l. The large fraction of the concentration was occupied in the form of NH_4 (ammonium-nitrogen) throughout the year (Table 2); the highest concentration of NH_4-N was 28.2 μg-at/l on 23 November 1984, which was approximately 80% of the total inorganic nitrogen concentration. Throughout the year, NO_3-N (nitrate-nitrogen) concentration fluctuated within a range from 0 up to 5 μg-at/l, whereas NO_2-N (nitrite-nitrogen) was dissolved in only trace amounts from 0.1 to 0.9 μg-at/l.

The concentration of PO_4-P (phosphate-phosphorus) also fluctuated within the mesotrophic range throughout the year (Figure 20). The concentration increased rapidly in June, and then stayed in a steady-state oscillation during the summer and early autumn. Then the concentration increased rapidly up to the annual maximum of 1.8 μg-at/l on 23 November 1984, when the inorganic nitrogen concentration also reached its annual maximum.

This simultaneous outbreak of the annual maxima showed a good periodical agreement with breakdown of the autumn bloom of phytoplankton (Liu & Seki, 1987). On the other hand, lowest levels were found in spring. The annual average was 0.4 μg-at/l (Table 2).

The DOC concentration increased in summer, reaching its annual maxmum of 11.5 mgC/l on 17 August 1984, when water temperature was the highest and the chlorophyll-a concentration was at the summer-peak (Figure 21). In the other seasons, the DOC concentration fluctuated within a steady range. The lowest DOC concentration was 0.4 mgC/l on 27 April 1984. The annual average was 3.8 mgC/l (Table 2).

The POC concentration had the spring and autumn maxima, each of which was composed of several pulses (Figure 21). The maximum pulse was as high as 16.5 mgC/l on 20 April 1984. Because each of these pulses corresponded to each pulse of chlorophyll-a in the bog water, the particulate organic matters consisted primarily of phytoplankters and phytodetritus. The lowest POC concentration was 1.1 mgC/l on 29 March 1985. The average POC concentration was 5.0 mgC/l (Table 2).

The concentration of chlorophyll-a fluctuated with each pulse corresponding to a POC pulse (Figure 21). The highest peak was of 101.5 μg/l on 13 October 1984. Other marked peaks were found in June, August, September, January and February. Dominant phytoplankton genera were Melosira and Fragilaria of diatom in

June; Microcystis and Merismopedia of blue-green algae in August; Fragilaria in September and October; Chlamydomonas of green algae in January; Navicula and Melosira of diatom in February (Liu & Seki, 1987). The annual average was 22.0 µg/l (Table 2).

Bacterioplankton in Main Basin of Doh-Hoh-Numa Bog

i) Population Density

The population densities of bacterioplankton increased progressively from the annual minimum of 1.1×10^9 cells/l on 20 April 1984 to the annual maximum of 6.2×10^9 cells/l on 2 November 1984 (Figure 22). Sharp decrease, however, was found just after the maximum, down to half of the maximum level, i.e., 3.1×10^9 cells/l. Then the density fluctuated within a rather steady range. The annual average was 3.1×10^9 cells/l (Table 2).

ii) Population Growth Rate

The population growth rates fluctuated within a steady range throughout the year (Figure 22). As a whole, the growth rates were around the average of 0.08/hr (Table 2), being higher in summer and lower in winter. Actually the highest rates of 0.18/hr was found on 27 July 1984, the lowest rate of 0.00/hr was found on 18 January 1985.

iii) Production Rate

The production rate of bacterioplankton was within a range of 0 to 165 μgC/l/day (Figure 22). The smallest production of 0 μgC/l/day on 18 January 1985 was due to the zero growth rate on that day. The greatest production was 165 μgC/l/day on 28 September when the population density and the growth rate of bacterioplankton attained the pulse peaks. A comparable peak of 160 μgC/l/day was found on 19 October 1984, and numerous smaller peaks were found in almost every month. The annual average production was 63 μgC/l/day (Table 2).

iv) DOC - Growth Relationship

The DOC concentration primarily affected on the population growth rates of bacterioplankton, when analyzed by the modified kinetic model of Monod (1949). For the natural populations of bacterioplankton in Matsumi-Ike Bog, this model was proved to be more practical when a threshold concentration for the bacterial growth-initiation is assumed. This has to be applied also for the DOC-growth analysis in Doh-Hoh-Numa Bog (Figure 23). Then two distinguished regressions were obtained in relation to the DOC concentration with the greatest statistical significance among environmental factors as follows:

(1) the DOC concentration below 3.0 mgC/l;

$$F[D] = 0.06\ D \exp(1 - 0.68\ D) \quad \text{---------- Equation 13}$$

$$(F = 6.1,\ F_{0.05} = 4.6)$$

(2) the DOC concentration above 3.0 mgC/l;

$$F[D] = 0.05(D-3.0)\exp\{1-0.37(D-3.0)\} \quad \text{----- Equation 14}$$

($F = 34.2$, $F_{0.01} = 7.6$)

where D represents the DOC concentration (mgC/l). From these regression, it is suggested that the natural bacterioplankton community in Doh-Hoh-Numa Bog was consisted mainly of two groups with different efficiency on nutrient assimilation; One is an oligotrophic group at the DOC concentrations below 3.0 mgC/l, and another is mesotrophic group at the DOC concentrations above 3.0 mgC/l. Then the optimum DOC concentration was calculated to be 1.5 mgC/l for the oligotrophic group and 5.7 mgC/l for the mesotrophic group. The oligotrophic bacteria was shown to develop in natural reservoirs where concentrations of dissolved organic substances do not exceed several miligrames per liter (Kuznetsov et al., 1979). As the oligotrophic bacteria, in some cases, do not grow in the nutrient-rich media, Kuznetsov et al. (1979) described "Why inhibition of the oligotroph growth occurs on the rich nutrient substrate is not clear at present. One reason may be the action of toxic products of metabolism, in particular, of hydrogen peroxide, which forms in a number of metabolic reactions". Although the trophic nature of bacteria may change relating to their physiological states and the ambient conditions (Kuznetsov et al., 1979), those two groups in Doh-Hoh-Numa Bog may be distinguished, as have been shown in other environments (Moaledj & Overbeck, 1980; Witzel et al., 1982).

The highest growth rate obtained from Equations 13 and 14 was 0.13/hr (i.e. generation time of 5.3 hours). The annual average DOC concentration was 3.8 mgC/l, at which the growth rate was 61% of the highest rate at the optimum DOC concentration.

v) Temperature - Growth Relationship

Population growth rates of bacterioplankton followed an Arrhenius equation throughout the year, over a thermal range of $32^{o}C$. The thermal function (F[T]) on the growth rates was statistically evident as the second most profound function among environmental factors based on the F-value, and could be expressed as a highly significant regression (Figure 24):

$$\log F[T] = -939/K + 2.13 \quad \text{---------- Equation 15}$$

$$(F = 11.8, F_{0.01} = 7.2)$$

where K is the absolute temperature of bog water.

vi) Density - Growth Relationship

Bacterial growth has been shown to be affected also by their own population density. This density effect (F[P]) on the population growth rates was statistically (F analysis) the third most profound among environmental functions, and could be expressed as a highly sgnificant regression (Figure 25):

$$F[P] = 0.02\ P \exp(1 - 0.25\ P) \quad \text{---------- Equation 16}$$

$$(F = 9.9, F_{0.01} = 4.1)$$

where P represents the population density of bacterioplankton

($x10^9$ cells/l). From this mathematical model of Equation 16, the population density of $4.0x10^9$ cells/l was shown to be the optimum for the bacterioplankton growth in Doh-Hoh-Numa Bog. The annual average density was $3.1x10^9$ cells/l, at which the growth rate was expected to be 97% of that at the opotimum density.

Mesotrophy of Main Basin of Doh-Hoh-Numa Bog

In classifying the oligotrophy, mesotrophy and eutrophy of bogs (see Section of Matsumi-Ike Bog), the trophic state of a water body is regulated not only by the nutrient concentration (or biological factor) concentration but also by the depth. As it is a shallow bog, the trophic state of Doh-Hoh-Numa Bog is just the same as that of Matsumi-Ike Bog; both bogs are with similar topography with water depth around one metre, although the eutrophication degree of Doh-Hoh-Numa Bog was slightly lower than that of Matsumi-Ike Bog (Tables 1 and 2).

Two DOC Sources: Two Energy Flows

A major ultimate source of the dissolved organic materials is known to be photosynthetic products of phytoplankton. Sharp (1977) interrogates "Excretion of organic matter by marine phytoplankton: Do healty cells do it?" Through this question, it was suggested that the phytoplankton excretion are mostly due to artifacts. Here there is evidence of extensive excretion by healthy populations of phytoplankton (Fogg, 1977, 1983): With

many algae, glycollic acid was mostly the single excreted substance. The mechanism of the excretion was thought to be as "overflow" of carbon when photosynthesis takes place rapidly. The "passive diffusion" hypothesis was also proposed (Bjornsen, 1988), where the calculated passive diffusion could be responsible for all of the observed excretion of low-molecular-weight compounds from healthy phytoplankton of 10 μm in size. These excreted organic materials are rapidly utilized by bacteria (Bell, 1980, 1983; Chrost & Faust, 1983; Cole et al., 1982; Conveney 1982; Jordan & Likens, 1980; Kato & Stable, 1984; Larsson & Hagstrom, 1979, 1982; Mague et al., 1980). The phytoplankton excretion is commonly estimated to be around 5% of total carbon fixation through the photosynthesis in eutrophic waters, and about 40% in oligotrophic waters. The bacterial utilization is rapid immediately after the excretion.

In the Main Basin of Doh-Hoh-Numa Bog, the DOC concentration was shown to have a statistically significant relationship with the concentration of chlorophyll-a in the bog water during the period from 13 April 1984 to 2 November 1984 (Figure 26); this period is designated the Period P (phytoplankton-relating). The relationship was expressed as:

$$[DOC]_{(mgC/l)} = 0.83 + 0.16\,[chlorophyll\text{-}a]_{(\mu g/l)} \quad \text{------ Equation 17}$$

$$(F = 10.14,\ F_{0.01} = 7.19)$$

It is thus suggested that during the Period P, seasonal

fluctuation of the DOC concentration was regulated primarily by the formation and destruction of phytoplankton blooms.

On the other hand, the negative relationship between DOC and chlorophyll-a was shown during the period from 9 November 1984 to 29 March 1985 (Figure 26); this period is designated Period N (not phytoplankton-relating). The regression of this relationship was:

$$[DOC]_{(mgC/l)} = 3.51 - 0.03\,[chlorophyll\text{-}a]_{(\mu g/l)} \quad \text{------ Equation 18}$$

$$(F = 3.24,\ F_{0.1} = 2.99)$$

During Period N, the organic materials from reeds and other littoral vegetations were accumulated on the bog bottom. This accumulation of slightly refractory substances provided the major source of the DOC concentration.

The DOC fluctuation and its DOC-chlorophyll relationship showed different profiles during Period P and Period N (Figure 27). The threshold DOC concentration of these two profiles was 3 mgC/l. Each fluctuation can be regressd as a high significant sine-curve as follows:

Period P (from 13 April 1984 to 2 November 1984);

$$[DOC]_{(mgC/l)} = 2.81 \sin\{(w-11)\pi/20\} + 3.99 \quad \text{------ Equation 19}$$

$$(F = 48.90,\ F_{0.005} = 9.28)$$

Period N (from 9 November 1984 to 29 March 1985);

$$[DOC]_{(mgC/l)} = 0.65 \sin\{(w-33)\pi/6\} + 2.95 \quad \text{------- Equation 20}$$

$$(F = 6.70,\ F_{0.025} = 5.92)$$

where w is the number of weeks elapsed since 13 April 1984. Differentials of Equations 19 and 20 show the oscillation rate in the DOC concentration as follows:

Period P;

$$d[DOC]/dw = 0.44 \cos\{(w-11)\pi/20\} \quad \text{---- Equation 21}$$

Period N;

$$d[DOC]/dw = 0.34 \cos\{(w-33)\pi/6\} \quad \text{----- Equation 22}$$

The fluctuation of the population density of bacterioplankton ($x10^9$ cells/l) shows an obvious break at the same time when the DOC concentration had the break in its oscillation (Figure 27):

Period P;

$$[Density] = 1.55 \sin\{(w-15)\pi/28\} + 3.16 \quad \text{---- Equation 23}$$

Period N;

$$[Density] = 0.50 \sin\{(w-33)\pi/12\} + 3.00 \quad \text{---- Equation 24}$$

where w is the number of weeks elapsed since 13 April 1984. The oscillation rates of the population density can be calculated by the differential of Equations 23 and 24:

Period P;

$$d[Density]/dw = 0.17 \cos\{(w-15)\pi/28\} \quad \text{----- Equation 25}$$

Period N;

$$d[Density]/dw = 0.13 \cos\{(w-33)\pi/12\} \quad \text{----- Equation 26}$$

Thus the DOC excreted from littoral vegetations was consumed by bacterioplankton not too slqwly, as has been observed on the fluctuation rate of the bacterioplankton population density upon

the decomposition of macrophytes in the marsh environment (e.g., Murray & Hodson, 1986).

3. Bacterioplankton Community in Main Basin of Doh-Hoh-Numa Bog -- After the Introduction of the Bio-filter System

Bio-filter System

The eutrophication and pollution of lakes and ponds has adverse effects on water use and poses serious social problem threatening our living environment. The recent increase in water demand, coupled with the incessant increase in the amount of pollution, makes the matter worse.

In spite of the extension of sewage systems, the efforts to expand quality control of waste water, the campaign for prohibiting the use of detergents, and other efforts to improve water quality, the increase in the artificial pollution load cannot be checked, and in fact the degree of pollution has exceeded the self-purification ability of the ecosystem.

The self-purification system of the natural world is a very complex mechanism incorporating not only the primary and secondary but also tertiary water treating processes, through which substances are in constant circulation. If this processing mechanism can be realized in a mechanical system, it would be possible to purify the water in a more ideal form.

A purification system utilizing aquatic plants, "Bio-filter System", have been devised, taking note of the tertiary water treating mechanism of the natural world. By making the most of aquatic plants that have an ability to grow rapidly, and by

artificially creating a living environment suitable for their growth, a purification rate higher than that of natural plants alone can be expected.

This system is a mechanical one in which aquatic plants are grown in particular areas of a lake or a pond or a inner lake or waterway in order to absorb and remove nutritive salts, and in which the natural aquatic plants are collected and effectively utilized as feed, solid fuel, compost, or methane gas. The system is expected to promote the natural purification through a new mechanically rationalized circulation system that incorporates human life.

A demonstration test plant was constructed in Doh-Hoh-Numa Bog. One section of the water area of the bog (i.e. the north-western part) is designated for culturing aquatic plants; mainly water hyacinth (Eichhornia crassipes), and water cress (Nasturtium officinale) in minor scale. Into this cultivation area, polluted (raw) water is pumped through a submerged pipeline. The water is pumped at a flow rate of 300 to 800 m^3/day through such a route, so that the BOD (biochemical oxygen demand; an indicator of water pollution) of the raw water is first reduced by the flow-type catalytic oxidation waterway. Next, the nutritive salts are absorbed and removed in the aquatic plant area. Finally, the purified water is returned to the main bog. The matured aquatic plants that have grown by absorbing the nutritive salts are removed by a recovery system and dried in a

solar drying system. The dried aquatic plants are converted into compost, feed or solid fuel in order to be reused as useful resources.

As a preliminary result, this Bio-filter System reduced the nutrients in the bog from a moderately mesotrophic level to a low mesotrophic level within a few weeks (Engineering Advancement Association of Japan, 1986). Such rapid oligotrophication should directly affect microbial communities within the time scale of one generation.

Surface water was collected at Station 1, near which a pump was set (Figure 28). Setting of this pump resulted in shallowness around Station 1, and boats were unable to be used for sampling. Hence the surface sampling was made with a polythene bottle holded at the tip of a pole (2 to 3 metre long).

Two Ecologically Different Periods of the Study Year

The study year was the period of post-introduction of the Bio-filter system, and was separated into two periods according to operation of the Bio-filter system. And the study year was separated between October and November, because the water temperature became lower than 15^{0}C below which water hyacinth, having the tropical origin, cannot maintain active growth and incorporation of nutritive salts:

Period I -- the operation period

from 21 June 1985 to the end of October 1985

Period II - the non-operation period

from 1 November 1985 to 14 June 1986

Corresponding periods in the previous study year (1984-1985) were also divided into Periods I' and II':

Period I' -- from 22 June 1984 to the end of October 1984

Period II' - the rest priod

The results during Periods I and II were compared with those during Period I' and II', respectively, and differences were examined by the statistical F-test (Sokal & Rohlf, 1973).

Limnological Characteristics of Main Basin of Doh-Hoh-Numa Bog

The water temperature fluctuated with almost the same profiles as that before the operation of the Bio-filter system, and could be expressed as a highly significant regression:

$$t = 13.0 \sin\{(w+7)\pi/26\} + 15.2 \quad \text{---------- Equation 27}$$

$$(F = 572.6, F_{0.001} = 12.3)$$

where t is surface water temperature (^{o}C) and w is the number of weeks elapsed since 21 June 1985 (Figure 29).

The dissolved oxygen (DO) concentration in the surface water showed fluctuations of between 5 and 7 mgO_2/l during summer, and between 10 and 14 mgO_2/l during winter after the operation of the Bio-filter system (Figure 29).

The concentration of inorganic nitrogen (nitrate, nitrite and ammonium) fluctuated over 2 to 4 week cycles (Figure 29). From 9 August 1985 to 7 February 1986, the fluctuation range

increased progressively and reached a maximum concentration of 19.9 µg-at/l on 24 January 1986. The concentration then decreased rapidly down to 0.3 µg-at/l by 7 February 1986; this was a decrease of the concentration of 19.6 µg-at/l within two weeks. The average concentration was 4.8 µg-at/l during Period I, and 6.7 µg-at/l during Period II (Table 3). Of the three forms of inorganic nitrogen studied, ammonium-nitrogen (NH_4-N) was the most abundant throughout the study year. Its average concentration was 2.4 µg-at/l during Period I, and 3.9 µg-at/l during Period II (Table 3).

The inorganic phosphorus (phosphate-phosphorus) concentration was prominently high during October and November in 1985 with the annual maximum of 1.9 µg-at/l on 19 October 1985 (Figure 29). A second high level of 1.5 µg-at/l and a smaller maximum of 0.8 µg-at/l were formed on 7 June 1986 and 14 March 1986, respectively (Figure 29). During the period from June to September, the concentration ranged from only 0.1 to 0.5 µg-at/l. However, the average concentration was 0.5 µg-at/l during Period I because of the effect of the high levels in October (Table 3).

The DOC concentration during the post-introduction period of the Bio-filter system (Periods I and II) showed the fluctuation with the same annual profile as that during the pre-introduction period (Periods I' and II'); the concentration was high in summer with an annual maximum of 18.5 mgC/l on 16 August 1985. It was

generally low in winter, although the annual maximum of 0.3 mgC/l occurred in spring on 17 May 1986 (Figure 30). The average concentration was 8.8 mgC/l during Period I, and 2.8 mgC/l during Period II (Table 3).

The POC concentration was below 10 mgC/l throughout the year with highly dynamic fluctuation of 2 to 4 week cycles from August until March, reaching maxima of 7.6 mgC/l on 20 September 1985, 7.4 mgC/l on 25 October 1985, 9.0 mgC/l on 15 November 1985, and 9.7 mgC/l on 17 January 1986 (Figure 30). This annual POC fluctuation was almost the same between the periods of pre-introduction and post-introduction of the Bio-filter system, with the exceptions of the occurrence of a winter maximum and the disappearance of a spring maximum during Period II. The average POC was 3.3 mgC/l during Period I, and 3.6 mgC/l during Period II (Table 3).

The concentration of chlorophyll-a dynamically fluctuated with 2 to 5 week cycles throughout the year (Figure 30). It is notable that the summer phytoplankton bloom did not occur in this year; the highest chlorophyll-a concentration of 32.4 µg/l was found on 6 December 1985 (Period II). The average concentration was slightly lower in summer than winter, i.e., 13.9 µg/l during Period I and 14.2 µg/l during Period II (Table 3).

Bacterioplankton in Main Basin of Doh-Hoh-Numa Bog

i) Population Density

The population densities of bacterioplankton were mostly between $1x10^9$ and $4x10^9$ cells/l throughout the year (Figure 31). A higher population density occurred during the summer: $3.8x10^9$ cells/l on 9 August 1985 and $4.6x10^9$ cells/l on 14 June 1986. The population denisty became lower levels from early September 1985 and attained the lowest during the final period of the system operation: $0.93x10^9$ cells/l on 19 October 1985 and $0.95x10^9$ cells/l on 1 November 1985. The average population density was $2.1x10^9$ cells/l during Period I, that is comparable to $2.3x10^9$ cells/l during Period II (Table 3).

ii) Population Growth Rate

The growth rates of bacterioplankton were similar both during Periods I and II (Figure 31). The average growth rate was 0.10/hr (generation time of 6.8 hours) during Period I, and it was slightly lower as 0.08/hr (8.7 hours) during Period II (Table 3). However, the sharp fluctuations occurred immediately after the operation was discontinued. The bacterial growth rate increased greatly in the middle of November when the water hyacinth declined in its physiological activities and started to decay. The specific growth rate was the highest as 0.23/hr (generation time of 3.0 hours) on 22 November 1985, but decreased

rapidly down to 0.02/hr (46 hours) on 29 November 1985, thereafter increased.

iii) Production Rate

The bacterioplankton production became at a higher level just after the introduction of the Bio-filter system (Figure 31). Then it decreased and constantly fluctuated within a range from 5 to 60 μgC/l/day, except 138 μgC/l/day on 22 November 1985 when the highest growth rate was observed. The average production was slightly higher during Period I than during Period II; 48 μgC/l/day during Period I, and 42 μgC/l/day during Period II (Table 3).

iv) DOC - Growth Relationship

The bacterioplankton growth rate was affected primarily by the DOC concentration even after the introduction of the Bio-filter system (Figure 32). In Doh-Hoh-Numa Bog, two bacterioplankton groups were obviously characterized in relation to the nutrient requirement with the nutrient threshold of 3 mgDOC/l. The DOC-growth analysis was made based on the growth response of the bacterioplankton community to a nutritional environment above and below this threshold concentration. The growth rate (F[D]; /hr) was expressed as a function of the DOC concentration (D; mgC/l) in the bog environment:

Period I:

(1) The DOC concentration below 3 mgC/l;

The analysis was impossible due to too small number of data.

(2) The DOC concentration above 3 mgC/l;

$$F[D] = 0.04\ (D-3)\ \exp(1 - 0.26(D-3)) \quad \text{----- Equation 28}$$

($F = 29.3$, $F_{0.005} = 11.4$)

Period II:

(1) The DOC concentration below 3 mgC/l;

$$F[D] = 0.07\ D\ \exp(1 - 0.95\ D) \quad \text{---------- Equation 29}$$

($F = 10.7$, $F_{0.01} = 9.3$)

(2) The DOC concentration above 3 mgC/l;

$$F[D] = 0.08\ (D-3)\ \exp(1 - 0.73(D-3)) \quad \text{----- Equation 30}$$

($F = 12.2$, $F_{0.01} = 12.2$)

These equations gave theoretically for the population growth of bacterioplankton community the optimal DOC concentrtions of 6.9 mgC/l with the DOC concentration above 3 mgC/l during Period I, 1.1 mgC/l with the DOC concentration below 3 mgC/l during Period II, and 4.4 mgC/l with the DOC concentration above 3 mgC/l during Period II.

v) Density - Growth Relationship

The ecological factor affecting secondly the bacterial growth was the bacterioplankton population density (Figure 33), when analyzed based on Allee's principle. The density-growth relationship for each period was highly significant:

Period I:

$$F[P] = 0.05\ P \exp(1 - 0.55\ P) \quad \text{---------- Equation 31}$$

$$(F = 9.2,\ F_{0.01} = 8.5)$$

Period II:

$$F[P] = 0.06\ P \exp(1 - 0.78\ P) \quad \text{---------- Equation 32}$$

where F[P] is the growth rate (/hr) as a function of the bacterioplankton population density (P; $x10^9$ cells/l). These equations show theoretically that the optimal densities of $1.8x10^9$ cells/l (Equation 30) and $1.3x10^9$ cells/l (Equation 32) for Periods I and II, respectively, were almost comparable without regard to the slight activation of the heterotrophic processes in the bog water during the system operation.

vi) Temperature - Growth Relationship

During the study periods, the water temperature did not affect the bacterioplankton growth rate, as the Arrhenius plot of temperature-growth relationship during each period was neither statistically (low F-value) nor theoretically (positve slope) significant (Figure 34):

Period I:

$$\log F[T] = -86.23/K - 0.76 \quad \text{---------- Equation 33}$$

$$(F = 0.007,\ F_{0.9} = 0.016)$$

Period II:

$$\log F[T] = 441.68/K - 2.79 \quad \text{---------- Equation 34}$$

(no significance due to the positive slope)

where F[T] is the growth rate (/hr) as a function of the absolute temperature (K).

Effect of the Bio-filter System in Main Basin of Doh-Hoh-Numa Bog

The Bio-filter system caused significant changes to the nutrient structure of Doh-Hoh-Numa Bog, and as the consequence the nature of the bacterioplankton community also changed. These environmental perturbations are represented by five patterns; for example, "down-level" represents the downwards shift in relative values of an environmental parameter from Period I' to Period I, and no change in relative values from Period II' to Period II (Table 3). These patterns of change were statistically (analysis of variance) analyzed using the F-test, and significant shifts are marked with an asterisks. Each parameter will be discussed in the order of their appearance in Table 3.

The change in average temperature was necessarily a "level-level" pattern (103-107), because the climate conditions were not different and the Bio-filter system has no thermal effect. However, there were naturally occurring differences of 3 to 7%, since the changes in relative values were 103-107, not exactly 100-100.

The change in average DO concentration was also a "level-level" pattern (116-93). The differences were somewhat larger than that of temperature, because the DO concentrtion is affected not only physico-chemically but also biologically. However, the

the Bio-filter system did not affect the DO concentration at Station 1, 150m distant from the aquatic plant cultivation area.

The change in levels of inorganic nitrogen followed a "down-level" pattern (60^{*}-106) with a highly significant decrease (*, F = 3.9, $F_{0.1}$ = 2.9) during the operation period. This decrease was chiefly brought about by the removal of ammonium-nitrogen (NH_4-N), being the dominant form of inorganic nitrogen throughout the year as 36^{*}-91 (*, F = 11.7, $F_{0.01}$ = 7.4). In contrast with ammonium-nitrogen, the nitrate-nitrogen (NO_3-N) concentration markedly increased as 232^{*}-151^{**}. This increase in nitrate concentration was due to the catalytic oxidation process in the waterway, a part of the Bio-filter system.

The phosphate-phosphorus (PO_4-P) concentration was a "level-up" pattern of 111-178^{*} (*, F = 10.2, $F_{0.01}$ = 7.1). This increase was probably due to a depression of phytoplankton that usually consumes phosphate leaching from the bog sediment; it has already been shown that phytoplankton decreased in abundance through the Bio-filter system operation (Liu & Seki, 1987; in press). The phosphate in the bog sediment should be constantly released before and after the introduction of the Bio-filter system under the same conditions of pH, oxygen content, and redox potential that control its solubility (e.g., Andersen, 1975; Hutchinson, 1957; Kawai et al., 1984); these act synergistically with wind (e.g., De Groot, 1981) and the effect of phosphate solubilizing bacteria (e.g., Jana & Patel, 1984).

The "up-level" pattern of the DOC concentration would have been brought about by depressing the heterotrophic processes over the autotrophic processes in the bog ecosystem due to the Bio-filter system operation. The shifting profile was 157^{*}-100 (*, F = 7.0, $F_{0.02}$ = 5.9). The high DOC concentration during the Period I is caused primarily by its release from the water hyacinth into bog water without consumption by the heterotrophic processes, as has been shown with other aquatic plants such as Scirpus (Rich et al., 1971), Phragmites (Banoub, 1975), and Nuphar and Nymphaea (Ward & Wetzel, 1984). The return of the DOC concentration to previous winter levels (Period II') can be attributed to the readily degradable nature of the detritus from dead water hyacinth (Ayyappan et al., 1986; Geller, 1983).

The POC concentration shows a "down-down" pattern as 70^{*}-71^{**} (*, F = 4.2, $F_{0.01}$ = 4.1; **, F = 3.3, $F_{0.1}$ = 2.8). This POC decrease was mostly due to a diminished abundance of phytoplankton. Detritus from the dead water hyacinth is readily degradable, and was taken out from the bog to have little contribution to the POC concentration.

Chlorophyll-a concentration also shows a "down-down" pattern as 46^{*}-83^{**} (*, F = 11.5, $F_{0.01}$ = 4.1; **, F = 1.8, $F_{0.1}$ =1.7). Largely decreased biomass of the phytoplankton was due to Melosira, which was dominant before and after the introduction of the Bio-filter system (Liu & Seki, 1987; in press). Although the limiting factor controlling the ecosystem of Doh-Hoh-Numa Bog is

phosphate as in most inland waters (e.g., Schindler, 1974; Vollenweider, 1968), nitrogen has shown to be the limiting factor for the growth and maintenance of the phytoplankton in Doh-Hoh-Numa Bog (Liu & Seki, 1987; in press).

The bacterioplankton population density shows a "down-down" pattern as 57^{*}-83^{**} (*, F = 27.9, $F_{0.001}$ =12.9; **, F = 6.9, $F_{0.02}$ = 5.7). Depression of the bacterial population density during Period I was possibly caused by the bacteriostatic (inhibiting or slowing down the bacterial growth) effect of phytoplankton (Kuznetsov, 1959) and the decreased abundance of interacting phytoplankters (Bell et al., 1982; Fiala & Vasata, 1982). The incomplete shift back to the level during Period II (84) must have followed the incomplete shift from oligotrophication to the moderately mesotrophy in the bog ecosystem.

In contrast with the shifting patterns in trophic parameters described above and below, the population growth rate of bacterioplankton showed the steady-state fluctuations throughout the study year without obvious influence of the Bio-filter system operation. The fluctuations are shown as a pattern of 95-116 (Period I, F = 0.2, $F_{0.5}$ = 0.5; Period II, F = 0.2, $F_{0.5}$ = 0.5). This unchangability of the bacterial growth rate during the oligotrophication is caused primarily by multiple relationships between DOC-growth and density-growth, although the degrees of oligotrophication of either the DOC concentration or

the bacterioplankton population density could not be so great as to force a shifting of the growth rate of bacterioplankton.

The bacterioplankton production followed a "down-level" pattern as 51^{*}-93 (*, $F = 19.6$, $F_{0.001} = 13.1$). The shift back to the previous level during Period II was responsible for returning the trophic state from oligotrophication to the moderate mesotrophy in Doh-Hoh-Numa Bog.

For the simulated growth rates, marked changes by the Bio-filter system were not found by remaining rather at the constant level as 110-80, as well as shown for the actually determined growth rates. Because the simulated growth rates are obtained from the DOC-growth and density-growth relationships, the steadiness of the simulated growth rates suggests that the in situ growth rates were kept unchanging by the function of these relationships.

Steadiness of Growth Rate during Oligotrophication

As the equations used in analyzing DOC-growth and density-growth relationships are transformed into

$$\ln (y/x) = (\ln (A) + 1) - B x$$

the slope B should be compared for differences between the periods. Since the inverse of B represents the optimal x-value of the x-y relationship, the shift in the optimal DOC concentration and the optimal population density can be analyzed for examining the ecological changes by this rapid

oligotrophication. For the pre-introduction of the Bio-filter system, Periods I' and II' are combined into a single period, because the DOC-growth and density-growth relationships were not seasonally affected and there is no need to divide the pre-introduction period, or pre-opration period of the Bio-filter system.

In the case of the DOC-growth relationship when the DOC concentration above 3 mgC/l, the optimal concentration was 6.9 mgC/l during Period I, and then it decreased to 4.4 mgC/l during Period II (Figure 32). The optimum during the pre-operation period being 5.7 mgC/l, the shifting pattern of the optimum DOC concentration is expressed as 120-76* (*, F = 3.3, $F_{0.1}$ = 3.0), as affected by the "up-level" pattern of the DOC concentration of 157-100 (Table 3). This suggests a rapid response of the bacterioplankton community to the DOC perturbation.

In the case of the density-growth relationship, the optimal density was 1.8×10^9 cells/l during Period I, decreasing slightly to 1.3×10^9 cells/l during Period II. The optimum during the pre-operation period being 3.9×10^9 cells/l, the shifting pattern of the optimum density is expressed as 45-32* (*, F = 7.3, $F_{0.01}$ = 7.0), which was affected by the shifting pattern of the population density as 57-83 (Table 3).

It is thus clear that the population growth rate of bacterioplankton was controlled to be steady by shiftings of both

the DOC- and density-growth relationships.

Two Sources of DOC: Two Energy flows

The concentrations of DOC (mgC/l) and chlorophyll-a (ug/l) were correlated differently between Periods I and II (Figure 35), and different lines were drawn for the two periods:

Period I:

$$[DOC] = 0.30[Chl\text{-}a] + 4.55 \quad (r^2 = 0.15) \quad \text{-- Equation 35}$$

Period II:

$$[DOC] = 0.08[Chl\text{-}a] + 1.57 \quad (r^2 = 0.08) \quad \text{-- Equation 36}$$

This difference suggests the two different DOC suppliers. Actually two modes of the DOC fluctuation were distinguished in October and November of 1985 (Figure 36). The sudden break in the DOC fluctuation occurred at the extreme end of Period I (operation period of the Bio-filter system), just the same week as in the previous study year. The DOC sources were, however, different; the DOC were released from both phytoplankton and water hyacinth during Period I, while it was mainly supplied by phytoplankton during the previous Period P (corresponding to Period I) in the previous year. By contrast, the DOC was chiefly from decaying bodies of littoral vegetation during Period II as well as Period N (correponding to Period II). These fluctuations in the DOC concentration (mgC/l) were regressed as:

Period I (corresponding to Period P in the previous year):

$$[DOC] = 4.49 \sin\{(w-3)\pi/20\} + 7.02 \quad \text{------- Equation 37}$$

Period II (corresponding to Period N in the previous year):

$$[DOC] = 1.41 \sin\{(w-25)\pi/8\} + 3.70 \quad \text{------- Equation 38}$$

where w is the number of weeks elapsed since 21 June 1985. Differentials of Equations 37 and 38 show the changing rate of the DOC concentration as:

Period I:

$$d[DOC]/dw = 0.71 \cos\{(w-3)\pi/20\} \quad \text{---------- Equation 39}$$

Period II:

$$d[DOC]/dw = 0.55 \cos\{(w-25)\pi/8\} \quad \text{---------- Equation 40}$$

Fluctuations in the population density of bacterioplankton ($x10^9$cells/l) were able to be divided into two modes at the same breaking week as shown in the DOC fluctuations, as follows:

Period I:

$$[Density] = 0.39 \sin\{(w-1)\pi/10\} + 2.10 \quad \text{--- Equation 41}$$

Period II:

$$[Density] = 0.28 \sin\{(w-17.5)\pi/5\} + 1.95 \quad \text{- Equation 42}$$

Differentials of Equations 41 and 42 show the changing rate of the population density as:

Period I:

$$d[Density]/dw = 0.12 \cos\{(w-1)\pi/10\} \quad \text{------ Equation 43}$$

Period II:

$$d[Density]/dw = 0.18 \cos\{(w-17.5)\pi/5\} \quad \text{---- Equation 44}$$

The fluctuation modes of the DOC concentration and the bacterial population density during Periods I and II were different from those during Periods P and N in the previous year. Taking their differentials, however, it is revealed that the fluctuation modes have rather constant amplitudes of their changing rates, in spite of differences in cycles. The steadiness of the amplitudes is shown below:

	1984 - 1985		1985 - 1986	
Amplitude	Period P	Period N	Period I	Period II
d[DOC]/dw	0.44	0.34	0.71	0.55
d[Density]/dw	0.17	0.13	0.12	0.18

Thus, not only the bacterial growth rate but also the changing rate of the DOC concentration and the bacterial population density were kept steady during the rapid oligotrophication by the Bio-filter system.

4. Effect of Water Hyacinth on the Bacterioplankton Community in the Plant Cultivation Area of Doh-Hoh-Numa Bog

Water Hyacinth Cultivation Area

As a principle part of the Bio-filter system (Figure 37), the marginal basin of Doh-Hoh-Numa Bog was used for cultivating the aquatic plants which remove nutritive salts from the bog water. The bog water was pumped from the main basin, through submerged pipes, into the flow-type catalytic oxidation waterway for converting organic debris into inorganic nutrients, located along the cultivation area. In this area, two kinds of plants were cultivated: one was the major plant, water hyacinth (Eichhornia crassipes), and the other was, in much smaller scale, water cress (Nasturtium officinales). Water hyacinth was introduced into this area in June 1985, covered almost all the water surface of the area in July, and began to decay in September. After October, the water temperature being below $15^{o}C$, water hyacinth could not actively grow and was unable to remove nutritive salts. Here the plant was removed from this area.

Surface water was sampled at Station 3 in the cultivation area of Doh-Hoh-Numa Bog. The study year was separated into Periods I and II as described in the previous section, based on the operation period of the Bio-filter system.

Limnological Characteristics of the Plant Cultivation Area

The fluctuation of surface water temperature was significantly expressed as an annual sine-curve:

$$t = 12.5 \sin\{(w+8)\pi/26\} + 15.4 \quad \text{---------- Equation 45}$$

where t is the water temperature (^{o}C) and w is the number of weeks elapsed since 21 June 1985 (Figure 38).

The DO concentration in the surface water showed fluctuation of between 1.5 and 7.0 mgO_2/l during summer and between 3.3 and 14.3 mgO_2/l during winter, after the operation of the Bio-filter system (Figure 38). The low DO concentrations throughout the year were the most marked feature of Station 3 in the plant cultivation area during and after the Bio-filter system operation. The average DO concentration was 4.4 mgO_2/l during Period I, and it was 8.6 mgO_2/l during Period II (Table 4).

The concentration of inorganic nitrogen reached the pulse peak of 27.9 µg-at/l in its seasonal oscillation at the beginning of Period I, then it decreased to maintain lower level between 1.5 and 5.8 µg-at/l during the rest of Period I (Figure 38). On the other hand, it fluctuated dynamically with the periodical duration of 2 to 4 week cycles, ranging from 1.3 µg-at/l of the annual minimum on 3 May 1986 to 29.0 µg-at/l of the annual maximum on 24 May 1986. The average concentrations were 5.4 µg-at/l during Period I, and 8.7 µg-at/l during Period II (Table 4). When the profile is compared with those of Station 1 and Matsumi-Ike Bog, the fraction of nitrate-nitrogen (NO_3-N) in

the toal inorganic nitrogen dissolved in the plant cultivation area was greater, and the NO_3 concentration was comparable to or higher than the ammonium-nitrogen (NH_4) concentration (Table 4).

The concentration of phosphate-phosphorus (PO_4-P) was kept at lower level between 0.1 and 0.4 ug-at/l until water hyacinth became to reduce their activities in October; thereafter it reached higher levels, and then fluctuated dynamically reaching its annual maximum of 2.9 µg-at/l on 24 May 1985 when the inorganic nitrogen also reached its highest concentration (Figure 38). The average concentrations were 0.4 µg-at/l during Period I, and 0.9 µg-at/l during Period II (Table 4).

The DOC concentration fluctuated wihtin a higher range during Period I, from the period minimum of 3.3 mgC/l at the beginning to the annual maximum of 15.8 mgC/l on 9 August 1985. The DOC concentration was the maximum when the concentrations of inorganic-nitrogen and inorganic-phophorus were at the lowest level (Figure 39). The DOC concentration fluctuated mostly at lower levels during Period II, except two pulse peaks of 8.4 mgC/l on 24 January 1986 and 8.8 mgC/l on 21 February 1986. Although the average concentrations were 8.9 mgC/l during Period I, it was 3.3 mgC/l during Period II (Table 4).

The level of POC concentration was low between 0.3 and 1.8 mgC/l during Period I, but fluctuated dynamically during Period II ranging from 0.4 mgC/l to the annual maximum of 4.7 mgC/l on 20 December 1985 (Figure 39). Each POC pulse peak

was related to the pulse peak of chlorophyll-a concentration, showing that the major fraction of POC was regulated by the formation and breaking-down of phytoplankton bloom. The average POC concentrations were 0.8 mgC/l during Period I, and 1.7 mgC/l during Period II (Table 4).

The concentration of chlorophyll-a was obviously low during Period I, and fluctuated by forming greater pulses during Period II (Figure 39). These phytoplankton pulses in winter had peaks of 33.1 µg/l on 13 December 1985, the annual maximum of 34.1 µg/l on 7 February 1986, and 25.3 µg/l on 14 March 1986. The average concentrations were 5.2 µg-at/l during Period I, and 13.4 µg/l during Period II (Table 4).

Bacterioplankton in the Plant Cultivation Area

i) Population Density

The bacterioplankton population density was high in summer as 2.1×10^9 cells/l at the beginning of Period I but decreased to the annual minimum of 5.3×10^8 cells/l on 27 September 1985. Thereafter it increased progressively by showing the steady-state fluctuation up to the annual maximum of 3.1×10^9 cells/l (Figure 40). The averages were 1.3×10^9 cells/l during Period I, and 1.4×10^9 cells/l during Period II (Table 4).

ii) Population Growth Rate

The bacterioplankton growth rates fluctuated with the periodicity of 2 to 8 week, and showed slight fluctuation range at the beginning of Period II (Figure 40). The annual highest of the bacterioplankton growth rate was 0.18/hr (generation time of 3.8 hours) on 19 April 1986, followed by the annual lowest of 0.004/hr (148 hours) on 26 April 1986. The averages rates were 0.10/hr (generation time of 6.8 hours) during Period I, and 0.09/hr (8.0 hours) during Period II (Table 4).

iii) Production Rate

The production rate of bacterioplankton showed evident seasonal fluctuations; it decreased progressively during early-Period I, holding low levels throughout late-Period I and early-Period II. Then it fluctuated dynamically during late-Period II (Figure 40). The annual maximum bacterial production was 85 µgC/l/day on 17 May 1986, and the annual minimum was 1.2 µgC/l/day on 26 April 1986 when the bacterial growth rate was the lowest. The average production was 32 µgC/l/day during Period I, and 30 µgC/l/day during Period II (Table 4).

iv) DOC - Growth Relationship

The bacterioplankton growth rate was affected primarily by the DOC concentration (Figure 41). Two bacterioplankton groups were classified at the nutrient threshold of the DOC

concentration of 3 mgC/l. The DOC-growth analysis was made based on the growth function of each group as affected by the ambient DOC conditions above and below this threshold concentration:

Period I:

(1) The DOC concentration below 3 mgC/l;

The analysis was impossible due to small number of data.

(2) The DOC concentration above 3 mgC/l;

$$F[D] = 0.05(D-3)\exp\{1-0.29(D-3)\} \quad \text{--------- Equation 46}$$

($F = 21.7$, $F_{0.005} = 11.3$)

Period II:

(1) The DOC concentration below 3 mgC/l;

$$F[D] = 0.11\ D\ \exp(1 - 0.90\ D) \quad \text{---------- Equation 47}$$

($F = 26.0$, $F_{0.005} = 11.4$)

(2) The DOC concentration above 3 mgC/l;

$$F[D] = 0.04(D-3)\exp\{1-0.42(D-3)\} \quad \text{--------- Equation 48}$$

($F = 8.8$, $F_{0.02} = 7.4$)

where F[D] is the growth rate (/hr) as a function of the DOC concentration (D: mgC/l). These equations gave the optimal DOC concentrations of 6.5 mgC/l at the DOC concentration above 3 mgC/l during Period I, 1.1 mgC/l at the DOC concentration below 3 mgC/l during Period II, and 5.4 mgC/l at the DOC concentration above 3 mgC/l during Period II.

v) Density - Growth Relationship

The second factor affecting the bacterioplankton growth rate was the bacterioplankton population density, when analyzed based on Allee's principle (Figure 42). The density-growth relationship during each period could be regressed highly significantly as follows:

Period I:

$$F[P] = 0.07\ P \exp(1 - 0.64\ P) \quad \text{---------- Equation 49}$$

$$(F = 4.9,\ F_{0.05} = 4.5)$$

Period II:

$$F[P] = 0.05\ P \exp(1 - 0.57\ P) \quad \text{---------- Equation 60}$$

$$(F = 6.1,\ F_{0.02} = 6.1)$$

where F[P] is the bacterial growth rate (/hr) as a function of the bacterioplankton population density (P: $x10^9$ cells/l). These equations show theoretically that the optimal densities of $1.6x10^9$ cells/l during Period I and $1.7x10^9$ cells/l during Period II were almost comparable, without being affected by the the Bio-filter system operation.

vi) Temperature - Growth Relationship

During the study periods, the water temperature seems to have not affected significantly the growth rate of bacterioplankton, because the Arrhenius plots were neither statistically nor theoretically significant (Figure 43) as:

Period I:

$$\log F[T] = -1707/K + 4.71 \quad \text{---------- Equation 51}$$

($F = 2.2$, $F_{0.1} = 3.0$, $F_{0.2} = 1.8$)

Period II:

$$\log F[T] = 696/K - 3.59 \quad \text{---------- Equation 52}$$

(no significance due to the positive slope)

where F[T] is the bacterial growth rate (/hr) as a function of the absolute temperature (K).

Oxygen Condition in the Plant Cultivation Area

One of the most remarkable limnological features at Station 3 was the decrease of the DO concentration in August and January. The DO concentration of 1.5 mgO_2/l on 2 August 1985 was the lowest among all values from Doh-Hoh-Numa Bog and Matsumi-Ike Bog; There it was so low as 3.3 mgO_2/l was even in winter. Since Station 3 was in the cultivation area of water hyacinth, this reduction of DO concentration at Station 3 relates to water hyacinth activities:

1) Water hyacinth has the photosynthetic shoots above water, and does not photosynthetically evolve the molecular oxygen (O_2) into water through the photosynthesis.
2) Phytoplankton and benthic algae were prevented from their active photosynthesis by water hyacinth, as the plant removed the nutritive salts from the water. Its shoots shaded these algae by preventing the light penetration into

the water.

3) Roots of water hyacinth consumed the dissolved oxygen.

4) Dead and older bodies of water hyacinth degradaded in the water; this degradation caused the removing of dissolved oxygen from the water column.

The DO decrease in summer was caused by reasons 1), 2) and 3), wheareas the DO decrease in winter took place most eminently by the reason 4). In fact, water hyacinth is reported to consume a significant amount of oxygen on death and decay at the rates of 0.123 to 0.159 mgO_2/g dry weight/hr (Olah et al., 1987).

Bacterioplankton Decrease

The lower population density was observed in the range of 0.5 to 1 ($x10^9$ cells/l) during late-Period I and early-Period II, when the water hyacinth started to decay. This bacterial decrease must be caused by the inhibitory effect of water hyacinth decomposition, as has been suggested on aqautic macrophytes (e.g., Murray & Hodson, 1986).

Another probable factor affecting the decrease of bacterioplankton population was caused by the association with phytoplankton. When the bacteriostatic effects of phytoplankton increased (Kuznetsov, 1959), the bacterioplankton decreases. The bacterial population decreases also when the abundance of interacting phytoplankton decreases (Bell et al., 1982; Fiala & Vasata, 1982). This could be indirect effect of water hyacinth,

because the water hyacinth can control the abundance and activities of phytoplankton. This effect must be the most eminent through the DOC-bacterioplankton relationship, because the DOC is released in large amount by water hyacinth.

CONCLUSION

As the intensity of primary production by phytoplankton relates directly to the nutrient loading of a water body, the primary production in the pelagic region of a natural water has been extensively shown to maintain the ecological equilibrium with the trophic system of the water. In this equilibrium, the bacterial contribution had not been yet fully clarified.

When the chlorophyll-a concentration is taken as a biologically established indicator of eutrophication level for analyzing the bog ecosystems, a certain meaningful correlation is shown among the bacterioplankton production and the chlorophyll-a concentration, by using the averages in Tables 1, 2, 3, and 4 of this study:

$$\log[\text{Bac Prod}] = 0.81(\log[\text{Chl-a}]) + 0.80 \quad \text{------- Equation 53}$$

$$(F = 3.9,\ F_{0.2} = 2.1)$$

where [Bac Prod] is the production rate of bacterioplanklton (μgC/l/day), and [Chl-a] is the concentration of chlorophyll-a (μg/l).

More significant correlation is evident in the relationship between the chlorophyll-a concentration and the population density of bacterioplankton [Bac Dens] ($x10^9$ cells/l):

$$\log[\text{Bac Dens}] = 0.81\{\log[\text{Chl-a}]\} - 0.54 \quad \text{------- Equation 54}$$

$$(F = 5.6, F_{0.1} = 3.8)$$

Thus the bacterioplankton activity relates obviously to the eutrophication process at least in the mesotrophic bog ecosystems, where the Short Circuit Metabolism (Ohle, 1984) of nutrient-phytoplankton-bacterioplankton is eminent in the geochemical cycles. On the other hand, the growth rate of bacterioplankton [Bac GR] (/hr) is not correlated to the eutrophic level expressed by the chlorophyll-a concentration:

$$\log[\text{Bac GR}] = 0.003\{\log[\text{Chl-a}]\} - 1.04 \quad \text{-------- Equation 55}$$

$$(F = 0.0005, F_{0.9} = 0.0172)$$

This consistence of the bacterial activity at different eutrophication levels in the mesotrophic bog systems results from the cybernetic adaptation of bacterioplankton. This adaptation is explained by the relationships of the DOC-growth and the density-growth of bacterioplankton (Figure 44): As the DOC concentration and the bacterioplankton population density at each eutrophication level are shown to affect the bacterioplankton growth, the optimal values of these factors give the most favorable condition affecting the growth rate of bacterioplankton. As these optimal and the average values could be significantly regressed as a line with a slope of 1, representing the average:optimum ratio of 1:1 (F= 34.4, $F_{0.001}$= 21.0; Figure 44), the bacterioplankton growth in the bog

ecosystems can be estimated as highly adapted to the environment. This evaluation is possible because the *in situ* average value and the theoreticaly optimum value of the bacterial growth rate were comparable in these bogs. This desirable equilibrium has been attained possibly through a number of cybernetic loops of the bog ecosystems.

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Table 1. Annual average values of environmental parameters at Stations 1 and 2 in Matsumi-Ike Bog in 1983-1984.

Parameter	Total	Station 1	Station 2
Water temperature (^{o}C)	15.4	15.3	15.6
D O (mgO_2/l)	10.1	10.5	9.6
Inorganic-N (µg-at/l)	17.6	15.1	20.0
NO3-N (µg-at/l)	8.2	7.1	9.3
NO2-N (µg-at/l)	1.2	1.2	1.3
NH4-N (µg-at/l)	7.6	6.8	8.4
Inorganic-P (µg-at/l)	1.3	1.2	1.4
D O C (mgC/l)	4.6	4.7	4.5
P O C (mgC/l)	2.0	2.0	1.9
Chlorophyll-a (µg/l)	20.9	23.0	18.9
Bacterioplankton			
density ($x10^9$cells/l)	8.9	9.2	8.6
growth rate (/hr)	0.12	0.12	0.12
generation time (hrs)	5.9	5.8	6.0
production (µgC/l/day)	250	238	225

Table 2. Annual average values of environmental parameters at Station 1 in Doh-Hoh-Numa Bog in 1984-1985.

Parameter		Average
Water temperature	(^{o}C)	15.0
D O	(mgO_2/l)	8.8
Inorganic-N	(µg-at/l)	6.9
NO3-N	(µg-at/l)	1.4
NO2-N	(µg-at/l)	0.3
NH4-N	(µg-at/l)	5.2
Inorganic-P	(µg-at/l)	0.4
D O C	(mgC/l)	3.8
P O C	(mgC/l)	5.0
Chlorophyll-a	(µg/l)	22.0
Bacterioplankton		
density	($x10^{9}$cells/l)	3.1
growth rate	(/hr)	0.08
generation time	(hrs)	8.3
production	(µgC/l/day)	63

Table 3. Average values of environmental parameters at Station 1 in Doh-Hoh-Numa Bog during Period I (operation period of the Bio-filter system), Period II (non-operation period) and each corresponding periods in the previous study (Periods I' and II'). Relative values (%) are in the parentheses: Periods I' and II to Periods I' and II', respectively).

Parameter	I'	II'	I		II	
Water Temperature (^{o}C)	23.8	9.4	24.4	(103)	10.1	(107)
D O (mgO_2/l)	6.2	10.3	7.2	(116)	9.6	(93)
Inorganic-N (µg-at/l)	8.0	6.3	4.8	(60)	6.7	(106)
NO3-N (µg-at/l)	1.0	1.7	2.3	(232)	2.6	(151)
NO2-N (µg-at/l)	0.2	0.4	0.1	(43)	0.2	(67)
NH4-N (µg-at/l)	6.7	4.3	2.4	(36)	3.9	(91)
Inorganic-P (µg-at/l)	0.5	0.4	0.5	(111)	0.7	(178)
D O C (mgC/l)	5.6	2.8	8.8	(157)	2.8	(100)
P O C (mgC/l)	4.8	5.1	3.3	(70)	3.6	(71)
Chlorophyll-a (µg/l)	30.2	17.1	13.9	(46)	14.2	(83)
Bacterioplankton						
density ($x10^{9}$cells/l)	3.6	2.8	2.1	(57)	2.3	(83)
growth rate (/hr)	0.11	0.07	0.10	(95)	0.08	(116)
generation time (hrs)	6.5	10.0	6.8		8.7	
production(µgC/l/day)	95	45	48	(51)	42	(93)

Table 4. Average values of environmental parameters at Station 3 in Doh-Hoh-Numa Bog during Period I (operation period of the Bio-filter system) and Period II (non-operation period).

Parameter	Period I	Period II
Water temperature (^{o}C)	23.7	10.8
D O (mgO_2/l)	4.4	8.6
Inorganic-N (µg-at/l)	5.4	8.7
NO3-N (µg-at/l)	2.7	5.8
NO2-N (µg-at/l)	0.9	0.3
NH4-N (µg-at/l)	2.7	2.6
Inorganic-P (µg-at/l)	0.4	0.9
D O C (mgC/l)	8.9	3.3
P O C (mgC/l)	0.8	1.7
Chlorophyll-a (µg/l)	5.2	13.4
Bacterioplankton		
density ($x10^9$cells/l)	1.3	1.4
growth rate (/hr)	0.10	0.09
generation time (hrs)	6.8	8.0
production (µgC/l/day)	32	30

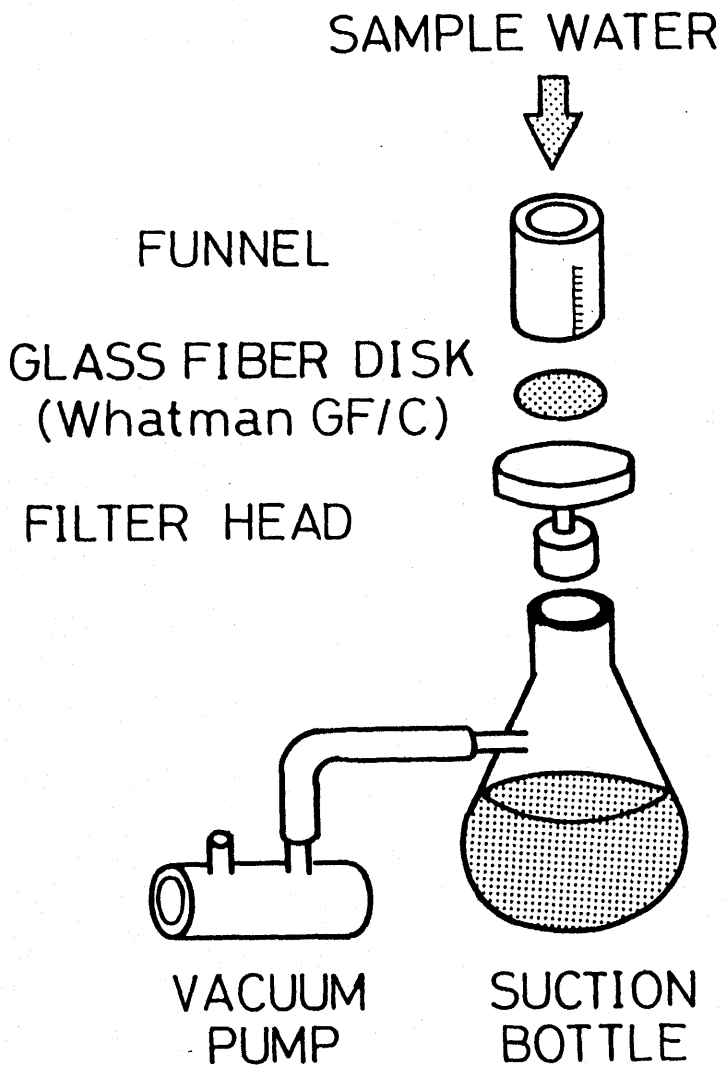


Figure 1. The filtration apparatus. In the cases of Matsumi-Ike Bog and Doh-Hoh-Numa Bog, 300 to 500ml of sample water was filtered per glass fiber filter.

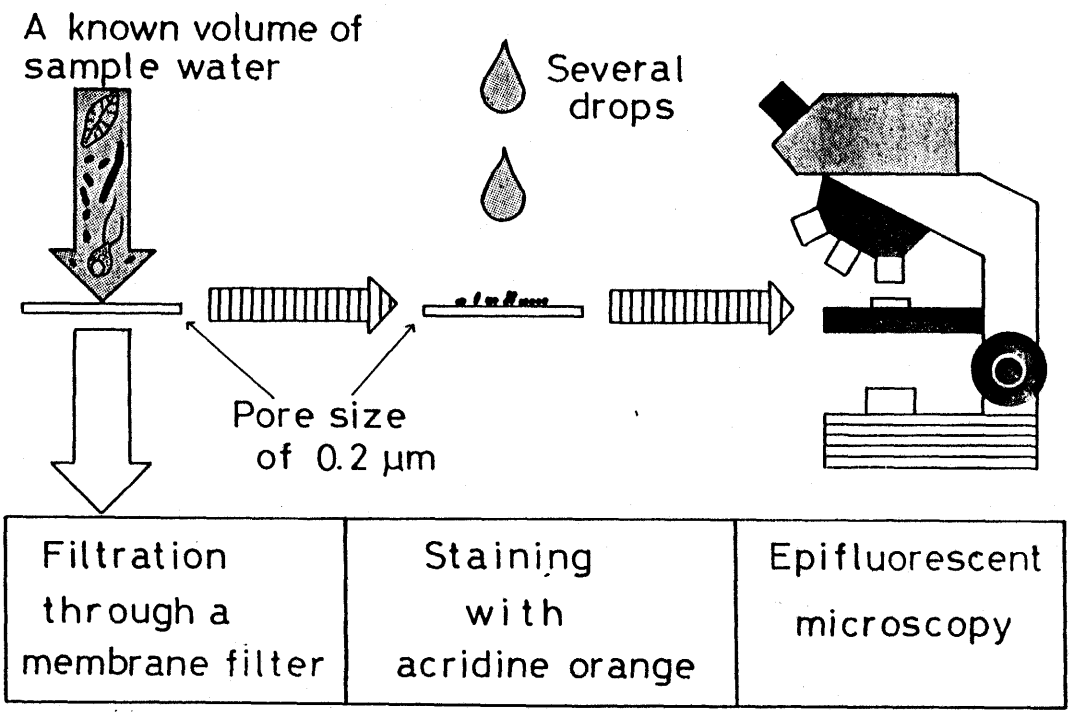


Figure 2 Procedure for counting the bacterioplankton cells. The combination of the membrane filter (e.g., Nuclepore, pore size of 0.2 um) and the epifluorescent microscopy gives the most reliable counts of total bacterioplankton cells at present.

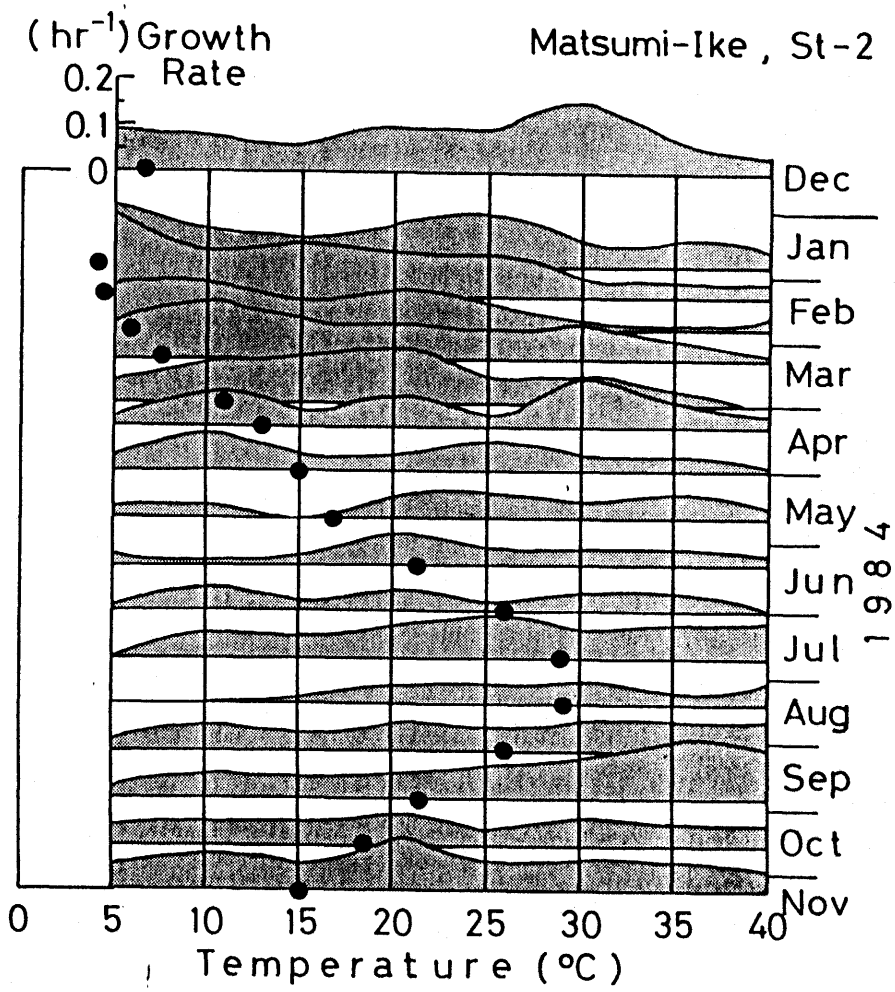


Figure 3. Temperature-spectrum of the bacterioplankton growth at Station 2 in Matsumi-Ike Bog. The growth rate was determined by increase in the cell number during the batch culture within 12 hours, using autoclaved bog water as the medium. The filled circles indicates the in situ temperature at each sampling time.

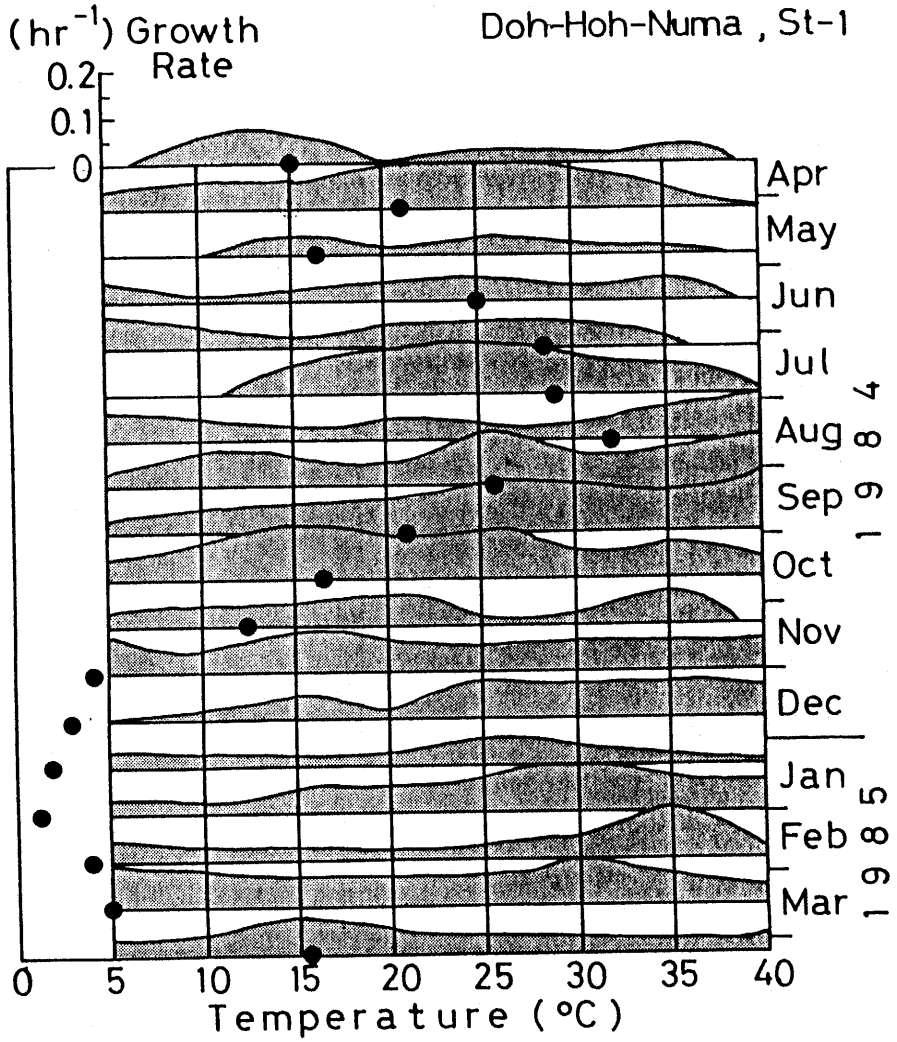


Figure 4. Temperature-spectrum of the bacterioplankton growth at Station 1 in Doh-Hoh-Numa Bog. The growth rate was determined by increase in the cell number during the batch culture within 12 hours, using autoclaved bog water as the medium. The filled circles indicates the in situ temperature at each sampling time.

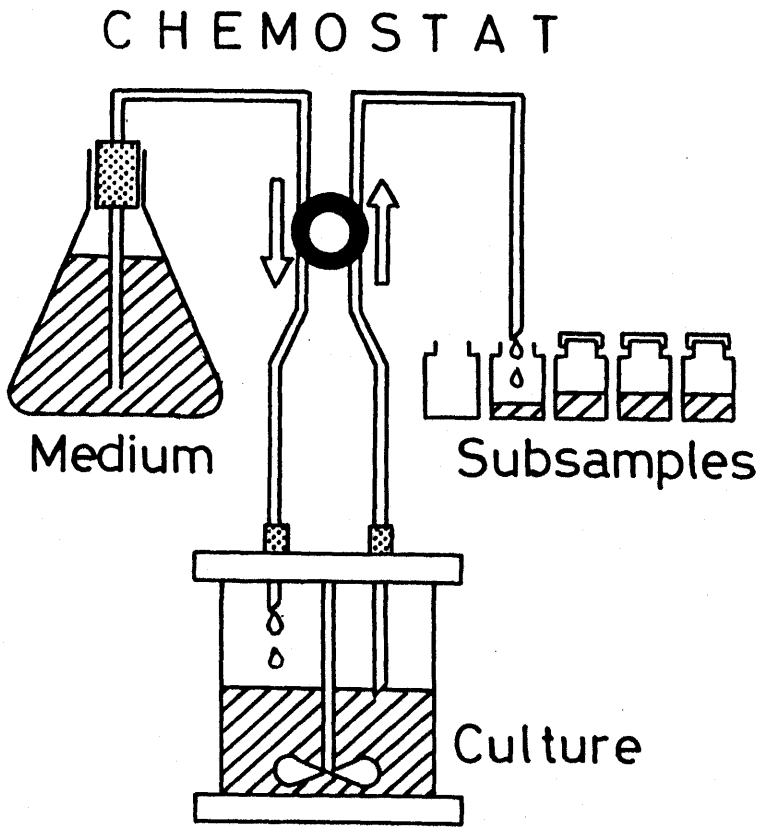


Figure 5. A simplified diagram of the chemostat culture. The medium is put into the culture vessel (jar-fermenter) at the same flow-out rate.

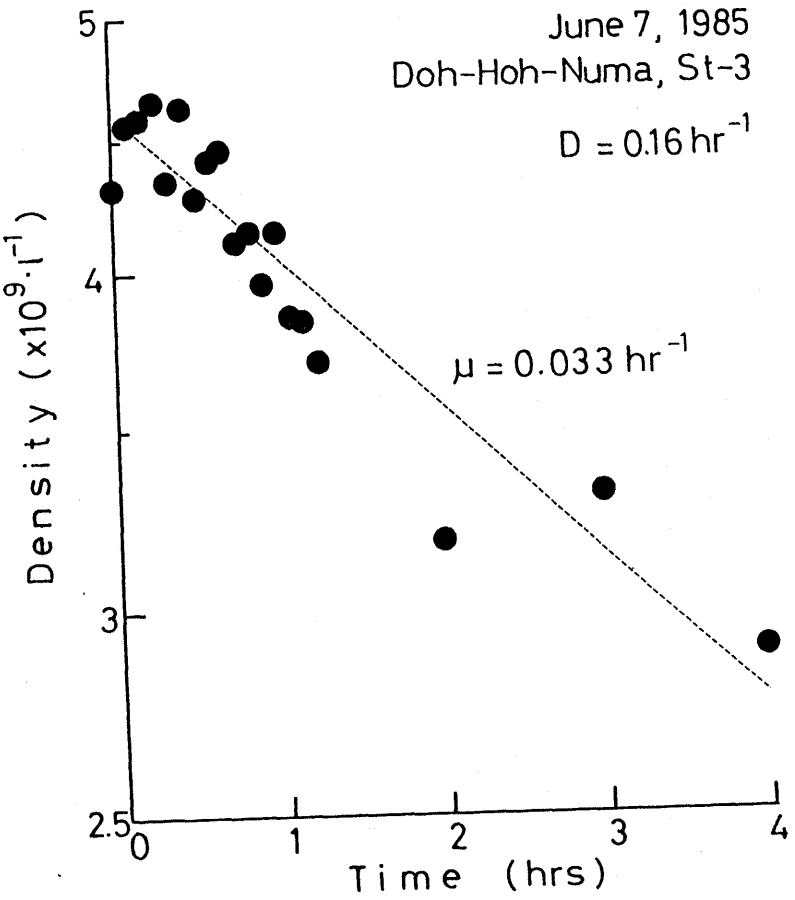


Figure 6. Plots of logarithm of the bacterioplankton population density against the time during the first 4 hours of the chemostat culture. The slope of the regressed line, i.e. the washout rate, was -0.127/hr, and the growth rate was calculated to be 0.033/hr; 0.033 = -0.127 (washout rate) + 0.160 (dilution rate).

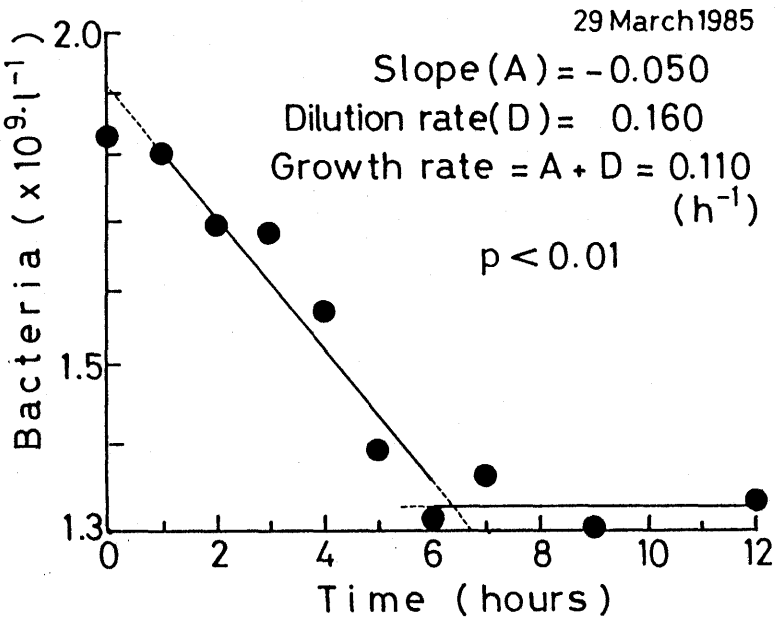


Figure 7. Plots of logarithm of the bacterioplankton population density against the time during the first 12 hours of the chemostat culture. The linear decrease in the population density lasted to 6 hours, thereafter the population density was reached a steady-state.

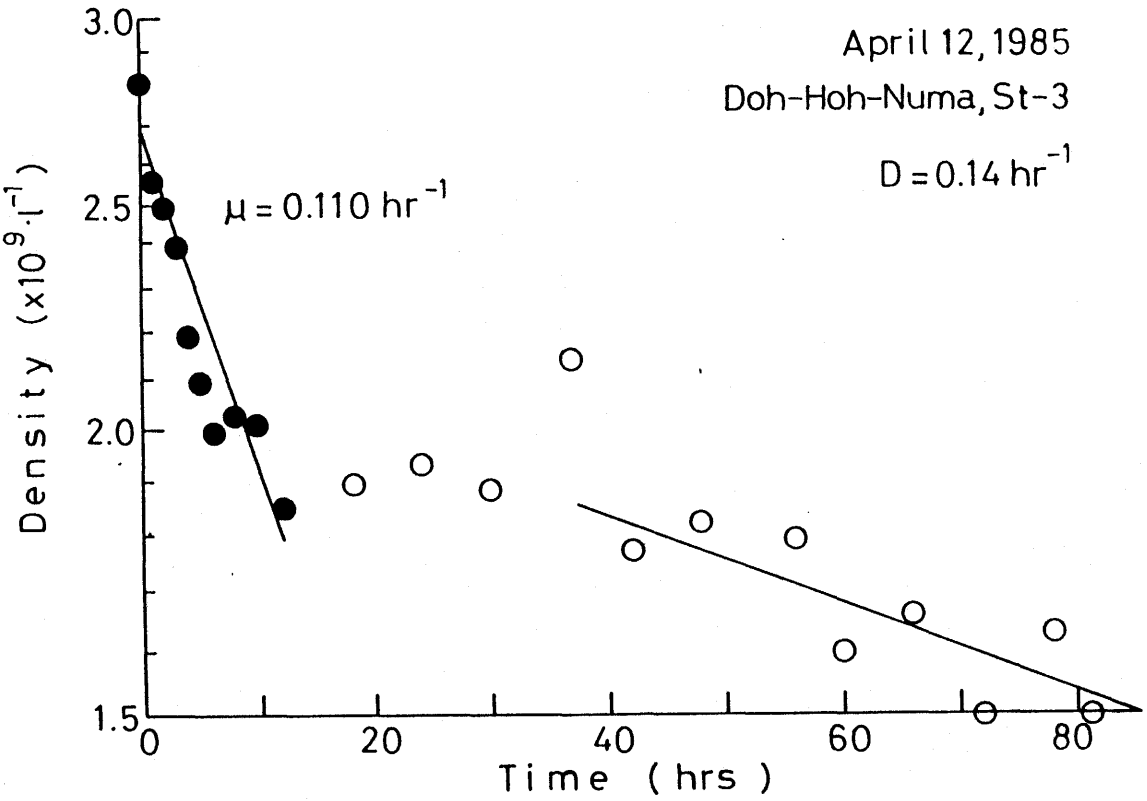


Figure 8. Plots of logarithm of the bacterioplankton population density against the time during the chemostat culture for more than 80 hours. The linear decrease in the population density (washout) was observed within the first 10 hours, and another washout was shown after 40 hours of the culture.

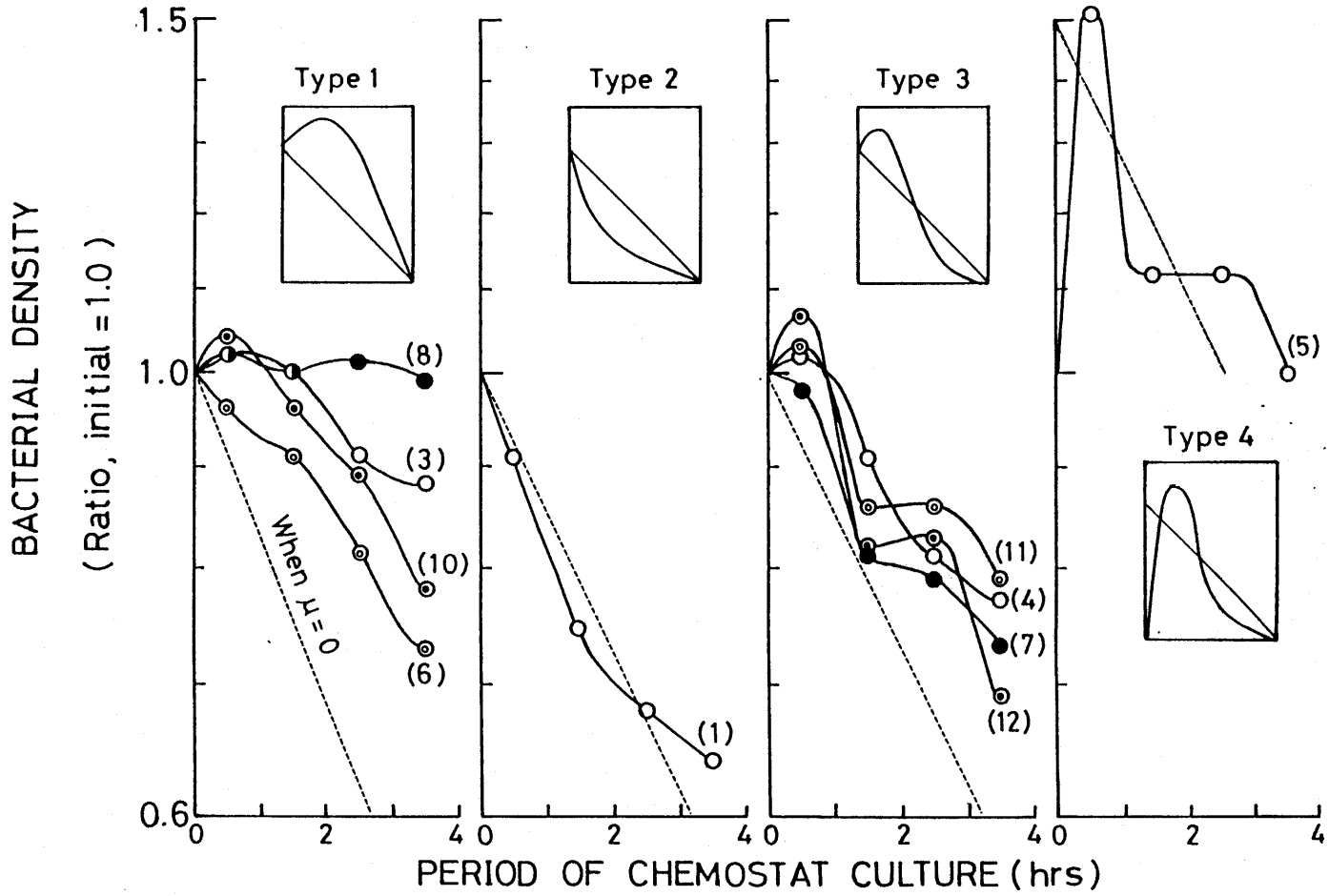


Figure 9. Four types of the washout during the first 4 hours of the chemostat culture. Each number in parenthses corresponding to the category number in Figure 10.

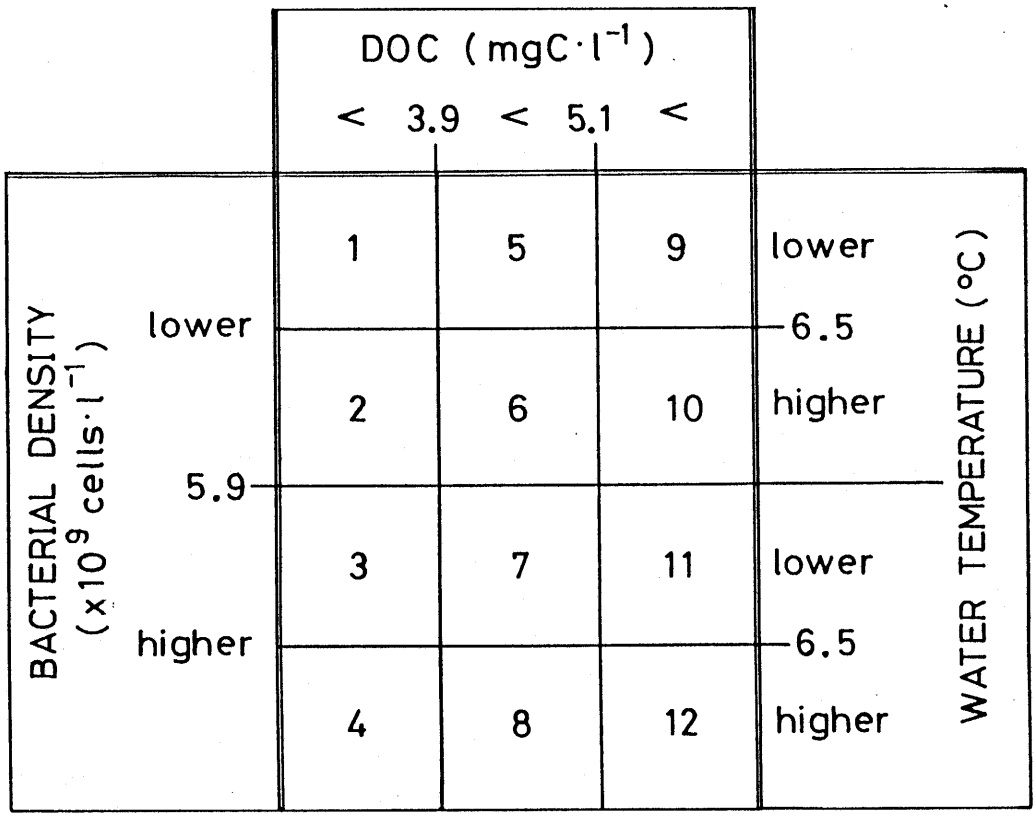
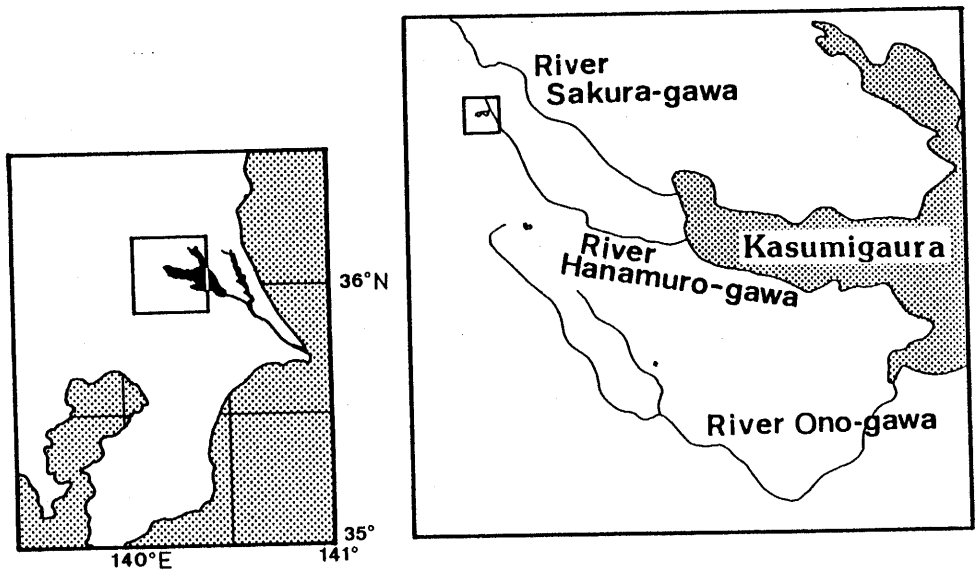


Figure 10. Environmental categories of Matusmi-Ike Bog, based on the environmental factors affecting the bacterioplankton population growth rates. Categories 2 and 9 were not actually observed during the study year.



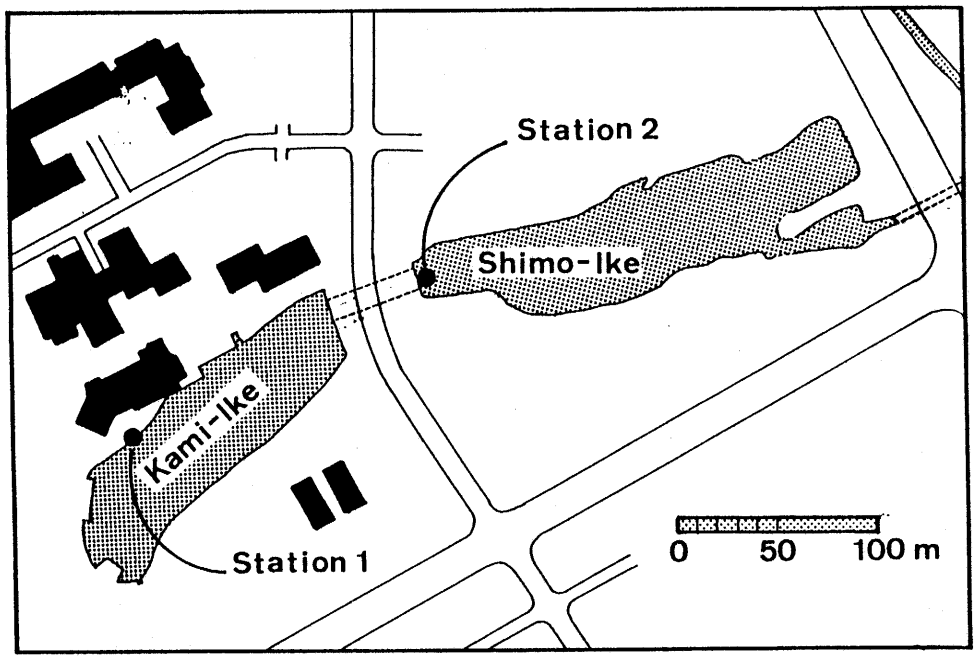


Figure 11. Two basins of Matsumi-Ike Bog, and locations of Stations 1 and 2 in the bog.

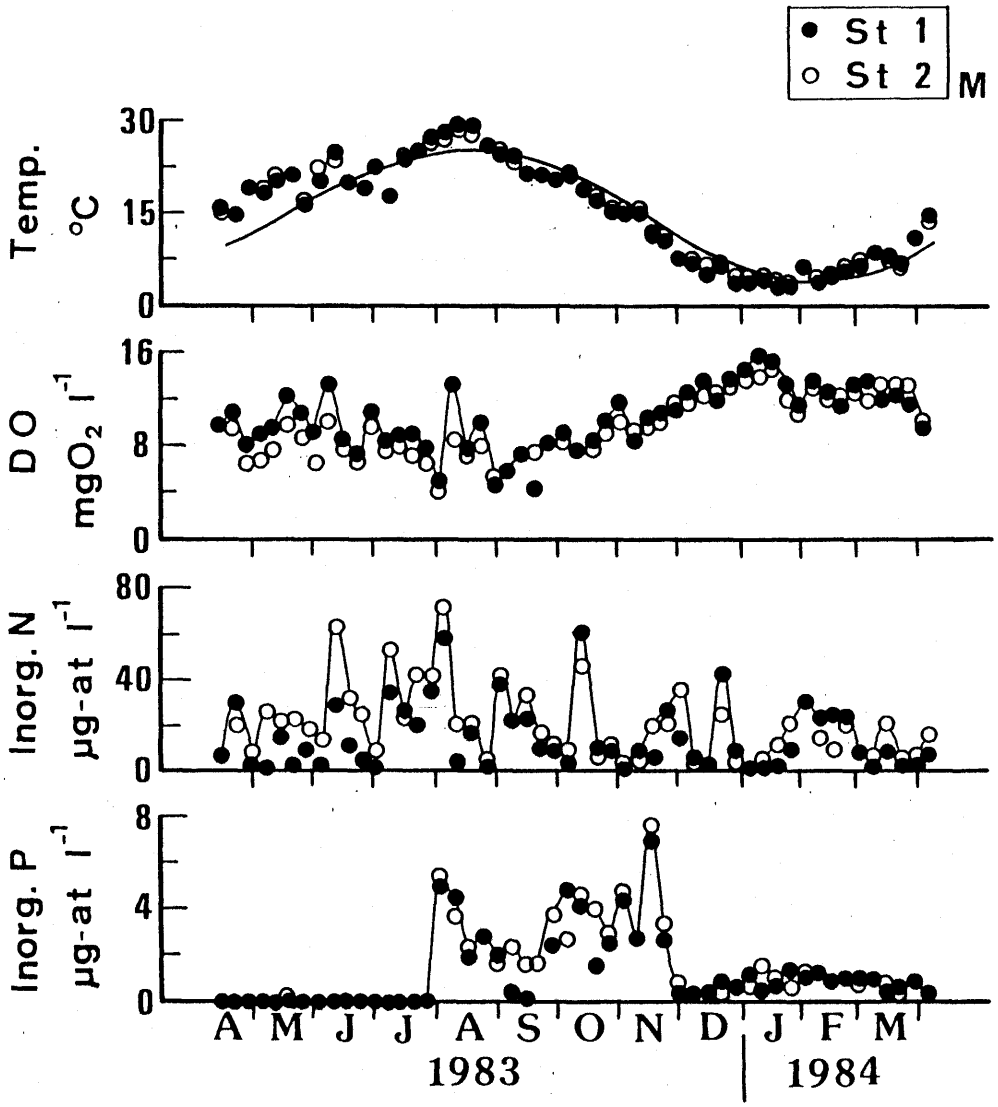


Figure 12. Seasonal variations in the physico-chemical parameters at Stations 1 and 2 in Matsumi-Ike Bog: (from top to bottom) temperature, concentration of dissolved oxygen, concentration of inorganic nitrogen, and concentration of inorganic phosphorus.

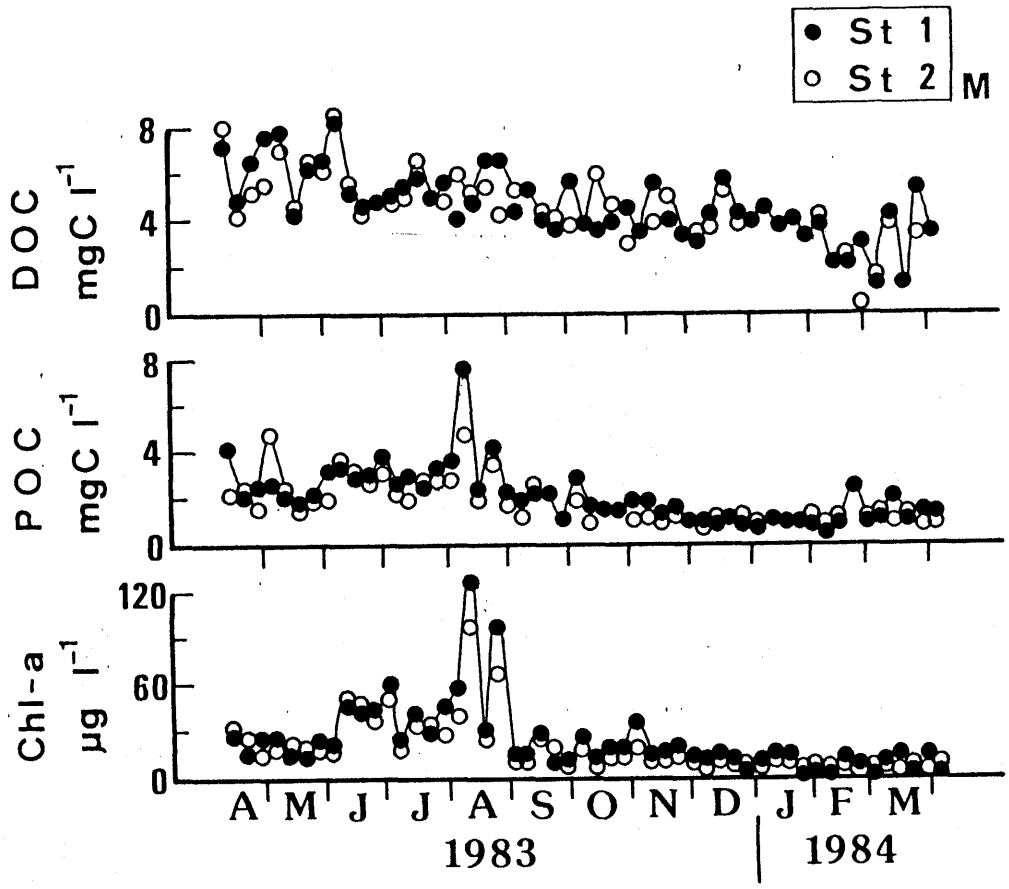


Figure 13. Seasonal variations in the concentrations of dissolved organic cabon (DOC), particulate organic carbon (POC) and chlorophyll-a (Chl-a) at Stations 1 and 2 in Matsumi-Ike Bog.

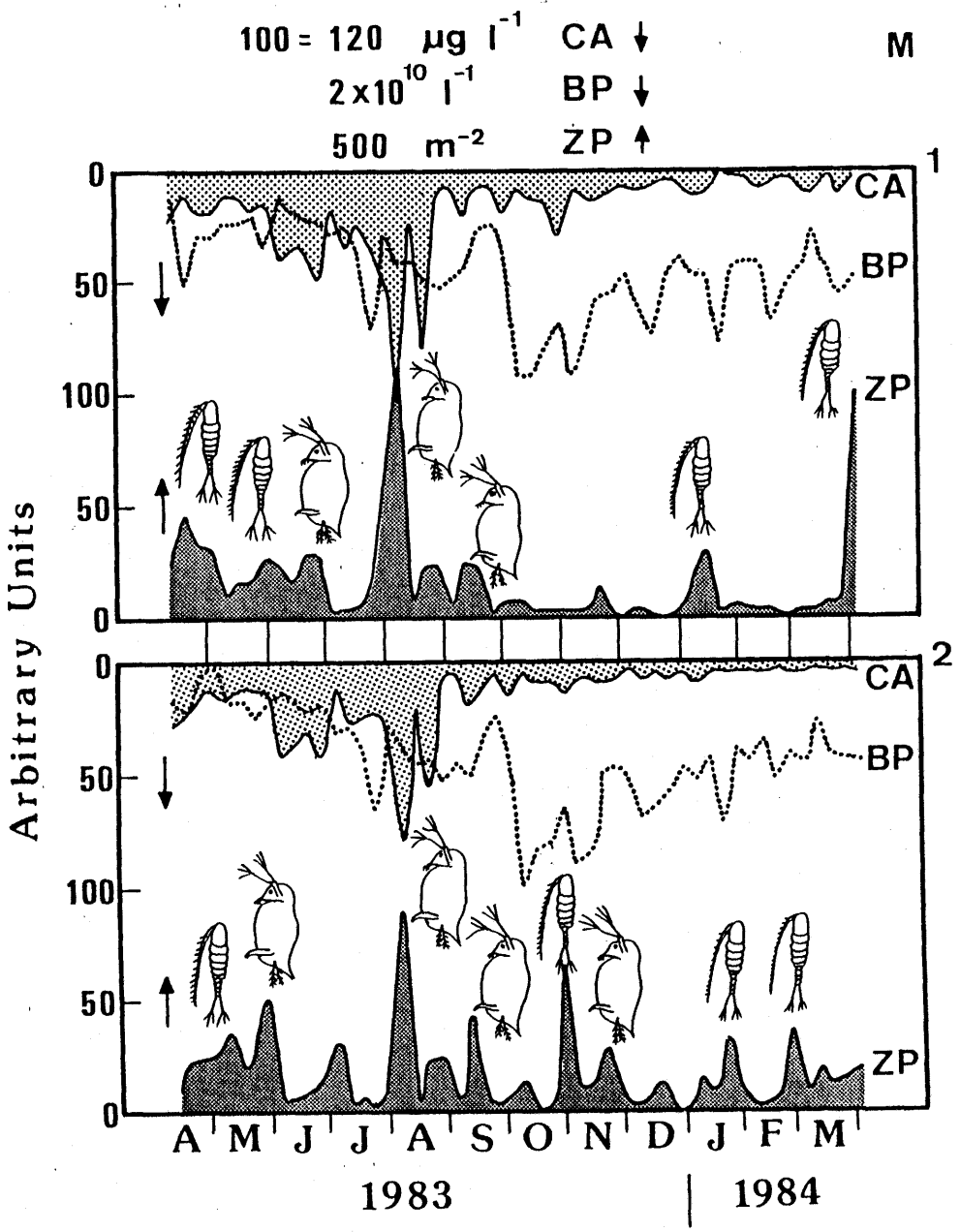


Figure 14. Seasonal variation in the number of zooplankton associated with variations in chlorophyll-a concentration and bacterioplankton population density at Stations 1 and 2 in Matsumi-Ike Bog. Cladcerans were abundant in summer, while copepods were numerous in autumn, winter and spring.

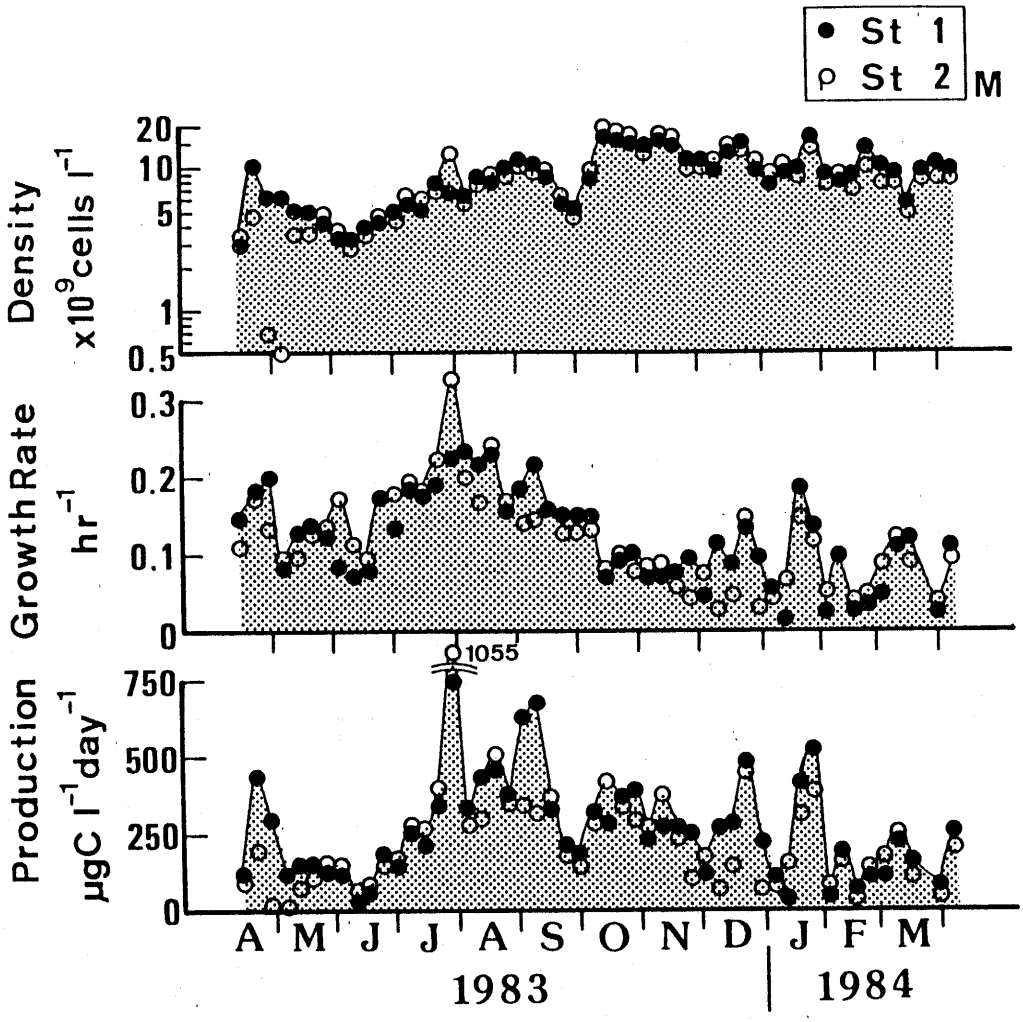


Figure 15. Seasonal variations of the bacterioplankton at Stations 1 and 2 in Matsumi-Ike Bog: (from top to bottom) the population density, the population growth rate, and the production rate.

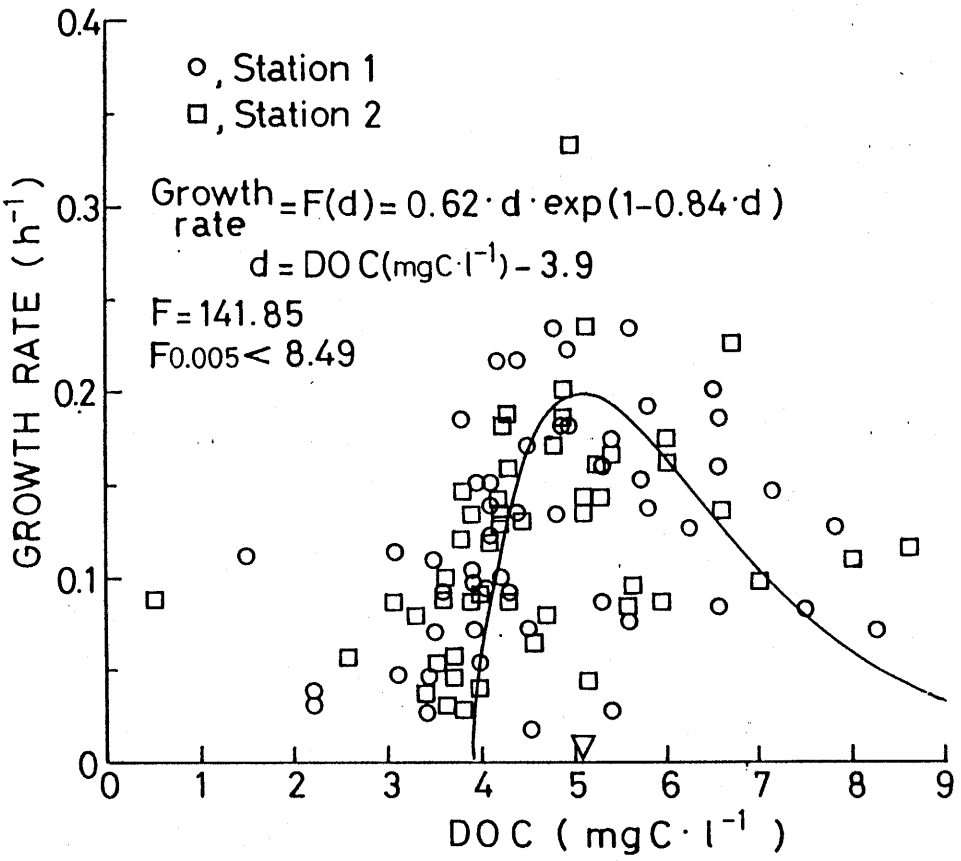


Figure 16. Effect of the DOC concentration on the population growth rate of bacterioplankton at Stations 1 and 2 in Matsumi-Ike Bog. The wedge indicates the optimal concentration of 5.1 mgC/l.

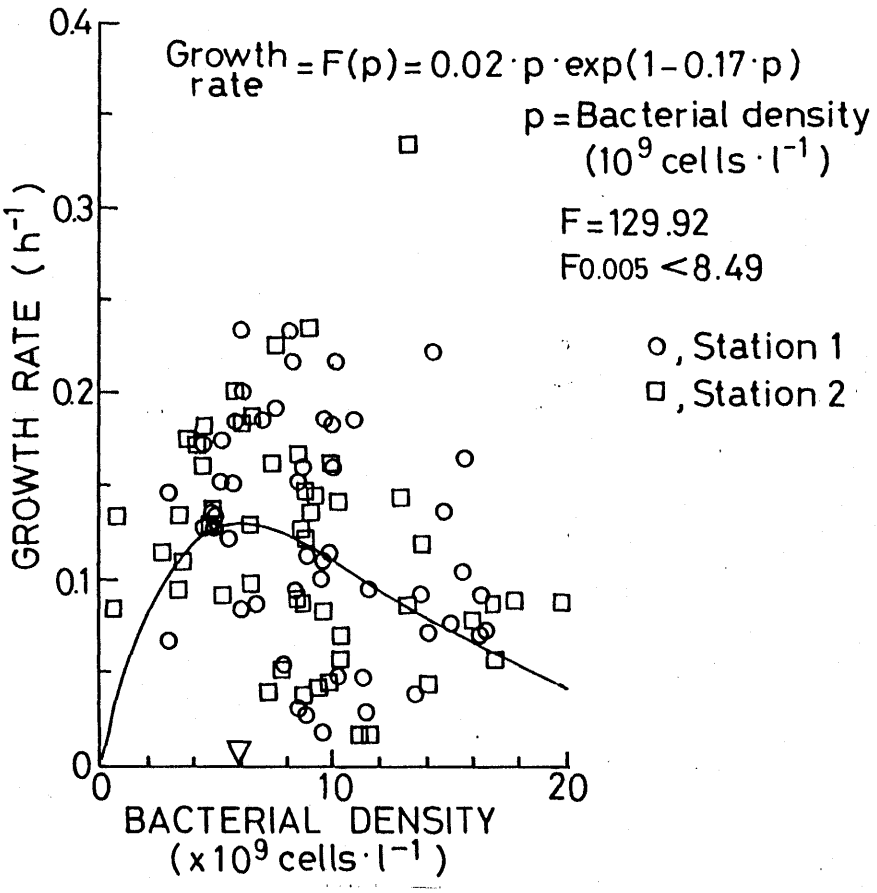


Figure 17. Effect of the bacterioplankton population density on their population growth rate at Stations 1 and 2 in Matsumi-Ike Bog. The wedge indicates the optimal density of 5.9×10^9 cells/l.

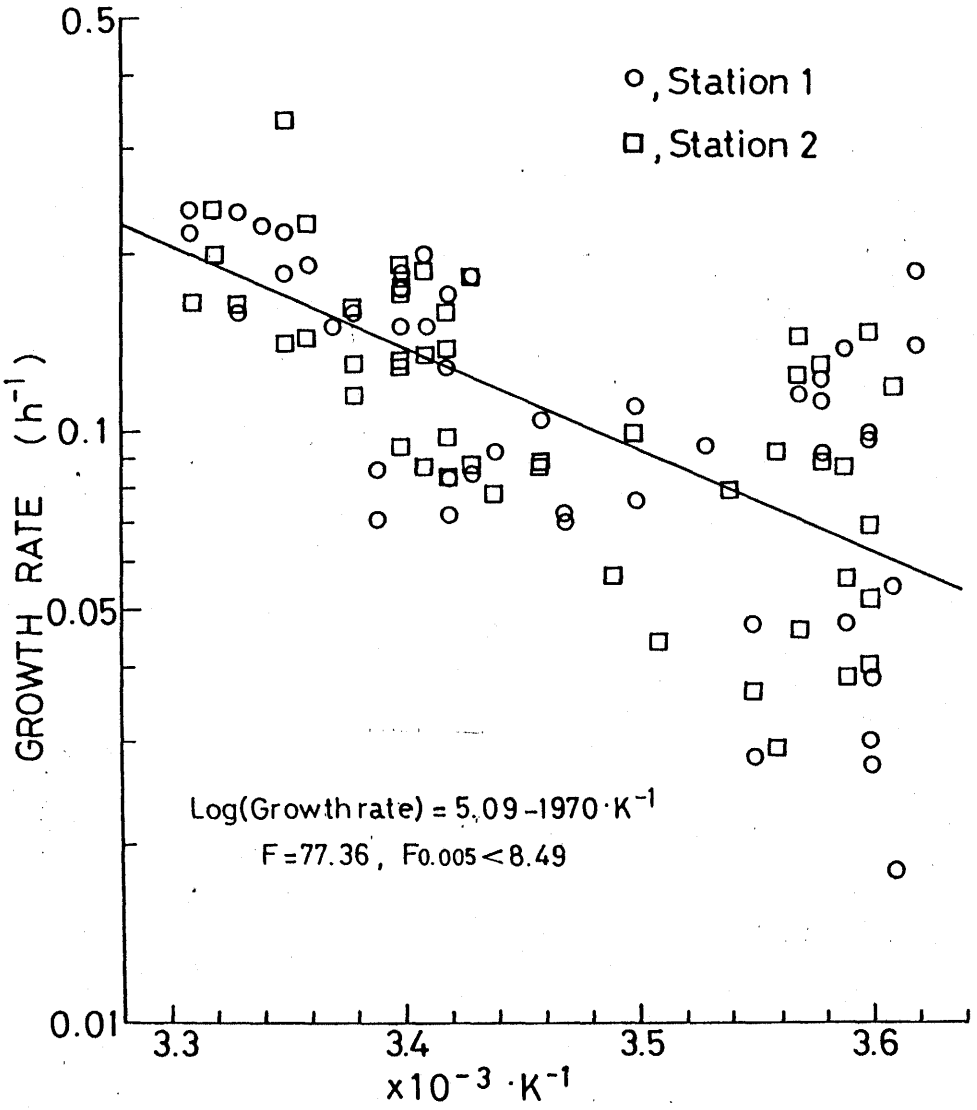


Figure 18. Thermal influence on the bacterioplankton growth rate at Stations 1 and 2 in Matsumi-Ike Bog, as shown by the Arrhenius plots of the logarithm of growth rate against the reciprocal of absolute temperature.

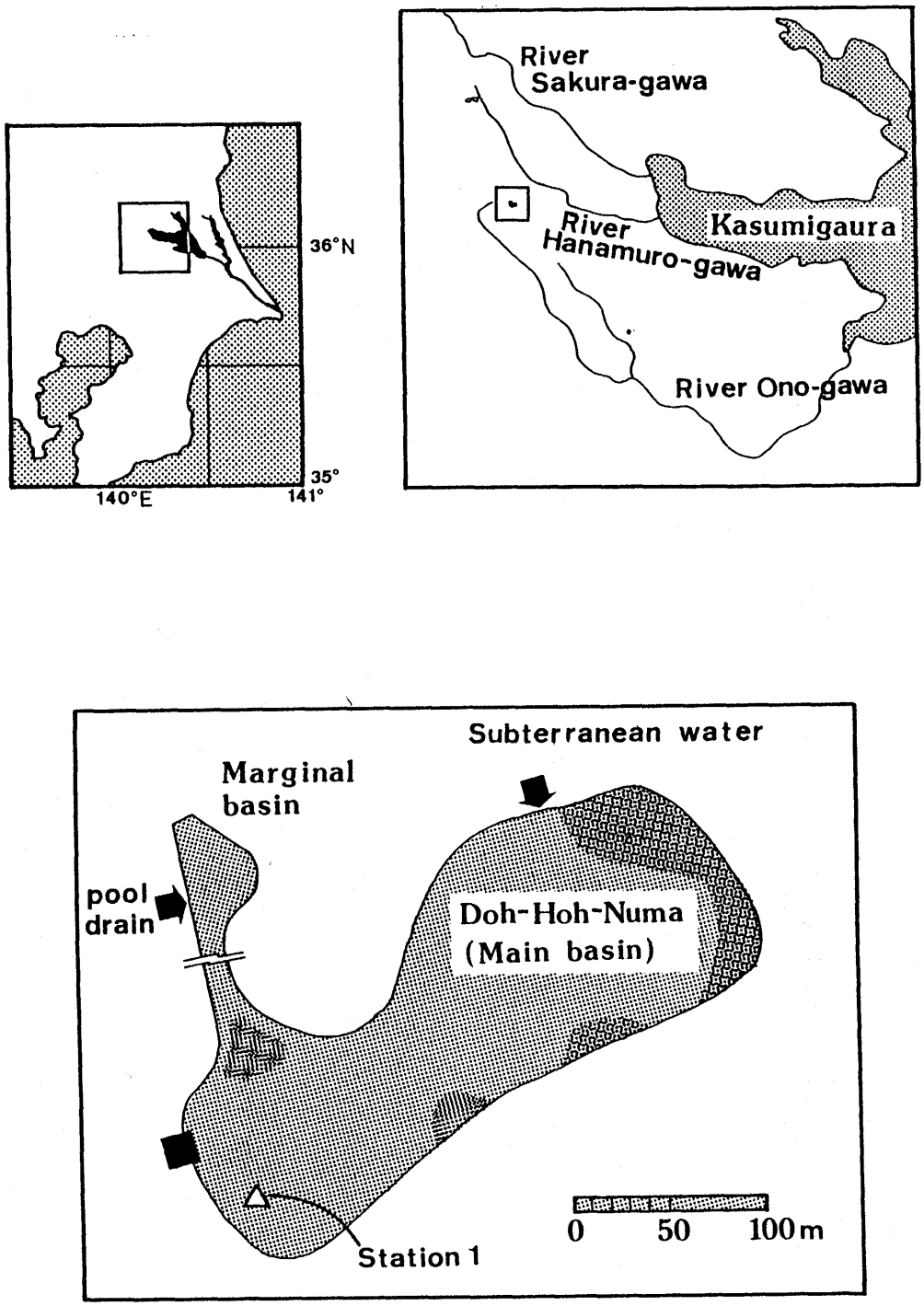


Figure 19. Location of Doh-Hoh-Numa Bog and Station 1 in the Main Basin of the bog. Reed communities colonized at the darkened areas.

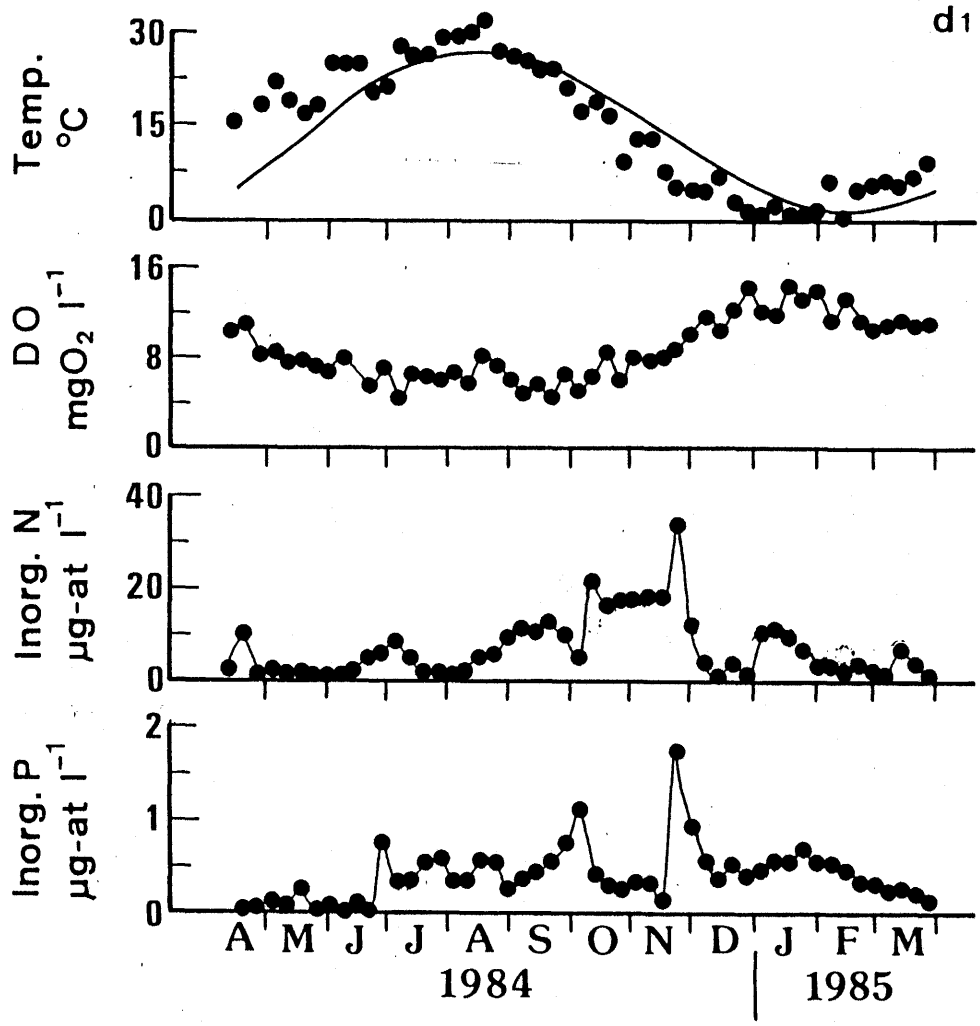


Figure 20. Seasonal variations in the physico-chemical parameters at Station 1 in Doh-Hoh-Numa Bog: (from top to bottom) temperature and the concentrations of dissolved oxygen, inorganic nitrogen and inorganic phosphorus.

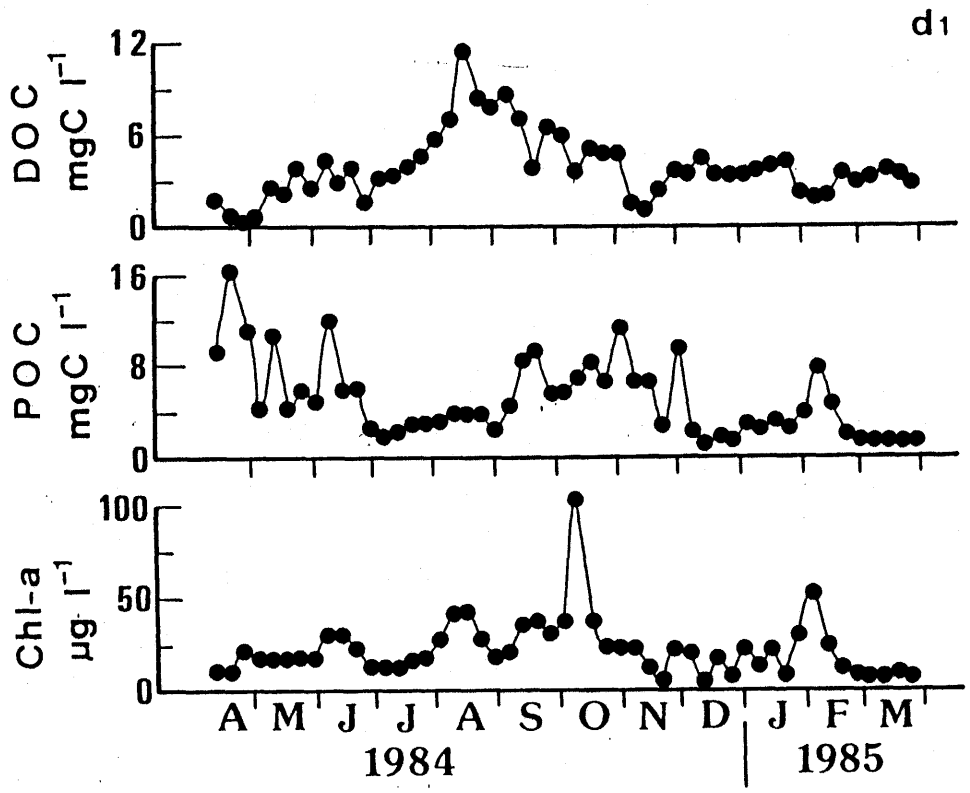


Figure 21. Seasonal variations in the concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC) and chlorophyll-a (Chl-a) at Station 1 in Doh-Hoh-Numa Bog.

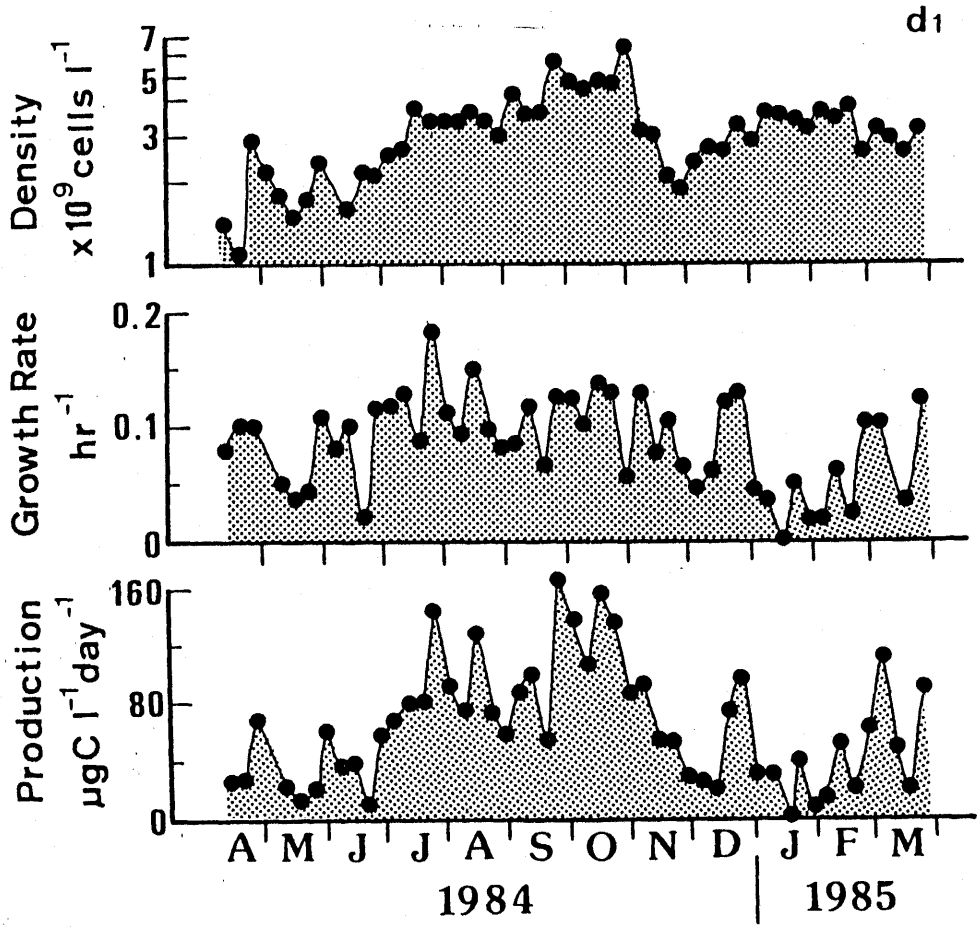


Figure 22. Seasonal variations of bacterioplankton at Station 1 in Doh-Hoh-Numa Bog: (from top to bottom) the population density, the growth rate and the production rate.

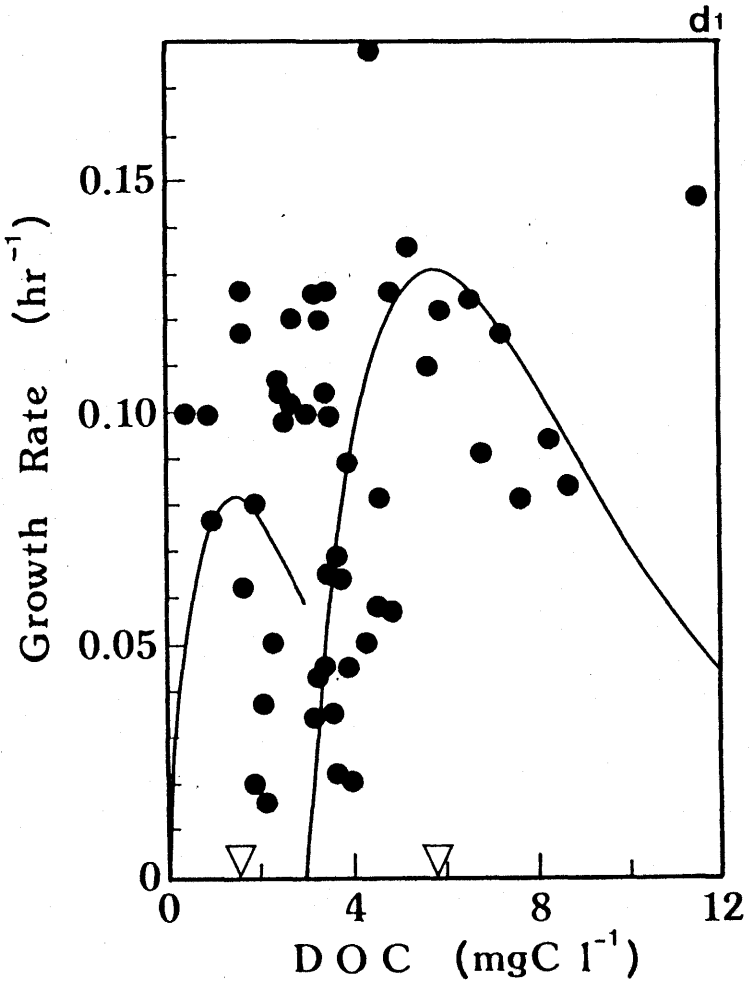


Figure 23. Effect of the DOC concentration on the population growth rate of bacterioplankton at Station 1 in Doh-Hoh-Numa Bog. Wedges indicates the optimal concentrations of 1.5 and 5.7 mgC/l.

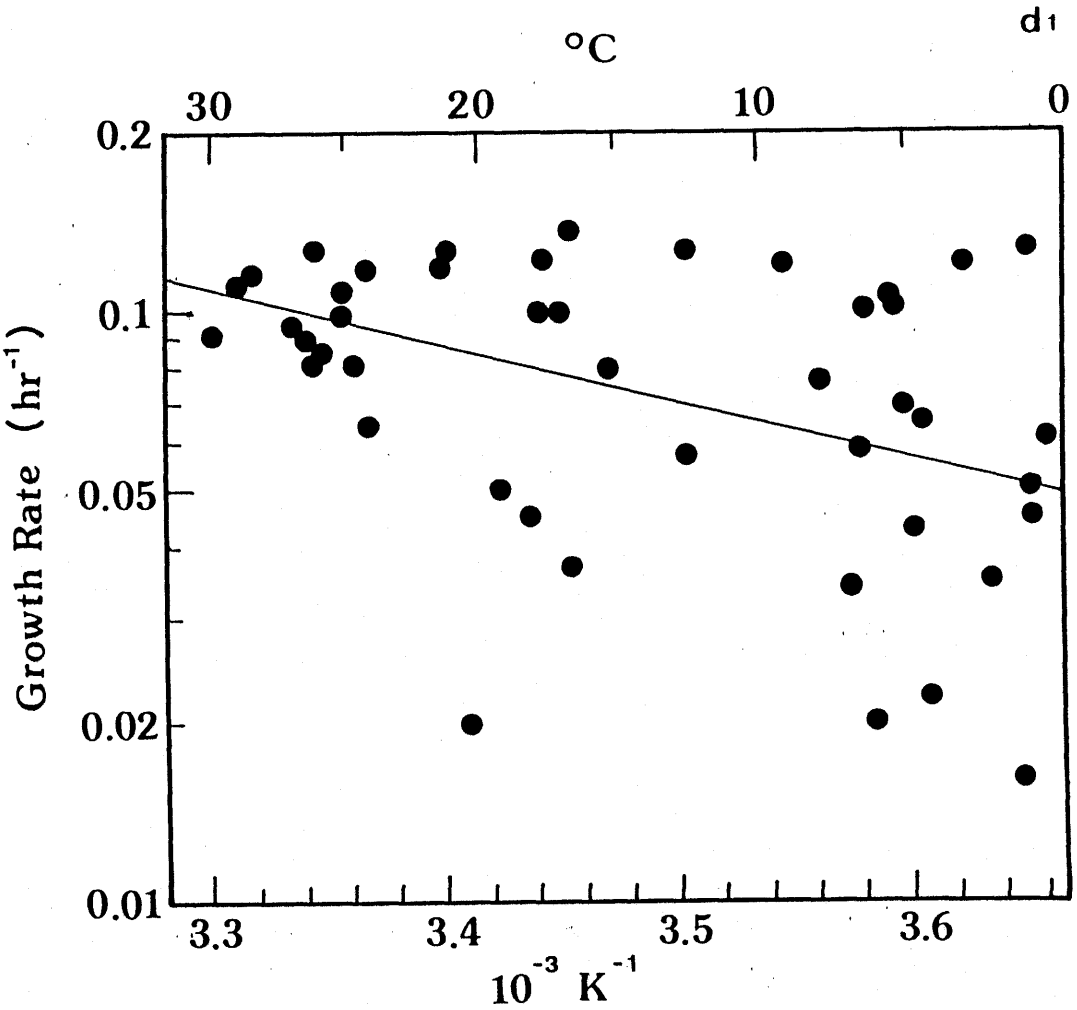


Figure 24. Effects of temperature on the population growth rate of bacterioplankton at Station 1 in Doh-Hoh-Numa Bog.

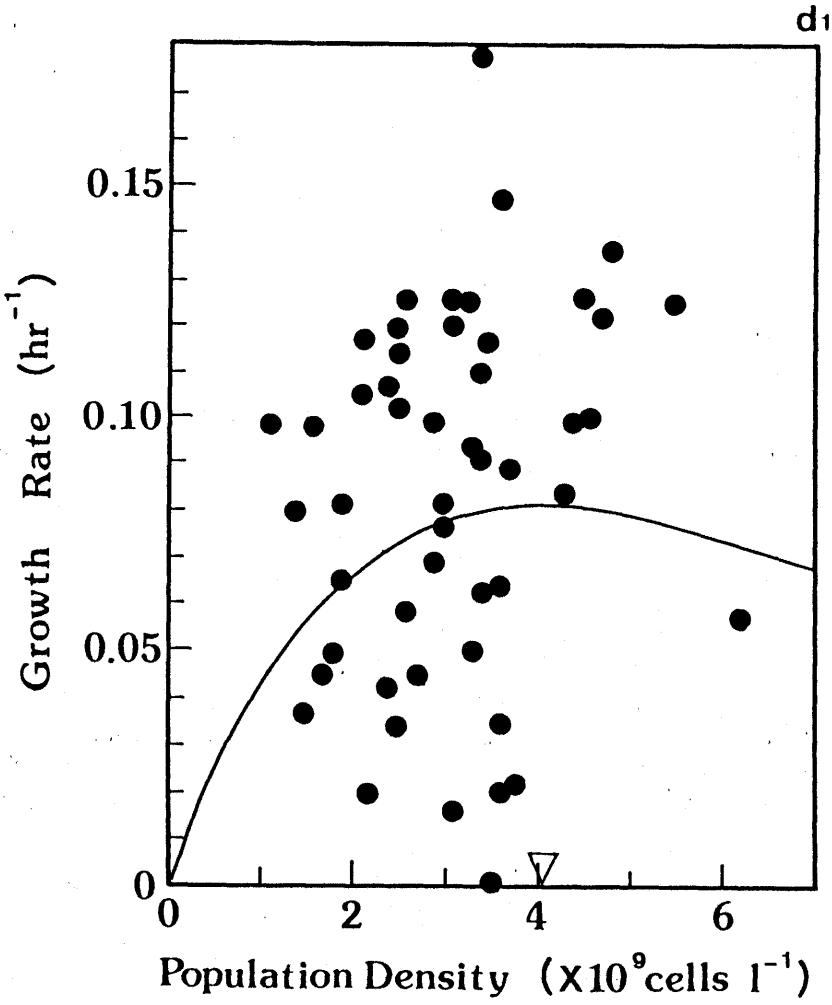


Figure 25. Influence of the bacterioplankton population density on their growth rate at Station 1 in Doh-Hoh-Numa Bog. The wedge indicates the optimal density of 4.0×10^{9} cells/l.

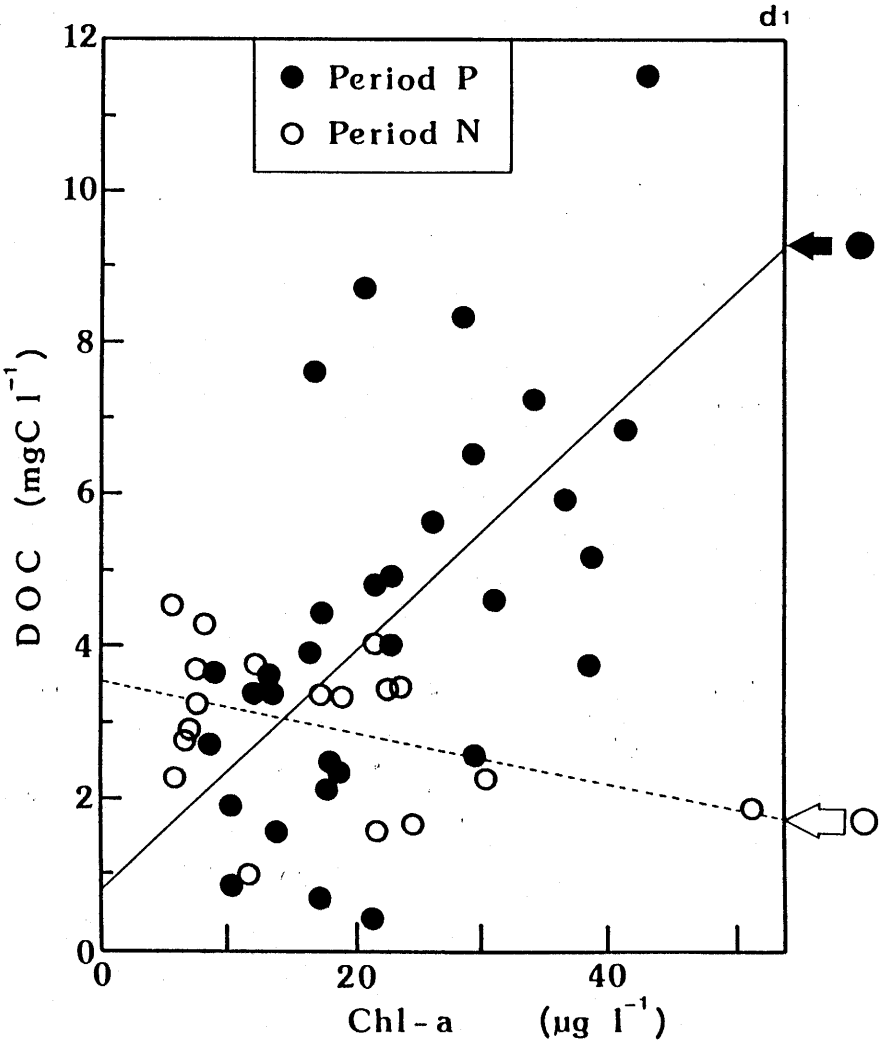


Figure 26. Relationship between the concentrations of chlorophyll-a and DOC at Station 1 in Doh-Hoh-Numa Bog. Filled circles are used for Period P (from 13 April 1984 to 2 November 1984); open circles are for Period N (from 9 November 1984 to 29 March 1985).

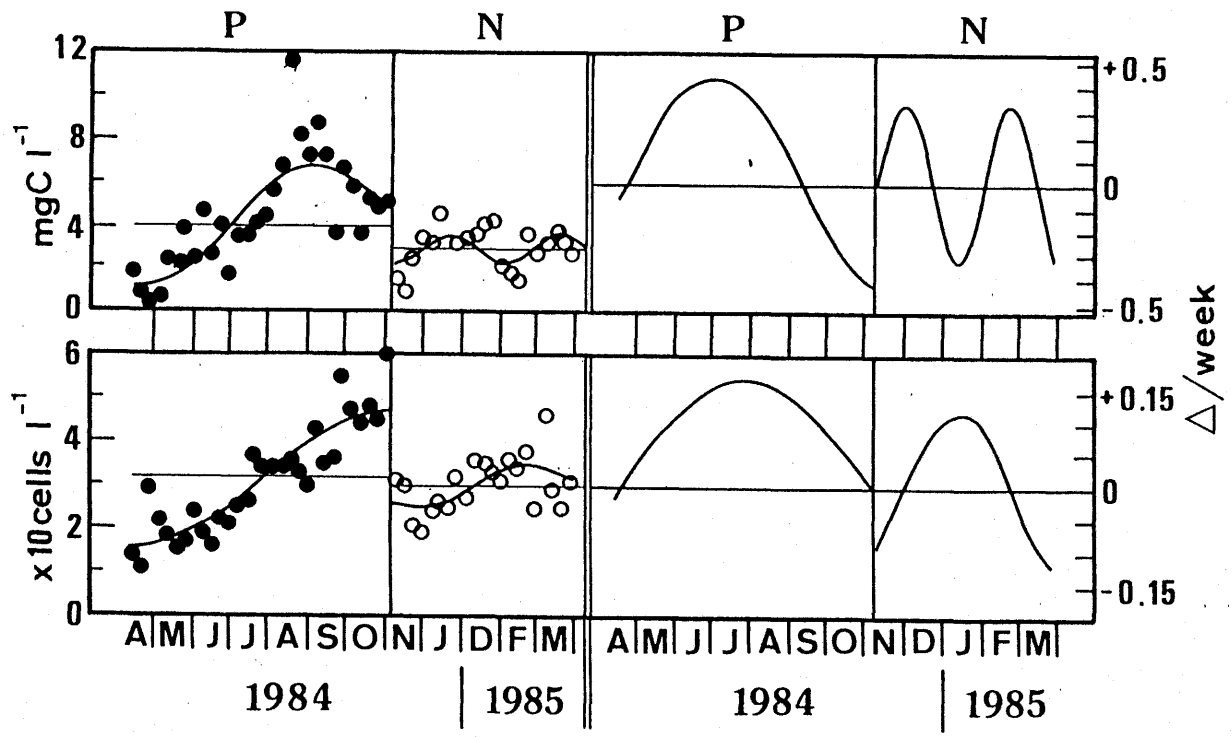


Figure 27. Seasonal fluctuations of the DOC concentration and the bacterioplankton population density (left two columns), and their differential curves (right two columns) at Station 1 in Doh-Hoh-Numa Bog during Periods P and N.

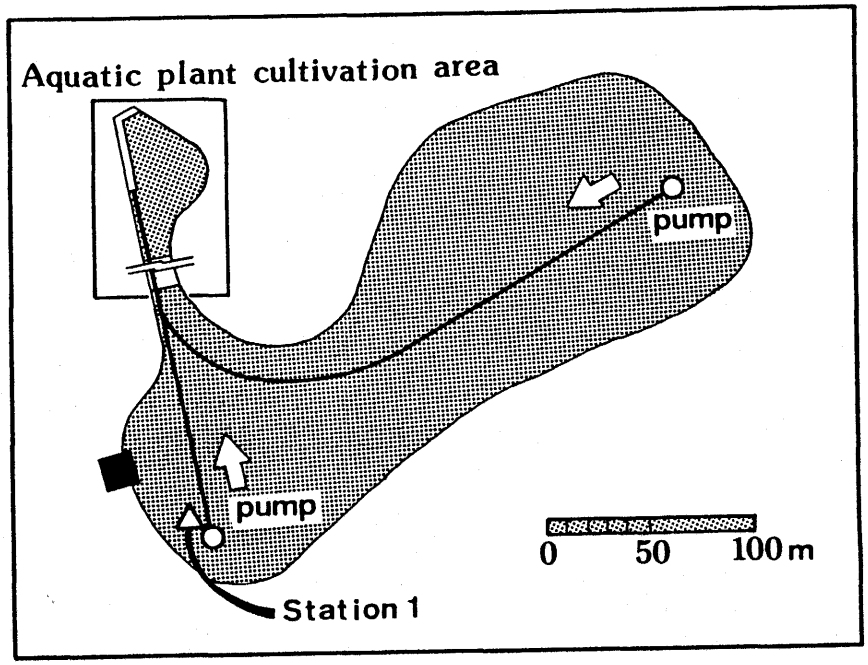


Figure 28. Location of Station 1 in Doh-Hoh-Numa Bog after the introduction of the Bio-filter system. Bog water is pumped from the Main basin through submerged pipes into the aquatic plant cultivation area.

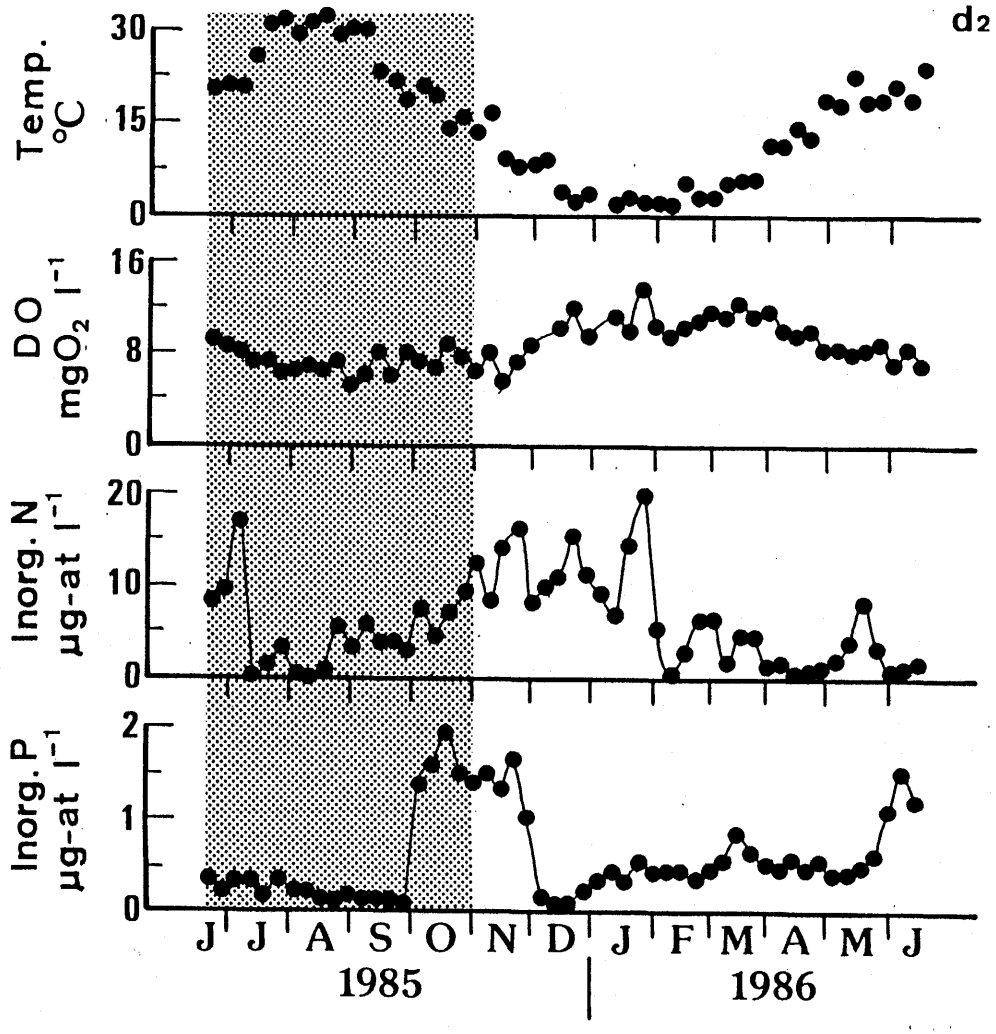


Figure 29. Seasonal variations of the physico-chemical parameters at Station 1 in Doh-Hoh-Numa Bog after the introduction of the Bio-filter system: (from top to bottom) the water temperature and the concentrations of dissolved oxygen, inorganic nitrogen and inorganic phosphorus. The system was operated during Period I (darkened period), thereafter it was discontinued.

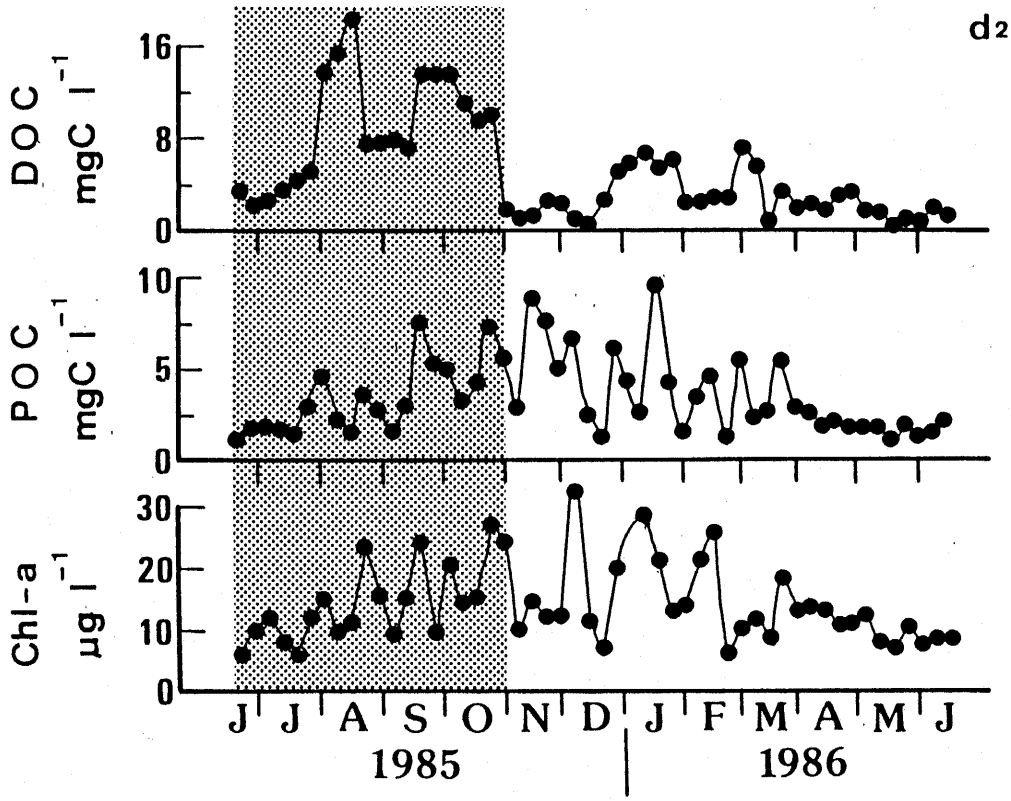


Figure 30. Seasonal variations of the concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC) and chlorophyll-a (Chl-a) at Station 1 in Doh-hoh-Numa Bog after the introduction of the Bio-filter system. The system was operated during Period I (darkend period), thereafter it was discontinued.

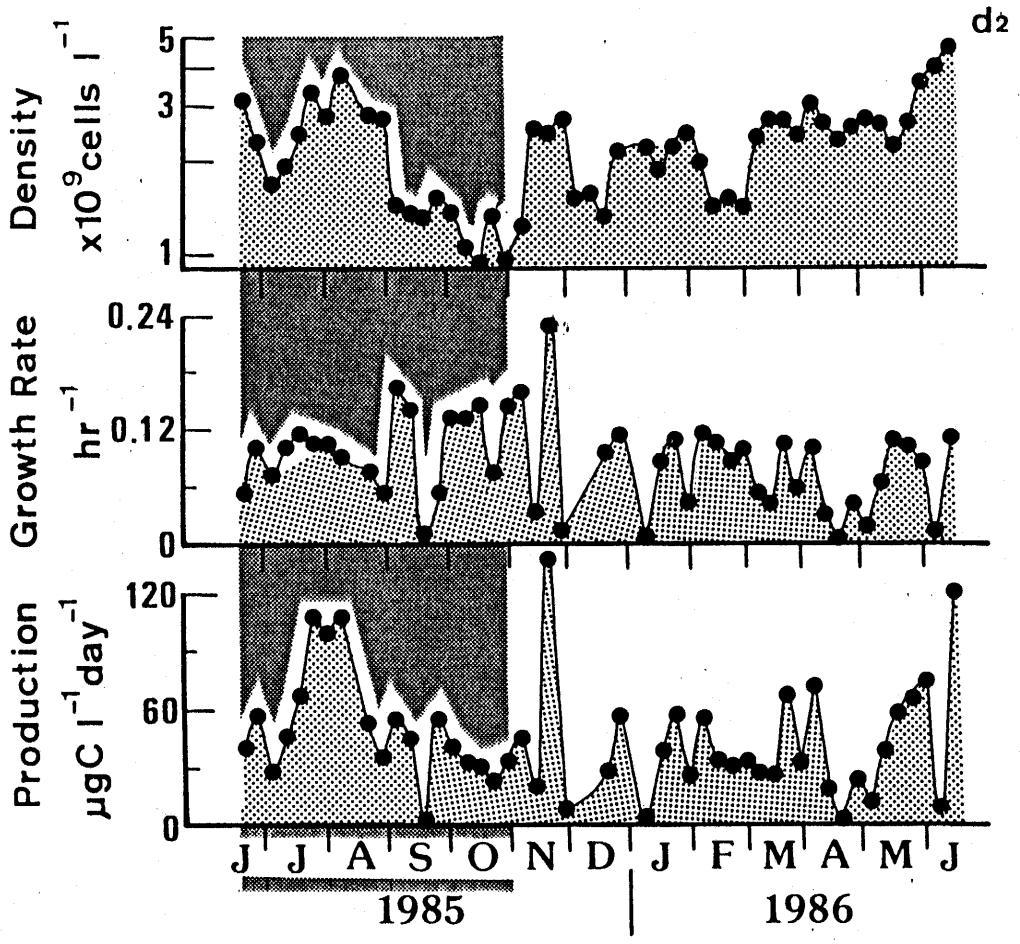


Figure 31. Seasonal variations of bacterioplankton at Station 1 in Doh-Hoh-Numa Bog after the introduction of the Bio-filter system: (from top to bottom) the population density, the growth rate and production rate. The system was operated during Period I (darkened period), thereafter it was discontinued.

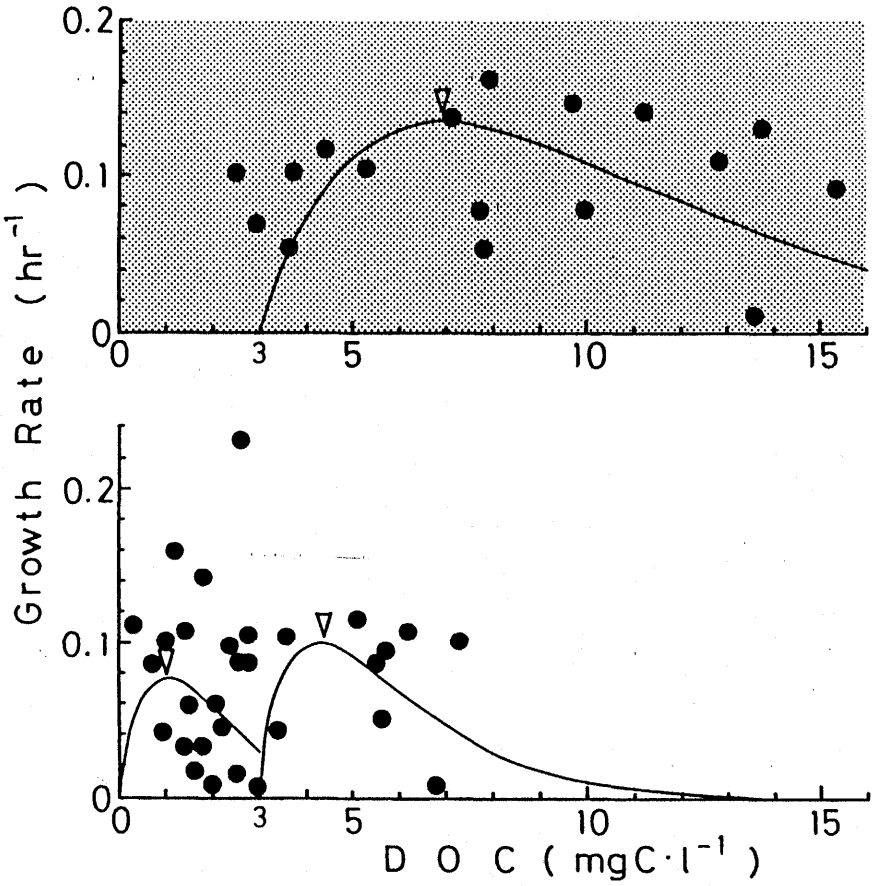


Figure 32. Effect of the DOC concentration on the population growth rate of bacterioplankton at Station 1 in Doh-Hoh-Numa Bog after the introduction of the Bio-filter system. Wedges indicates the optimal concentrations of 6.9 mgC/l during the system operation (upper, Period I), and 1.1 and 4.1 mgC/l after the system was discontinued (lower, Period II).

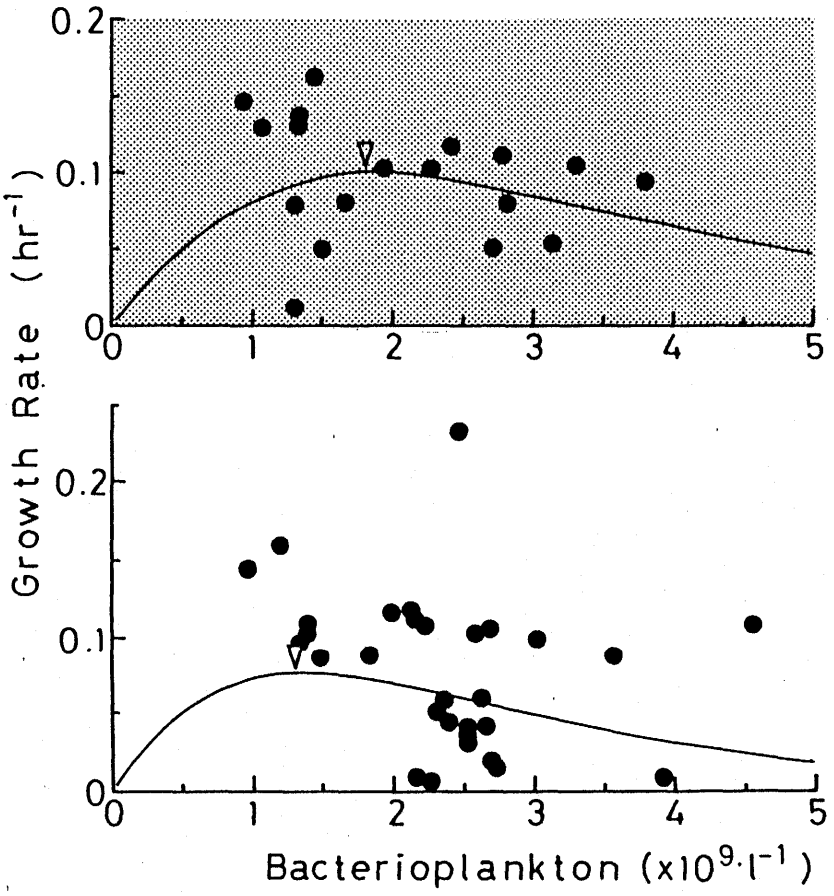


Figure 33. Effect of the bacterioplankton population density on their population growth rate at Station 1 in Doh-Hoh-Numa Bog after the introduction of the Bio-filter system. Wedges indicates the optimal densities of 1.8×10^9 cells/l during the system operation (upper, Period I), and 1.3×10^9 cells/l after the system was discontinued (lower, Period II).

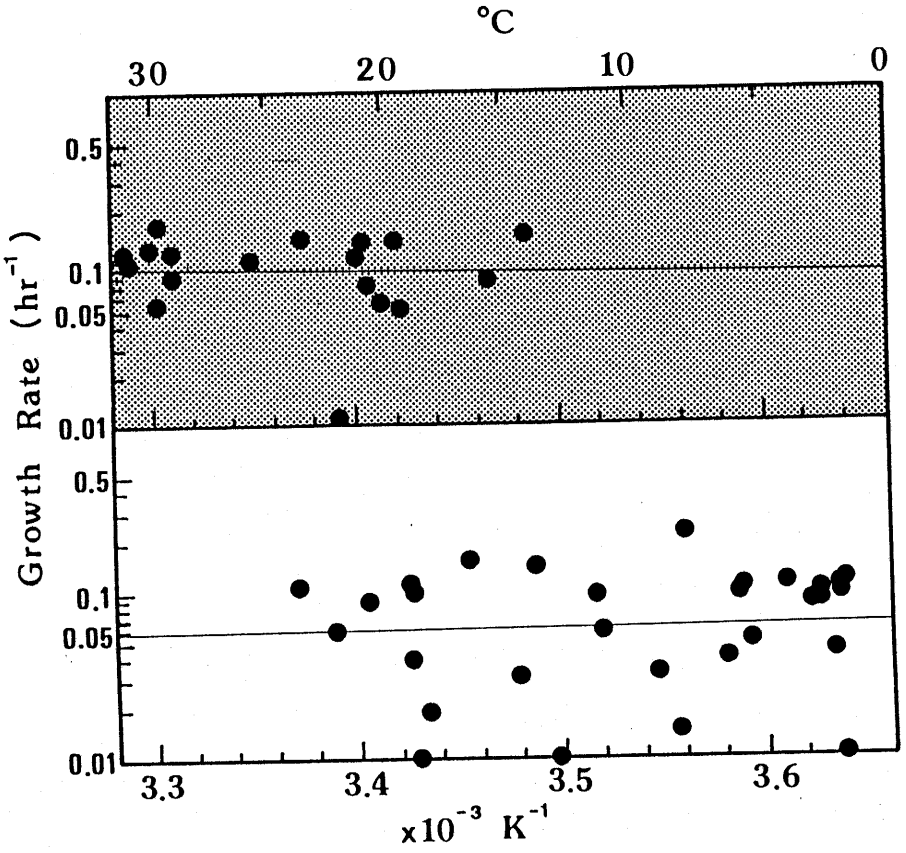


Figure 34. Influence of temperature on the population growth rate of bacterioplankton at Station 1 in Doh-Hoh-Numa Bog during the operation of the Bio-filter system (upper, Period I) and after the system was discontinued (lower, Period II).

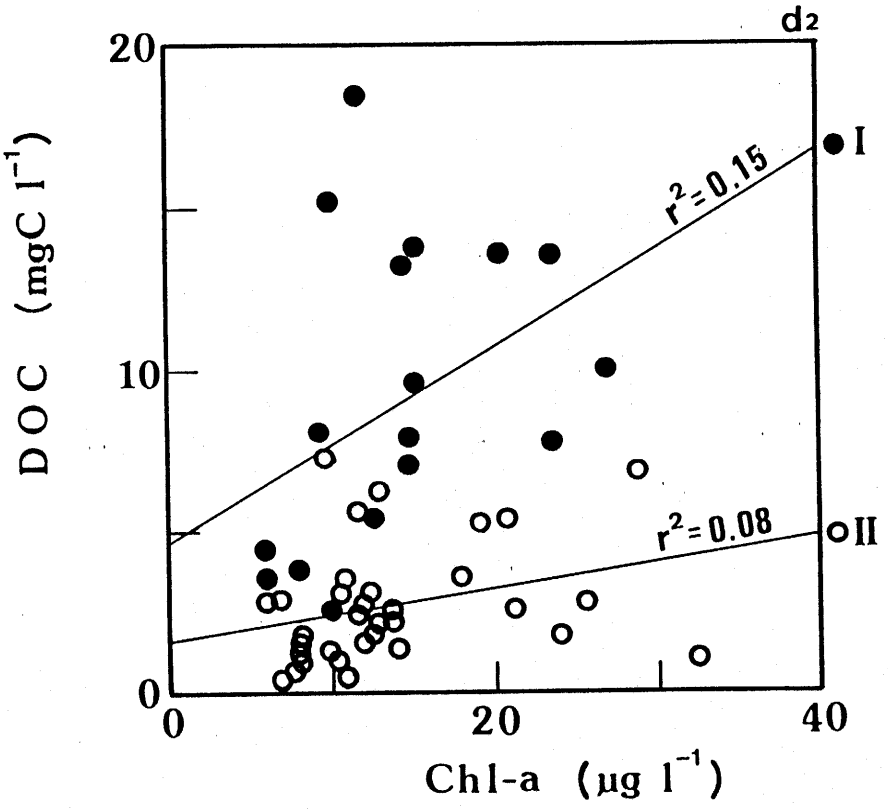


Figure 35. Relationship between the concentrations of chlorophyll-a and DOC at Station 1 in Doh-Hoh-Numa Bog during the operation of the Bio-filter system (filled circle, Period I) and after the system was discontinued (open circle, Period II).

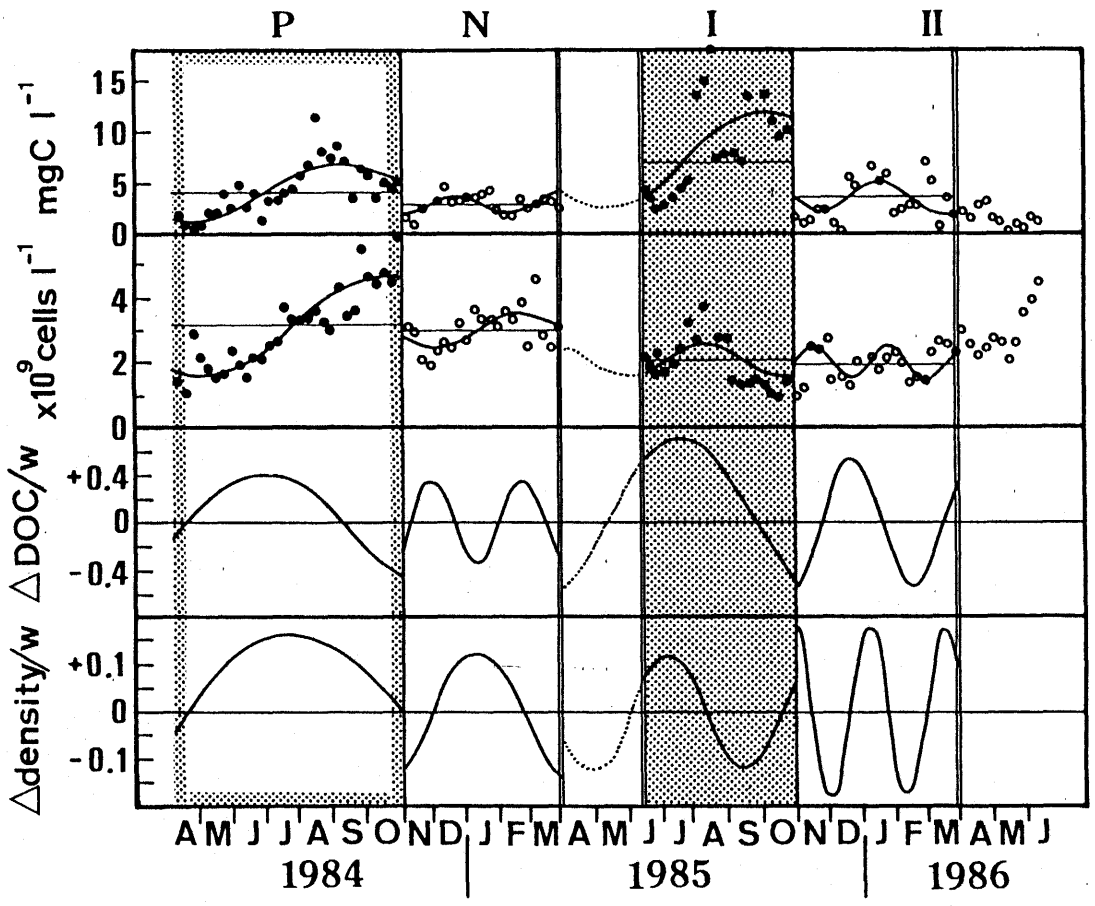


Figure 36. Fluctuations of the DOC concentrations (top) and the bacterioplankton population density (upper-middle), and their differential curves (lower-middle and bottom), at Station 1 in Doh-Hoh-Numa Bog before and after the introduction of the Bio-filter system. Periods P and N are the same as in Figure 27. The system was operated during Period I (darkened period), thereafter it was discontinued (Period II).

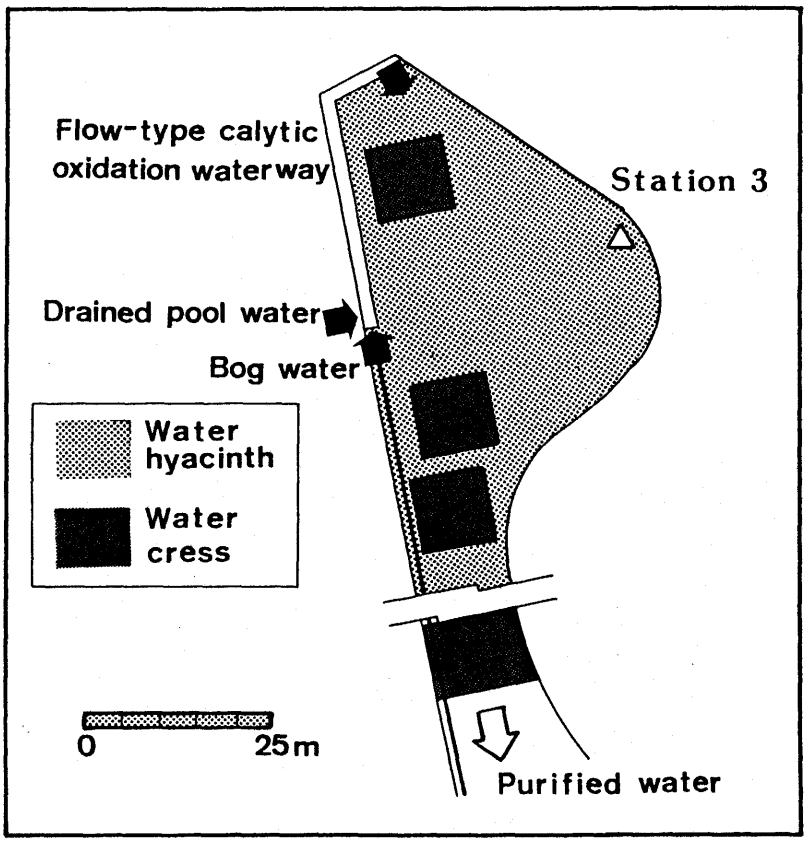


Figure 37. Aquatic plant cultivation area of the Bio-filter system in Doh-Hoh-Numa Bog, and the location of Station 3 in the bog.

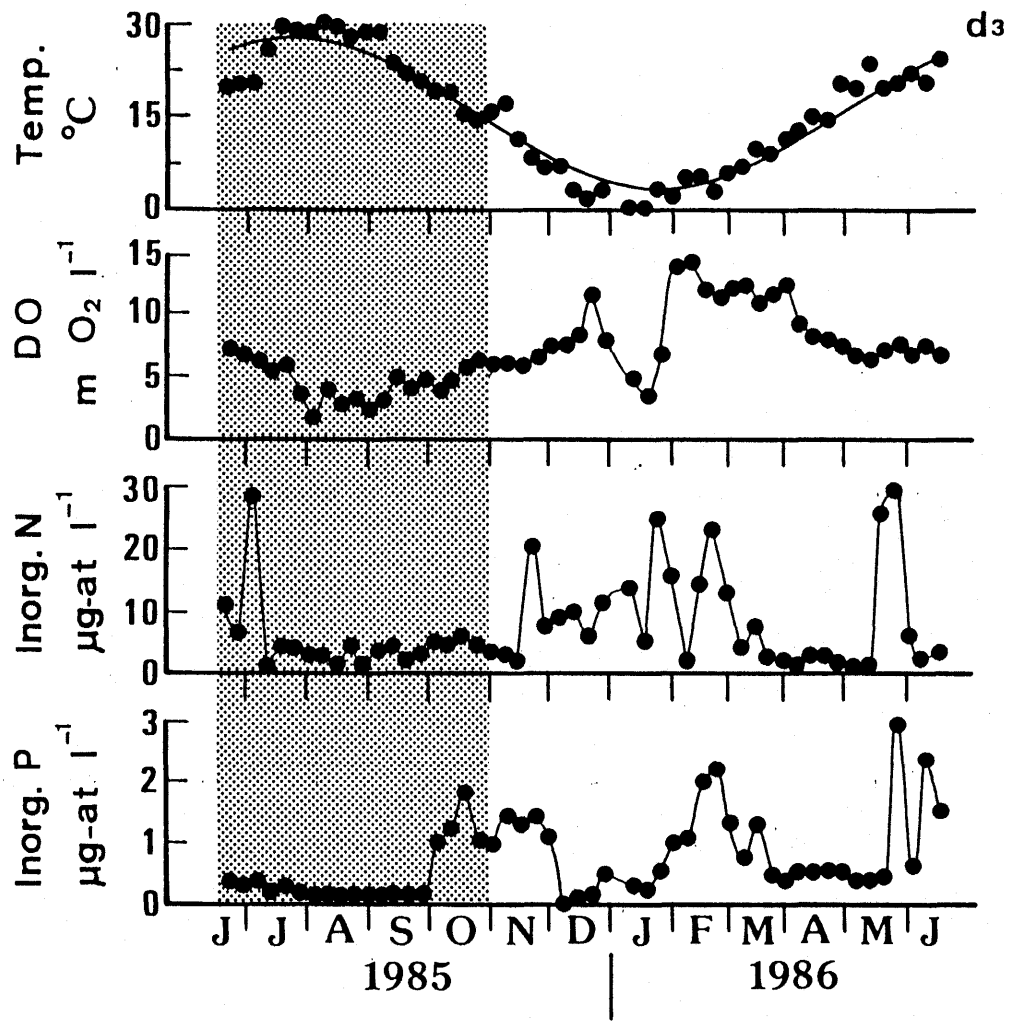


Figure 38. Seasonal variations of the physico-chemical parameters in the surface water at Station 3 in the Doh-Hoh-Numa Bog: (from top to bottom) the water temperature, and the concentrations of dissolved oxygen, inorganic nitrogen and inorganic phosphorus. The system was operated during Period I (darkened period), thereafter it was discontinued (Period II).

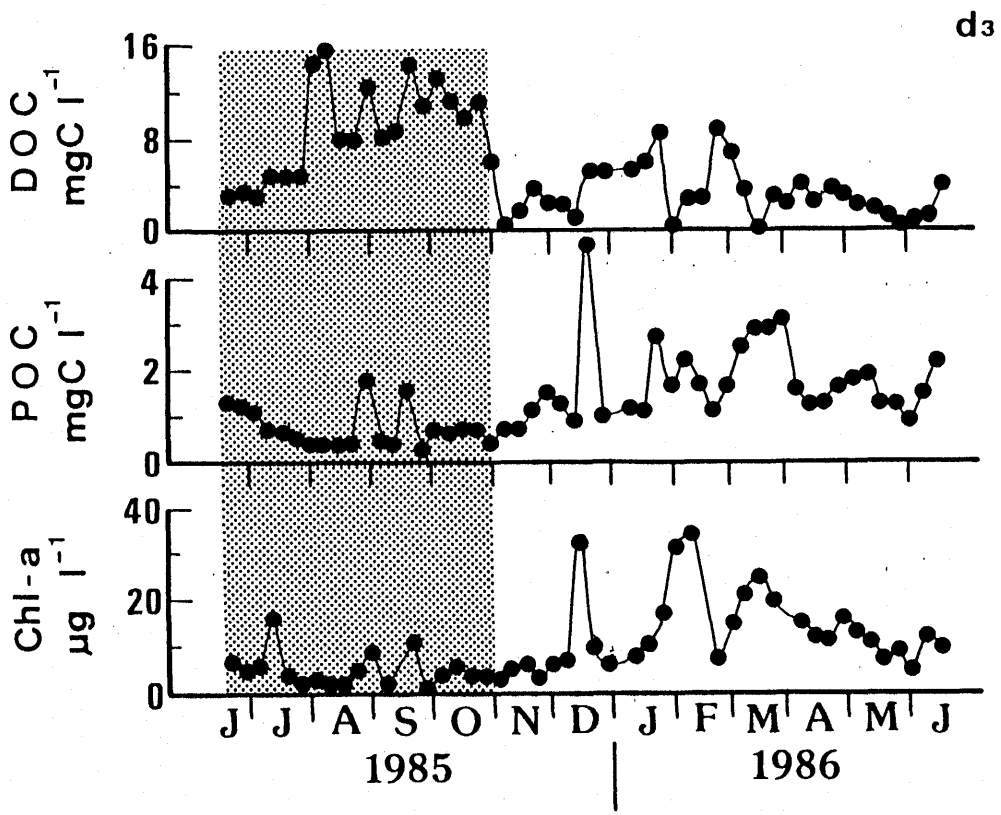


Figure 39. Seasonal variations of the concentrations of dissloved organic carbon (DOC), particulate organic carbon (POC) and chlorophyll-a (Chl-a) at Station 3 in Doh-Hoh-Numa Bog. The Bio-filter system was operated during Period I (darkened period), thereafter it was discontinued (Period II).

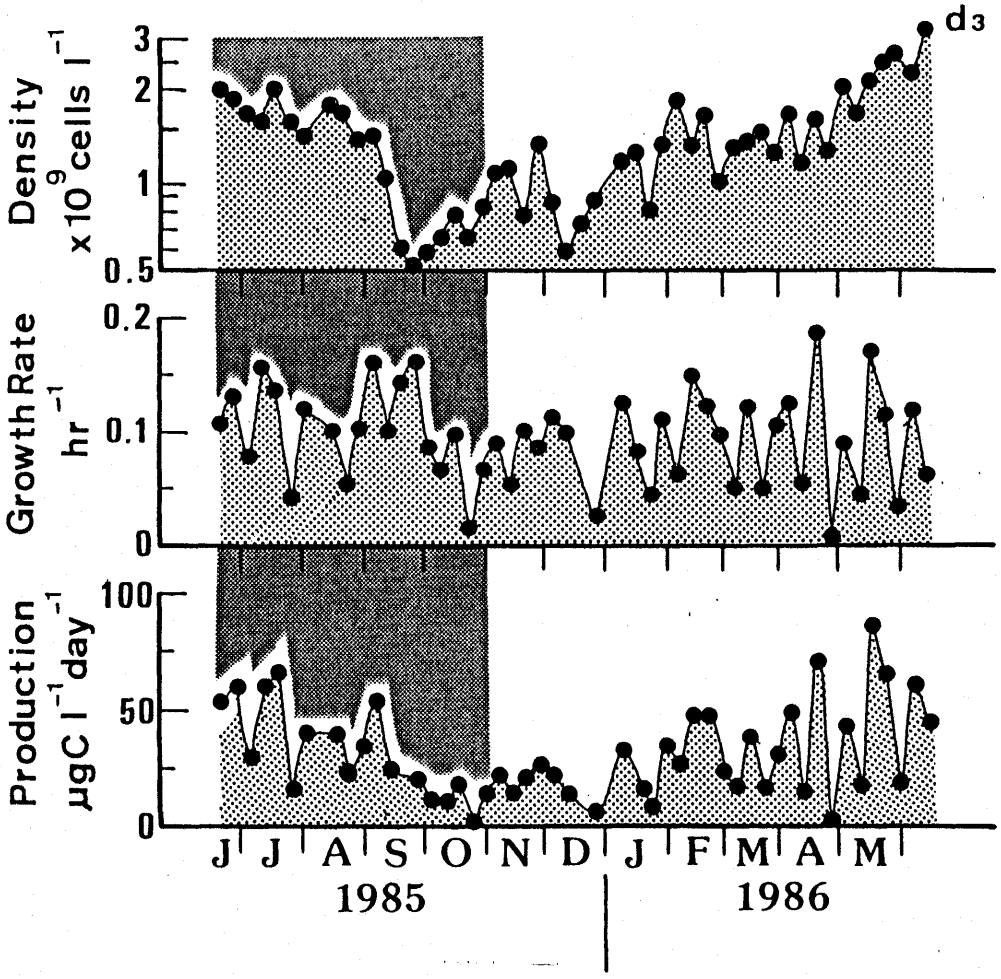


Figure 40. Seasonal variations of bacterioplankton at Station 3 in Doh-Hoh-Numa Bog. The Bio-filter system was operated during Period I (darkened period), thereafter it was discontinued (Period II).

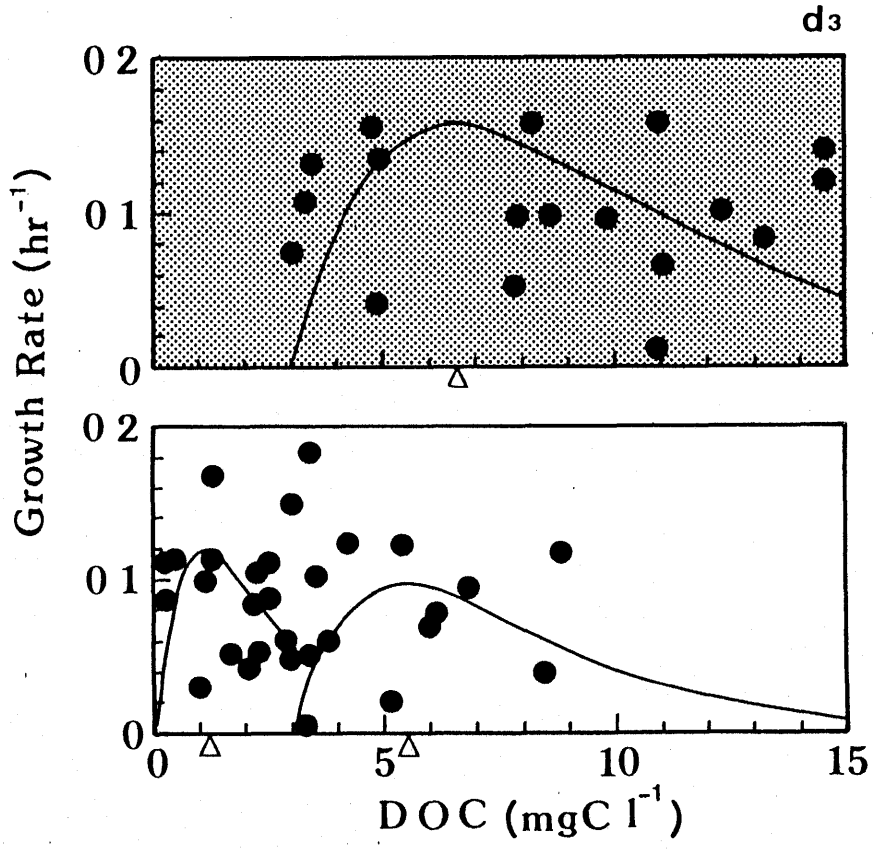


Figure 41. Effect of the DOC concentration on the population growth rate of bacterioplankton at Station 3 in Doh-Hoh-Numa Bog. Wedges indicate the optimal concentrations of 6.5 mgC/l during the Bio-filter system operation (upper, Period I), and 1.1 and 5.4 mgC/l after the system was discontinued (lower, Period II).

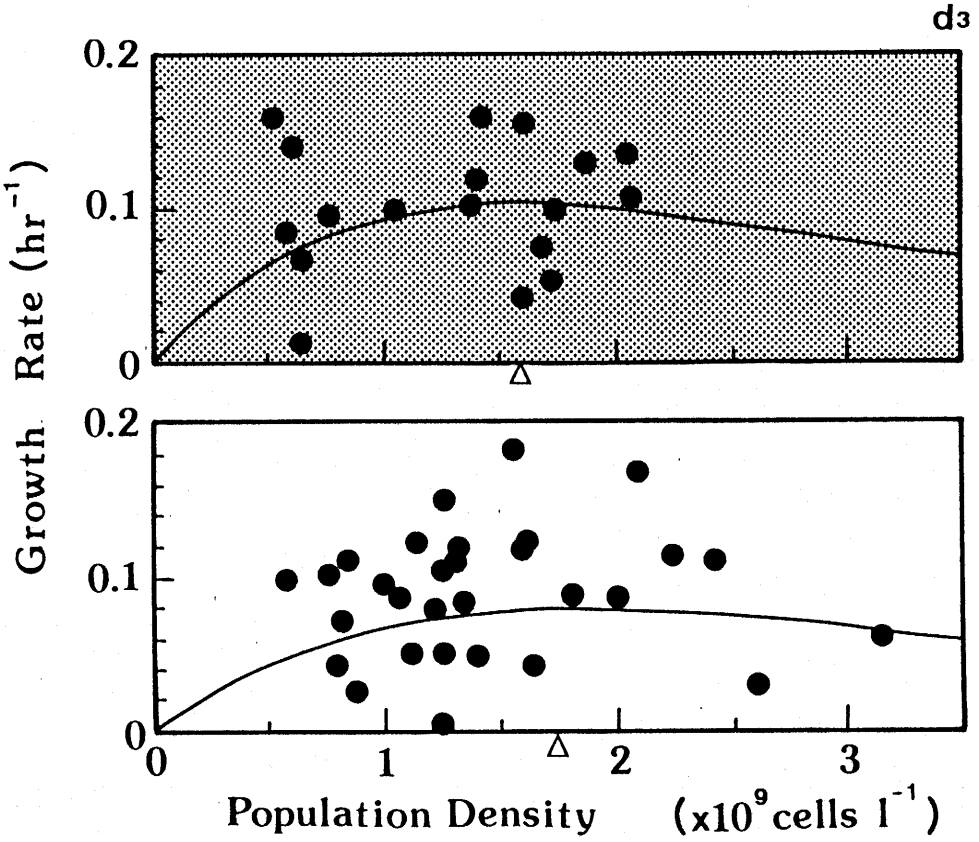


Figure 42. Effects of the bacterioplankton population density on their population growth rate at Station 3 in Doh-Hoh-Numa Bog. Wedges indicate the optimal densities of 1.6×10^9 cells/l during the Bio-filter system operation (upper, Period I), and 1.7×10^9 cells/l after the system was discontinued (lower, Period II).

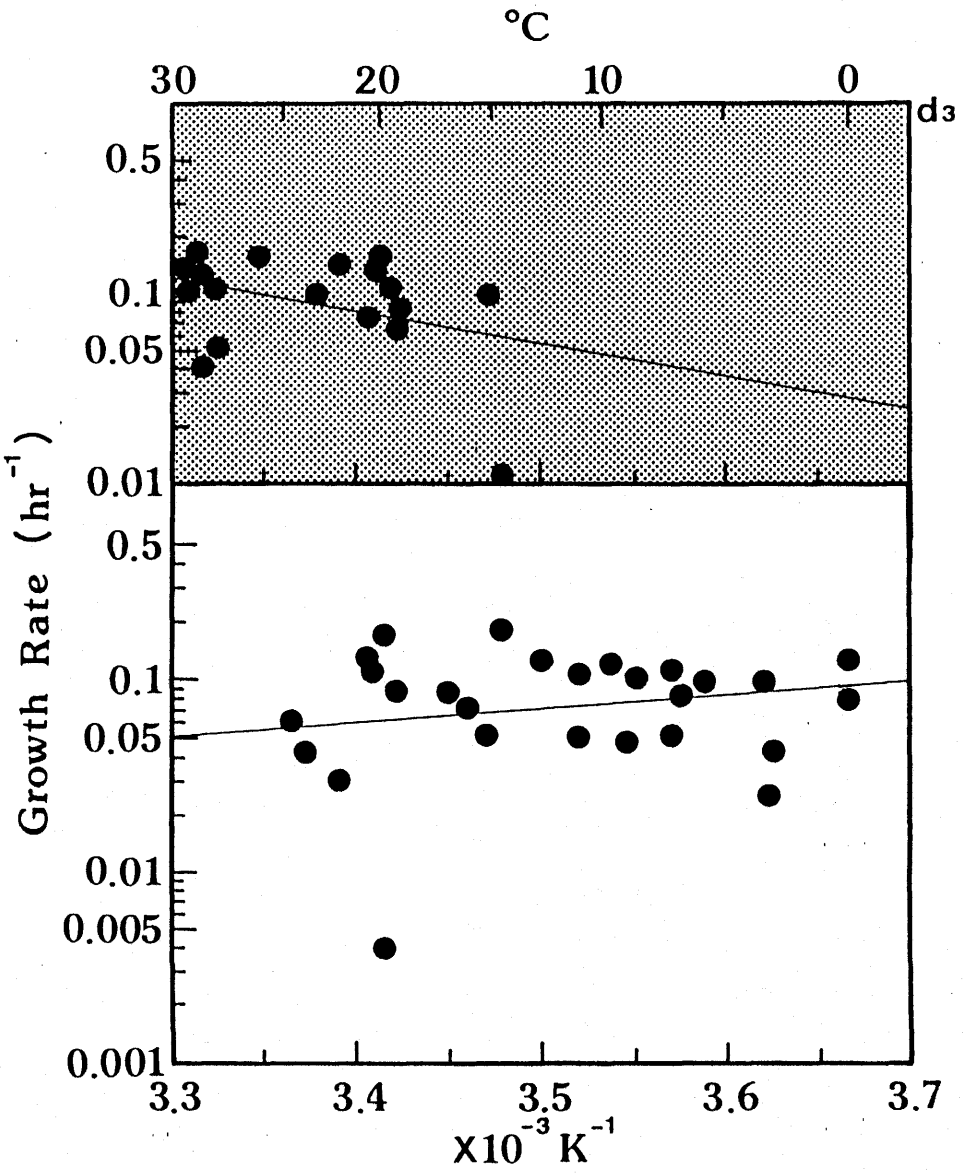


Figure 43. Influence of temperature on the population growth rate of bacterioplankton at Station 3 in Doh-Hoh-Numa Bog. The Bio-filter system was operated during Period I (upper), thereafter it was discontinued (lower, Period II).

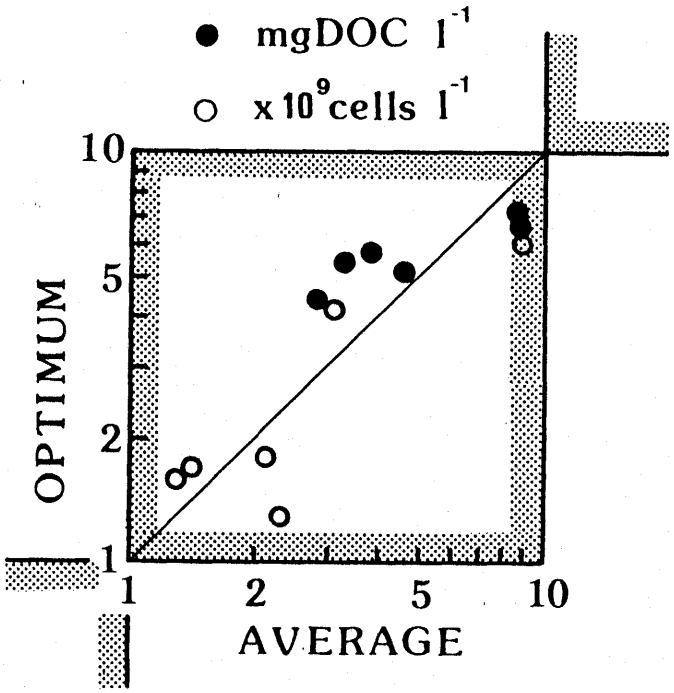


Figure 44. The relationship between the averages and the optima of the DOC concentration and the bacterioplankton population density. The line with the slope of 1, shows the average-optimum ratio of 1:1.

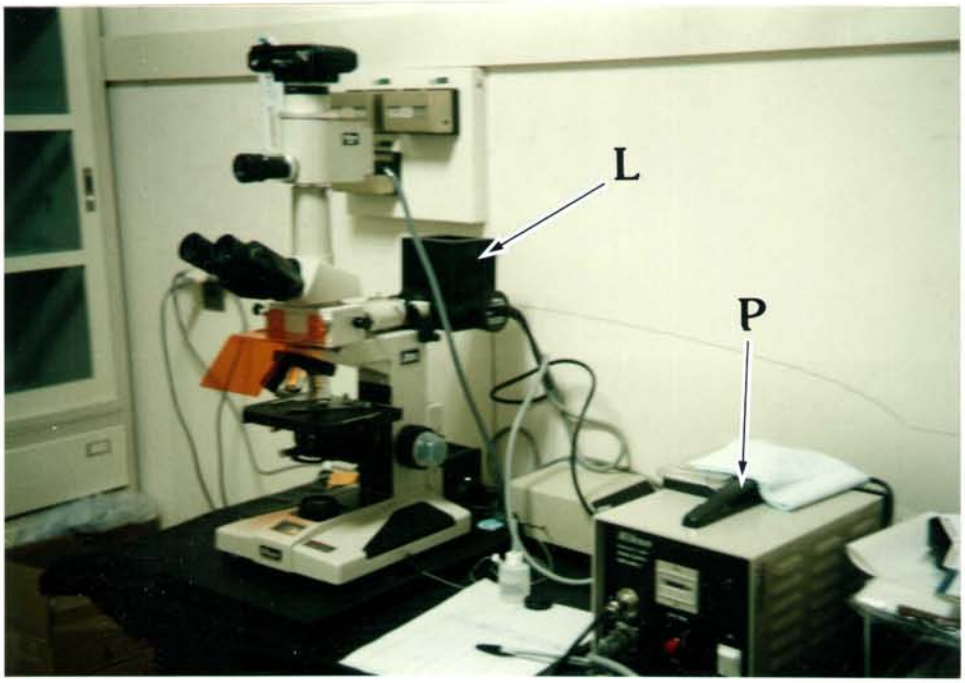


Plate 1. The epifluorescence microscope (Type EFD-2, Nikon, Tokyo): P, the power supply unit; L, the lamp house.

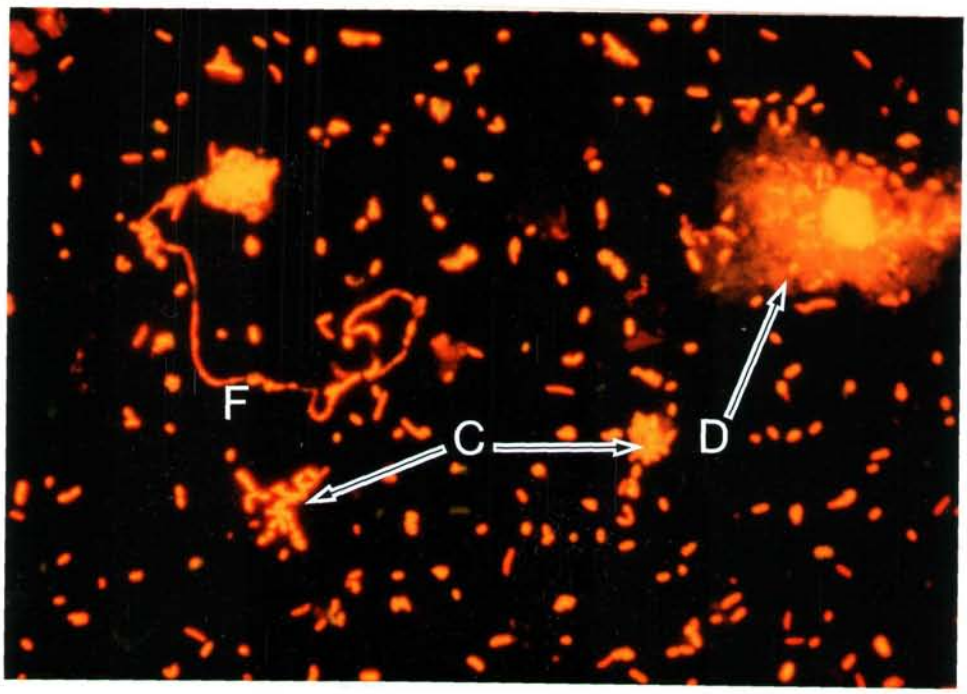


Plate 2. The epifluorescence micrograph of bacterioplankters stained with acridine orange (x 1,000). Bacterial microcolonies (C), detritus attached by bacteria (D), and filamentous bacteria (F) are also shown.