Control of bacterioplankton activity in a eutrophic lake emphasizing relationships among bacteria, cyanobacteria and nutrients

by Lizhu Wang

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences
Montana State University
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Abstract:

This study examined epilimnetic bacterioplankton abundance and production and their relationships with ambient environmental factors in a eutrophic reservoir. Its major objectives were (1) to quantify epilimnetic bacterial abundance and production, (2) to identify roles of temperature, inorganic and organic nutrients in regulating bacterial abundance and activity, and (3) to investigate interactions between bacterioplankton and phytoplankton. Field data were gathered for two consecutive years during the ice-free season. Field and laboratory experiments were based on nutrient enrichment and isotopic tracer (14C, 3H) methodologies, plankton size-fractionation and metabolic inhibition techniques. Bacterial abundance and production averaged 1.7±0.6 (±1 SD) x 10^6 cell ml^-1 and 0.28±0.19 (+1 SB) µg C 1^-1 h^-1, which were strongly correlated with water temperature, chlorophyll a, and particulate N. Both field data and results from in situ and laboratory experiments showed that the dominant factors regulating bacterioplankton varied seasonally. Field and laboratory experiments collectively demonstrated that inorganic nutrients directly limited bacterial growth when insufficient inorganic nutrient were present and indirectly limited bacterial growth by limiting phytoplankton growth when phytoplankton products were necessary for bacterial growth. Bacterial growth was enhanced in the presence of phytoplankton photosynthesis and was stimulated by an increase in phytoplankton density. The movement of motile bacterioplankton toward phytoplankton was also stimulated by an increase in phytoplankton density. Bacterial optimal growth temperatures from different seasons were always higher than in situ temperature. These findings collectively suggested that temperature and nutrient collaboratively regulated bacterioplankton growth. Inorganic nutrient regulated bacterial growth both directly and indirectly via phytoplankton products. Enhancement of bacterioplankton growth by dissolved organic carbon occurred only under, inorganic nutrient replete environment. Phytoplankton products were important nutrient sources of bacterioplankton. Temperature, inorganic nutrients, and phytoplankton products were all factors regulating bacterioplankton growth in the studied system. This conclusion does not support the common view that growth rates of bacterioplankton are always limited by the availability of reduced carbon substrates.

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24 August 1992
Date

Chairperson, Graduate Committee

Approved for the Major Department

24 August 1992
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ABSTRACT

This study examined epilimnetic bacterioplankton abundance and production and their relationships with ambient environmental factors in a eutrophic reservoir. Its major objectives were (1) to quantify epilimnetic bacterial abundance and production, (2) to identify roles of temperature, inorganic and organic nutrients in regulating bacterial abundance and activity, and (3) to investigate interactions between bacterioplankton and phytoplankton. Field data were gathered for two consecutive years during the ice-free season. Field and laboratory experiments were based on nutrient enrichment and isotopic tracer (^{14}C, ^{3}H) methodologies, plankton size-fractionation and metabolic inhibition techniques. Bacterial abundance and production averaged 1.7±0.6 (±1 SD) x 10^6 cell ml^{-1} and 0.28±0.19 (+1 SD) μg C l^{-1} h^{-1}, which were strongly correlated with water temperature, chlorophyll a, and particulate N. Both field data and results from in situ and laboratory experiments showed that the dominant factors regulating bacterioplankton varied seasonally. Field and laboratory experiments collectively demonstrated that inorganic nutrients directly limited bacterial growth when insufficient inorganic nutrient were present and indirectly limited bacterial growth by limiting phytoplankton growth when phytoplankton products were necessary for bacterial growth. Bacterial growth was enhanced in the presence of phytoplankton photosynthesis and was stimulated by an increase in phytoplankton density. The movement of motile bacterioplankton toward phytoplankton was also stimulated by an increase in phytoplankton density. Bacterial optimal growth temperatures from different seasons were always higher than in situ temperature. These findings collectively suggested that temperature and nutrient collaboratively regulated bacterioplankton growth. Inorganic nutrient regulated bacterial growth both directly and indirectly via phytoplankton products. Enhancement of bacterioplankton growth by dissolved organic carbon occurred only under inorganic nutrient replete environment. Phytoplankton products were important nutrient sources of bacterioplankton. Temperature, inorganic nutrients, and phytoplankton products were all factors regulating bacterioplankton growth in the studied system. This conclusion does not support the common view that growth rates of bacterioplankton are always limited by the availability of reduced carbon substrates.

CHAPTER 1

GENERAL INTRODUCTION

During the past decade, the ecological role of heterotrophic bacterioplankton in both marine and freshwater ecosystems has received unprecedented attention (e.g., Van Es and Mayer-Reil 1982; Wetzel 1983; Azam et al. 1983; Williams 1984; Ducklow et al. 1986; Sherr et al. 1987; Sherr and Sherr 1988; Scavia and Laird 1987; Cole et al. 1988; Pomeroy and Wiebe 1988; Christiffersen et al. 1990; Gebre-Mariam and Taylor 1990; Riemann and Bell 1990; Gomes et al. 1991; Robarts and Wicks 1990; Mariazzi et al. 1991; Sorokin and Mamaeva 1991; Toolan et al. 1991). It is now increasingly recognized that heterotrophic bacterioplankton are not only responsible for the degradation of organic matter and recycling of nutrients, but also are important producers of particulate organic carbon, through the consumption and assimilation of dissolved organic matter released by phytoplankton. Thus, they may provide a route for the assimilation of dissolved organic carbon into the classical food chain. Such advancements were made possible by (1) new fluorometric techniques for the enumeration of natural bacteria (Zimmermann and Meyer-Reil 1974; Hobbie et al. 1977), and (2) the development of new techniques for assessing growth rates of natural bacterial assemblages (Fuhrman and Azam 1980; Karl 1986; Moriarty 1986).

In the years since 1946, when ZoBell (1946) published his seminal book on marine microbiology, it has become evident that heterotrophic bacteria play an important role in nutrient cycling in the sea (Pomeroy 1970; Sorokin 1978). The importance of heterotrophic bacteria in freshwater cycling has also been recognized as stated by Wetzel (1983): "In aquatic ecosystems, most organic detritus is metabolized by bacteria. The organic matter brought to a lake would quickly fill the basin if it were not decomposed by bacteria, and the lake would cease to exist". The views of these authors consider bacteria only as a sink of organic carbon.

The new microbial paradigm that implicates bacteria and small algae and protozoa in major pathways for carbon flow was first developed for the pelagic food webs of oligotrophic oceans (Azam et al. 1983; Pomeroy 1974, 1984; Sherr and Sherr 1988), and is now being recognized in the plankton of lakes and rivers (Porter 1984; Meyer et al. 1985; Stockner and Antia 1986). These views stress the role of bacteria as sources of organic carbon. Heterotrophic bacterioplankton are a large and dynamic component of aquatic ecosystems. Bacterial production can be as high as 150 μg carbon l^{-1} d^{-1} and reach 50% of the planktonic primary production in both fresh- and saltwater ecosystems (Cole et al. 1988). In a Russian reservoir, bacteria provided 75% of the food consumed by animals, while other planktonic organic matter provided only 25% (Sorokin 1972).

Although the importance of bacteria as mineralizers and biomass producers is now unquestioned, field data on the quantification of parameters that describe specific roles of bacteria are still scarce (Van Es and Meyer-Reil 1982). The ultimate role bacteria play in food chains is an issue at the forefront of aquatic ecology (Azam et al. 1983; Wright and Coffin 1984; Sherr et al. 1987; Gebre-Mariam and Taylor 1990). Fundamental questions concerning relationships between heterotrophic bacterioplankton and their environment in aquatic ecosystems which remain unresolved are: What environmental factors regulate seasonal patterns in heterotrophic bacterioplankton abundance and production? How do these factors interact? What role does organismal interaction play in regulating heterotrophic bacterioplankton abundance and production? The understanding of the role of heterotrophic bacterioplankton as mineralizers and biomass producers in aquatic ecosystems and wise management of such ecosystems will depend upon how well these questions are answered.

Literature Overview

Bacterioplankton Abundance and Production

The observation and preliminary description of microorganisms in natural habitats date back to the seventeenth century with the initial microscopic studies of

Van Leeuwenhoek (1677). However, the founding principles of microbial ecology as a specialized branch of science were not established until the late nineteenth century. The study of microorganisms in natural habitats represents one of the least developed and most poorly quantitated areas of microbiological research (Karl 1986). The entire subject of aquatic microbial ecology is a neglected frontier in limnology (Wetzel 1983).

Early attempts to determine the abundance of bacterioplankton in aquatic ecosystems used culture techniques which underestimated abundance by two to three orders of magnitude (Williams 1984). Direct staining techniques originally used erythrosin, but subsequently used fluorescence stains such as acridine orange (Hobbie et al. 1977) and DAPI (Coleman 1980), which have been adopted as virtual standards. Many studies have been done to investigate bacterioplankton abundance in both marine (e.g., Ferguson and Rublee 1976; Goulder 1977; Palumbo and Ferguson 1978; Wright 1978; Ferguson and Palumo 1979; Meyer-Reil et al. 1979; Fuhrman et al. 1980; Fuhrman and Azam 1980; Wright and Coffin 1984; Sorokin and Mamaeva 1991; Gomes et al. 1991) and freshwater (e.g., Pedrós-Alió and Brock 1982; Bell et al. 1983; Scavia and Laird 1987; Chrzanowski and Hubbard 1988; Marvalin et al. 1989; Gebre-Mariam and Taylor 1990; Robarts and Wicks 1990; Mariazzi et al. 1991). Reported bacterial numbers were greater than 5.0 X 10^9 cells l^{-1} in estuarine interiors, 1.0 X 10^9 to 5.0 X 10^9 cells l^{-1} in tidal inlets and coastal regions, and ranged from 0.05 x 10 ^{9} to 1.0 X 10^9 cells l^{-1} in eutrophic offshore and ocean waters (Van Es and Meyer-Reil 1982). Reported bacterial numbers in freshwater ranged from 0.1 X 10^9 to 1.0 X 10^9 cells l^{-1} in oligotrophic lakes (Pedrós-Alió and Brock 1982; Scavia and Laird 1987) and from 1.0 X 10^9 to 10.0 10^9 cells l^{-1} in eutrophic lakes (Coveney et al. 1977; Hobbie and Wright 1979; Riemann et al. 1982). Bacterioplankton abundance in a given environment varies in a relatively small range (10^7 - 10^9 cells l^{-1}); it is generally thought that grazing limits the upper limit (Azam et al. 1983).

Bacterioplankton activity measurements stemmed from the introduction of ^{14}C-tracer technique by Parson and Strickland (1962) during the late 1960's and early 1970's. This technique enables the uptake of selected organic substrates by natural communities to be studied under in situ conditions. As the information provided by such a technique is restricted to the fate of selected substrates, several new methods for the determination of heterotrophic bacterial activity have developed. One such new method developed by Hagström et al. (1979) used the frequency of dividing cells to convert bacterial culture data into growth rates of natural populations. Another new method developed by Fuhrman and Azam (1980, 1982) utilized the incorporation of [^{3}H]-thymidine into DNA to measure bacterioplankton DNA doubling time and to convert it into production rate. Contrary to [^{3}H]-thymidine, [^{3}H]-adenine has been proposed to measure total microbial production, although [^{3}H]-adenine may be incorporated into RNA and DNA by both prokaryotes and unicellular algae (Karl 1981). These methods were developed first in marine environments and have been considered as the most promising methods that are currently available (Azam at al. 1983; Hagström 1984; Moriarty 1986). Now they are widely used in freshwater environments (e.g., Pedrós-Alió and Brock 1982; Riemann et al. 1982; Bell et al. 1983; Bell and Kuparinen 1984; Güde et al. 1985; Lovell and Konopka 1985; Scavia et al. 1986, Nagata 1987; Chrzanowski and Hubbard 1988; Gegre-Mariam and Layor 1990; Robarts and Wicks 1990; Mariazzi et al. 1991). Reported bacterioplankton production ranged from 0.08 to 5.60 μg C l^{-1} h^{-1} in saltwater (see review by Moriarty, 1986) and 0.1-13.7 μg C l^{-1} h^{-1} in freshwater (Bell et al. 1983; Chrzanowski and Hubbard 1988; Cole et al. 1988; Robarts and Wicks 1990; Mariazzi et al. 1991).

Despite current wide use of bacterioplankton enumeration and growth measurement techniques, it is still difficult to evaluate precisely the ecological significance of estimates from different areas and periods of time because of the combined uncertainty of these measurements (Hagström 1984). For example, the methods designed to measure the bacterial growth rate to the nearest order of magnitude has been regarded as a considerable advance (Hagström 1984). Although a significant body of data on bacterioplankton abundance and production have been emerged, the complexities of natural ecosystems and their resident microbial populations still overwhelm our technological and analytical capabilities (Karl 1986). It is evident that a thorough understanding of the flow of the nutrients and carbon in aquatic ecosystems will require knowledge of the parameters that regulate bacterioplankton abundance and production. Both abundance and production are likely affected by a mosaic of environmental factors in both fresh and saltwater ecosystems. These factors have long been a matter of considerable speculation (Cole et al. 1988; White et al. 1991). It is certainly evident that more studies are needed to identify such factors and to relate these factors to bacterioplankton abundance and production in natural environments.

Factors Regulating Bacterioplankton Abundance and Production

Much of the microbial literature on aquatic systems maintains the notion that bacterioplankton abundance and production are controlled, or directly related to, the supply of decomposable organic matter, but little quantitative data exist (Hobbie and Rublee 1977; Cole 1983; Cole et al. 1988). Because of the difficulties of precisely quantifying the supply of labile organic matter in a system, many studies have tried to estimate variables, such as primary production, which would be related to that supply. Although many studies have traced products from algae or cyanobacteria to heterotrophic bacteria (e.g., Bell and Lang 1974; Paerl 1976; Cole et al. 1982; Bell 1983; Jensen 1983; Chrost and Faust 1983; Jensen and Sødergaard 1985; Feulillade et al. 1988; Sundh 1989; Chrzanowski and Hubbard 1989; Gomes et al. 1991), direct tests showing an increase in bacterioplankton production as a function of organic matter supplied from phytoplankton are scarce. Among the few studies addressing this subject, Peterson et al. (1985), working with arctic benthic algae, found that heterotrophic bacterial activity incubated in light was significantly higher than in the dark. Murray et al. (1986), in a study of an algal-bacterial biofilm, also found that bacterial thymidine incorporation was four to sixteen-fold greater in the presence of diatoms than in their absence. These studies imply that a strong coupling between bacterioplankton and phytoplankton will exist in pelagic systems. Such speculation has been indirectly indicated by the positive correlations between bacterioplankton abundance and phytoplankton biomass, between bacterioplankton production and primary production from certain lakes (Pedrós-Alió and Brook 1982; Bell and Kuparinen 1984; Lovell and Konopka 1985; Riemann and Søndergaard 1986; Scavia et al. 1986; Douglas et al. 1987; Chrzanowski and Hubbard 1988; Bjørnsen et al. 1989; Marvalin et al. 1989; Jonas and Tuttle 1990; Robarts and Wicks 1990) and large scale empirical models (Bird and Kalff 1984; Cole et al. 1988; Currie 1990; White et al. 1991). However, lack of correlation between bacterioplankton and phytoplankton production has also been reported (Wright and Coffin 1984; Coffin and Sharp 1987; Joint and Pomeroy 1987; Nagata 1987), which indicates that phytoplankton products may not be the only factor regulating bacterioplankton abundance and production.

Net consumption of dissolved inorganic nitrogen and phosphorus by bacterioplankton have been reported in both laboratory experiments (Fuhrman et al. 1988; Horrigan et al. 1988) and natural water studies (Wheeler and Kirchman 1986: Vadstein et al. 1988; Vadstein and Olsen 1989). The limitation of bacterioplankton growth by dissolved inorganic nutrients has received little attention. Because bacterioplankton have a relatively high surface to volume ratio, they should be strong competitors for mineral nutrients, relative to phytoplankton. Indeed, studies in both marine and freshwater systems have shown that bacteria can consume more of the available dissolved inorganic nitrogen and phosphorus than phytoplankton (Currie and Kalff 1984; Wheeler and Kirchman 1986; Vadstein et al. 1988). The question to what extent either organic carbon or inorganic nutrients may limit bacterioplankton production has remained unanswered.

Temperature has been considered one of the most important factors influencing bacterioplankton activity and production (Hagström and Larsson 1984; Wiebe 1984; Pomeroy and Deibel 1986; White et al. 1991). Positive correlations between bacterioplankton production and temperature have been reported from various marine and freshwater studies (Wright and Coffin 1984; Coffin and Sharp 1987; Joint and Pomeroy 1987; Nagata 1987; Chrzanowki and Hubbard 1988; Marvalin et al. 1989; Autio 1990; Robarts and Wicks 1990; Mariazzi et al. 1991; White et al. 1991). The question as to whether these correlations indicate direct effect of temperature on bacterioplankton production remains to be answered. Only a few studies have been done on the temperature regulation of bacterial production occurring naturally in water columns. Ducklow and Kirkman (1983) showed a 4-fold increase in [^{3}H]-thymidine incorporation when water temperature was raised from 3.5 to 18 °C. Pomeroy and Deibel (1986), in a study of Newfoundland coastal water, found that particulate materials that would be utilized by microorganisms in 2 to 3 days at 20 to 25 °C required 11 days at 4 °C and 18 days at -0.2 °C. Servais and Billen (1989) also found that up to 85% of the increase in bacterial activity could be explained by higher temperature in the River Meuse at Tihange. In some cases, both phytoplankton production and bacterioplankton production were positively correlated with temperature (e.g., Chrzanowski and Hubbard 1988; Robarts and Wicks 1990; Mariazzi et al. 1991). In other cases, only bacterial production was correlated with temperature (e.g., Wright and Coffin 1984; Coffin and Sharp 1987; Joint and Pomerory 1987). Whether temperature directly affects bacterioplankton production or temperature affects substrate supply from phytoplankton, which indirectly affects bacterioplankton production, is not clear. The role of water temperature as a factor regulating the growth of microbial population has received relatively little attention (Lovell and Konopka 1985). There is an increasing demand to study the regulation mechanism of temperature on bacterial growth, especially the possible interactions with phytoplankton and the supply of organic carbon to bacterial production (Pomeroy and Wiebe 1988; Robarts and Wicks 1990).

Hypotheses

A unifying theme in this study is the relationship between the abundance and production of heterotrophic bacterioplankton and ambient environmental factors in a eutrophic ecosystem. The following hypotheses were tested.

1) One or more ambient environmental factors that regulate bacterioplankton growth can be identified by statistical correlation analysis from data collected during an extensive field survey.

2) Bacterioplankton activity is not always regulated by

organic carbon supply.

Inorganic nitrogen and inorganic phosphorus are as important as organic carbon in controlling bacterioplankton growth. The dominant limiting nutrient varies seasonally, depending on conditions such as organic carbon, inorganic nitrogen and inorganic phosphorus supply. Nutrient supply from river inflow plays an important role in regulating epilimnetic bacterioplankton growth.

3) Inorganic nutrients can limit bacterioplankton growth directly when inorganic nutrients are in short supply.

Inorganic nutrients can also limit bacterioplankton growth indirectly by limiting phytoplankton growth when phytoplankton products are necessary for bacterioplankton growth. Phytoplankton products (presumably via extracellular release) are important substrates for bacterioplankton growth.

4) Water temperature is an important physical factor controlling bacterioplankton growth in nature. Temperature and nutrients regulate bacterioplankton activity collaboratively.

Objectives.

The major objectives of my research were, firstly to quantify seasonally epilimnetic heterotrophic bacterioplankton abundance and production in a eutrophic lake, secondly to evaluate the role of organic carbon, inorganic nitrogen, and inorganic phosphorus in regulating bacterioplankton abundance and production through both laboratory and field experiments, thirdly to investigate the interactions between bacterioplankton and phytoplankton and, finally to identify the role of temperature in regulating bacterioplankton abundance and activity. The specific objectives of this study were to:

1) Assess the importance of bacterioplankton abundance and production in Hebgen Lake relative to other eutrophic ecosystems.

2) Correlate bacterioplankton parameters with ambient variables to identify important factors related to bacterioplankton and test whether these factors change seasonally.

3) Investigate the importance of nutrient input from river flows in regulating bacterioplankton growth.

4) Test if inorganic nitrogen and inorganic phosphorus stimulate bacterioplankton activity and whether such stimulations are direct or indirect via phytoplankton products.

5) Verify the relationship between bacterioplankton activity and phytoplankton biomass and photosynthesis activity.

6) Determine the optimal growth temperature of natural bacterioplankton for summer, winter, and for an isolate, and document the potential limitation of temperature on

bacterioplankton growth.

7) Examine the effect of temperature on the nutrient regulation of bacterioplankton.

Test of Hypotheses and Organization of Report

The research was carried out in a eutrophic reservoir, Hebgen Lake, in southwestern Montana. Most of the drainage of Hebgen Lake lies within Yellowstone National Park. The unique feature of this area is that it receives minimal impact from human activities, i.e. it is naturally eutrophic.

As part of an extensive study aimed at defining the role of organic and inorganic nutrients on bacterioplankton and phytoplankton in Hebgen Lake, I approached this study from both a monitoring and an experimental perspective.

The remainder of this dissertation is presented in 7 chapters. Chapter 2 presents baseline data collected during the 1988 and 1989 ice-free seasons in Hebgen Lake. The baseline data have been used to develop a correlation data base, which revealed important environmental factors closely related to bacterioplankton parameters (test of hypotheses 1). The monitoring data have also been used to determine ambient conditions at the time of the field and laboratory experiments and to corroborate and expand upon the experimental results (supporting data for hypotheses 2; cf. Chapter 3 - 7). Chapter 2 also presents results from laboratory experiments conducted with lake water enriched with river water during various times of the year in 1989. These experiments have been used to test the effect of external nutrient loading on bacterioplankton growth (hypotheses 2).

Chapter 3 describes how natural assemblage of bacterioplankton and phytoplankton communities were enriched with inorganic and organic nutrients in 20-liter microcosms incubated in the lake and the laboratory at different seasons of 1988 and 1989. Such experiments have provided information on interactions between bacterioplankton and nutrients, phytoplankton and nutrients, and bacterioplankton and phytoplankton. These experiments have also revealed information on whether organic and inorganic nutrients stimulate bacterioplankton activity, and whether the stimulation is different at different seasons or years (hypotheses 2).

Chapter 4 presents laboratory experiments describing natural bacterioplankton communities enriched with organic and inorganic nutrients in the presence and absence of photosynthetically active phytoplankton. These experiments have been designed to demonstrate whether inorganic nutrients can directly stimulate bacterioplankton activity without the effect of phytoplankton (hypotheses 2).

Chapter 5 presents laboratory experiments separating different concentrations of two species of cyanobacteria from heterotrophic bacteria by dialysis tubing. These experiments were designed to test whether phytoplankton products are important substrates for heterotrophic bacteria growth and to provide evidence as to whether high phytoplankton biomass can support high heterotrophic bacterial abundance and activity (hypotheses 3). Chapter 6 also presents laboratory experiments designed to test the chemotactic movement of heterotrophic bacteria toward different concentrations of three cyanobacterial species (hypotheses 3).

Chapter 7 describes laboratory experiments designed to test temperature effect on the activity of both natural bacterioplankton assemblage and an isolated single bacterial species (hypotheses 4).

Chapter 8 summarizes my major conclusions and management implications resulting from the research.

CHAPTER 2

EPILIMNETIC BACTERIOPLANKTON ABUNDANCE AND PRODUCTION: RELATIONSHIP WITH BIOTIC AND ABIOTIC FACTORS

Introduction

The significance of the heterotrophic bacterial community, both as a potential food resource for larger consumer organisms and in the regeneration of nutrients necessary to support primary production, has attracted considerable attention in recent years. A number of studies have shown a close coupling between heterotrophic bacterial growth or biomass and phytoplankton primary production or chlorophyll a (Fuhrman et al. 1980; Chrzanowski and Hubbard 1989; Marvalin et al. 1989). Other studies have attempted to quantify the transport of organic carbon from phytoplankton to bacterioplankton (e.g., Cole et al. 1982; Jensen 1983; Jensen and Sondergaard 1985; Feuillade et al. 1988; Chrzanowski and Hubbard 1989; Sundh 1989; Tranvik 1989). It has been suggested that bacterial production is controlled by, or is directly related to, the supply of decomposable matter (Cole et al. 1988), which has been confirmed by the study of a hypertrophic lake (Robarts and Wicks 1989). In studies emphasizing factors controlling bacterial production, Marvalin et al. (1989) found that epilimnetic phytoplankton productivity and temperature played major roles in controlling bacterial development. Bjornsen et al.

(1989), studying a eutrophic estuary, found that phytoplankton was the dominate source of bacterial substrate. Gebre-Mariam and Taylor (1989) also found that no significant positive correlation existed between bacterial variables and inorganic phosphorus, nitrogen, and total dissolved solids. However, Vadstein et al. (1988) showed that bacteria have a substantially higher phosphorus requirement than do phytoplankton and act as net consumers of inorganic phosphorus. Bacterial abundance and production have also been shown to be correlated with particulate organic carbon and nitrogen in the Chesapeake Bay (Jonas and Tuttle 1990). Other studies have shown that bacteria are net consumers of inorganic nitrogen and phosphorus, and they may compete for such nutrients with phytoplankton (Rhee 1972; Parker et al. 1975; Wheeler and Kirchmen 1986; Horrigan et al. 1988). In general, these studies have revealed that bacterioplankton production may be limited by inorganic phosphorus and nitrogen in addition to organic carbon.

The objectives of this study were to determine the major factors controlling bacterioplankton production in a natural eutrophic system, with an emphasis on the effects of nutrient supply.

Study Site

Date were collected at three permanently buoyed stations

(Figure 1) in Hebgen Lake, located on the upper Madison River at latitude 44°51'55' and longitude 111°20'05' (Martin 1967). The maximum depth was 6.5 m for station I, 20 m for station II, and 10 m for station III during the study period. Based on temperature profiles, the upper 5 m depths were chosen to represent the epilimnion depth for the stations. Lake water samples were collected biweekly at 0, 3, and 5 m for bacterial variables, and at 0, 1, 3, and 5 m for other variables during the 1988 ice-free season at station I, II, and III. Lake water samples were collected every 2 to 3 weeks at 0, 1, 3, and 5 m for all variables during the 1989 ice-free season at station I, and on 8 June, 19 July, and 8 October 1989 at station II. River inflow samples were collected at the time of lake sampling during 1988, and on 21 May, 26 July, 13 August, 20 September, and 8 October during 1989. The sampling sites are shown in Figure 1.

Materials and Methods

Field Procedures.

Samples were collected between 1100 and 1200 h using a 4-l Van-Dorn water sampler. $^{14}CO_2$ based nutrient bioassays were incubated at the location of water collection for 4-6 h followed by filtration (Whatman GF/C). ^{3}H-thymidine based nutrient bioassays were incubated in a dark box at the temperature of collection for 20-30 minutes.

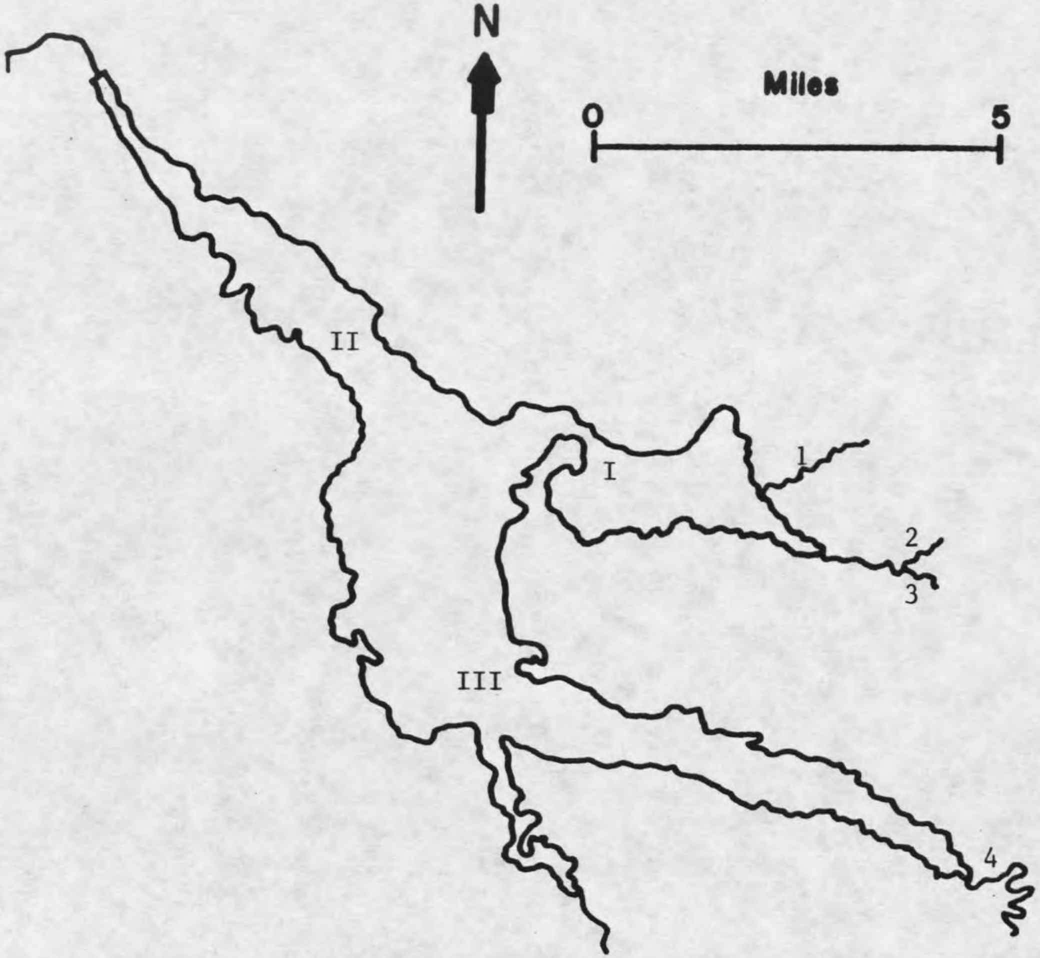

Figure 1. Map of Hebgen Lake showing the three permanent sampling stations (I, II, III) and the four river sampling sites (1, 2, 3, 4). The map is modified from Martin (1967).

Samples for bacterial abundance were fixed with particle-free formaldehyde (3% final concentration) immediately after collection. All samples were transported on ice to the laboratory for final analysis.

Laboratory Procedures

Dissolved Nutrients. Nitrate was determined by cadmium reduction (Eppley 1985), ammonium by the phenol hypochlorite method (Solorzano 1969), and soluble reactive phosphorus (SRP) by the molybdate method modified for arsenate interference (Downes 1978). Total dissolved phosphorus (TDP) was measured using the acid hydrolysis procedure of Solorzano and Sharp (1980) followed by orthophosphate determination (Stainton et al. 1977); dissolved organic phosphorus (DOP) was computed from the difference between TDP and SRP. Total dissolved nitrogen (TDN) was measured by persulfate digestion (D'Elia et al. 1977) followed by determination of nitrate by Cd reduction. Dissolved organic nitrogen (DON) was computed by subtracting the sum of nitrate and ammonium from TDN. All of the above samples were prefiltered in the field through Whatman GF/C filters and frozen before analysis.

Particulate Matter. Chlorophyll a was determined by fluorometry on 90% acetone extracts (Strickland and Parsons 1972). Fluorescence was measured with a Turner model 112 fluorometer on pre- and post-acidified samples and compared to a chlorophyll a standard (Sigma) treated in the same manner. Particulate organic carbon (POC) and particulate organic nitrogen (PON) were measured with a Carlo-Erba model 1106 elemental analyzer calibrated with standard amounts of acetanilide. Particulate organic phosphorus (PP) was determined with the acid hydrolysis procedure of Solorazano and Sharp (1980) followed by orthophosphate measurement of the digestion (Stainton et al. 1977). Dissolved organic carbon (DOC) was analyzed using Dorhmann Carbon Analyzer. Total nitrogen (TN) was the sum of TDN and PON; total phosphorus (TP) was the sum of TDP and PP; and total organic carbon (TOC) was the sum of DOC and POC.

Radioisotope Bioassay. Phytoplankton $^{14}CO_2$ uptake was measured by adding ^{14}C-NaHCO$_3$ stock (ICN Radiochemical Inc.) to lake water to a final ^{14}C activity of about 0.05 μCi ml^{-1}. The photosynthetic reaction was terminated by filtration of the entire sample through Whatman GF/C filters. Seven ml of CytoScint scintillation cocktail (ICN Radiochemical, Irvine, CA) was added to the filter in a 20-ml scintillation vial and counted with a Beckman LS-100C scintillation spectrometer. Efficiency was computed by the external standard channels ratio.

Bacterial ^{3}H-thymidine incorporation was determined by adding high activity (55 Ci mmole^{-1}) methyl-^{3}H-thymidine (in 70% ethanol) (ICN Radiochemical INC.) to a 10 ml water sample (final concentration 10 nM) in a 20-ml glass scintillation vial. The thymidine was evaporated to dryness and rehydrated with deionized water before use to eliminate products of self radiolysis and to remove ethanol. Thymidine incorporation was terminated by adding 10 ml ice-cold 10% (w/v) trichloroacetic acid (TCA) to each vial followed by extraction overnight at 4 ^{o}C. The extracted samples were then filtered onto 0.2 μm membrane filters (Poretics Co.). After rinsing the filter 5 times (2 ml each rinse) with 5% ice-cold TCA, the filter was transferred to a 20 ml polyethylene scintillation vial with 7.0 ml CytoScint scintillation cocktail (ICN Radiochemical, Irvine, CA). Radioactivity in each sample was determined by standard scintillation spectrometery using a Beckman LS-100C liquid scintillation counter. The counting efficiency was determined by the external standard ratio method using ^{3}H-toluene as a reference and acetone as the quench agent.

Data Expression and Bacterial Conversions

Data Expression. All data used for correlation analysis were expressed as l^{-1} (biomass, abundance, physical and chemical data) or l^{-1} h^{-1} (production data) at each sampling depth. For other analysis, bacterioplankton and phytoplankton biomass and abundance, physical and chemical data from the first 5 m of Hebgen Lake were integrated and expressed as m^{-2} (5 m^{-3}). Bacterioplankton and phytoplankton production data from the first 5 m depth were integrated and expressed as m^{-2} h^{-1} (5 m^{-3} h^{-1}). All the integrated units were then converted and presented as depth integrated mean

(l^{-1} or l^{-1} h^{-1}).

Bacterial Conversions. The portion of ^{3}H-thymidine incorporated into DNA was determined by the method of Servais et al. (1987). A conversion factor from mole ^{3}H-thymidine incorporated into DNA to number of cells produced was $1x10^{18}$ (Moriarty 1988). Bacterial cell counts were made using the acridine orange direct count technique (Hobbie et al, 1977) with a Nikon Labophot epifluorescence microscope. Bacteria were filtered onto irgalan-black-stained 0.2 μm pore sized Nuclepore polycarbonate filters and stained with acridine orange. At least 200 cells were counted in a minimum of 20 fields. Bacterial size was estimated with a calibrated ocular micrometer. The corresponding biomass and production were calculated, in terms of carbon, using the coefficient of 560 fg C μm^{-3} (Bratbak 1985).

Nutrient and River Water Enrichment Experiments

Three laboratory experiments were conducted during May (experiment 1), July (experiment 2), and October (experiment 3) 1989. Surface water enriched with ammonium, phosphorus, mannitol, and river water (singly and in various combinations, see Table 1) were incubated at the temperature of collection (Table 1) in a laboratory incubator in 1-l polyethylene bottles. A photon flux density of 150 μE $m^{-2}s^{-1}$ was provided by cool-white fluorescent lamps with 12 h light/dark cycle. The incubation period for May was 5 days and for June and October was 4 days. River water used to

Table 1. Nutrient and river water enrichments in the laboratory nutrient enrichment experiments. Control presents ambient levels, NH_4^+ and PO_4^{-3} are in units of µg N or P l^{-1}, mannitol in mg l^{-1}, and river in % of lake water. NA=treatment not applicable.

Treatment	May	July	October
Temp (o^C)	14	18	15
Control	0	0	0
NH_4^+-N	NA	NA	200
PO_4^{-3}-P	NA	NA	50
NH_4^+-N & PO_4^{-3}-P	NA NA	NA NA	200 50
Mannitol	91	91	91
River water	50	50	50
NH_4^+-N & mannitol	100 91	100 91	NA NA
PO_4^{-3}-P & mannitol	50 91	50 91	NA NA
NH_4^+-N & PO_4^{-3}-P & mannitol	100 50 91	100 50 91	NA NA NA

enrich the lake water was a mixture from sampling sites 1 and 2 (1:1) for experiment 1 and 2, and was a mixture from sampling sites 1, 2, and 3 (1:1:1) for experiment 3 (see Figure 1 for sampling sites). The mixed river water was filtered through a 0.45 μm filter to remove particles before mixing with lake water collected from the surface of station I. Bacterial ^{3}H-thymidine incorporation and cell number were measured before starting the incubation for all 3 experiments, on days 1, 3, and 5 for experiment 1, and on days 2 and 4 for experiments 2 and 3 using the methods described above.

Results

Bacterioplankton Production, Biomass, Abundance, and Doubling Time

Depth integrated mean epilimnion bacterioplankton production varied throughout the sampling seasons. The minimum production (<0.1 μg C l^{-1} h^{-1}) was found in both early spring and late fall; the maximum (>0.8 μg C l^{-1} h^{-1}) was found in late July and early August for all the three sampling stations in both 1988 and 1989 (Figure 2). The minimum depth integrated mean bacterioplankton biomass (<20 μg C l^{-1}) occurred in the spring (May and June) and the maximum (>50 μg C l^{-1}) in mid-summer (early August). Depth integrated mean biomass was about 40 μg C l^{-1} during fall 1988 and 1989 in all three stations (Figure 2).

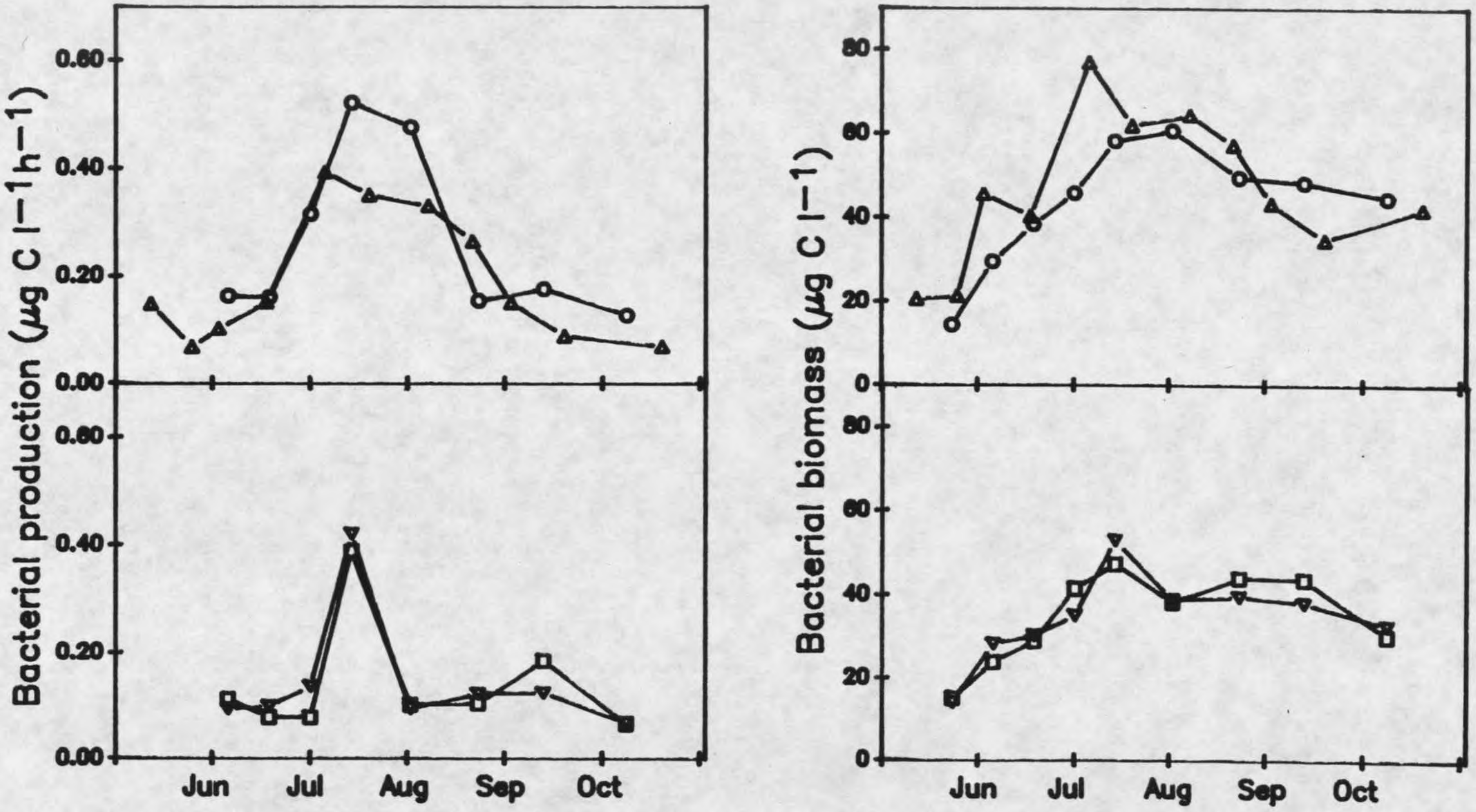

Figure 2. Hebgen Lake seasonal distribution of epilimnetic bacterioplankton production and biomass during 1988 and 1989. ○ Station I 1988, △ Station I 1989, ▽ Station II 1988, □ Station III 1988.

Depth integrated mean bacterioplankton abundance had the same trends as biomass (Figure 3) owing to the presence of similar sized bacterial cells over the season. Depth integrated mean bacterioplankton doubling time was highest during late spring and fall, and lowest during early spring and summer of both 1988 and 1989 at all three stations (Figure 3).

Phytoplankton Production, Chlorophyll a, and Organic and Inorganic Nutrients

Depth integrated mean epilimnetic phytoplankton production was 12.4 μg C l^{-1} h^{-1} (range 2.5-36.6) at station 1, 8.1 μg C l^{-1} h^{-1} (range 1.8-13.6) at station 2, and 8.9 μg C l^{-1} h^{-1} (range 3.9-13.1) at station 3 over the sampling period. Depth integrated annual mean epilimnion chlorophyll a concentration was 9.8 μg l^{-1} (range 1.5-21.1) at station 1, 5.84 μg l^{-1} (range 1.2-12.0) at station 2, and 5.8 μg l^{-1} (range 0.7-27.2) at station 3 during the sampling season. The seasonal patterns of phytoplankton depth integrated mean production and chlorophyll a are shown in Figure 4. Seasonal trends of inorganic nitrogen and phosphorus, organic carbon, and organic nitrogen and phosphorus are shown in Figures 5, 6, and 7. Phosphorus parameters (SRP, DOP, TDP, PP) had similar seasonal patterns for the 3 stations during 1988 (Figure 5); SRP was less than 10 μg l^{-1}, DOP and PP were less than 30 μg l^{-1} during most of the study period (Figure 5). Nitrogen parameters (NH_4^+, NO_3^-, TDN, PON, DON) varied substantially throughout the season;

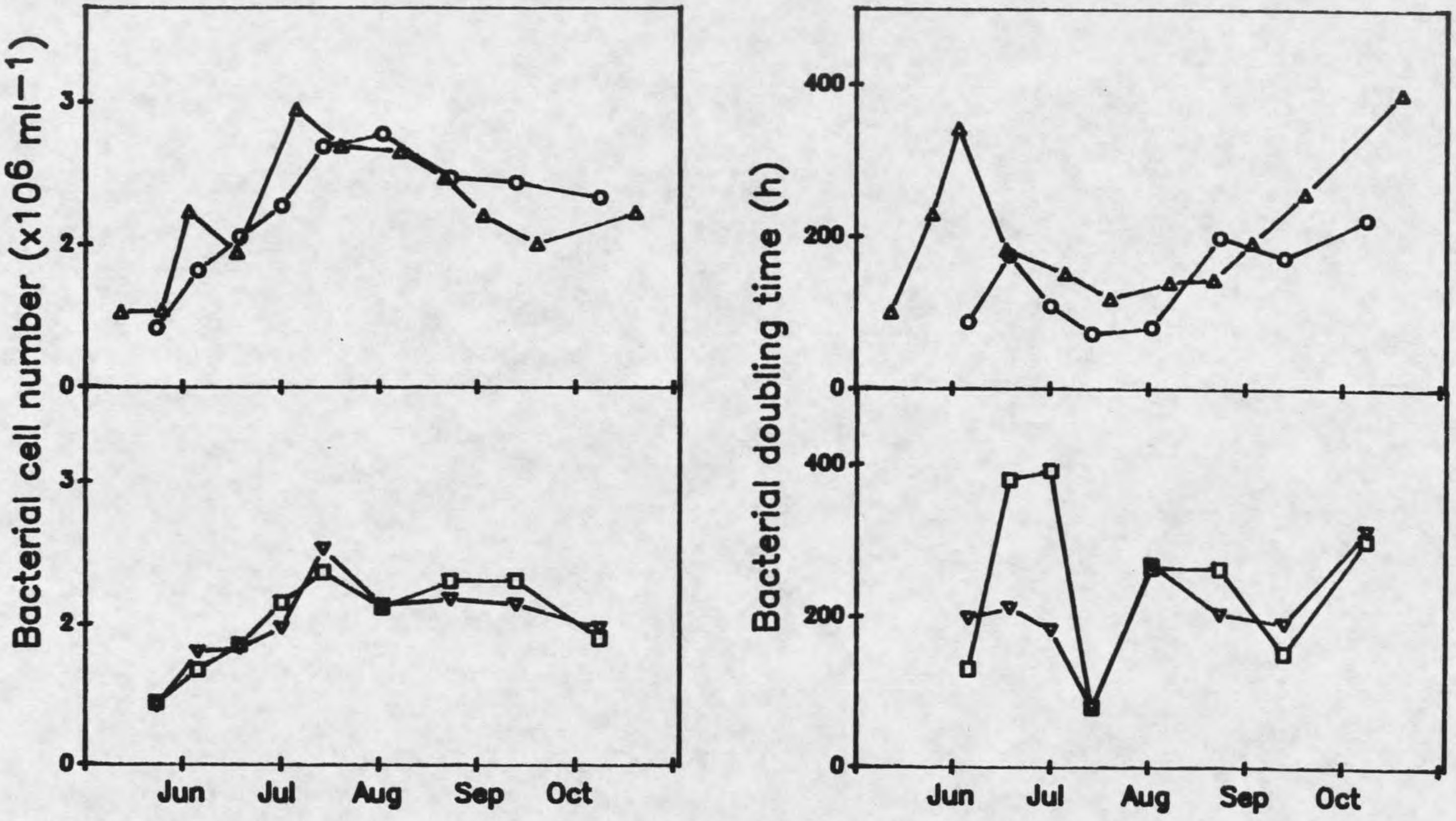

Figure 3. Hebgen Lake seasonal distribution of epilimnetic bacterioplankton cell number and doubling time during 1988 and 1989. Symbols are the same as in Figure 2.

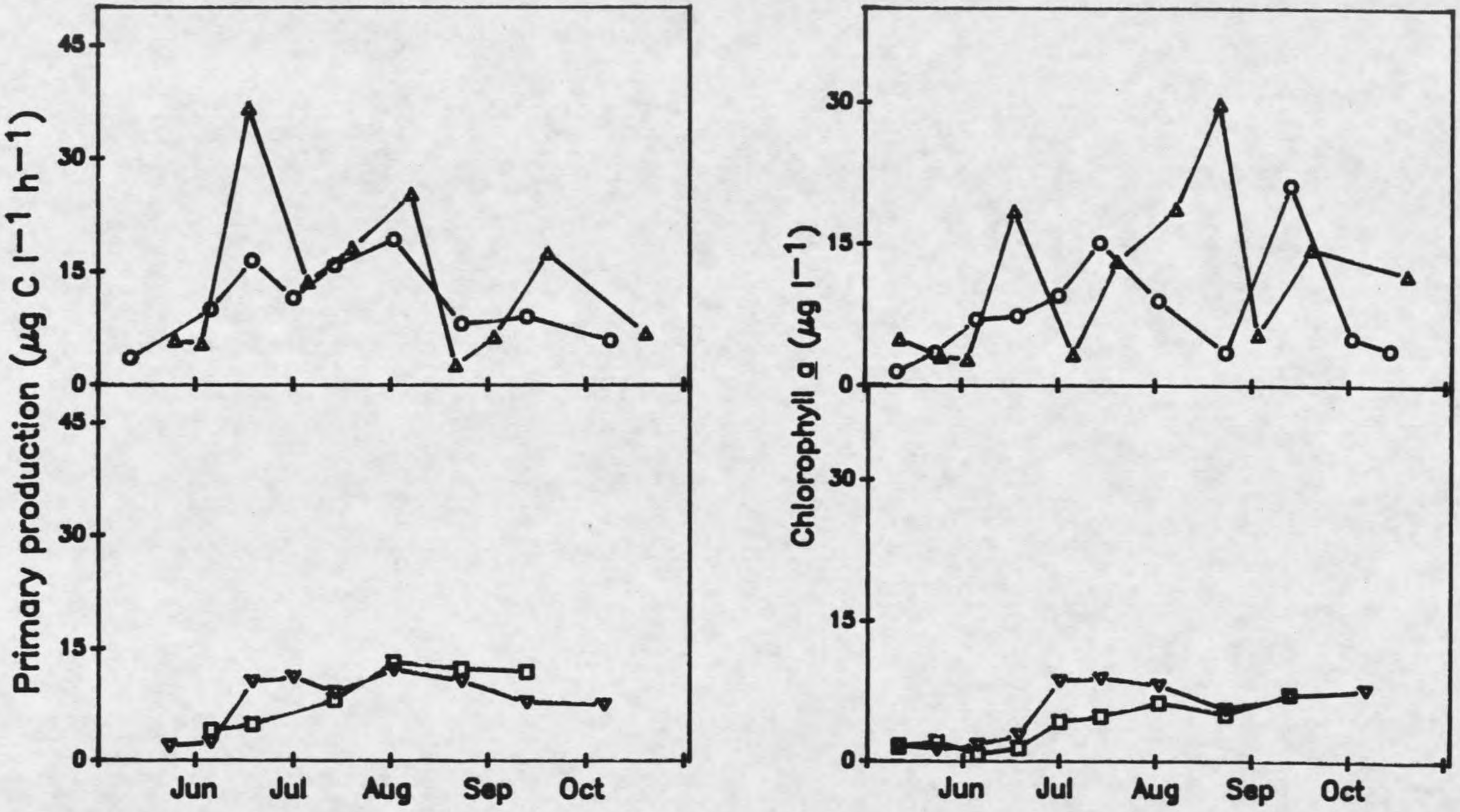

Figure 4. Hebgen Lake seasonal distribution of epilimnetic phytoplankton production and chlorophyll a concentration during 1988 and 1989. Symbols are the same as in Figure 2.

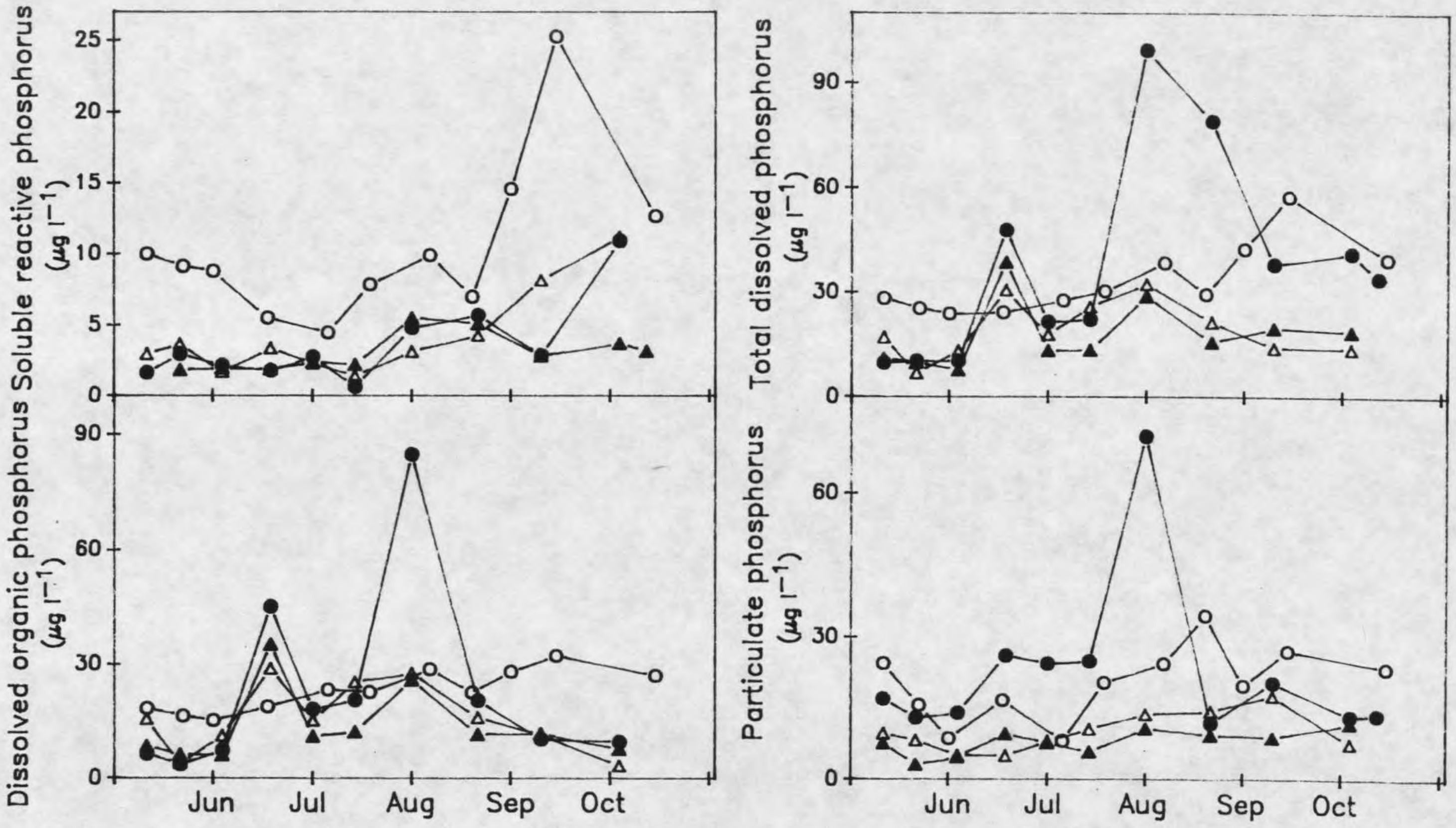

Figure 5. Hebgen lake seasonal distribution of epilimnetic phosphorus variables (SRP, DOP, TDP, PP) during 1988 and 1989. ○ Station I 1988, ● Station I 1989, △ Station II 1988, ▲ Station III 1988.

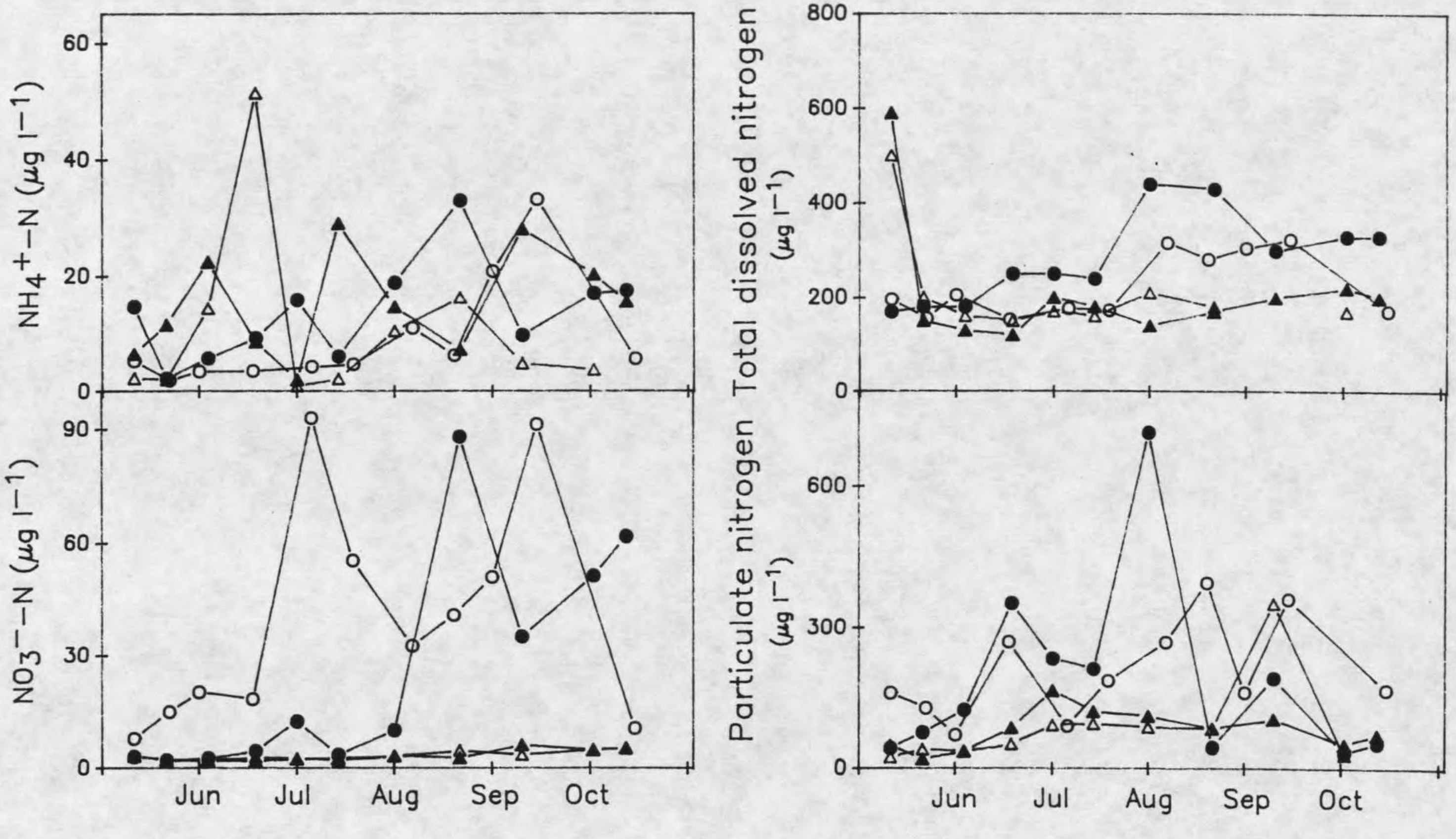

Figure 6. Hebgen Lake seasonal distribution of epilimnetic nitrogen variables (NH_4^+, NO_3^-, TDN, PON) during 1988 and 1989. Symbols are the same as in Figure 5.

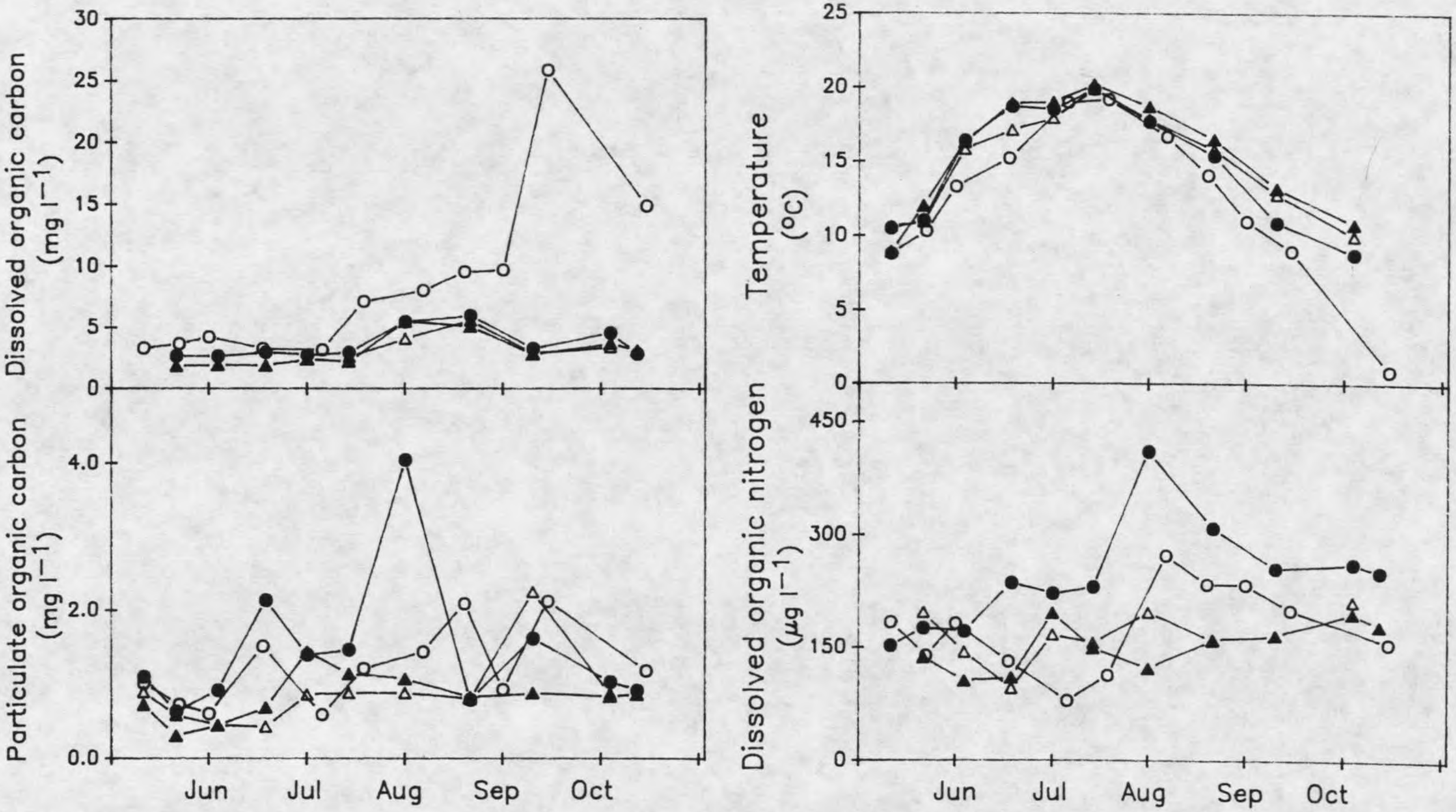

Figure 7. Hebgen Lake seasonal distribution of epilimnetic DOC, POC, DON, and temperature during 1988 and 1989. Symbols are the same as in Figure 5.

their concentrations were higher at station 1 during most of the study period (Figure 6, 7). Maximum DOC concentration occurred during late summer and fall and did not have the same seasonal pattern as POC (Figure 7). Water temperature showed the same seasonal pattern in all the three stations in both 1988 and 1989 (Figure 7).

Correlations Among Bacterioplankton Variables, Phytoplankton Variables, and Other Biotic and Abiotic Nutrients

Bacterioplankton production and specific activity (thymidine uptake per bacterial cell) were most strongly correlated to water temperature ($r>0.5$, $p<0.001$) followed by PON ($r>0.26$, $p<0.05$) and chlorophyll a ($r>0.23$, $p<0.05$). Bacterioplankton production and specific activity were inversely correlated with TOC:TN and TOC:TP ratios ($p<0.001$) followed by DOC and SRP ($p<0.05$, Table 2). Bacterioplankton biomass and abundance were most strongly correlated with chlorophyll a ($r>0.39$, $p<0.001$) followed by water temperature ($r>0.36$, $p<0.01$). No significant negative correlations were found between bacterioplankton biomass or abundance and other variables (Table 2).

PP, PON, POC, TN, TP, and TC were strongly correlated ($p<0.01$) to chlorophyll a concentration and phytoplankton biovolume. The ratio of TN:TP was also correlated with chlorophyll a and phytoplankton biovolume ($p<0.05$) while water temperature, inorganic nitrogen and inorganic phosphorus showed no significant correlation with

Table 2. Linear correlations (r) of bacterioplankton and phytoplankton variables against inorganic and organic nutrients in Hebgen Lake during 1988 and 1989 (n=86). BP=bacterial production; BAPC= bacterial activity/cell; BB=bacterial biomass; BA= bacterial abundance; CHL=chlorophyll; PHYB= phytoplankton biovolume; PPR=phytoplankton production rate. NS not significant at $p>0.05$; r values are listed only when $p<0.05$. All data were transformed (ln) before analysis.

Parameter	BP	BAPC	BB	BA	CHL	PHYB	PPR
Temp	0.60	0.52	0.40	0.36	NS	NS	0.35
CHL	0.28	0.23	0.41	0.39	1.00	0.62	0.41
PHYB	NS	NS	NS	NS	0.62	1.00	0.36
PPR	NS	NS	NS	NS	0.41	0.36	1.00
SRP	-0.25	-0.35	NS	NS	NS	NS	NS
TDP	NS	NS	0.28	0.28	0.83	0.45	0.26
DOP	NS	NS	0.32	0.29	0.30	NS	NS
PP	0.28	NS	0.29	0.28	0.83	0.45	0.26
NH4+-N	NS	NS	NS	NS	NS	NS	NS
NO3--N	NS	NS	0.26	NS	NS	NS	NS
TDN	NS	NS	NS	NS	0.41	0.24	NS
DON	NS	NS	NS	NS	NS	NS	NS
PON	0.33	0.26	0.30	0.24	0.83	0.56	0.31
DOC	-0.28	-0.43	NS	NS	0.29	NS	NS
POC	0.28	NS	0.30	0.29	0.89	0.61	0.42
TN	0.30	NS	0.30	0.28	0.79	0.51	NS
TP	0.28	NS	0.29	0.29	0.83	0.45	0.26
TOC	NS	NS	0.26	0.29	0.72	0.41	NS
TN:TP	NS	NS	NS	NS	0.25	0.40	NS
TOC:TN	-0.44	-0.54	NS	NS	NS	NS	NS
TOC:TP	-0.40	-0.47	NS	NS	NS	NS	NS

chlorophyll a or phytoplankton biovolume (Table 2). Phytoplankton production was significantly ($p<0.01$) correlated with water temperature, POC, and PON. Chlorophyll a, phytoplankton production and phytoplankton biovolume were all correlated strongly ($p<0.01$, Table 2).

Effects of Stratification on Epilimnion Bacterioplankton Growth and Abundance

Integrated average epilimnetic bacterioplankton production and biomass during the summer (July-August) were significantly higher ($p<0.05$) at station I (with unstable thermal stratification) than at station II (with stable thermal stratification). Bacterioplankton doubling time was significantly shorter ($p<0.01$) at station I than station II (Figure 8). Phytoplankton variables (chlorophyll, biovolume, PPR) were significantly higher ($p<0.05$) at station I than at station II (Table 3). All inorganic nitrogen, inorganic phosphorus, organic nitrogen, organic phosphorus and organic carbon were consistently higher at station I than at station II, except ammonium which showed little difference between stations (Table 3). The ratios of TOC:TP and TOC:TN were higher at station II than at station I (Table 3).

Seasonal Variation of Factors Regulating Bacterioplankton Activity and Abundance

Correlation among bacterial variables, phytoplankton variables, nutrient variables and temperature. During spring (May-June), bacterioplankton production, biomass, and numerical abundance were significantly ($p<0.05$) correlated

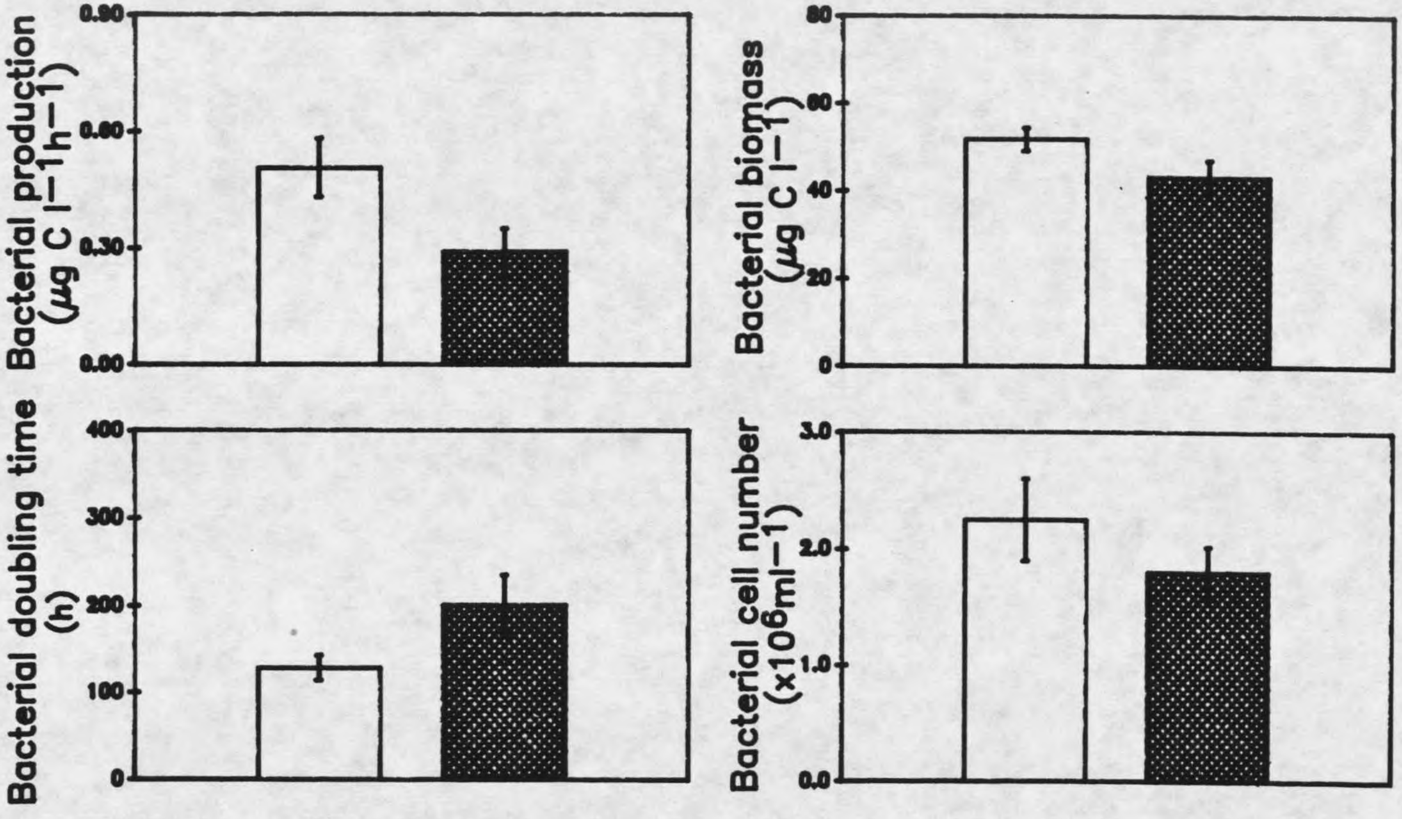

Figure 8. Mean summer (Jul-Aug) epilimnetic bacterioplankton production, doubling time, biomass, and cell number at station I and II of Hebgen Lake during 1988 and 1989. Open boxes are station I, and crossed boxes are station II.

Table 3. Comparison of mean (± 1 SE) summer season epilimnon phytoplankton variables and nutrient concentrations at station I and II in Hebgen Lake during 1988 and 1989. Symbols are as in Table 2. Errors on TP, TN, and TOC were obtained by propagating errors associated with each form.

Parameter	Station I	Station II
Temp (^oC)	18.1±0.5	19.2±0.3
CHL ($\mu g\ l^{-1}$)	10.8±1.8	7.5±1.2
PHYB ($mg\ l^{-1}$)	6.4±1.2	1.8±0.8
PPR ($\mu g\ C\ l^{-1}\ h^{-1}$)	19.5±2.8	11.3±0.8
SRP ($\mu g\ l^{-1}$)	6.3±1.5	2.7±0.4
TDP ($\mu g\ l^{-1}$)	38.9±9.2	23.9±4.8
PP ($\mu g\ l^{-1}$)	20.6±2.3	8.3±0.8
NH_4^+-N ($\mu g\ l^{-1}$)	9.2±2.0	11.0±5.1
NO_3^--N ($\mu g\ l^{-1}$)	28.7±11.0	3.6±1.2
TDN ($\mu g\ l^{-1}$)	249.9±33.1	146.7±19.4
PON ($\mu g\ l^{-1}$)	290.9±66.0	127.7±14.8
DOC ($\mu g\ l^{-1}$)	4470 ±750	2890 ±660
POC ($\mu g\ l^{-1}$)	1733.4±362.5	1136.4±130
TP ($\mu g\ l^{-1}$)	59.5±9.5	32.2±4.9
TN ($\mu g\ l^{-1}$)	540.8±73.8	274.4±24.2
TOC ($\mu g\ l^{-1}$)	6203.4±833	4026.4±673
TOC:TN:TP (weight)	103:9:1	126:9:1

with water temperature. All other measured variables showed no significant ($p>0.05$) correlation with bacterial variables (Table 4). During summer (July-August), bacterial variables were most strongly correlated to phosphorus variables. Bacterial variables were significantly negatively correlated to the TN:TP ratio whereas carbon variables showed no significant correlation with bacterial variables (Table 4). During autumn (September-November), bacterial production and specific activity were strongly correlated with nitrogen variables ($r>0.41$, $p<0.05$) and temperature ($r>0.43$, $p<0.01$), and bacterial biomass and abundance were strongly correlated with certain nitrogen variables (PON, TN) ($r>0.32$, $p<0.05$) and phytoplankton biovolume and chlorophyll a ($r>0.39$, $p<0.05$). Phosphorus variables showed no significant correlation ($p>0.05$) with bacterial variables (Table 4).

Nutrient and River Water Enrichment Experiment. During the May experiment, river water and combinations of DOC, NH_4^+, and PO_4^{-3} enrichments increased both bacterial thymidine uptake and cell numbers (Figure 9A, 10A). All nutrient (singly and in combinations) and river water enrichments stimulated bacterial thymidine uptake and cell numbers during the July experiment (Figure 9B, 10B). PO_4^{-3} enrichments did not stimulate bacterial thymidine uptake or cell number during the October experiment (Figure 9C, 10C).

Nutrient Differences Between River Inflow and Lake Water

The annual mean concentration of phosphorus in lake

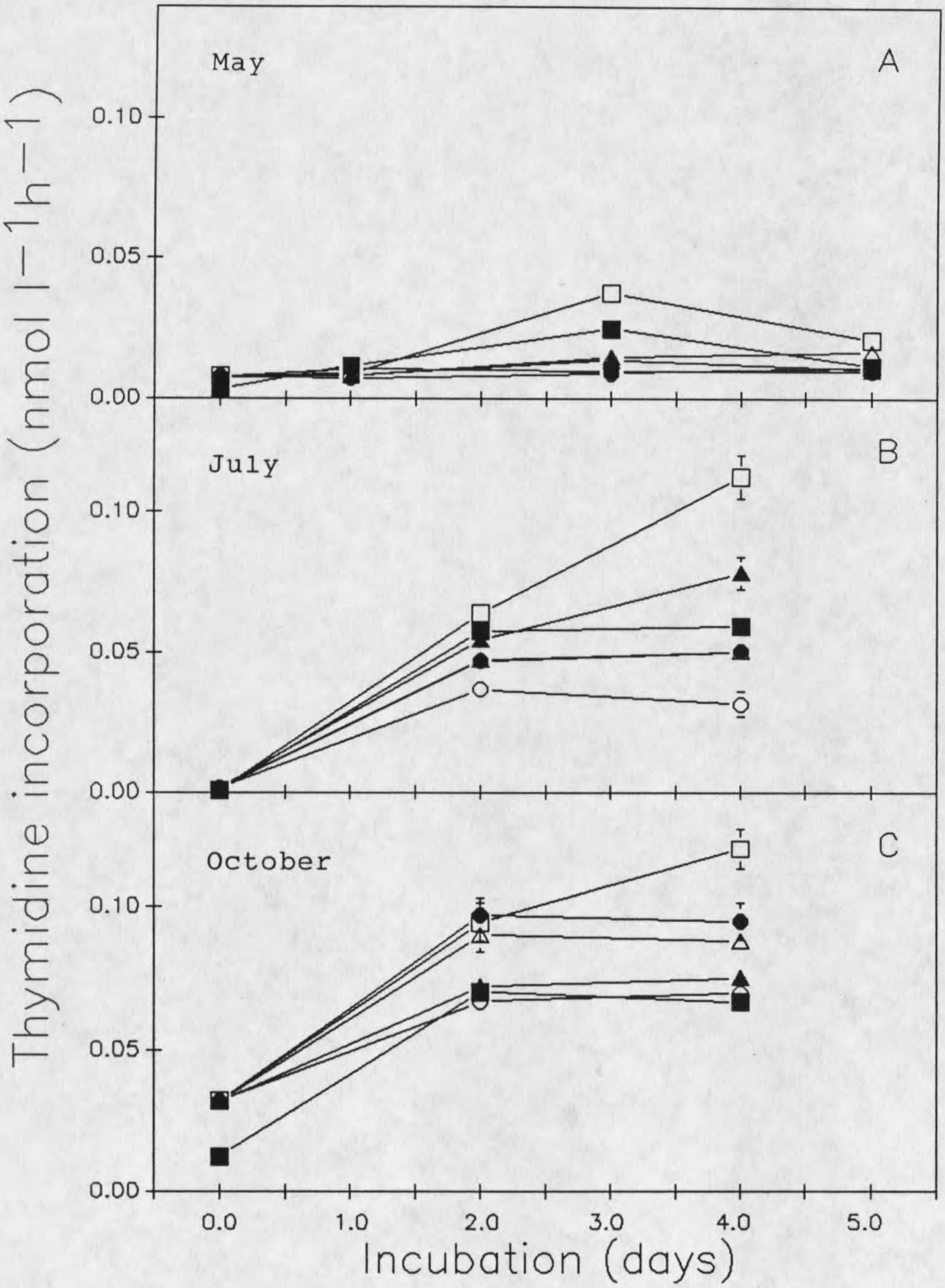

Figure 9. Bacterioplankton ^{3}H-thymidine incorporation in nutrient enriched lake water. In A and B, ○ +control, ● +C, △ +C+N, ▲ +C+P, □ +C+N+P, ■ +50 river water. In C, ○ control, ● +C, △ +N, ▲ +P, □ +N+P, ■ +50% river water.

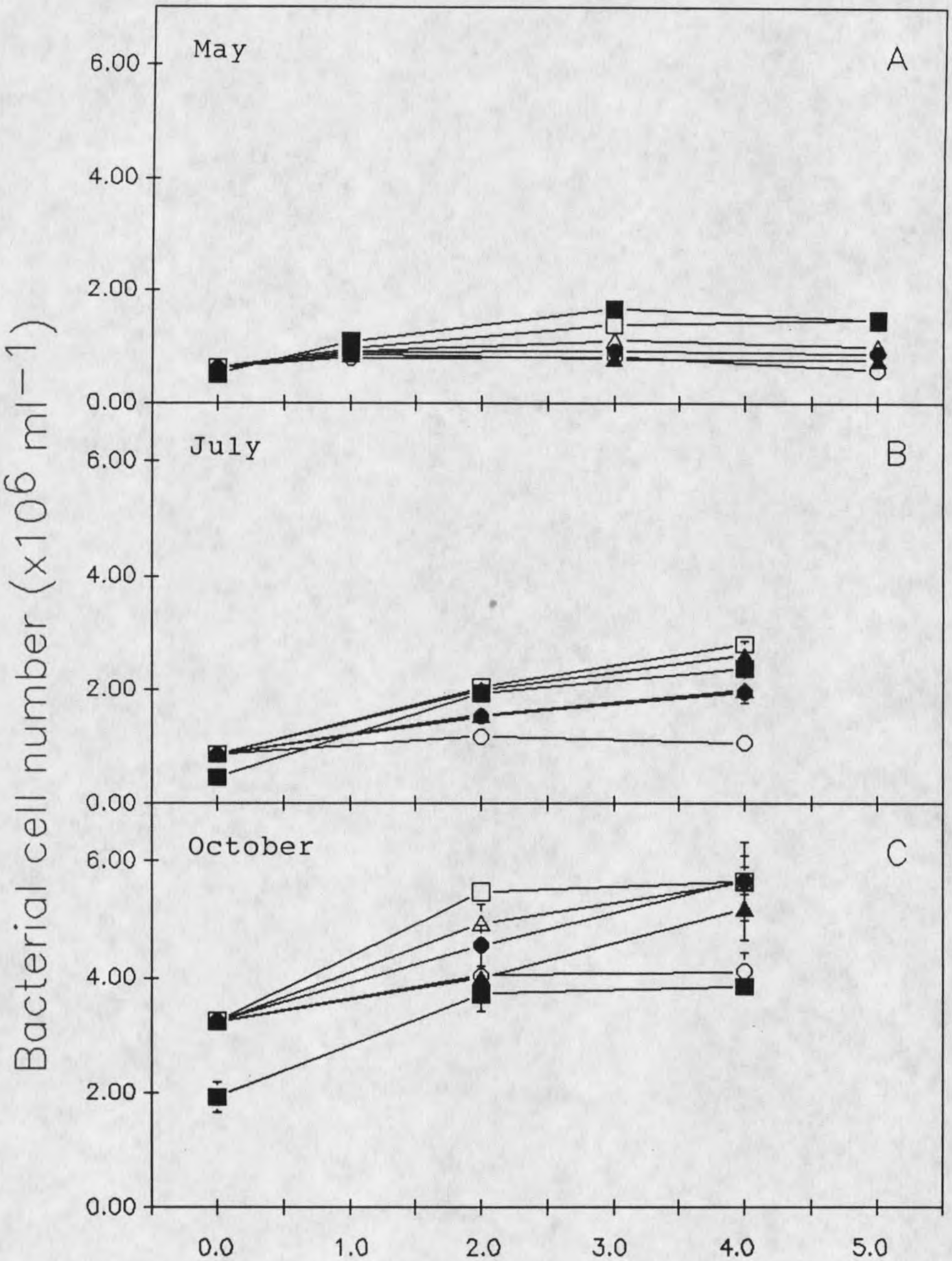

Figure 10. Bacterioplankton cell number in nutrient enriched lake water. In A and B, ○ control, ● +C, △ +C+N, ▲ +C+P, □ +C+N+P, ■ 50% river water. In C, ○ control, ● +C, △ +N, ▲ +P, □ +N+P, ■ 50% river water.

Table 4. Linear correlations of bacterial variables against various biotic and abiotic variables for different seasons in Hebgen Lake. Symbols are as in Table 2. NS not significant at $p>0.05$; r values are listed only when they are significant at $p<0.05$. All data were transformed (ln) before analysis.

	May-Jun (N=32)				Jul-Aug (N=34)				Sep-Oct (N=38)			
Parameter	BP	BAPC	BB	BA	BP	BAPC	BB	BA	BP	BAPC	BB	BA
Temp	0.48	NS	0.35	0.38	NS	NS	NS	NS	0.43	0.40	NS	NS
CHL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.47	0.43
PHYB	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.45	0.39
PPR	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SRP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TDP	NS	NS	NS	NS	0.38	NS	0.44	0.44	NS	NS	NS	NS
DOP	NS	NS	NS	NS	0.39	NS	0.45	0.47	NS	NS	NS	NS
PP	NS	NS	NS	NS	0.34	0.38	NS	NS	NS	NS	NS	NS
NH_4^+-N	NS	NS	NS	NS	0.34	0.42	NS	NS	NS	NS	-0.36	NS
NO_3^--N	NS	NS	NS	NS	NS	NS	0.39	NS	0.41	0.42	NS	NS
DON	NS	NS	NS	NS	NS	NS	NS	NS	0.54	0.51	NS	NS
TDN	NS	NS	NS	NS	NS	0.38	NS	NS	0.53	0.57	NS	NS
PON	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.45	0.34
DOC	NS	NS	NS	NS	NS	NS	NS	NS	-0.49	-0.53	NS	NS
POC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.43	0.40
TP	NS	NS	NS	NS	0.41	NS	NS	NS	NS	NS	NS	NS
TN	NS	NS	NS	NS	NS	NS	-0.40	-0.35	0.51	0.41	0.41	0.32
TOC	NS	NS	NS	NS	NS	NS	NS	NS	-0.43	-0.51	NS	NS
TN:TP	NS	NS	NS	NS	-0.32	NS	-0.34	-0.34	0.48	0.43	NS	NS
TOC:TN	NS	NS	NS	NS	NS	NS	NS	NS	-0.66	-0.68	NS	NS
TOC:TP	NS	NS	NS	NS	-0.30	NS	NS	NS	-0.56	-0.60	NS	NS

water was essentially the same as river water. However, the concentrations of nitrogen and organic carbon in lake water were consistently higher than the river water. The ratio of TOC:TN:TP (by weight) was 147.5:8.0:1 in lake water and 80.6:3.7:1 in river water (Table 5).

Discussion

Bacterioplankton Variables

The mean epilimnetic bacterial production (0.28 μg C l^{-1} h^{-1}, range 0.09-0.82) of Hebgen Lake is low compared with that for the freshwater and saltwater systems (average 1.1, range 0.02-6.38 μg l^{-1} h^{-1}) summarized by Cole et al. (1988). The average epilimnion bacterial biomass and abundance (41.49 μg C l^{-1}; 1.86 x 10^9 cell l^{-1}) are also low compared with other eutrophic lake data of 80-130 μg C l^{-1} (Riemann et al. 1982) and 3.9-31 X 10^9 cells l^{-1} (Gebre-Mariam and Taylor 1989; Marvalin et al. 1989; Robarts and Wicks 1990). Bacterioplankton production, as a percent of phytoplankton production, in Hebgen lake (2%) is much lower than the reported mean value (20%) from a range of aquatic systems (Cole et al. 1988) although it is within the range reported by Robarts and Wicks (1990) of 2% for Hartbeesport Dam, a hypertrophic reservoir in Africa.

Relation Between Bacterioplankton And Phytoplankton

The strong correlation ($r>0.23$, $p<0.05$) between bacterial variables and chlorophyll a over the entire study period

Table 5. The mean (± 1 SE) nutrient concentrations ($\mu g\ l^{-1}$) of the epilimnetic water column and river inflows in Hebgen Lake during 1988 and 1989. Symbols are the same as in Table 2.

Nutrient	River inflow	Epilimnetic lake water
SRP	10.8±1.9	9.4±2.7
TDP	23.9±2.9	29.0±4.6
PP	15.1±4.3	15.9±3.0
NH_4^+-N	9.6±3.1	11.6±4.0
NO_3^--N	14.2±3.7	27.2±10.3
TDN	109.0±11.0	200.9±19.9
PON	35.9±6.5	157.4±38.1
DOC	2515 ±475	5490 ±364
POC	626 ±79	1140 ±165
TP	39.0±5.2	44.9±5.5
TN	144.4±12.8	358.3±43.0
TOC	3141 ±482	6630 ±399
TOC:TN:TP (weight)	80.6:3.7:1	147.5:8.0:1

implies a close coupling between bacterioplankton and phytoplankton biomass in the epilimnion of Hebgen Lake. The lack of a significant correlation between bacterial variables and phytoplankton production may be due to the variable effect of underwater irradiation, the regression was done on discrete samples down the water column which does not take light attenuation into effect. However, the non-significant correlation between bacterial and all phytoplankton variables during spring and summer in concert with a significant correlation during the fall indicates that epilimnetic bacterial production is affected by phytoplankton during only part of the ice-free season. Positive correlations between bacterial and phytoplankton variables have been reported for a number of other marine and freshwater environments (Fuhrman et al. 1980; Chrzanowski and Hubbard 1989; Marvalin et al. 1989; Robarts and Wicks 1990). Lack of correlation between bacterial and phytoplankton variables were also found in some marine environments in which water temperature was strongly correlated with bacterial variables (Coffin and Sharp 1987; Joint and Pomroy 1987). Our findings support these latter results in that bacterial variables were significantly ($p<0.05$) correlated with temperature but not phytoplankton variables during the spring.

Factors Regulating Bacterioplankton

Entire Study Period.

That bacterial biomass and

abundance were highly correlated with organic phosphorus and particulate organic nitrogen and carbon (Table 2.2) infers that these are the main substrates regulating bacterioplankton in Hebgen Lake. The strong correlation between these organic nutrients and phytoplankton variables (Table 2) further implies that phytoplankton are an important source of such nutrients. The negative correlations between bacterial production and specific activity with SRP and DOC imply that high bacterial activity can effectively reduce these nutrients. Therefore, high concentrations of essential nutrients do not necessarily indicate high bacterial production. The correlations between bacterial variables and TOC, TN, and TP suggest that they are important bacterial substrates. When these three nutrients are compared, TN and TP appear to be the most deficient forms. This is supported by the strong negative correlations between bacterial production or specific activity and ratios of TOC:TN and TOC:TP.

<u>Seasonal</u> <u>Difference</u>. Our correlations infer that bacterioplankton in Hebgen Lake are most closely associated with water temperature during spring, with phosphorus followed by nitrogen during summer, and nitrogen followed by organic carbon during fall. This conclusion is supported by our laboratory experiments, which showed essentially the same results (Figure 9, 10). Seasonal variation in factors controlling bacterial production and abundance has also been

reported for other aquatic environments (Jonas and Tuttle 1990).

Influence of Nutrient Input from River on Lake Bacterioplankton and Nutrient Composition

External nutrient input from river inflow is an important bacterial nutrient source to Hebgen Lake. This conclusion is supported by all of our river water bioassays which showed increased bacterial thymidine incorporation and bacterial abundance with the addition of river water (Figure 9, 10).

The lower ratio of TOC:TN:TP in river water relative to lake water (Table 5) indicates that lake water has relatively more organic carbon. It has been reported that bacteria are phosphorus limited when the C:P ratio is 8.3-58.8 by weight (Vadstein and Olsen 1989). Reported bacterial C:N and N:P weight ratios are 2.3-8.3 and 4.7-8.7 (Linley and Newell 1984; Vadstein and Olsen 1989). Hebgen Lake water had lower proportions of nitrogen and phosphorus compared with organic carbon (TOC:TN=19:1; TOC:TP=148.1) than the reported ratios. However, the ratio of TN:TP (8:1) of the lake water is in the range of the reported values. Difference in these ratios can be used to explain why river water enrichments to Hebgen Lake water stimulated bacterial activity and abundance in our laboratory experiments.

CHAPTER 3

HETEROTROPHIC BACTERIOPLANKTON NUTRIENT DEFICIENCY

Introduction

Heterotrophic bacteria are largely responsible for the degradation of organic matter and regeneration of minerals in aquatic ecosystems. It has been suggested that bacterial production is controlled by, or is directly related to, the supply of decomposable organic matter (Cole et al. 1988). Correlations between mean bacterioplankton production and chlorophyll a concentration or photosynthesis (e.g. Fuhrman et al. 1980; Bird and Kalff 1984; Bjørnsen et al. 1989; Marvalin et al. 1989; Roberts and Wicks 1990) suggest that phytoplankton products may be an important source of substrate for bacterial growth. However, bacterioplankton effectively compete with phytoplankton for inorganic nitrogen and phosphorus in both oligotrophic and eutrophic systems (Priscu and Downes 1985; Vadstein et al. 1988; Vadstein and Olsen 1989). These findings indicate that inorganic nutrients may be as important as organic nutrients in regulating bacterioplankton growth in aquatic systems. Although direct stimulation of bacterioplankton growth by inorganic nutrient enrichments has been demonstrated in laboratory studies (e.g. Horrigan et al. 1988), nutrient stimulation of bacterioplankton activity in natural environments is generally indirect, transmitted via phytoplankton (Riemann and Søndergaard 1986; Bjørnsen et al. 1989).

The purpose of our study was to examine the response of bacterioplankton and phytoplankton to experimental manipulation of dissolved organic carbon (DOC), inorganic nitrogen and inorganic phosphorus in a eutrophic lake. The following basic questions were addressed: Can inorganic nutrient enrichment stimulate bacterioplankton activity? If so, is this stimulation direct or indirect (resulting from stimulation of phytoplankton)?

Materials and Methods

Study Site

Experiments were conducted in the Grayling Arm of Hebgen Lake, a eutrophic reservoir, located on the upper Madison River, Montana. The Grayling Arm is one of the three bays of Hebgen Lake with its own water inlet and a narrow connection with the main lake. The Grayling Arm had an area of 8 km^2 and a maximum depth of 6 m at the time of our experiments. Phytoplankton in this portion of the lake were dominated by cyanobacteria over most of the ice-free season. A detailed physical, chemical and biological description of Hebgen lake is given by Miller (1991).

Experimental Procedures

Bioassay experiments were conducted during June, August, and October of 1988 and 1989, each extending up to 5 days.

Water from 0.5 m depth was placed in 20-L polyethylene collapsible carboys attached to floats anchored at 0.5 m near the deepest part of the Grayling Arm. For both October experiments, containers were incubated in the laboratory at an irradiance of 150 μE m^{-2} s^{-1}, provided by cool white fluorescent lamps on a 12:12 h light:dark cycle, at the temperature of collection.

Water for our experiments was filtered with 280 μm pore size Nitex mesh to remove large grazers except during October 1988, and August and October 1989 when large cyanobacteria filaments and aggregates were present. Three nutrient treatments were used in 1988 and six treatments in 1989 (Table 6). Unamended controls were included with each experiment. Nutrients were added at time zero during the 1988 experiments and daily during the 1989 experiments (see Table 6 for schedule). This continuous addition of nutrients during 1989 was adopted to ensure that nutrient depletion did not occur and to simulate more closely advected nutrient addition which dominates the nutrient budget in Hebgen Lake. Water chemistry, $^{14}CO_2$ uptake rate, chlorophyll _a_ concentration, ^{3}H-thymidine incorporation rate, and bacterial cell concentration were determined on each day of the experiment. Bottles for $^{14}CO_2$ uptake (3 light and 1 dark) were incubated in the lake alongside each cubitainer; ^{3}H-thymidine incorporation samples were incubated in the dark at _in situ_ temperatures.

Table 6. Nutrient additions ($\mu g\ L^{-1}$ for NH_4^+ and PO_4^{-3}, mg L^{-1} for mannitol) for experiments in the Grayling Arm of Hebgen Lake. See Table 2 for ambient concentrations before each addition. NA = not applicable. Nutrients were added on day 0 only during 1988 experiments; nutrients were added every sampling interval during 1989 experiments except mannitol which was added on day 0 and day 3 only.

	Control	NH_4^+-N	PO_4^{-3}-P	NH_4^+-N & PO_4^{-3}-P	Mannitol	NH_4^+-N & Mannitol	PO_4^{-3}-P & Mannitol
1988 Experiments	0	100	50	NA NA	91	NA NA	NA NA
1989 Experiments	0	140	93	140 93	91	140 91	93 91

Water Chemistry.

Water was filtered through Whatman GF/C filters and frozen for later analysis of the following "dissolved" constituents. Concentrations of NO_3^- and NH_4^+ were determined by Cd reduction (Eppley 1978) and the phenol hypochlorite method (Solorzano 1969), respectively. Soluble reactive phosphorus (SRP) was measured using the molybdate method modified for AsO_4^- interference (Downes 1978). Total dissolved phosphorus (TDP) was measured according to Stainton et al. (1977) after acid hydrolysis (Solorzano and Sharp 1980). Dissolved organic phosphorus (DOP) was estimated from the difference between TDP and SRP. Total dissolved nitrogen (TDN) was determined as NO_3^- using the Cd reduction method (as above) following persulfate digestion (D'Elia et al. 1977) . Dissolved organic nitrogen (DON) was estimated by subtracting the sum of NO_3^- and NH_4^+ from TDN. Dissolved organic carbon (DOC) was analyzed with a Dorhmann Carbon Analyzer standardized with glucose.

Particulate Matter Analysis.

Particulate material was collected on precombusted Whatman GF/C filters and frozen for later analysis. Chlorophyll a was determined by fluorometry on 90% acetone extracts. Fluorescence of samples before and after acidification was measured with a Turner model 112 fluorometer standardized with pure chlorophyll a standard (Strickland & Parsons 1972). Particulate organic carbon (PC) and particulate organic nitrogen (PN) concentrations were measured with a Carlo-Erba model 1106 elemental analyzer calibrated with standard amounts of acetanilide. Particulate phosphorus (PP) concentration was determined using the same methods described for TDP. Total organic carbon (TOC) is the sum of DOC and PC; total nitrogen (TN) is the sum of TDN and PN, and total phosphorus (TP) is the sum of TDP and PP.

Bacterial cell concentrations were determined on samples filtered onto 0.2 μm polycarbonate filters, stained with acridine orange, and counted using epifluorescence microscopy (Hobbie et al. 1977).

Biological Rate Measurements

Photosynthetic rate was determined in 150 ml borosilicate bottles by adding $^{14}C\text{-}NaHCO_3$ to a final activity of ca. 0.05 μCi ml^{-1}. Samples were incubated for 4 h (near midday when *in situ*). Uptake was terminated by filtration through Whatman GF/C filters. After acidification and drying, 8 ml of Scintiverse E (Fisher) was added to the filter in a 20-ml scintillation vial and counted with a Beckman LS-100C scintillation spectrometer. Efficiency was computed by the external standard, channels ratio. Dissolved inorganic carbon, required for photosynthetic rate calculation, was computed from alkalinity titrations during each sampling interval.

Thymidine incorporation was determined by adding methyl-^{3}H thymidine (ICN Radiochemical Inc.) to 10 ml lake water in a 20 ml glass scintillation vial yielding a final concentration of 10 nM thymidine. The ^{3}H-thymidine stock (in 70% ethanol) was evaporated to dryness and rehydrated with deionized water before use to eliminate volatile products of self radiolysis and to remove ethanol. The inoculated sample was incubated at in situ temperature in the dark for 20 to 30 minutes. Activity was terminated by adding 10 ml of 10% ice-cold trichloroacetic acid (TCA) to each vial. The sample was then extracted overnight at 4 °C followed by filtration onto 0.2 μm polycarbonate filters which were rinsed 5 times with 2 ml each of ice-cold 5% TCA (Fuhrman and Azam 1980). Radioactivity was determined by liquid scintillation counting as described above. We use the term "incorporation" to denote the activity remaining in the TCA insoluble fraction. Microautoradiography showed that ^{3}H-thymidine was incorporated exclusively by the bacteria; no activity was noted in cyanobacteria or eukaryotic algal fractions (H.W. Paerl, unpublished data).

Statistical Analysis

To determine the effects of nutrient enrichments, bacterioplankton thymidine incorporation and cell number, phytoplankton $^{14}CO_2$ uptake and chlorophyll a from each treatment were compared with controls by multifactor analysis of variance (Neter et al. 1985). The comparisons were done on triplicate subsamples from each treatment for all time-course data. To overcome potential grazing influences on bacterioplankton abundance, bacterioplankton specific activity (thymidine incorporation/cell number) for each treatment was also compared with the control using the same statistical method. Simple linear regression analysis was conducted to test relationships between bacterioplankton and phytoplankton in each treatment of each experiment.

Results

Initial Conditions of the Experiments

Initial conditions for all six experiments are presented in Table 7. The dominant phytoplankton, in percent of total biovolume were *Anabaena spiroides* (73%) in June 1988, *A. circinalis* (92%) in August 1988, *Aphanizomenon flos-aquae.* (52%) and *Asterionella sp.* (35%) in October 1988, *A. circinalis* (64%) in June 1989, *Fragilaria sp.* (47%) and *Aphanizomenon flos-aquae* (34%) in August 1989, and *Aphanizomenon flos-aquae* (97%) in October 1989. The maximum chlorophyll *a* concentration occurred in October of both years. Physical and chemical conditions varied considerably among experiments providing a unique environmental setting for each experiment.

Nutrient Responses of Bacterioplankton and Phytoplankton

June 1988 Experiment. PO_4^{-3} addition significantly ($p<0.01$) increased both bacterioplankton and phytoplankton production. The maximum rates for thymidine incorporation and $^{14}CO_2$ uptake were 1.6 and 1.2 times higher than those of

Table 7. Initial temperature (°C), chlorophyll a (Chl a) (μg l^{-1}) and nutrient levels (μg l^{-1}) for the microcosm experiments in the Grayling Arm of Hebgen Lake. Symbols are defined in "Material and Methods", Phytoplankton refers to the dominant genera representing more than 60% of the biovolume. Ana = *Anabaena*, Aph = *Aphanizomenon*, Ast = *Asterionella*, Fra = *Fragilaria*.

	1988			1989		
Nutrient	Jun 22	Aug 21	Oct 23	Jun 20	Aug 8	Oct 19
Temperature	17	22	12	16	19	10
Chl a	30.3	15.6	144.5	4.5	5.0	48.5
Phyto-plankton	Ana.	Ana.	Aph. Ast.	Ana.	Aph. Fra.	Aph.
NH_4^+-N	3.8	61.1	12.5	9.9	4.2	6.0
NO_3^--N	2.6	64.0	5.6	10.2	7.9	94.1
TDN	140.0	570.0	390.0	176.0	146.0	255.0
DON	133.7	444.9	371.9	155.9	133.9	155.0
PN	514.8	299.1	1192.2	137.2	74.2	244.9
SRP	2.8	39.1	7.7	18.1	5.1	18.1
TDP	10.4	57.1	22.5	28.0	17.8	38.4
DOP	8.2	18.0	14.8	9.9	12.7	20.2
PP	14.7[a]	166.8[a]	72.1[a]	16.9	9.9	45.7
DOC	2834	7012	7749	2839	6960	6552
POC	3416	2288	7751	761	559	1364
TN:TP	26	4	17	9	8	6
TOC:TN	10	11	10	9	34	16
TOC:TP	249	42	164	80	271	94

a. Data were collected from the surface of lake water within 10 days of the experiment.

the control, respectively. Maximum thymidine incorporation occurred 1 day before that of maximum $^{14}CO_2$ uptake. NH_4^+ addition reduced thymidine incorporation ($p<0.01$) and did not have a significant effect on $^{14}CO_2$ uptake (Figures 11, 12; Table 8). PO_4^{-3} addition increased bacterioplankton cell concentration ($p<0.01$) and chlorophyll a concentration ($p<0.01$); other treatments induced no significant effects on bacterial cell concentration compared with the control (Figures 11, 12; Table 8). None of the treatments had a significant effect on bacterioplankton specific activity.

August 1988 Experiment. Mannitol addition significantly ($p<0.05$) stimulated bacterioplankton ^{3}H-thymidine incorporation and specific activity. NH_4^+ addition increased ($p<0.01$), and mannitol addition decreased ($p<0.01$), phytoplankton $^{14}CO_2$ uptake. NH_4^+ and PO_4^{-3} additions significantly ($p<0.01$) increased bacterial cell concentration (Figures 13, 14; Table 8).

October 1988 Experiment. Mannitol addition elevated both ^{3}H-thymidine incorporation and bacterial cell concentration ($p<0.01$) but not specific activity. PO_4^{-3} addition decreased ^{3}H-thymidine incorporation but showed no significant effect on bacterial cell concentration and specific activity. NH_4^+ addition enhanced ($p<0.01$) $^{14}CO_2$ uptake but none of the bacterioplankton parameters. $^{14}CO_2$ uptake decreased significantly ($p<0.01$) with mannitol addition. (Figures 15, 16; Table 8).

Table 8. Effect of nutrients on bacterioplankton and phytoplankton in the microcosm experiments conducted in the Grayling Arm of Hebgen Lake. Significance refers to comparison with unamended controls by multi-factor Analysis of Variance. NS = not significant at $p>0.05$, + and - = significant increase and decrease at $p<0.01$, $-^*$ = significant decrease at $p<0.05$, NA = not applicable. SPA = specific bacterioplankton ^{3}H-thymidine incorporation; Thym = bacterioplankton ^{3}H-thymidine incorporation; Cell = bacterial cell concentration; PPR = phytoplankton $^{14}CO_2$ uptake; Chl = chlorophyll a concentration.

Treatment		NH_4^+	PO_4^{-3}	NH_4^+ & PO_4^{-3}	Mannitol	NH_4^+ & mannitol	PO_4^{-3} & mannitol
1988 experiments							
Jun:	SPA	NS	NS	NA	$-^*$	NA	NA
	Thym	-	+	NA	NS	NA	NA
	Cell	N	+	NA	NS	NA	NA
	PPR	NS	+	NA	NS	NA	NA
	Chl	NS	+	NA	NS	NA	NA
Aug:	SPA	NS	NS	NA	+	NA	NA
	Thym	NS	+	NA	+	NA	NA
	Cell	+	+	NA	NS	NA	NA
	PPR	+	NS	NA	-	NA	NA
	Chl	NS	NS	NA	NS	NA	NA
Oct:	SPA	NS	NS	NA	NS	NA	NA
	Thym	NS	-	NA	+	NA	NA
	Cell	NS	NS	NA	+	NA	NA
	PPR	+	NS	NA	-	NA	NA
	Chl	NS	$-^*$	NA	-	NA	NA
1989 experiments							
Jun:	SPA	+	NS	+	+	+	+
	Thym	+	NS	+	+	+	+
	Cell	+	NS	+	+	+	+
	PPR	+	NS	+	NS	+	NS
	Chl	+	NS	+	NS	+	NS
Aug:	SPA	+	+	+	+	+	+
	Thym	+	+	+	+	+	+
	Cell	+	+	+	+	+	+
	PPR	+	+	+	NS	+	NS
	Chl	+	NS	+	NS	+	NS
Oct:	SPA	+	+	+	+	+	+
	Thym	+	+	+	+	+	+
	Cell	NS	NS	+	+	+	+
	PPR	NS	-	NS	NS	NS	$-^*$
	Chl	+	NS	NS	$-^*$	NS	NS

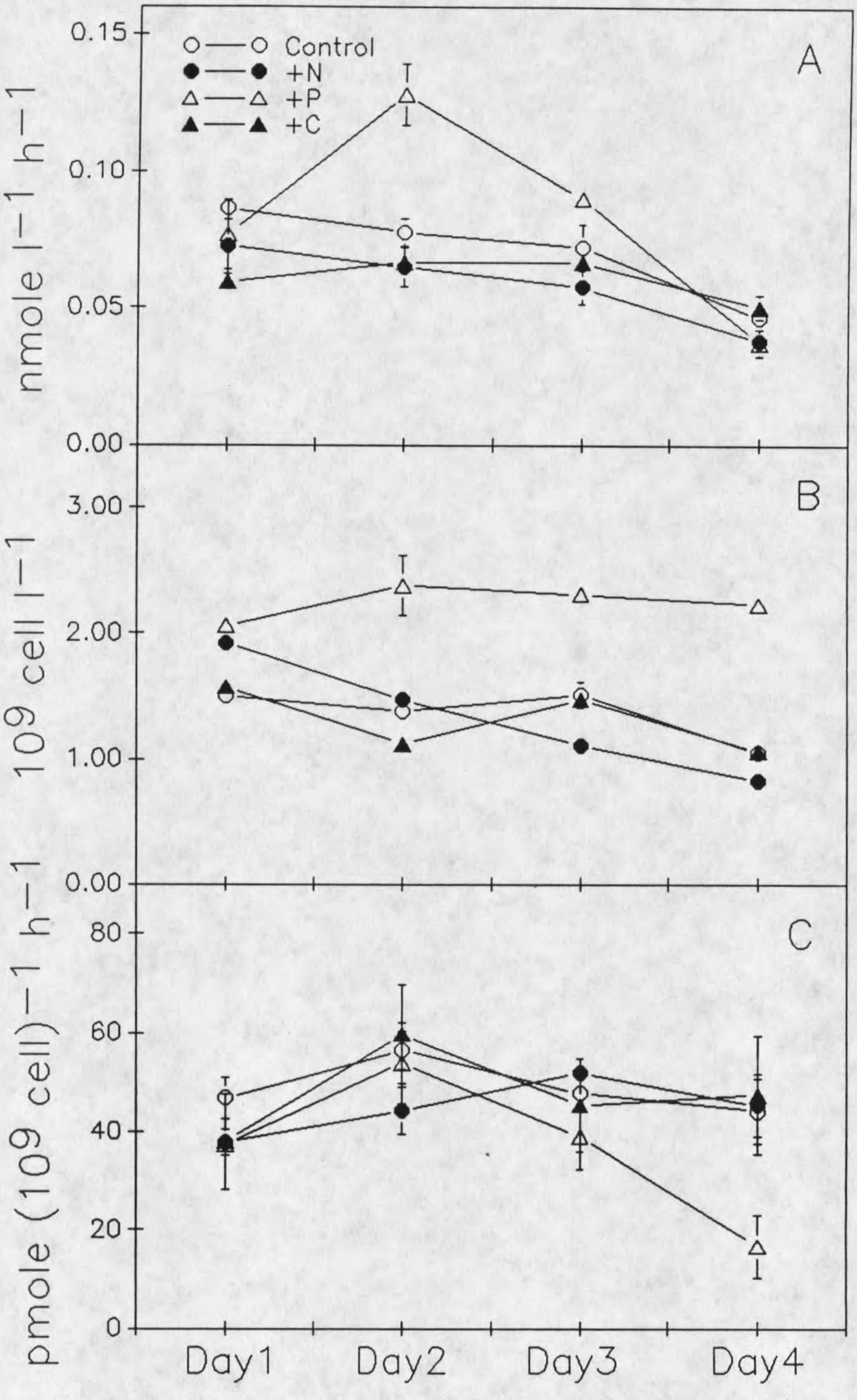

Figure 11. Bacterioplankton thymidine incorporation (A), bacterial cell number (B), and bacterioplankton specific activity (C). The mean of three observations ± 1 SE is presented.

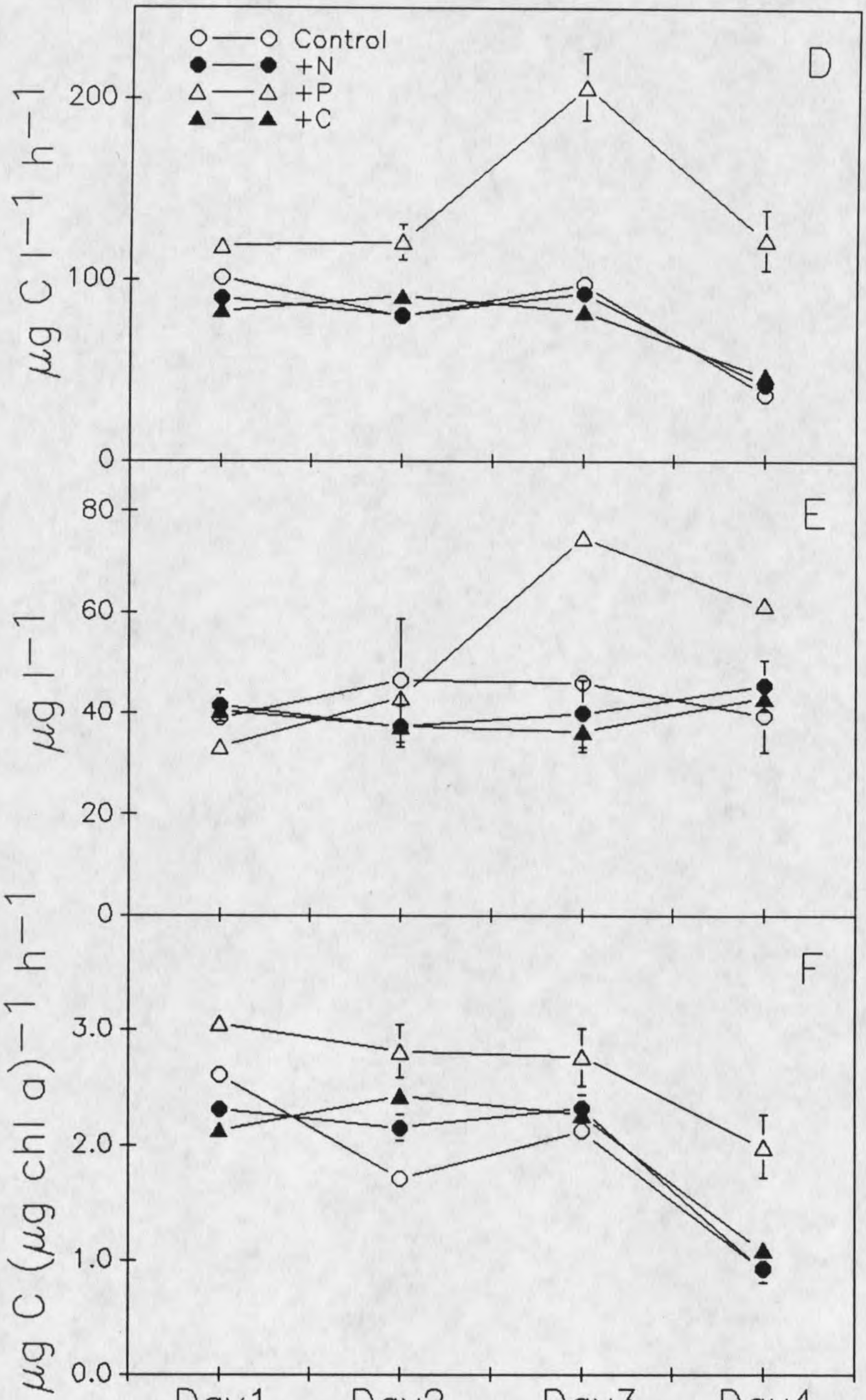

Figure 12. Phytoplankton $^{14}CO_2$ uptake (D), chlorophyll a concentration (E), and chlorophyll specific $^{14}CO_2$ uptake (F) in June 1988 experiment.The mean of three observations ± 1 SE is presented.

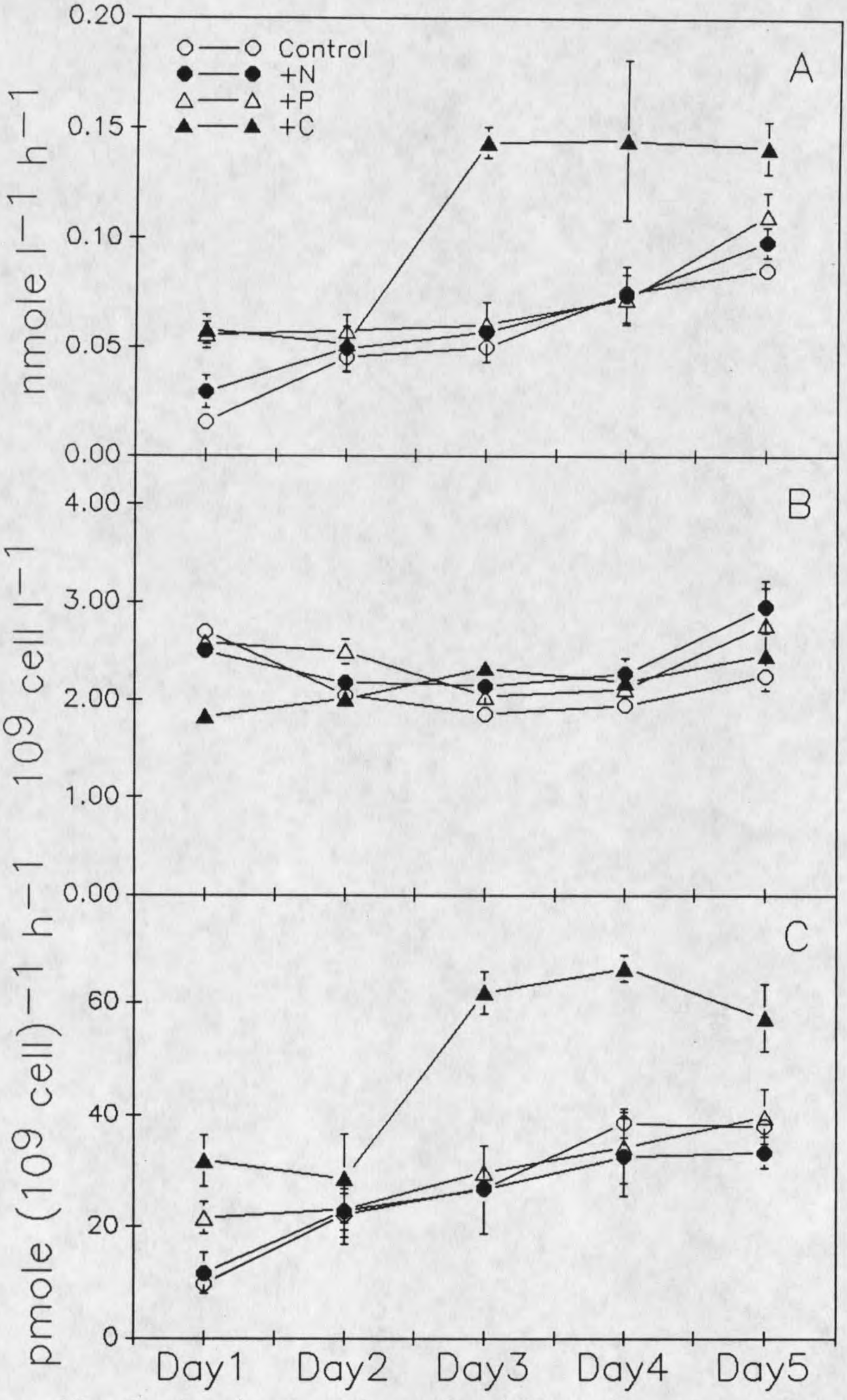

Figure 13. Bacterioplankton thymidine incorporation (A), bacterial cell number (B), bacterioplankton specific activity (C). The mean of three observations ± 1 SE is presented.

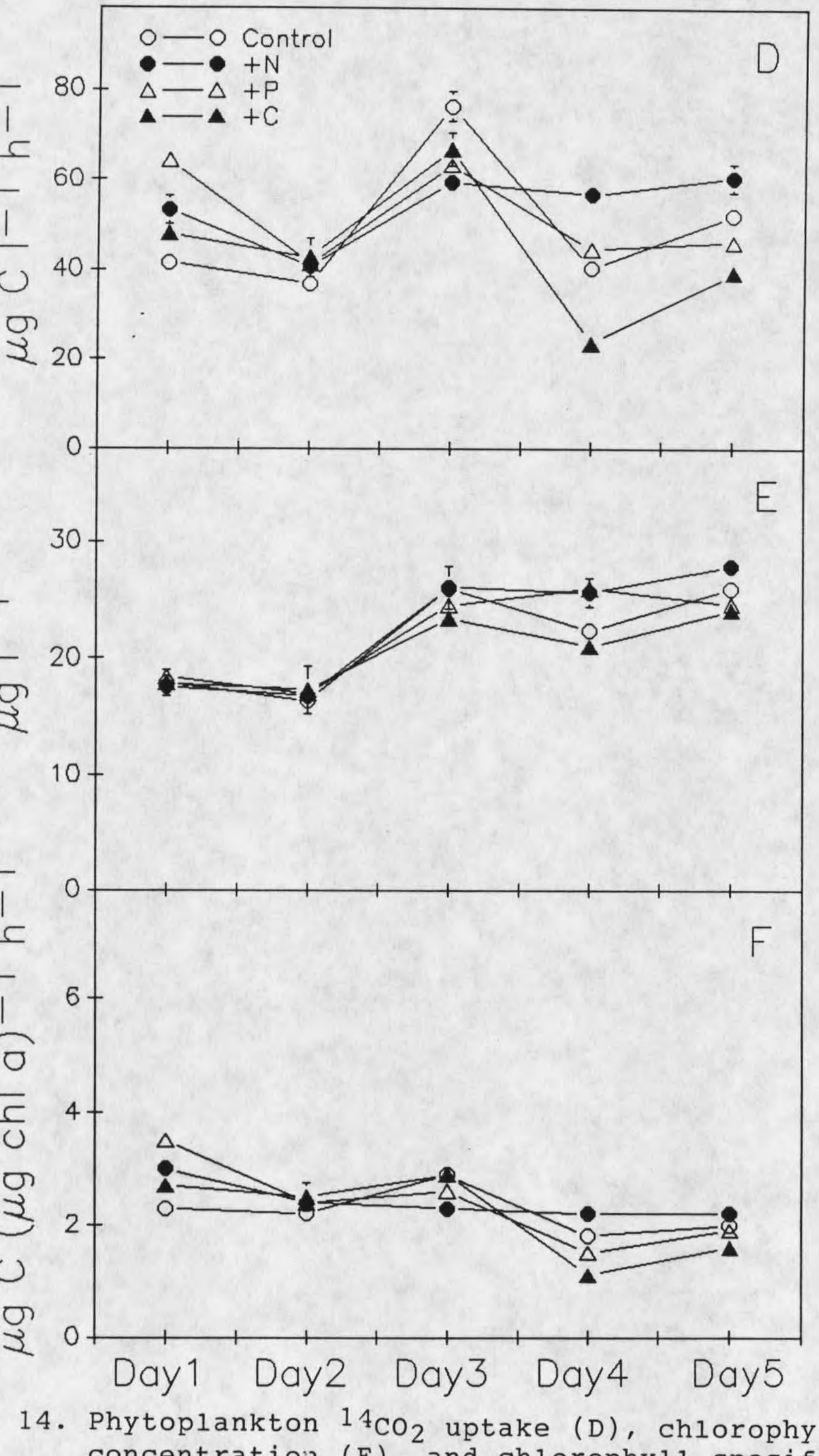

Figure 14. Phytoplankton $^{14}CO_2$ uptake (D), chlorophyll _a_ concentration (E), and chlorophyll specific $^{14}CO_2$ uptake (F) in the August 1988 experiment. Mean of three observations ± 1 SE is presented.

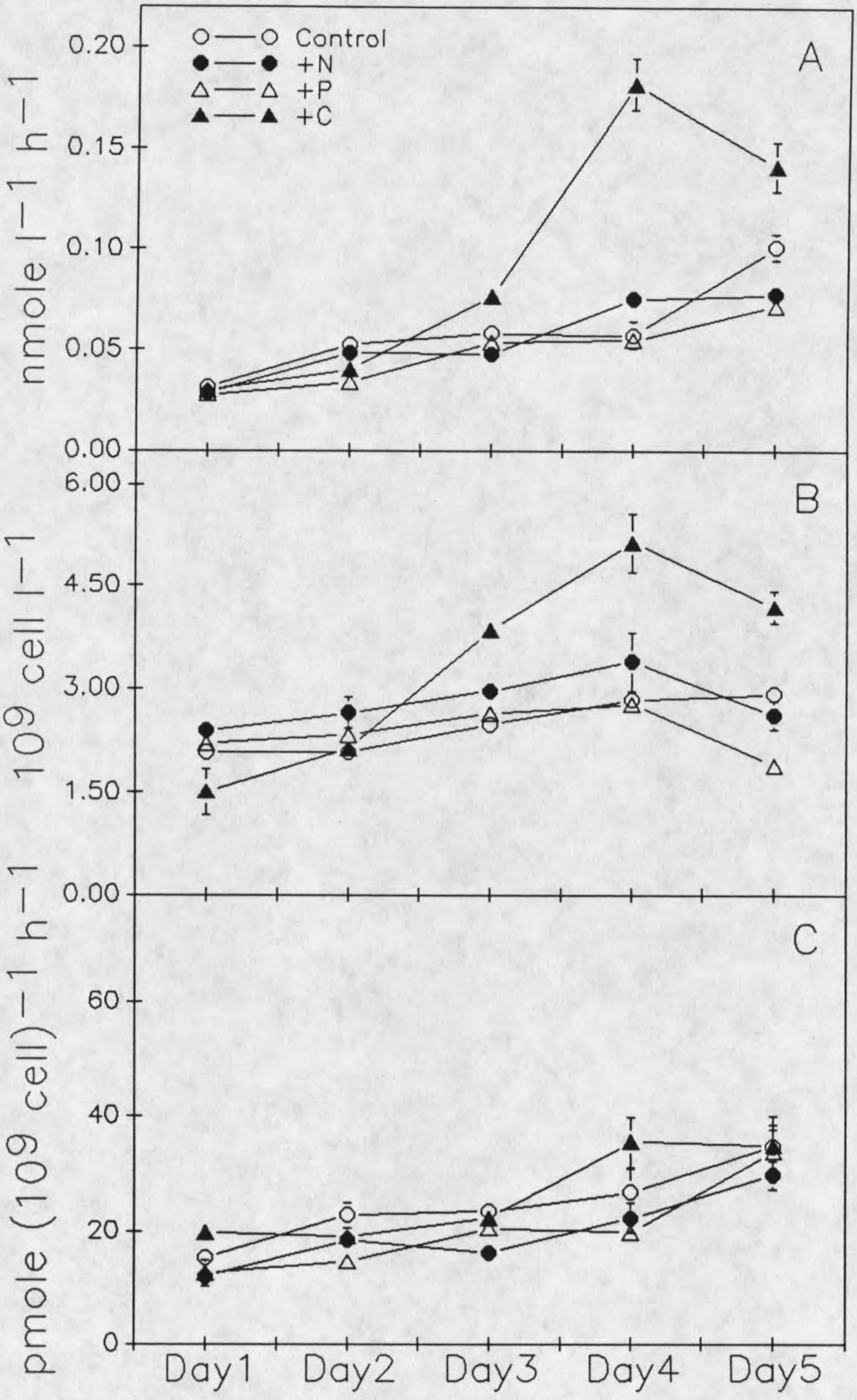

Figure 15. Bacterioplankton thymidine incorporation (A), bacterial cell number (B), bacterioplankton specific activity (C)in the October 1988 experiment. Statistic errors are as in Fig. 14.

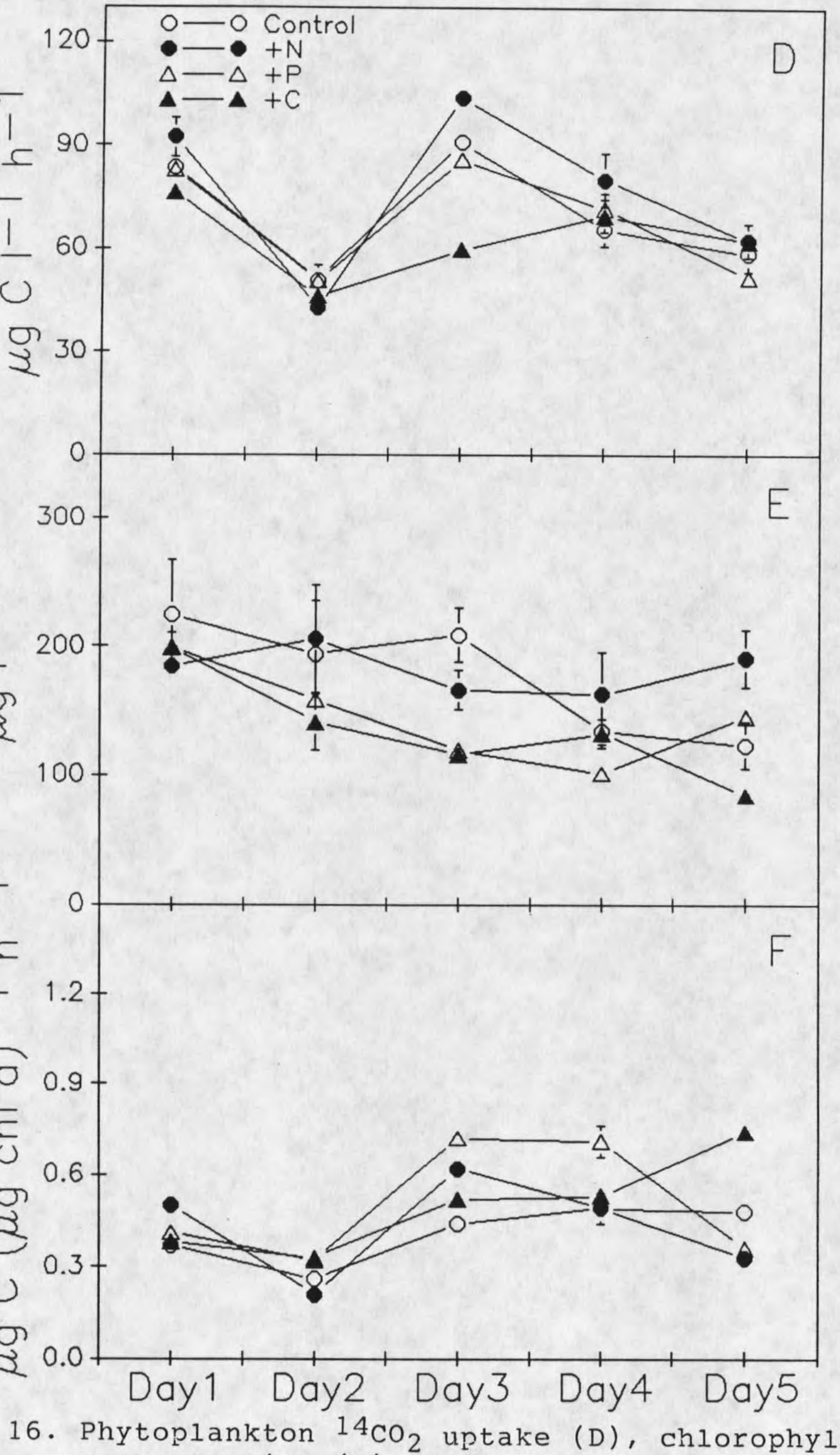

Figure 16. Phytoplankton $^{14}CO_2$ uptake (D), chlorophyll *a* concentration (E), and chlorophyll specific $^{14}CO_2$ uptake (F) in October 1988 experiment. Statistic errors are as in Fig. 14.

June 1989 Experiment. All treatments except PO_4^{-3} increased ^3H-thymidine incorporation, bacterial cell concentration, and specific activity ($p<0.01$). Maximum stimulation of bacterioplankton parameters occurred in the mannitol plus NH_4^+ treatment (Figures 17, 18; Table 8). All treatments containing NH_4^+ significantly ($p<0.01$) increased both $^{14}CO_2$ uptake and chlorophyll a concentration (Figures 17, 18; Table 8).

August 1989 Experiment. All nutrient additions significantly ($p<0.01$) increased ^3H-thymidine incorporation, bacterial cell concentration, and specific activity. Nutrient additions containing NH_4^+ elevated ($P<0.01$) $^{14}CO_2$ uptake and chlorophyll a concentration (Figures 19, 20; Table 8). Bacterioplankton and phytoplankton densities increased to the greatest extent in the treatment containing PO_4^{-3} plus NH_4^+.

October 1989 Experiment. All nutrients, except PO_4^{-3} and NH_4^+, significantly ($p<0.01$) increased ^3H-thymidine incorporation, bacterial cell concentration, and specific activity (Figures 21, 22; Table 8). $^{14}CO_2$ uptake was negatively influenced by PO_4^{-3} ($p<0.01$) and chlorophyll a was positively influenced ($p<0.01$) by NH_4^+ addition (Figures 21, 22; Table 8).

Relationships Between Bacterioplankton and Phytoplankton

Correlations, grouped by treatment and experiment, showed that, in 13 of the 132 cases, the bacterioplankton

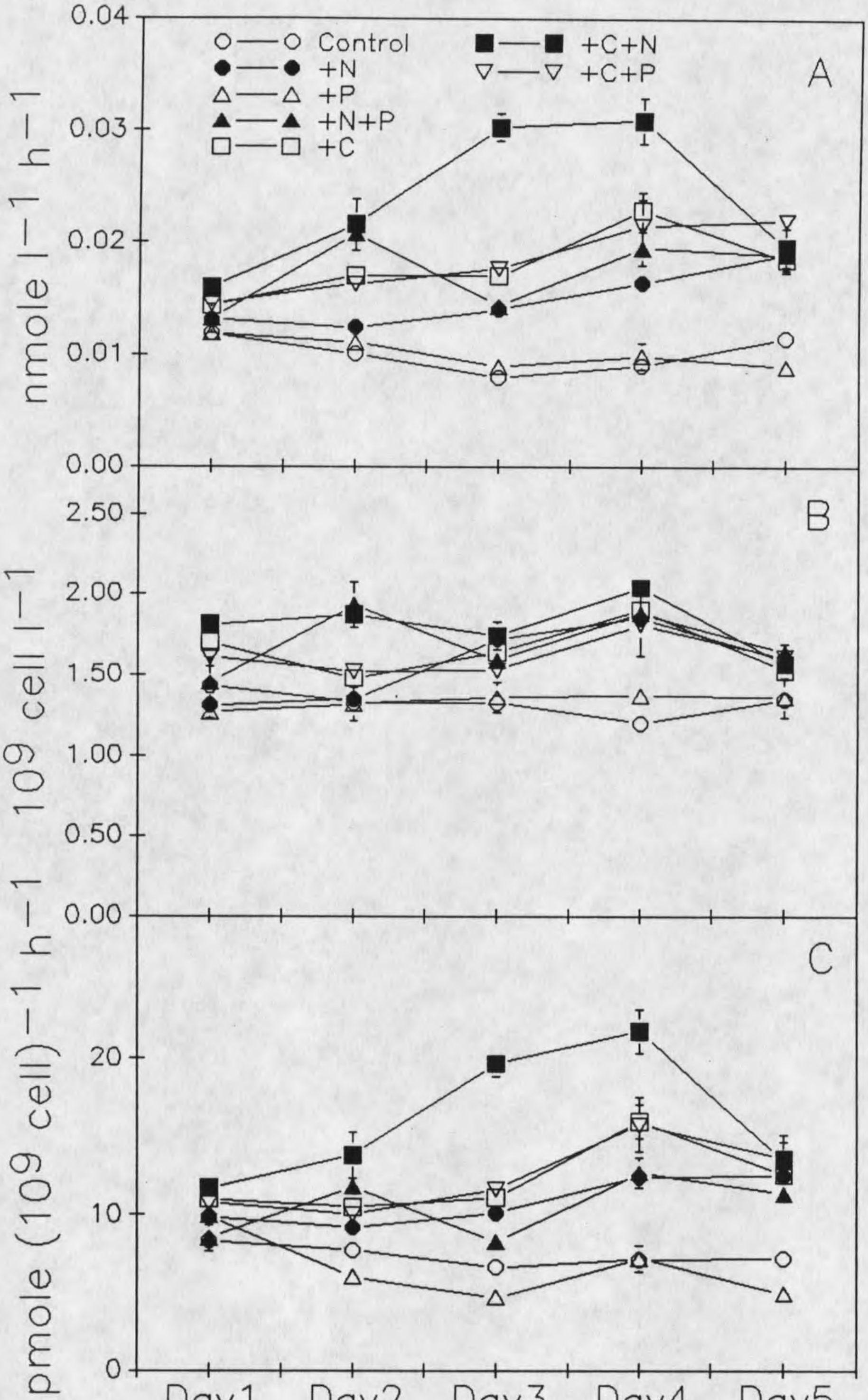

Figure 17. Bacterioplankton thymidine incorporation (A), bacterial cell number (B), bacterioplankton specific activity (C) in June 1989 experiment. Mean of 3 observations ± 1 SE is presented.

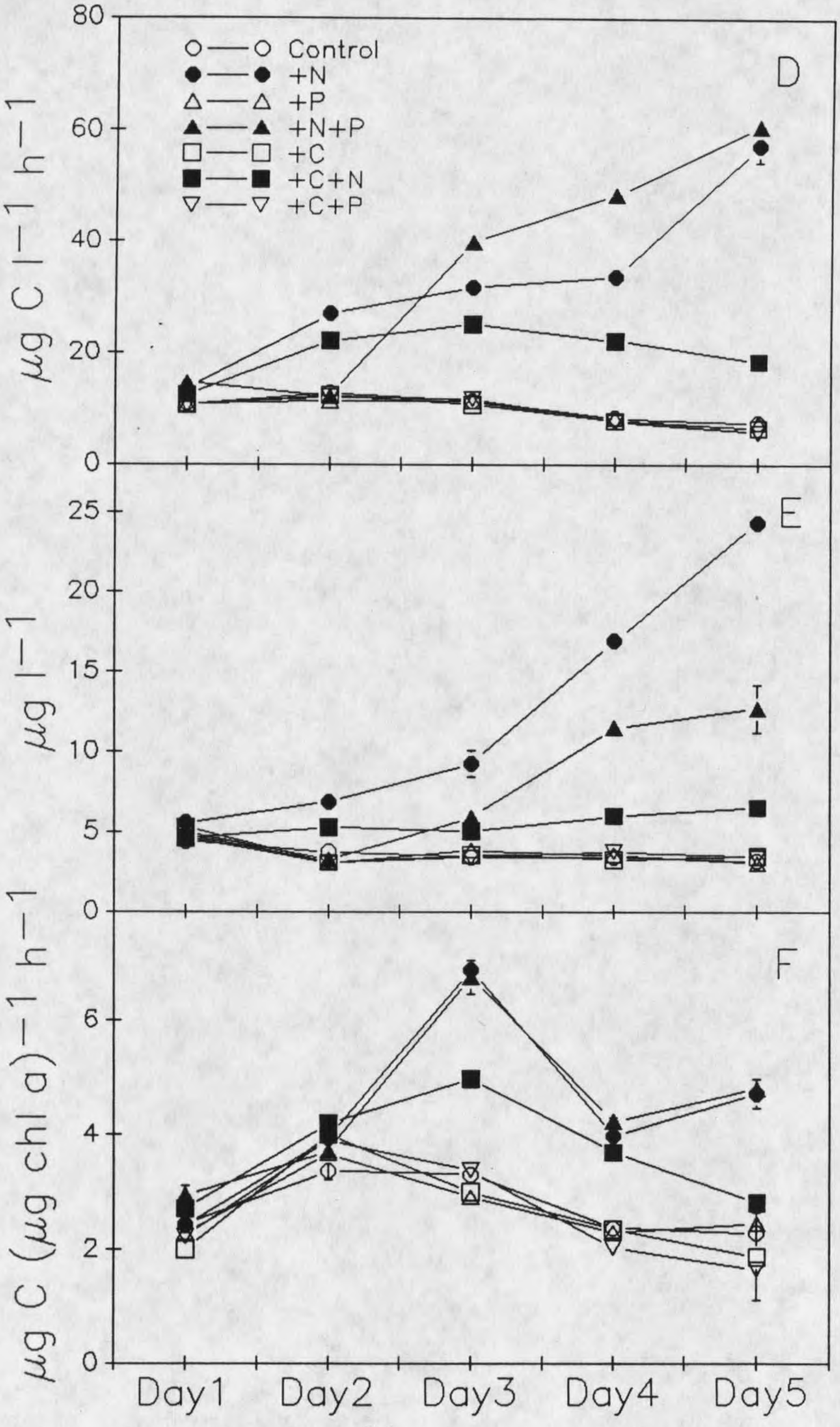

Figure 18. Phytoplankton $^{14}CO_2$ uptake (D), chlorophyll a concentration (E), and chlorophyll specific $^{14}CO_2$ uptake in June 1989 experiment.The mean of three observations ± 1 SE is presented.

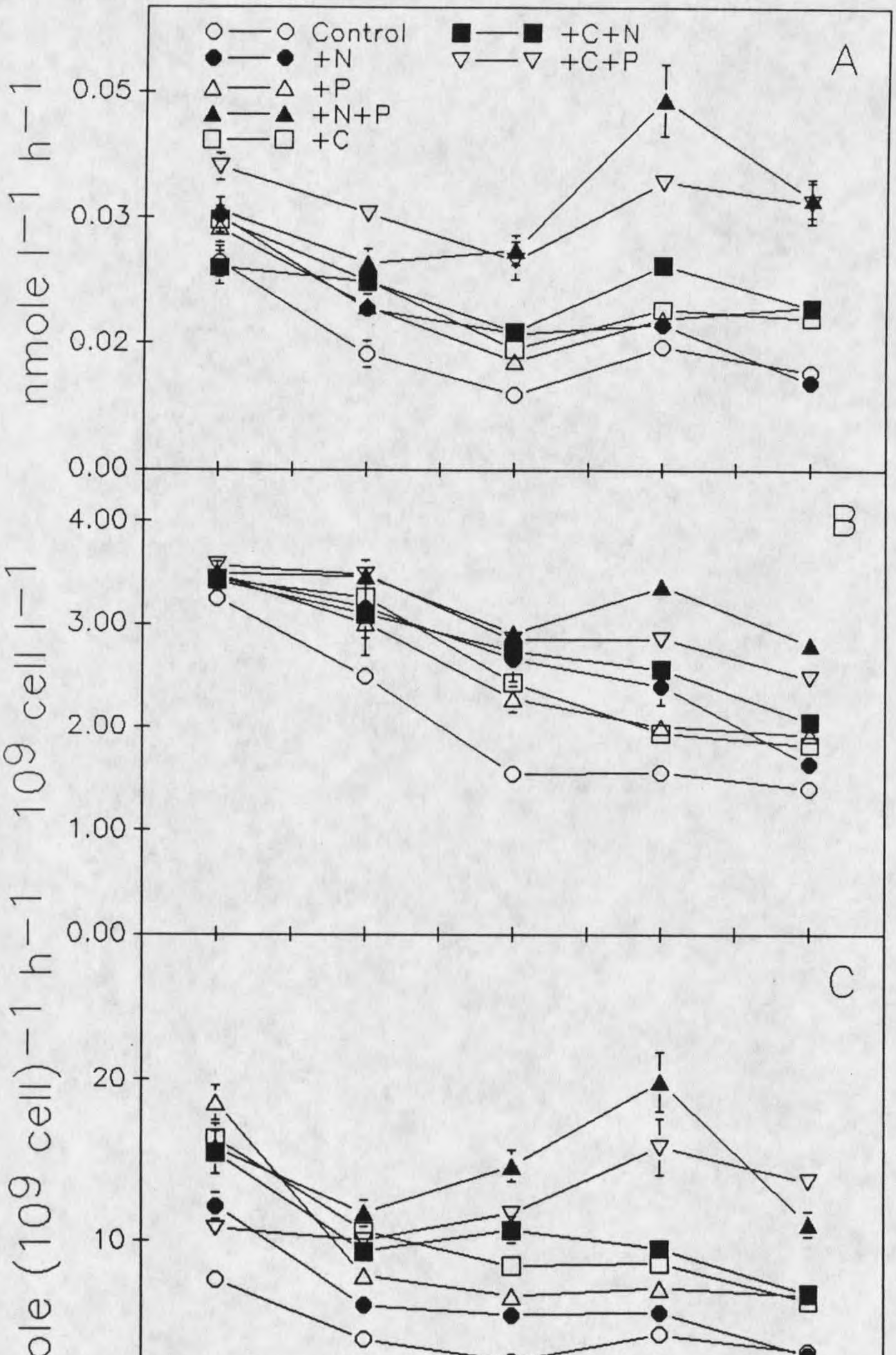

Figure 19. Bacterioplankton thymidine incorporation (A), bacterial cell number (B), and bacterioplankton specific activity (C) in August 1989 experiment. Mean of 3 observations ± 1 SE is presented.

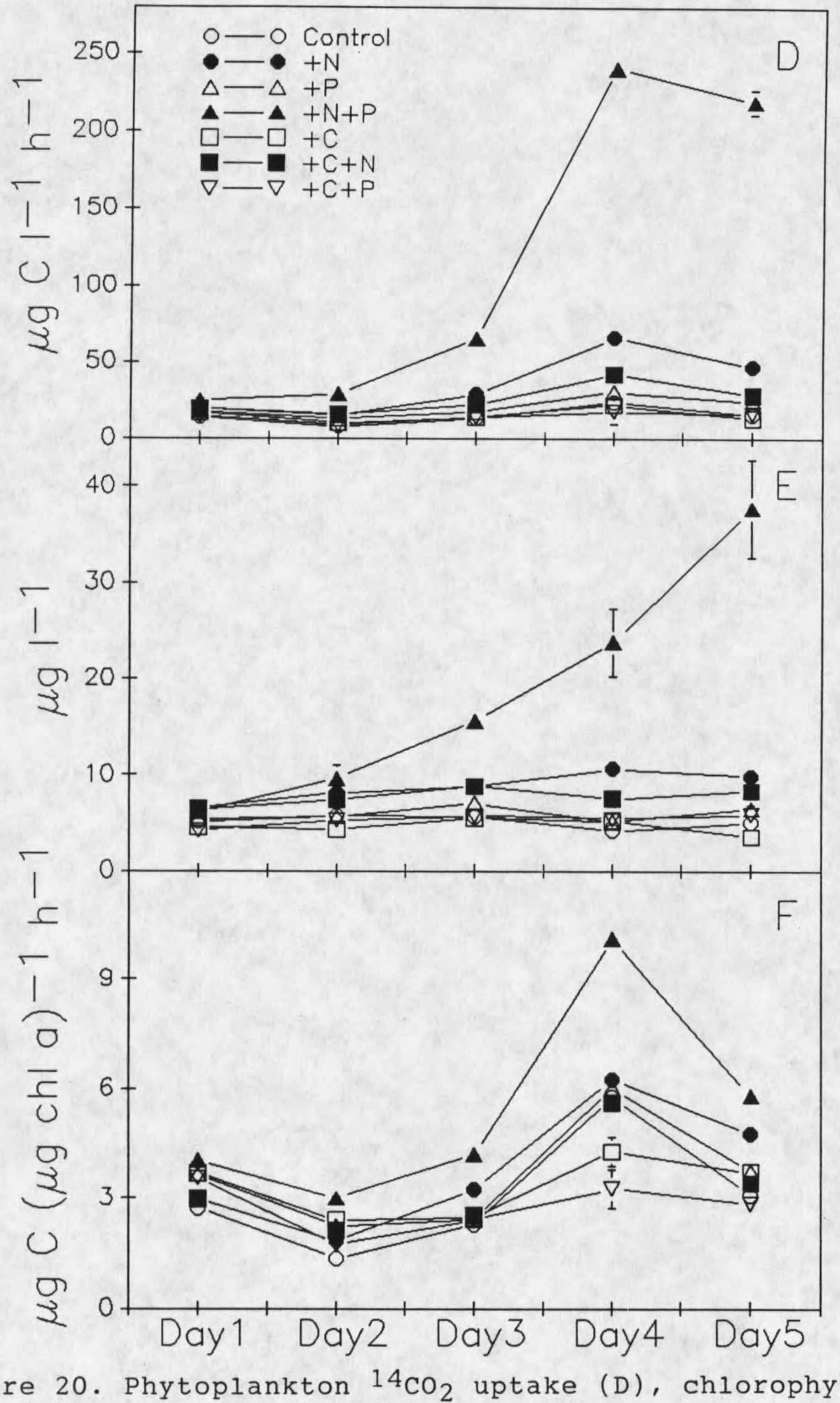

Figure 20. Phytoplankton $^{14}CO_2$ uptake (D), chlorophyll a concentration (E), and chlorophyll specific $^{14}CO_2$ uptake (F) in August 1989 experiment. Mean of 3 observations ± 1 SE is presented.

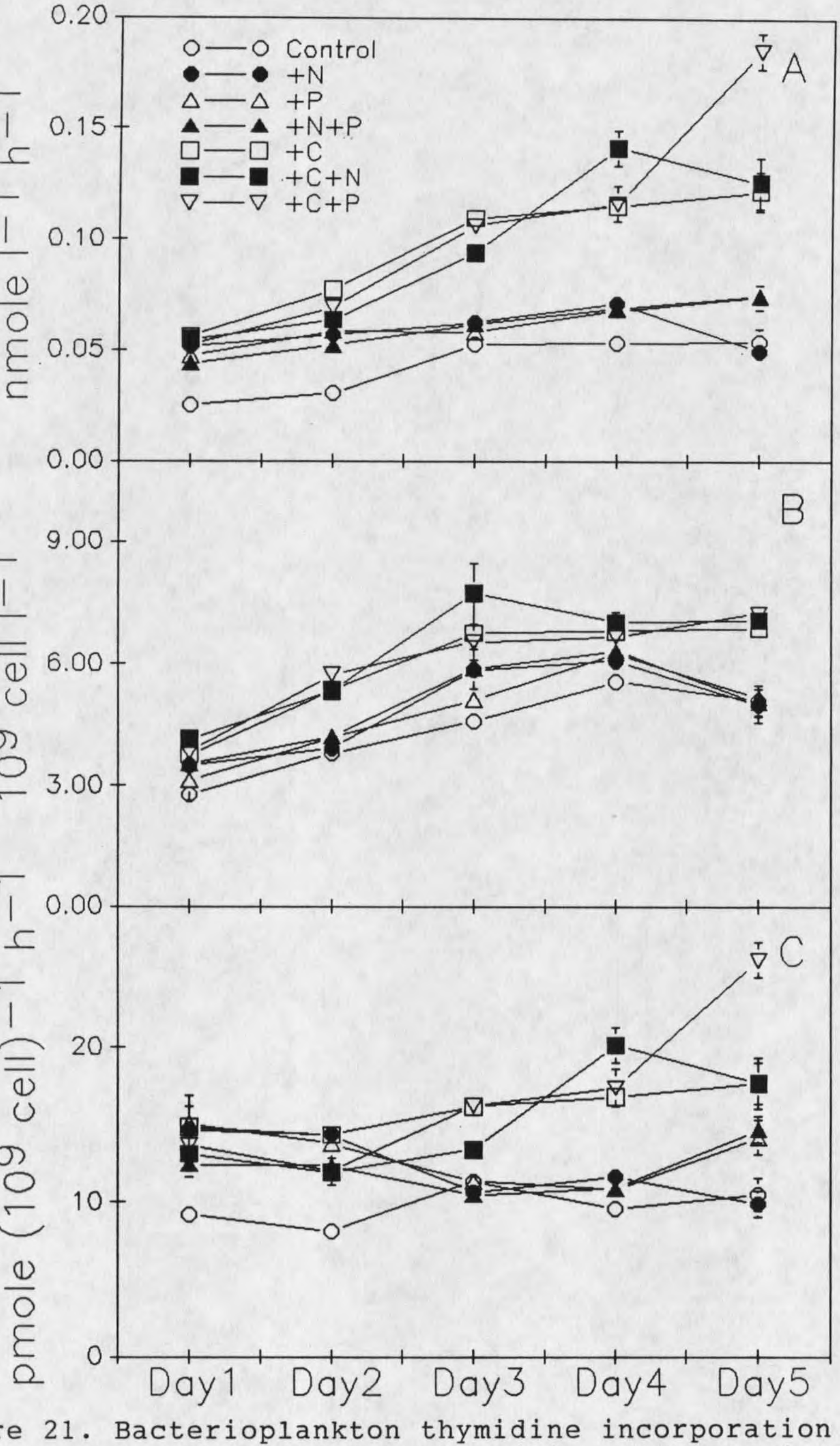

Figure 21. Bacterioplankton thymidine incorporation (A), bacterial cell number (B), and bacterioplankton specific thymidine activity (C) in October 1989 experiment. Statistical errors are as in Fig. 20.

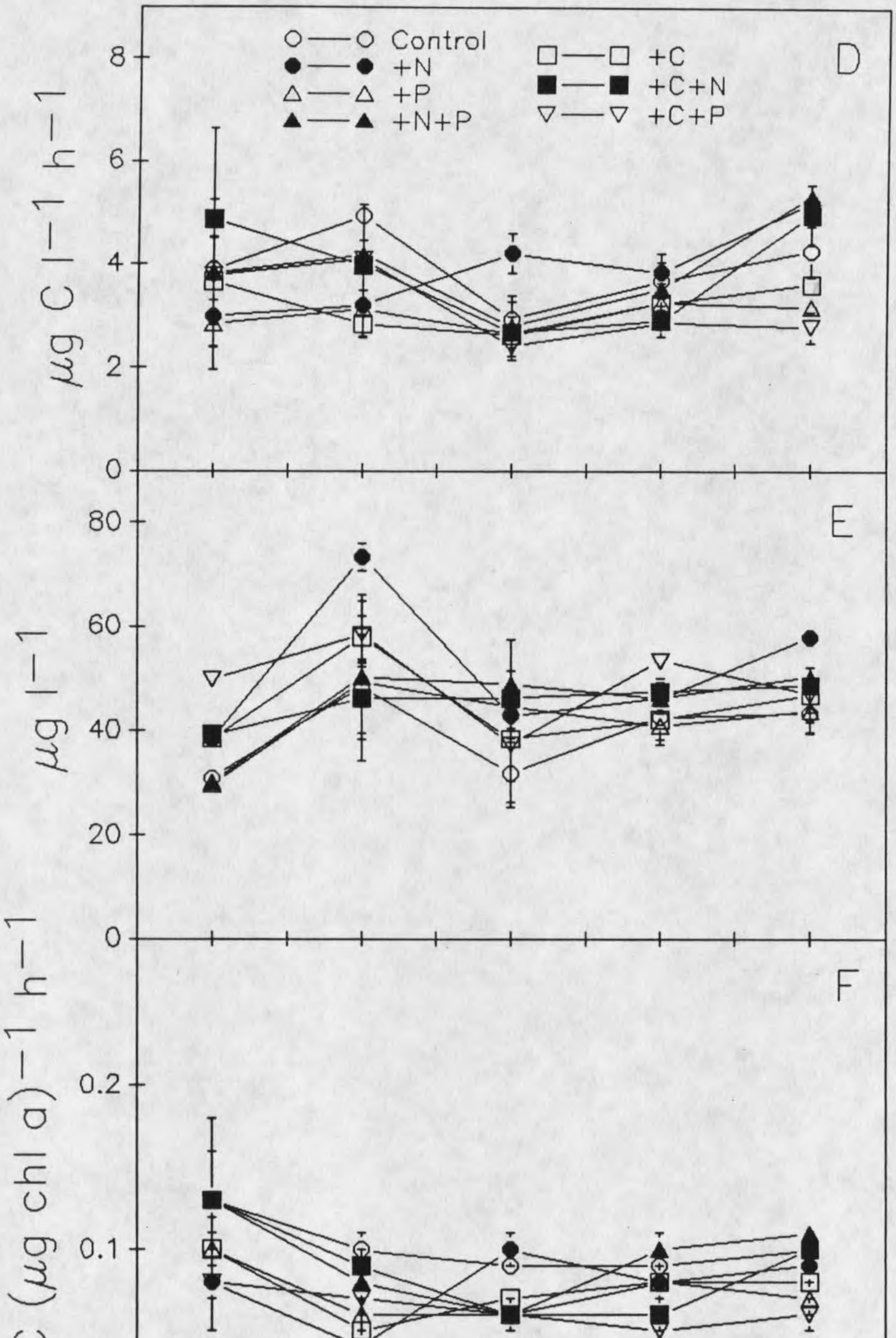

Figure 22. Phytoplankton $^{14}CO_2$ uptake (D), chlorophyll a concentration (E), and chlorophyll specific $^{14}CO_2$ uptake (F) in October 1989 experiment. Mean of 3 observations ± 1 SE is presented.

parameters (^{3}H-thymidine incorporation, cell concentration) were significantly ($p<0.05$) correlated with the phytoplankton parameters ($^{14}CO_2$ uptake, chlorophyll a) (Table 9). Among these, 2 of the 33 regressions between bacterial ^{3}H-thymidine incorporation and $^{14}CO_2$ uptake showed positive correlations ($p<0.05$). Two of the 33 regressions between bacterial cell concentration and $^{14}CO_2$ uptake showed positive correlations ($p<0.05$). Five (2 positive, 3 negative) of the 33 regressions between bacterial ^{3}H-thymidine incorporation and chlorophyll a, and 3 (1 positive, 2 negative) of the 33 regressions between bacterial cell concentration and chlorophyll a showed significant ($p<0.05$) correlations (Table 9).

Discussion

In a number of our experiments, nutrient enrichment increased bacterioplankton cell number and thymidine incorporation. Because changes in cell number and thymidine incorporation may result from both growth and losses via grazing, they may not necessarily reflect the actual growth response to nutrient addition. For this reason, we included bacterioplankton cell specific activity, defined as pmole thymidine incorporation 10^9 cell^{-1} h^{-1}, as a bacterial growth parameter which is independent of grazing and is consequently a more realistic estimator of actual response to nutrient addition.

Table 9. Correlation coefficients (r) between bacterioplankton variables (thymidine incorporation, bacterial cell concentration), and phytoplankton variables ($^{14}CO_2$ uptake, chlorophyll a) from the experiments in the Grayling Arm of Hebgen Lake.

Treat-ment	Bacterio-plankton	1988						1989					
		June		August		October		June		August		October	
		PPR	CHL	PPR	CHL	PPR	CHL	PPR	CHL	PPR	CHL	PPR	CHL
Control	Thym	0.93*	0.25	0.11	0.67	-0.47	-0.93*	-0.22	0.46	0.05	-0.20	0.54	0.10
	Cell	0.99*	0.41	-0.44	-0.23	-0.16	-0.89*	0.37	0.59	-0.43	0.28	-0.26	-0.26
NH_4^+	Thym	0.87	-0.72	0.52	0.83	-0.32	-0.21	0.88*	0.99*	-0.51	-0.82	-0.11	-0.22
	Cell	0.64	-0.40	0.42	0.22	0.21	0.49	0.50	0.60	-0.73	-0.94*	0.59	-0.25
PO_4^{-3}	Thym	0.11	-0.27	-0.40	0.55	-0.26	-0.64	0.58	0.51	-0.10	-0.61	0.67	-0.02
	Cell	0.08	0.17	-0.21	-0.43	0.52	-0.65	-0.47	-0.73	-0.67	-0.39	0.89*	0.47
NH_4^+ &	Thym	NA	NA	NA	NA	NA	NA	0.21	0.31	0.80	0.44	0.32	0.68
PO_4^{-3}	Cell	NA	NA	NA	NA	NA	NA	0.12	0.04	-0.40	-0.72	-0.38	0.61
Mannitol	Thym	-0.92*	-0.98*	-0.07	0.90*	0.22	-0.61	0.01	-0.64	0.08	-0.29	-0.16	-0.08
	Cell	0.51	-0.49	0.02	0.89*	0.29	-0.72	-0.35	0.18	-0.35	0.17	-0.34	0.01
NH_4^+ &	Thym	NA	NA	NA	NA	NA	NA	0.85	0.19	0.24	-0.89*	-0.33	0.75
mannitol	Cell	NA	NA	NA	NA	NA	NA	0.18	-0.17	-0.59	-0.71	-0.55	0.86
PO_4^{-3} &	Thym	NA	NA	NA	NA	NA	NA	-0.85	-0.39	-0.27	-0.67	-0.70	-0.31
mannitol	Cell	NA	NA	NA	NA	NA	NA	-0.62	0.28	-0.63	-0.84	-0.67	-0.21

PPR=phytoplankton $^{14}CO_2$ uptake; CHL=chlorophyll a; Thym=bacterioplankton thymidine incorporation; Cell=bacterial cell concentration; NA=not applicable; *=significant correlation at $p \leq 0.05$.

Inorganic phosphorus and nitrogen enrichments increased bacterioplankton thymidine incorporation, cell number, and specific activity in many of our experiments indicating that inorganic nutrient addition can stimulate bacterial activity. Interestingly, the limiting nutrient differed among experiments and showed no clear trends over the season. The nutrients stimulating bacterioplankton activity were organic carbon, inorganic phosphorus, and inorganic nitrogen in 2 of the 6 experiments and were inorganic nitrogen and organic carbon in 1 of the 6 experiments. Inorganic phosphorus alone increased bacterioplankton activity in 1 of the 6 experiments as did organic carbon. The variable response to nutrient addition presumably reflects differences in ambient water chemistry and physiology of the bacterioplankton at the beginning of the experiment. Vadstein and Olsen (1989), working with chemostats, reported that bacterial growth becomes phosphorus limited when TOC:TP is between 8.3 and 58.8 (by weight). During our experiments, all initial TOC:TP ratios exceeded 80, except in August 1988 when the ratio was 42. Hence, it is not surprising that 4 of our 6 PO_4^{-3} additions showed significant stimulation of bacterioplankton activity.

NH_4^+ and amino acids are generally considered to be the primary nitrogen sources for bacterioplankton in natural waters (e.g. Billen 1984, Wheeler and Kirchman 1986). When ambient concentrations of free amino acids are low, bacteria may be forced to utilize NH_4^+ as a nitrogen source (e.g. Wheeler and Kirchman 1986). Chemostat studies have also shown that NH_4^+ and NO_3^- additions stimulated heterotrophic bacterial activity (Horrigan et al. 1988). The reported range in bacterial C:N (by weight) content of 2.3-8.3 (Linley and Newell 1984) is below the initial ambient TOC:TN level in all of our experiments implying that nitrogen was in short supply for bacterioplankton relative to organic carbon. Relatively high TOC:TN in Hebgen Lake may explain why most NH_4^+ additions significantly stimulated bacterioplankton activity.

My results indicate that inorganic nutrients can stimulate bacterioplankton activity through direct utilization. In one case, following PO_4^{-3} addition (June 1988), bacterioplankton ^3H-thymidine incorporation was out of phase (1 day before) with phytoplankton $^{14}CO_2$ uptake and chlorophyll a concentration. That 95% (125 out of 132) of the correlations between bacterioplankton and phytoplankton variables were insignificant or negative supports our conclusion of direct bacterial stimulation by inorganic nutrient addition. Our results corroborate previous reports that bacterioplankton growth can be limited by inorganic nitrogen in natural waters (Wheeler and Kirchman 1986, Horrigan et al. 1988, Vadstein et al. 1988, Vadstein and Olsen 1989, Toolan et al. 1991).

Although inorganic nutrient enrichments seemed to directly stimulate bacterioplankton activity in certain cases, indirect stimulation via phytoplankton products also appeared to exist. That 5% of the correlations between bacterioplankton and phytoplankton variables were significantly positive supports this contention. Bacterioplankton growth and phytoplankton production has been shown to be correlated in both coastal waters (Fuhrman et al. 1980) and fresh waters (Chrzanowski and Hubbard 1989, Marvalin et al. 1989, Robarts and Wicks 1990). The roughly equal number of positive and negative correlation coefficients obtained in our study when bacterial activity was compared with phytoplankton production indicates that indirect enhancement of bacterial growth by phytoplankton products may be important at certain times only; direct uptake of added inorganic nutrients is presumably responsible for the lack of a consistent trend.

Microcosm nutrient enrichment experiments certainly have drawbacks for studying bacterioplankton growth limitation, although nutrient enrichment experiments have come into wide use in studies of phytoplankton nutrient limitation (e.g. Goldman 1978, Elser and Kimmel 1986, Dodds and Priscu 1990). Results from nutrient enrichment bioassays may be misleading with respect to actual <u>in situ</u> responses owing to isolation of bacterioplankton from external sources of nutrients and by alteration of physical, chemical, and biological environment during incubation (Elser and Kimmel 1986, Dodds and Priscu 1990). Despite these limitations, our microcosm nutrient bioassay approach offers perhaps the best method known for estimating in situ conditions (Elser et al. 1990, Dodds and Priscu 1990). Our use of semi-continuous nutrient addition (year 2) and relatively large containers (20 l) should have alleviated many of the artifacts resulting from containment.

Our experimental results show that organic carbon, inorganic nitrogen and inorganic phosphorus enrichments significantly increased bacterioplankton ^{3}H-thymidine incorporation, cell concentration, and specific activity at various times of the year in a eutrophic lake. Inorganic nutrient enrichments appeared to stimulate bacterioplankton activity both directly and indirectly via phytoplankton products. It is not clear why some single nutrient additions (2 cases of carbon and 1 cases of phosphorus addition) increased bacterioplankton and decreased phytoplankton growth. It is also not clear either why some other single nutrient additions (1 case of nitrogen and 1 case of phosphorus addition) increased phytoplankton activity while concomitantly decreasing bacterioplankton growth. Competition for a deficient nutrient may explain these results, although further experiments on these interactions are required before final conclusions can be drawn.

It has been increasingly recognized that bacteria, flagellates, and small ciliates are significant components of freshwater food webs (Porter et al. 1985, Porter et al. 1988). These microheterotrophic organisms feed on bacterioplankton and form a "microbial loop" to the traditional grazer food chain (Azam et al. 1983, Sherr and Sherr 1988, Christoffersen et al. 1990). Inorganic nutrient regulation of bacterioplankton growth is one of the important aspects in microbial ecology that has only recently been considered. The present study reveals how the growth of bacterioplankton, a primary component of the "microbial loop", is potentially regulated in a freshwater ecosystem. Further studies on the direct regulation of bacterioplankton activity are required for a more thorough understanding of bottom-up control of aquatic foodwebs.

CHAPTER 4

INFLUENCE OF PHYTOPLANKTON ON THE RESPONSE OF BACTERIOPLANKTON GROWTH TO NUTRIENT ENRICHMENT

Introduction

Heterotrophic bacterioplankton are not only responsible for the degradation of organic matter and recycling of nutrients, but also are important producers of particulate organic matter (Azam et al. 1983). Thus, they may provide a route for the assimilation of dissolved organic matter into classical food chain. Because of the ecological importance of bacterioplankton in aquatic ecosystems, knowledge of the factors regulating their activity will lead to a more thorough understanding of ecosystem processes.

It is commonly believed that phytoplankton growth is limited by inorganic phosphorus and nitrogen (Eler et al. 1990) but that bacterioplankton growth is limited by organic carbon (Azam et al. 1983; Bjørnsen et al. 1989; Riemann and Søndergaard 1986). Phytoplankton are thought to be the main suppliers of the carbon used by bacterioplankton (Currie 1990). Inorganic phosphorus and nitrogen concentrations therefore may determine phytoplankton abundance, which in turn, can regulate bacterioplankton growth (Currie 1990). Such a relationship is supported by the positive correlation between bacterioplankton production and phytoplankton biomass (Cole et al. 1988; Fuhrman et al. 1980; White et al.

1991). This relationship is also consistent with the fact that bacterioplankton inorganic phosphorus and nitrogen uptake systems have higher affinities for phosphorus and nitrogen than those of phytoplankton (Currie et al. 1986; Currie and Kalff 1984; Vadstein and Olsen 1989). Recent studies have shown that bacterioplankton growth rates can be stimulated directly by inorganic phosphorus enrichment in both oligotrophic lake water (Coneney and Wetzel 1992) and mesotrophic lake water (Toolan et al. 1991). These findings indicate that bacterioplankton growth is not controlled solely by the availability of organic carbon substrates.

To address the questions of whether inorganic phosphorus and nitrogen or organic carbon limit bacterioplankton growth, and whether this limitation is influenced by phytoplankton in a eutrophic ecosystem, we conducted two types of experiments. In the first, bacterioplankton growth response to nutrient enrichment in whole lake water samples was compared with samples from which phytoplankton were removed. In the second, bacterioplankton growth response to nutrient enrichments in lake water with a photosynthetically viable phytoplankton assemblage was compared with treatments where photosynthesis was inhibited.

Material and Methods

Initial Conditions

Organisms were collected from Hebgen Lake, a 50 km^2

eutrophic reservoir located on the upper Madison River 33 km west of Yellowstone National Park on 22 September 1989 (phytoplankton exclusion experiment) and 19 June 1990 (inhibition of photosynthesis experiment). In both experiments, 900 ml lake water was placed in duplicate autoclaved one-liter flasks on a G10 Gyrotory Shaker at 60 rpm. An irradiance of 120 μE m^{-2} s^{-1} was provided by 40 W "cool-white" fluorescent lamps. Water temperature was maintained at approximately 25 ^{o}C.

Phytoplankton Exclusion Experiment

Two treatments, one with and one without phytoplankton, were used in the experiment. Water was filtered through 1.0 μm membrane filters, then examined under an epifluorescence microscope to verify the absence chlorophyll autofluorescence in phytoplankton-free treatments. In treatments with phytoplankton, water was filtered through 280 μm size Nitex mesh to remove large grazers and detritus. In both treatments (with and without phytoplankton), three initial nutrient enrichments were established with the following additions: control (ambient levels), 140 μg NH_4^+-N liter^{-1} plus 93 μg PO_4^{3-}-P liter^{-1}, and 91 mg mannitol liter^{-1}.

Chlorophyll a subsamples were collected from each flask at the start of the incubation and at the end of the experiment on day 5. Chlorophyll a concentrations were measured by initial extraction in 95% ethanol heated to boiling (79 °C) followed by overnight extraction at 4 °C (Sartory and Grobbelaar 1984). Fluorescence of the extract was measured with a Turner model 112 fluorometer and compared to a chlorophyll a standard curve made using pure Anacystis chlorophyll a [Sigma Chemical Co.].

Bacterial ^{3}H-thymidine incorporation and cell number were measured 4 h after starting the incubation, and on days 1, 3, and 5. Bacterial thymidine incorporation was determined by adding high activity (55 Ci mmole^{-1}) methyl-^{3}H]thymidine (ICN Radiochemical Inc.) to 10 ml water samples (final concentration 10 nM) in 20 ml glass scintillation vials. ^{3}H-thymidine was evaporated to dryness and rehydrated with deionized water before use to eliminate products of self radiolysis and to remove ethanol. The inoculated sample was incubated at 25 °C in the dark for 30 minutes. Activity was terminated by adding 10 ml ice-cold 10% trichloroacetic acid (TCA) to each vial. Following overnight extraction at 4 °C, samples were filtered onto 0.2 µm membrane filters (Poretics Corporation). After rinsing 5 times (2 ml each rinse) with ice-cold 5% TCA, the filter was transferred to a 20 ml scintillation vial and 7 ml Cytoscint scintillation cocktail (ICN Radiochemical, Irvine, CA) was added. Radioactivity in each sample was determined by standard liquid scintillation spectrometry using a Beckman LS-100C counter. Counting efficiency was determined by the external standard ratio method using ^{3}H-toluene as reference and acetone as the quenching agent. Samples for bacterial counts were fixed with formaldehyde (3% final concentration) and stored at 4 °C. Bacterial cell counts were made using the acridine orange direct count technique (Hobbie et al. 1977) with a Nikon epifluorescence microscope.

Inhibition of Photosynthesis Experiment

Three treatments (light, + photosynthetic inhibitor, dark) were started with water filtered through 280 μm size Nitex mesh to remove large zooplankton and detritus. The photosynthetic inhibitor, 3-(3,4-Dichlorophenyl)-1, 1-Dimethylurea (DCMU), was added to a final concentration of 5×10^{-6} M in one treatment. Flasks were covered with two layers of aluminum foil for the dark treatment. All three treatments were subjected to three nutrient addition levels: control (ambient levels); +140 μg NH_4^+-N liter^{-1} plus 93 μg PO_4^{3-}-P liter^{-1}; and +90 mg glucose liter^{-1}. Chlorophyll *a* concentrations were measured 4 h after the start of incubation and on day 5; bacterial thymidine incorporation and cell number were measured daily over the entire 5 days of the experiment. Analytical methods were the same as described for the phytoplankton exclusion experiment.

Test of Significance

To determine the effect of inorganic P and inorganic N and organic C, bacterioplankton specific activity (thymidine incorporation per cell number) from each nutrient addition was compared with controls by multi-factor analysis of variance (Snedecor and Cochran 1980). The comparisons were done on replicate samples from each treatment for all time course data. A t-test was used to compare the influence of phytoplankton on bacterioplankton cell number, bacterioplankton thymidine incorporation and bacterioplankton specific activity after 72 h incubation in whole lake water with samples from which phytoplankton were removed or photosynthesis was inhibited. The nutrient influences on phytoplankton chlorophyll a at the end of the experiments (120 h) for different nutrient additions were also compared with controls using a t-test. Because grazing by protozoans can influence cell number and thymidine incorporation, specific activity should yield the most realistic estimate of bacterial response to experimental manipulation.

Results

Background Conditions

During the phytoplankton exclusion experiment, the dominant phytoplankton species in the whole lake water treatment were the cyanobacterium *Anabaena spiroides* (69% of total volume) and the chrysophyte *Ochromonas sp.* (13% of total biovolume). Before starting the incubation, the experimental water had the following conditions: chlorophyll a = 3.5 μg liter^{-1}, soluble reactive phosphorus (SRP) = 10 μg liter^{-1}, NH_4^+-N = 11 μg liter^{-1}, and dissolved organic carbon (DOC) = 66 mg liter^{-1}. During the inhibition of photosynthesis experiment, the dominant phytoplankton in the experimental water were the chlorophytes _Schroederia sp._ (48% of total biovolume) and _Closteridum sp._ (43% of total biovolume). At the beginning of the incubation, the experimental water had the following conditions: chlorophyll _a_ = 178 μg l^{-1}, SRP = 8 μg liter^{-1}, NH_4^+-N = 9 μg liter^{-1}, and DOC = 75 mg liter^{-1}.

Nutrient Effects on Bacterioplankton

Organic carbon enrichments significantly increased bacterioplankton specific activity in both whole water and filtered lake water treatments in the phytoplankton exclusion experiment ($p<0.01$). At the end of the incubation, bacterial specific activities in organic carbon treatments were 2 to 10 times higher compared with controls (Figure 23A, 23B; Table 10). Inorganic phosphorus and nitrogen stimulated bacterioplankton specific activity in the whole lake water treatment only ($p<0.01$), which was 1.5 times higher than control (Figure 23B, Table 10). Bacterioplankton specific activities were increased only by inorganic P and N enrichments in the photosynthetic inhibition experiment, ($p<0.01$). Inorganic P and N enrichment increased bacterioplankton specific activity 4 times more than the control in the light treatment and 2 to 3 times more in the light treatment than in the photosynthetic inhibitor and dark treatments by the end of the experiment (Figure 24,

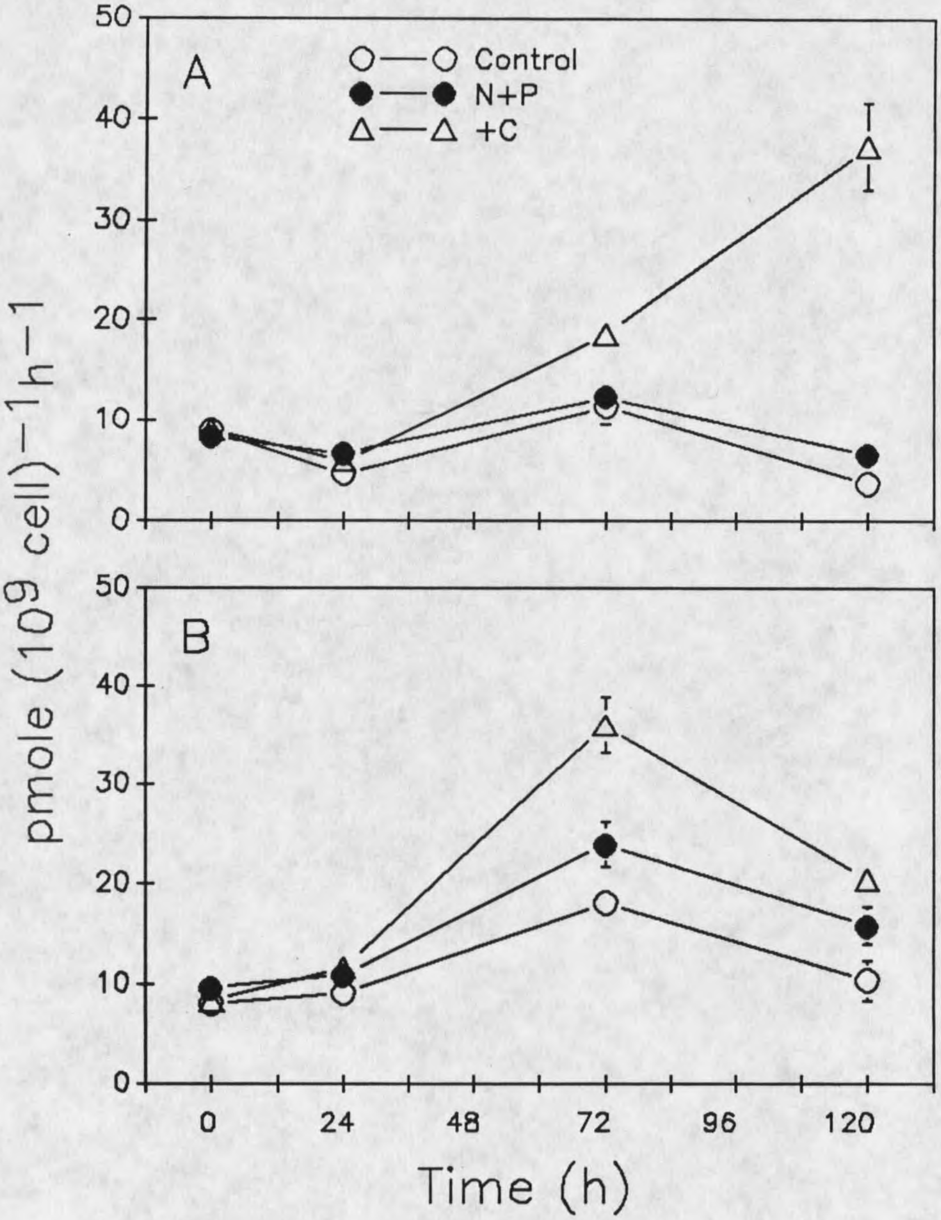

Figure 23. Bacterioplankton specific activity in filtered lake water (A) and whole lake water (B) treatments in the phytoplankton exclusion experiment. Error bars are ± 1 SE (n=2) and are shown only if larger than the symbol.

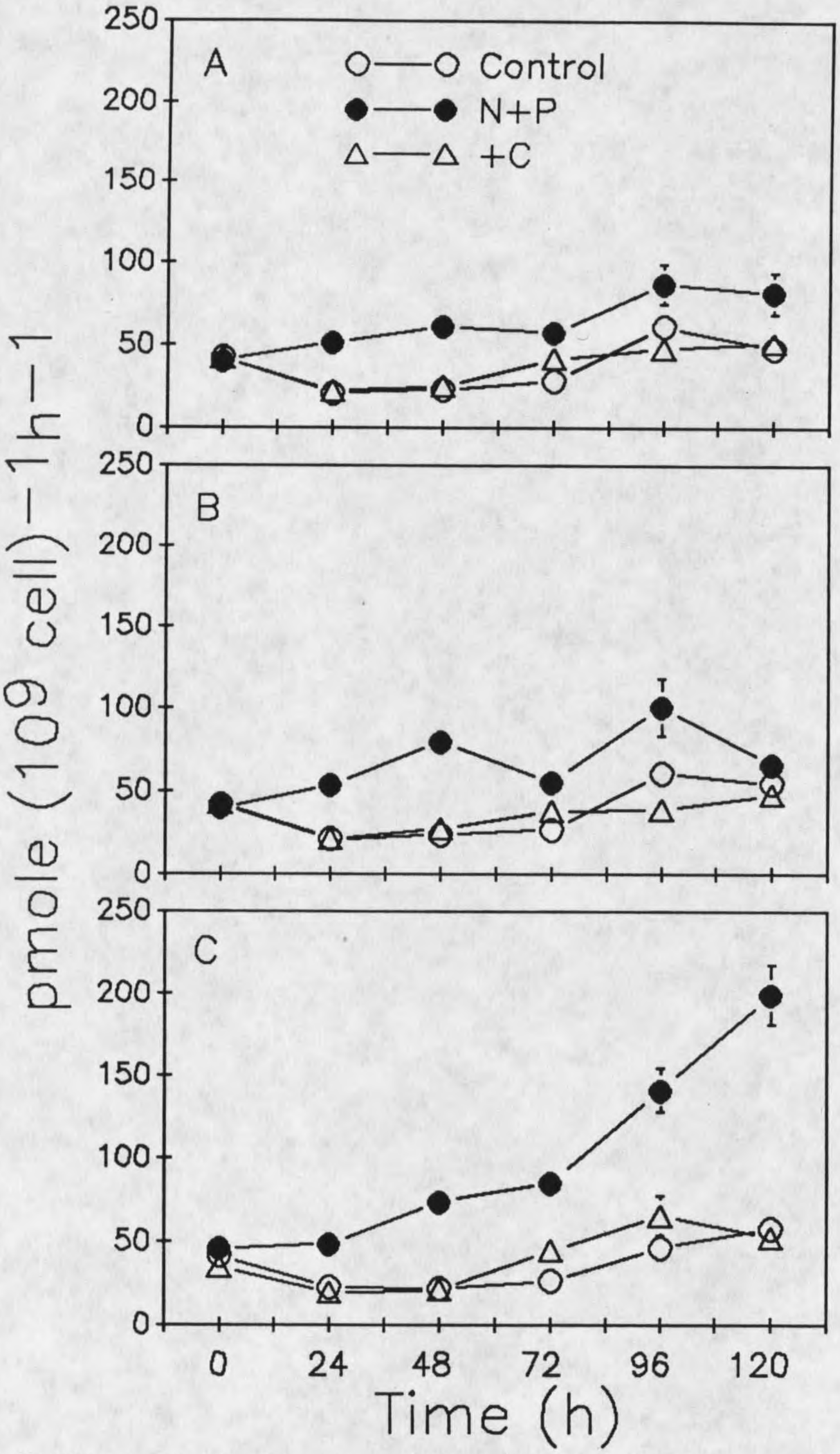

Figure 24. Bacterioplankton specific activity in the DCMU (A), dark (B), and light (C) treatments in the inhibition of photosynthesis experiment. Error bars are ± 1 SE (n=2) and are shown only if larger than the symbol.

Table 10. Time-course means of bacterial thymidine incorporation (^{3}H-THY, nmole liter^{-1} h^{-1}), bacterial cell number (CELL, 10^6 ml^{-1}), and specific activity (SP-ACT, pmol [10^9 cell]$^{-1}$ h^{-1}) in the phytoplankton exclusion experiment and the photosynthesis inhibition experiment. All measured points were used in the multi-factor analysis of variance to test the significant difference between control and the other treatments.

	Phytoplankton exclusion experiment						Photosynthesis inhibition experiment								
	Algae presence			Algae absent			Light			Light+DCMU			Dark		
Nutrient additions	3H-THY	CELL	SP-ACT	3H-THY	CELL	SP-ACT	3H-THY	CELL	SP-ACT	3H-THY	CELL	SP-ACT	3H-THY	CELL	SP-ACT
Control	0.04	3.23	11.3	0.02	2.54	7.12	0.08	2.07	36.3	0.08	2.16	37.3	0.08	2.08	38.2
P + N	0.06	3.78	15.0	0.03	3.18	8.55	0.43	2.71	99.1	0.18	3.12	63.4	0.19	3.12	66.0
+ C	0.08	3.82	19.0	0.06	3.51	17.6	0.09[a]	2.04[a]	39.7[a]	0.08[a]	2.17[a]	38.3[a]	0.08[a]	2.20[a]	36.0[a]

[a] Not significantly different from control at $p>0.05$

All the other nutrient enrichments are significantly different from control at $p<0.05$

Table 10).

Phytoplankton Effect on Bacterioplankton

Bacterioplankton thymidine incorporation, cell number and specific activity in all nutrient enrichments with phytoplankton present were significantly higher ($p<0.05$) than in the same enrichments with phytoplankton absent during the phytoplankton exclusion experiment (Figure 25). Time-course average thymidine incorporation, specific activity and cell number were respectively 1.8, 1.4 and 1.2 times higher in the treatment with phytoplankton.

Photosynthetic activity increased thymidine incorporation and bacterial specific activity in the inorganic phosphorus plus nitrogen enrichments only during the photosynthetic inhibition experiment (Figure 26). Controls and organic carbon enrichments were not significantly influenced by photosynthetic inhibition (Figure 26).

Nutrient Effect on Phytoplankton

Phytoplankton chlorophyll a was increased significantly in the inorganic phosphorus plus nitrogen ($p<0.01$) and organic carbon ($p<0.05$) enrichments compared with controls in the whole lake water treatment during the five-day phytoplankton exclusion experiment (Figure 27A). Phytoplankton chlorophyll a was increased significantly ($p<0.01$) by inorganic phosphorus and nitrogen enrichment only in the light treatment during the five-day

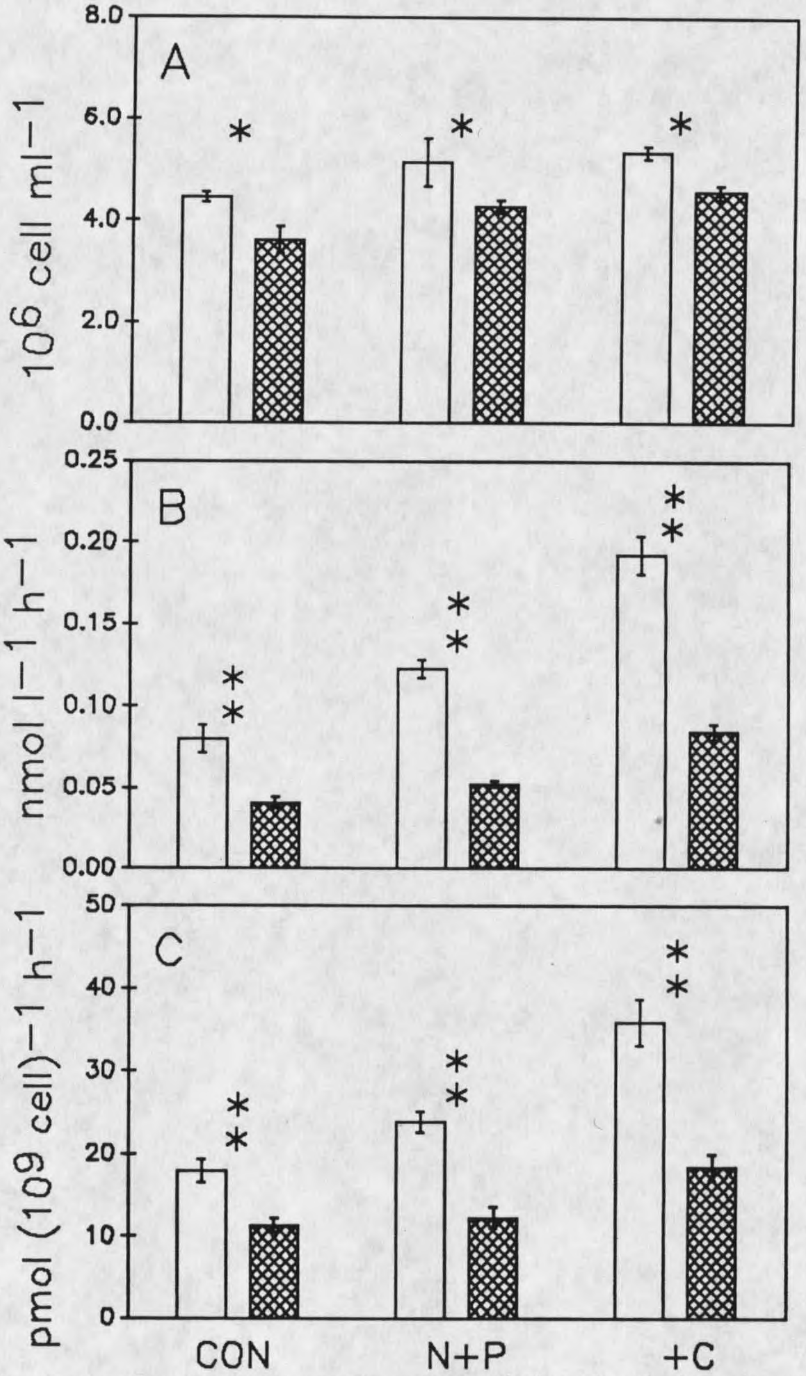

Figure 25. Bacterioplankton cell number (A), thymidine incorporation (B) and specific activity (C) after 72 h of incubation in the phytoplankton exclusion experiment. Open bars indicate whole lake water (phytoplankton presence); crossed bars represent filtered lake water (phytoplankton absent). Error bars show ± 1 SE (n=2) and are shown only if larger than the symbol. * = p 0.05; ** = p 0.01.

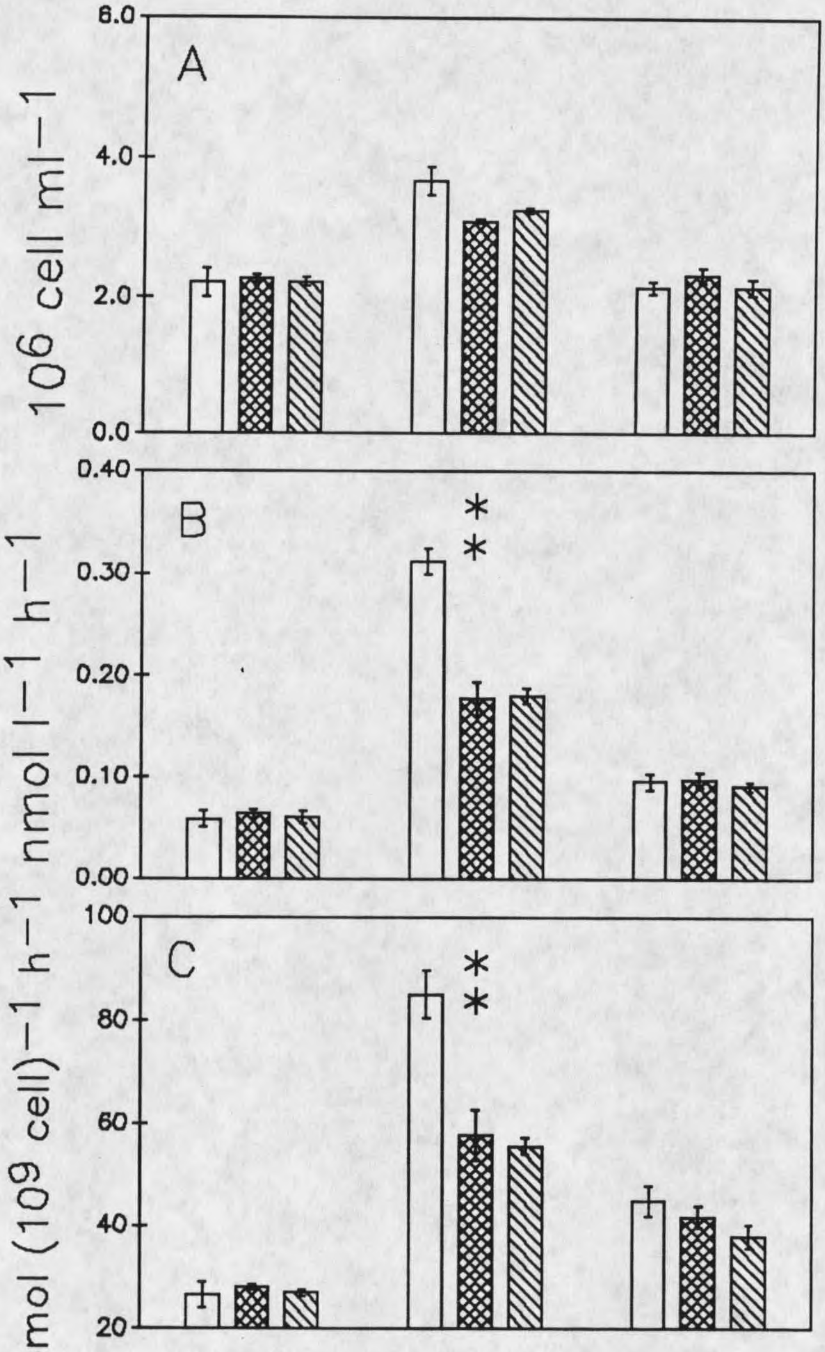

Figure 26. Bacterioplankton cell number (A), thymidine incorporation (B), and specific activity (C) after 72 h of incubation in the photosynthesis inhibition experiment. Open bars = light incubation (photosynthesis presence); crossed bars = +DCMU (photosynthesis inhibited); hatched bars = dark (photosynthesis inhibited). Error bars show ± 1 SE (n=2) and are shown only if larger than the symbol. ** = significant at p 0.01.

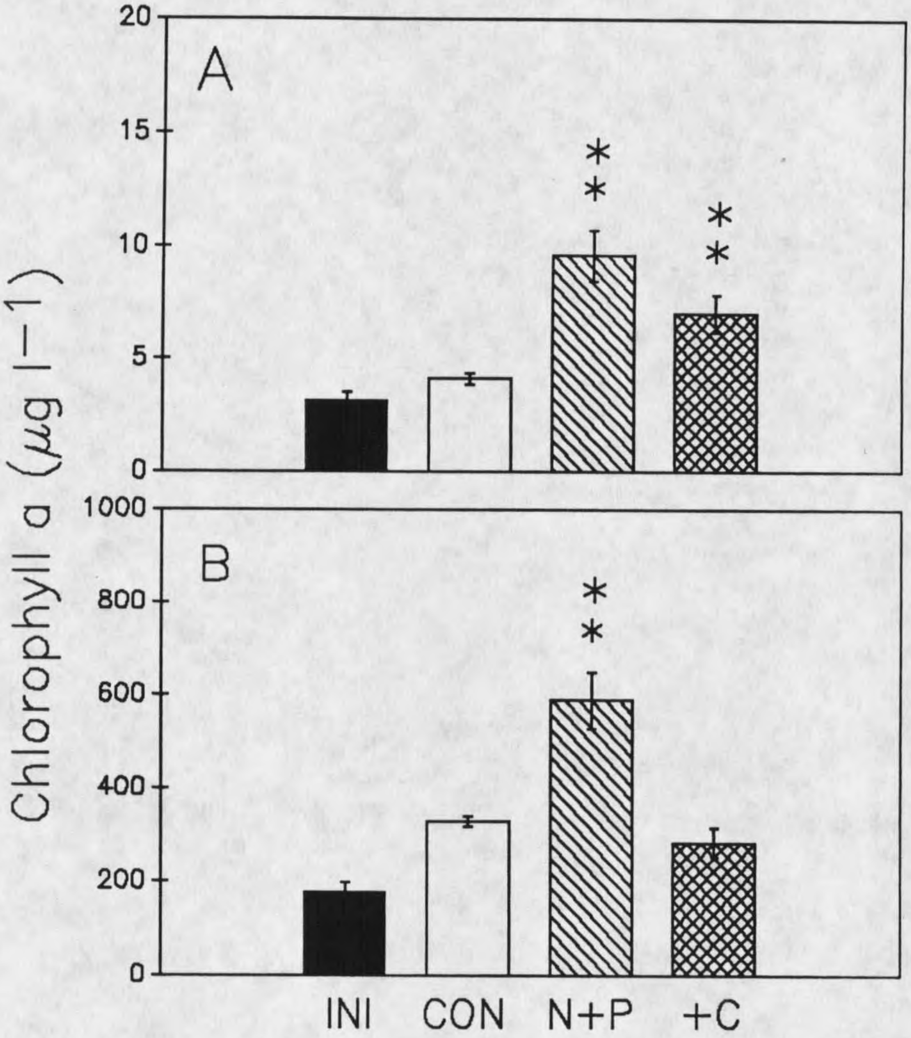

Figure 27. Initial (INI) and final chlorophyll a concentrations in the whole lake water treatment of the phytoplankton exclusion experiment (A) and in the light treatment of the photosynthesis inhibition experiment (B). Error bars are ± 1 SE (n=2) and are shown only if larger than the symbol. * = different from control (CON) at p 0.05; ** = different from control at p 0.01.

photosynthetic inhibition experiment (Figure 27B).

Discussion

Stimulation of bacterial activity by organic C addition occurred during the 1989 experiment only, implying that ambient organic carbon levels limited bacterial growth at this time. Conversely, lack of stimulation by organic carbon during the 1990 experiment indicated an organic carbon replete system. The result from 1990 are different from those in 1989 in that organic carbon addition showed no stimulation on the specific growth rate. Lack of DOC stimulation of bacterial activity has also been shown for an oligotrophic lake (Conveney and Wetzel 1992) but differs from DOC enrichment experiments conducted on Lake Michigan bacterioplankton (Pernie et al. 1990). Considering results from these other systems, my results may be related to the ambient phytoplankton biomass (i.e. trophic state) at the time of the experiment, which was almost 50 fold greater in 1990. The large particulate organic carbon pool (mostly phytoplankton) in 1990 presumably increased the supply of dissolved organic carbon and supported relative high bacterioplankton specific activity (5 fold higher in 1990 than in 1989). Although I contend that the phytoplankton DOC supply rate was greater in 1990, the DOC pool size was similar to that in 1989. Similar DOC pool sizes can occur, despite different supply rates, if DOC is in a dynamic equilibrium between uptake and supply. Such an equilibrium concept is supported by the data of Herbst and Overbeck (1978) who found that algal products never accumulated in growth medium because bacterial utilization of DOC balanced algal release. Bell and Sakshaug (1980) also reported that a 4-fold increase in bacterial activity was enough to prevent a large accumulation of dissolved extracellular products during an algal bloom in Trondheimsfjord, Norway.

Inorganic phosphorus and nitrogen enrichments stimulated bacterioplankton specific activity in four of our five treatments where phytoplankton activity was absent or suppressed (Figure 23B, 24A, B, C), indicating that inorganic phosphorus and nitrogen can directly limit bacterioplankton growth in our study Lake. Such results are supported by recent reports of inorganic P stimulation of bacterioplankton growth in an oligotrophic lake (Coveney and Wetzel 1992) and in a mesoeutrophic lake (Toolan et al. 1991). Inorganic phosphorus and nitrogen limitation in Hebgen Lake have also been found for phytoplankton growth (Figure 27). My results do not support the common view that the growth of bacterioplankton in nutrient-deficient lakes is always limited by the availability of reduced carbon substrates (Currie and Kalff 1984).

Phytoplankton stimulated bacterioplankton specific activity during our 1989 experiment, an apparent organic carbon insufficient situation, regardless of nutrient enrichments (Figure 25). The higher bacterioplankton activity in the whole water compared to the filtered water, was apparently not the result of filtering injury of bacteria because high bacterial activity was observed in the organic carbon enrichment after filtration (Figure 23A). This result implies that materials released from phytoplankton might have compensated the shortages of organic carbon for bacterioplankton. This contention is supported by the results from other studies showing enhanced bacterial activity in the presence of algae (Murray et al. 1986; Peterson et al. 1985). However, the stimulation of bacterioplankton by phytoplankton during our 1990 experiment, an apparent inorganic phosphorus and nitrogen deficient situation, occurred only in the inorganic phosphorus and nitrogen enrichment (Figure 26). This result indicates that phytoplankton products control bacterial growth only when excess phosphorus and nitrogen are available, implying that the commensualistic relationship between phytoplankton and bacterioplankton, with respect to DOC exchange, occurs only under inorganic phosphorus and nitrogen replete situations. This contention is supported by our results showing that inorganic phosphorus and nitrogen enrichment stimulated bacterioplankton activity in the 1989 experiment (apparent carbon deficient environment) when phytoplankton were present (Figure 23B).

We have used specific activity as an index of bacterioplankton growth. The use of this index has several advantages in interpreting our results. Firstly, specific activity (a physiological parameter) would respond rapidly and directly to a limiting nutrient enrichment. Secondly, specific activity should be independent of small changes in initial cell density, allowing direct comparison between whole-water and filtered-water treatments. Finally, direct grazing effects on bacterioplankton cell density are eliminated.

In summary, my experiments demonstrated that bacterioplankton growth can be stimulated by direct additions of inorganic phosphorus and nitrogen without involving phytoplankton, and that both bacterioplankton and phytoplankton can be stimulated by inorganic phosphorus and nitrogen enrichments. Indirect stimulation of bacterioplankton from inorganic phosphorus and nitrogen via phytoplankton products occurred only when organic carbon was in short supply. Collectively, our results from two seasons indicate that nutrient limitation of phytoplankton and bacterioplankton varies temporally in Hebgen Lake. Moreover, the commensualistic relationship between phytoplankton and bacterioplankton is not always present. The intensity of this latter relationship apparently depends on ambient DOC levels.

CHAPTER 5

STIMULATION OF BACTERIOPLANKTON BY AQUATIC CYANOBACTERIA

Introduction

Phytoplankton cells can release a substantial portion of their photosynthate to their environment under certain conditions (Larsson and Hagström 1979; Feuillade et al. 1988; Sundh 1989). Such release can result from actively growing and senescing phytoplankton and may arise from mechanical breakage by grazers (Nalewajko 1977; Lampert 1978). Operational distinctions between production by healthy cells and other modes of organic carbon loss by phytoplankton are controversial (Cole et al. 1982). The term "photosynthetically produced dissolved organic carbon" (PDOC) has been used by Wiebe and Smith (1977), Cole et al. (1982), and Sundh (1989) to include releases from phytoplankton. We use the term "phytoplankton products" to include all soluble products released during both active growth and senescence. Studies have shown that phytoplankton products can be used by bacteria (Larsson and Hagström 1979; Bell and Sakshaug 1980; Cole et al. 1982; Feuillade et al. 1988; Chrzanowski and Hubbard 1989; Sundh 1989) and can account for 30-90% of bacterial carbon uptake (Coveny 1982; Larsson and Hagström, 1982). Therefore, phytoplankton products are potentially important substrates and energy sources for bacterioplankton growth.

Methods to evaluate the importance of phytoplankton products to bacteria growth include correlations between <u>in situ</u> phytoplankton and bacterial activity (Larsson and Hagström 1982; Marvalin et al. 1989; Robarts and Wicks 1990), size fractionation and radioisotope labeling of bacteria and phytoplankton (Derenbach and Williams 1974; Larsson and Hagström 1982; Cole et al. 1982; Feuillade et al. 1988; Chrzanowski and Hubbard 1989; Sundh 1989) and antibiotic inhibition of bacterial activity (Chrost 1978; Jensen 1983; Jensen and Sondergaard 1985). There are, however, problems associated with these methods (Jensen and Sondergaard 1985; Sundh 1989). Problems associated with size fractionation techniques include size overlap of bacterioplankton and phytoplankton, and difficulties in separating attached bacteria from phytoplankton (Jensen and Sondergaard 1985). The use of antibiotics is often complicated by secondary effects on phytoplankton (Derenbach and Williams 1974; Chrost 1978), which makes calculations of natural release and uptake difficult (Jensen 1983; Jensen and Sondergaard 1985). Factors influencing direct activity or biomass correlations between phytoplankton and bacterioplankton include losses owing to grazing on bacterioplankton and phytoplankton, both of which can yield misleading relationships.

Despite methodological problems, a substantial amount of work has been done on the release of phytoplankton products and its subsequent assimilation by bacteria (Cole et al. 1982; Jensen 1983; Jensen and Sondergaard 1985; Feuillade et al. 1988; Sundh 1989). Most of the studies have been qualitative in their approach or have compared the heterotrophic utilization of phytoplankton products with primary production; few have treated the quantitative role of phytoplankton products in relation to bacterial growth (Riemann and Sondergaard 1984). The purpose of our study was to examine the dependence of bacterial activity and growth on cyanobacterial density using organisms collected from a eutrophic lake dominated by cyanobacteria. Our experimental approach utilized dialysis membrane to physically separate the test bacteria from cyanobacteria.

Materials and Methods

Sample Collection

The cyanobacteria, _Lyngbya_ _birgei_ and _Aphanizomenon_ _flos-aquae_, were collected from the surface water of Hebgen Lake, Montana, a 50 km^2 eutrophic reservoir located on the upper Madison River near Yellowstone National Park (see Miller 1991 for a detailed description). The cyanobacteria were transported at the temperature of collection under darkness to the laboratory. The organisms were transferred to sterile flasks and maintained in filter-sterilized (0.2 μm) lake water for 3 to 5 days at 25 ^{o}C and 120 μE m^{-2} s^{-1}

to acclimate them to the experimental conditions. Surface lake water containing natural bacterioplankton populations was also collected and transported in the dark to the laboratory and used for experimental bacterioplankton inoculation within 20 h of collection. Samples were collected during September 1989 and August 1990.

Experimental Conditions

All incubations were at room temperature (25 ± 1 °C) on a G10 Gyrotory Shaker (New Brunswick Scientific Co.) set at 60 rpm. Light was provided at a 12 h light/dark cycle by four 40 W cool white fluorescent tubes with a photosynthetic photon flux density of 120 $\mu E\ m^{-2}\ s^{-1}$. Incubations were conducted in 1000 ml (experiments 1 and 2) and 250 ml (experiment 3) flasks for 5 days. Duplicate flasks were used for each treatment.

Experimental Design

Three experiments were designed to test the response of free living and attached bacteria to cyanobacterial density. The response of natural, free-living bacterial populations to cyanobacteria was determined by thymidine incorporation and direct counts of free-living bacteria (Experiment 1). The response of bacteria isolated from cyanobacterial aggregates was also determined by thymidine incorporation and direct counts of free-living bacteria (Experiment 2). The response of natural, free-living bacterial populations to cyanobacteria was measured as free-living bacteria, and as attached bacteria associated to the surfaces of the tubing that were used to separate the test bacteria and cyanobacteria (Experiment 3).

Experiment 1 (September 1989): L. birgei and A. flos-aquae were filtered (20 mm Hg vacuum) onto 8.0 μm polycarbonate membrane filters and resuspended in filter-sterilized (0.2 μm) lake water to obtain chlorophyll a concentrations of 368 and 507 μg l^{-1}, respectively. The cyanobacteria were pipetted into spectra/por molecular-porous membrane tubing (2.5 cm diameter; MW cut off of 12,000-14,000) to partition them from the test bacteria. Control tubes were filled with lake water filtered through 1.0 μm membrane filters to eliminate cyanobacteria and other phytoplankton (confirmed by microscope observation).

The experiment was conducted in 1-l flasks filled with 900 ml filtered (1.0 μm) lake water each containing 4 individual dialysis tubes. Cyanobacterial concentration was adjusted by varying the number of tubes filled with cyanobacteria; tubes not receiving cyanobacteria were filled with 0.2 μm filter sterilized lake water. Using this method, we achieved 5 cyanobacterial densities for each species ("control", "low", "mid-low", "mid-high", and "high") while maintaining the same number of tubes per flask. The final concentration of chlorophyll a at each cyanobacterial density was expressed as the combined mass of chlorophyll a in all tubes of each flask per unit water volume within the flask. The purpose of using the same number of tubes per flask was to eliminate the effect of tubing surface area on bacterial activity at different cyanobacterial densities.

Experiment 2 (September 1989): L. birgei and A. flos-aquae were concentrated to 147 and 206 $\mu g\ l^{-1}$ chlorophyll a, respectively, as described in experiment 1. The cyanobacterial tubes were prepared and the final chlorophyll a concentrations were determined as in experiment 1 except that the "high" cyanobacterial density was omitted. The bacteria employed in experiment 2 were those physically associated with cyanobacteria isolated as follows. L. birgei and A. flos-aquae samples were rinsed three times with filtered lake water (0.2 μm) on 8 μm filters and resuspended in filtered lake water. The cyanobacterial suspensions were then expressed three times through 22, 23, and 26 gauge needles to remove as many bacteria attached to the cyanobacteria as possible. The suspensions of bacteria and cyanobacteria (0.5 ml) were plated on amended lake water agar (ALWA) using a spread plate technique. The ALWA contained 0.1% bacto-yeast extract, 0.25% bacto-dextrose, 0.2% bacto-peptone, and 0.2% bacto-agar in lake water filtered through a 0.2 μm filter. The agar medium was autoclaved at 1.1 kg cm^{-2} pressure for 15 minutes before the inoculation. The inoculated agar plates were incubated at room temperature (25 ± 1 ^{o}C) for 48 h. Two morphologically distinct bacterial colony types, which accounted for >95%

total colony forms, were isolated from each of L. birgei and A. flos-aquae cultures. The 2 types of colonies were transferred separately into amended lake water broths (ALWA without bacto-agar). The broth cultures were incubated at room temperature for 48 h. Lake water filtered through 1.0 μm membrane filters and autoclaved under 1.1 kg cm^{-2} pressure for 40 minutes was prepared in two 10-l containers. Equal amounts of both broth cultures isolated from the same cyanobacteria were pipetted into the autoclaved lake water to obtain a bacterial density of 1.6×10^6 cells ml^{-1} (determined microscopically). Nine hundred ml lake water with inoculated bacteria was transferred to each of the experimental flasks.

Experiment 3 (August 1990): L. birgei and A. flos-aquae were concentrated into 4 different densities by filtering as described in experiments 1 and 2. Thirty ml of each density of each cyanobacterial species was added to each of four spectra/por molecularporous membrane tubes, which had the same MW cutoff as in experiment 1 and 2 but a diameter of 2.86 cm. Lake water (200 ml), prepared as in experiment 1, was added to each of five 250-ml flasks. The four tubes containing different concentrations of each cyanobacterial species were then suspended in each of the 4 flasks. One additional tube filled with 1.0 μm filtered lake water was suspended in a fifth flask as a control for each cyanobacterial species treatment.

Routine Procedures

In experiments 1 and 2, bacterial cell numbers and bacterial activities were determined at 6 h, and on days 1, 3, and 5 after the beginning of incubation. The bacterial cell numbers in the surrounding medium, and the outer surface of the tubes in experiment 3, were enumerated on day 5. To enumerate bacteria attached to the surface of the tubes, 1 cm from each end of the tube was tied and cut off to reduce the effect of the ends of the tube. The tubes were then placed into autoclaved 40 ml 0.2% sodium dodecyl sulfate (SDS) in 3% aqueous formaldehyde followed by mixing on a G10 Gyrotory Shaker at 175 rpm for 5 minutes. Microscopic examination showed that this procedure removed virtually all the bacteria from the surface of the tubes.

NH_4^+ and dissolved organic carbon (DOC) were measured on day 1 and day 5 using the methods described below. To avoid significant reduction of the water volume in the flasks, aliquots of sample from replicate flasks were mixed before analysis.

Determination of Nutrients, Chlorophyll a, and Bacterial Parameters

NH_4^+ and DOC were determined by the phenol-hypochlorite method (Solorzano 1969) and a Dorhmann Carbon Analyzer, respectively. All the samples were prefiltered through Whatman GF/C filters before analysis. Chlorophyll a was measured fluorometrically after extraction in 95% ethanol (Sartory and Grobbelar 1984).

Bacterial ^{3}H-thymidine incorporation was determined by adding high activity (55 Ci [mmol]$^{-1}$) methyl-^{3}H thymidine (ICN Radiochemical INC.) to 10 ml samples (final thymidine concentration 10 nM) in 20 ml glass scintillation vials. The ^{3}H-thymidine stock (in 70% ethanol) was evaporated to dryness and rehydrated with deionized water before use to eliminate products of self radiolysis and to remove ethanol. The inoculated samples were incubated at room temperature (25 ± 1 °C) in the dark for 30 minutes. Bacterial activity was terminated by adding 10 ml of ice-cold 10% (W/V) trichloroacetic acid (TCA) to each vial. Following overnight extraction in TCA at 4 °C, the samples were filtered onto 0.2 μm polycarbonate filters. After rinsing 5 times with 2 ml each of ice-cold 5% TCA, the filters were transferred to a 20 ml polyethylene scintillation vial with 7.0 ml Cytoscint scintillation cocktail (INC Radiochemical, Irvine, CA.). Radioactivity in each sample was determined by standard scintillation spectrometry using a Beckman LS-100C liquid scintillation counter. Counting efficiency was determined by the external standard ratio method using ^{3}H-toluene as a reference and acetone as the quenching agent. Bacterial cell samples were fixed with formaldehyde (3% final concentration) and stored at 4 °C until analysis. Bacterial cell numbers were determined on samples filtered onto 0.2 μm polycarbonate filters counted with a Nikon Labophot epifluorescence microscope using the acridine orange direct count technique (Hobbie et al. 1977).

Statistical Analysis.

To determine the effect of cyanobacterial density on bacterial growth, simple linear regressions between cyanobacterial chlorophyll and bacterial thymidine incorporation or cell number were made. Such regressions were done at each time-point for both cyanobacterial species treatments. To test the effect of cyanobacteria on bacterial activity, time-course bacterial thymidine incorporation and cell number at each density of each cyanobacterial species was compared with the control by multifactor variance analysis (Neter et al., 1985). To determine the response of bacteria from different origins to each cyanobacterial species, the slopes of regressions between bacterial parameters and cyanobacterial density for each cyanobacterial species in experiment 1 (free-living bacteria) was compared with that in experiment 2 (bacteria associated with cyanobacteria) using a t-test (Dixon and Massey 1983). The difference between bacteria attached to the outer surface of the membrane tubes and that in the bulk water was also tested by comparison of the regression slopes using a t-test (Dixon and Massey 1983).

Results

Bacterial thymidine incorporation and cell number for all 3 experiments over the time-course were usually significantly ($p<0.05$) correlated with chlorophyll a concentrations of both L. birgei and A. flos-aquae (Figures. 28-33, Table 11). The only exception was that bacterial cell number did not significantly correlate with L. birgei chlorophyll a 6 h after incubation in experiment 1 (Figure 29, Table 11). The degree of correlation increased with time of incubation for both cyanobacterial species (Figures 28, 29; Table 11). Thymidine incorporation and cell number of the 2 unidentified attached bacteria isolated from L. birgei and A. flos-aquae in experiment 2 was highly correlated ($p<0.05$) with the chlorophyll a of each species at all incubation periods (Figures 30, 31; Table 11). Bacterial numbers in the bulk water outside and attached to the cyanobacterial tubes were also significantly ($p<0.01$) correlated with chlorophyll a of both A. flos-aquae and L. birgei (Figures 32, 33; Table 11). The slopes of linear regressions between bacterial parameters (thymidine incorporation and cell number) and cyanobacterial biomass (chlorophyll a) at each measured incubation length were significantly higher ($p<0.01$) in experiment 2 than in experiment 1 for both cyanobacterial species (Table 12). The slope of the regression between chlorophyll a and attached bacterial number was 2.8 and 2.5 times greater for A. flos-aquae and L. birgei, respectively, than for planktonic bacteria in the bulk water phase outside the cyanobacterial tubes (Table 12).

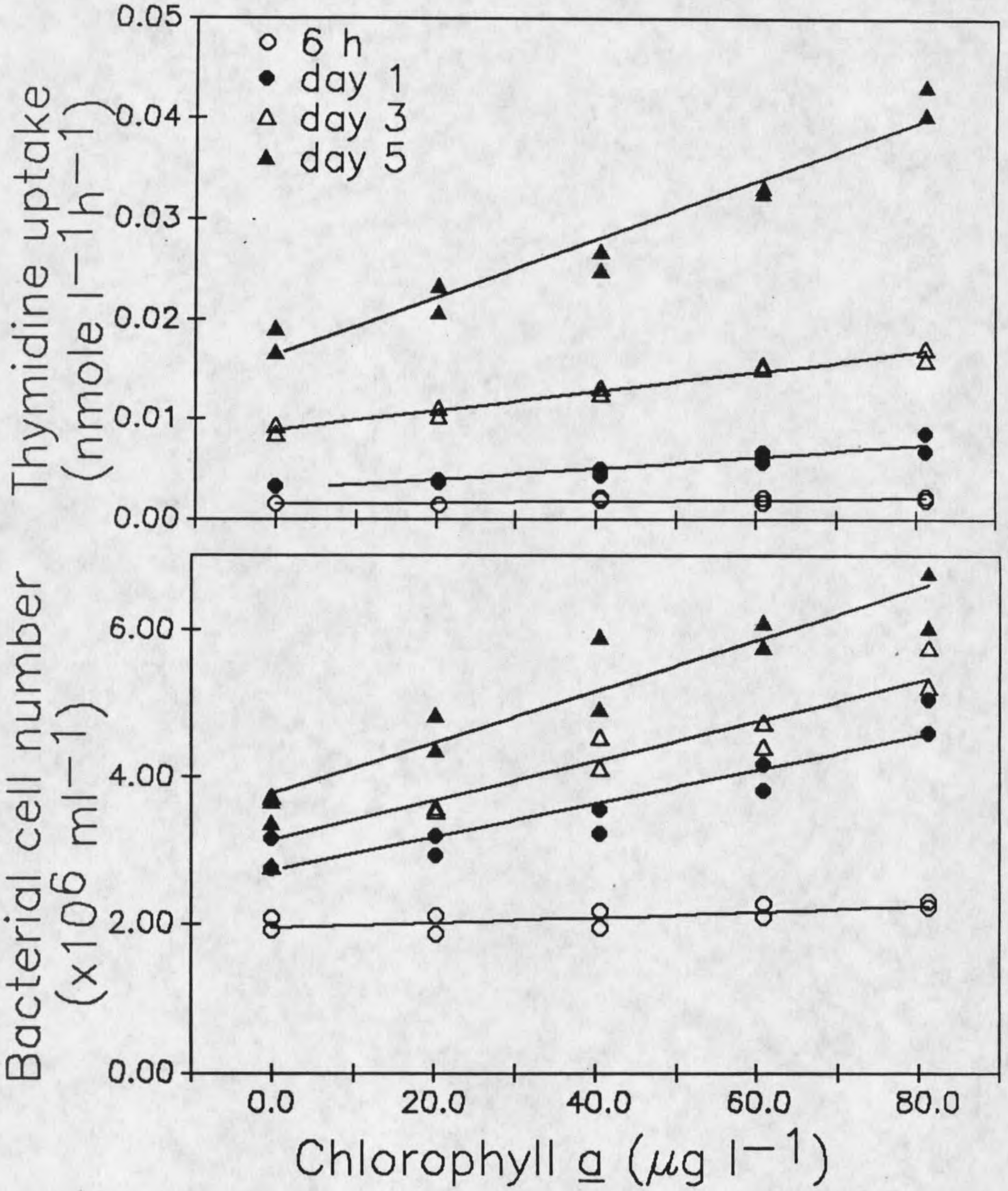

Figure 28. Bacterial thymidine incorporation and cell number at different densities of A. *flos-aquae* at different length of incubation in experiment 1. The line is a least-squares fit through the data.

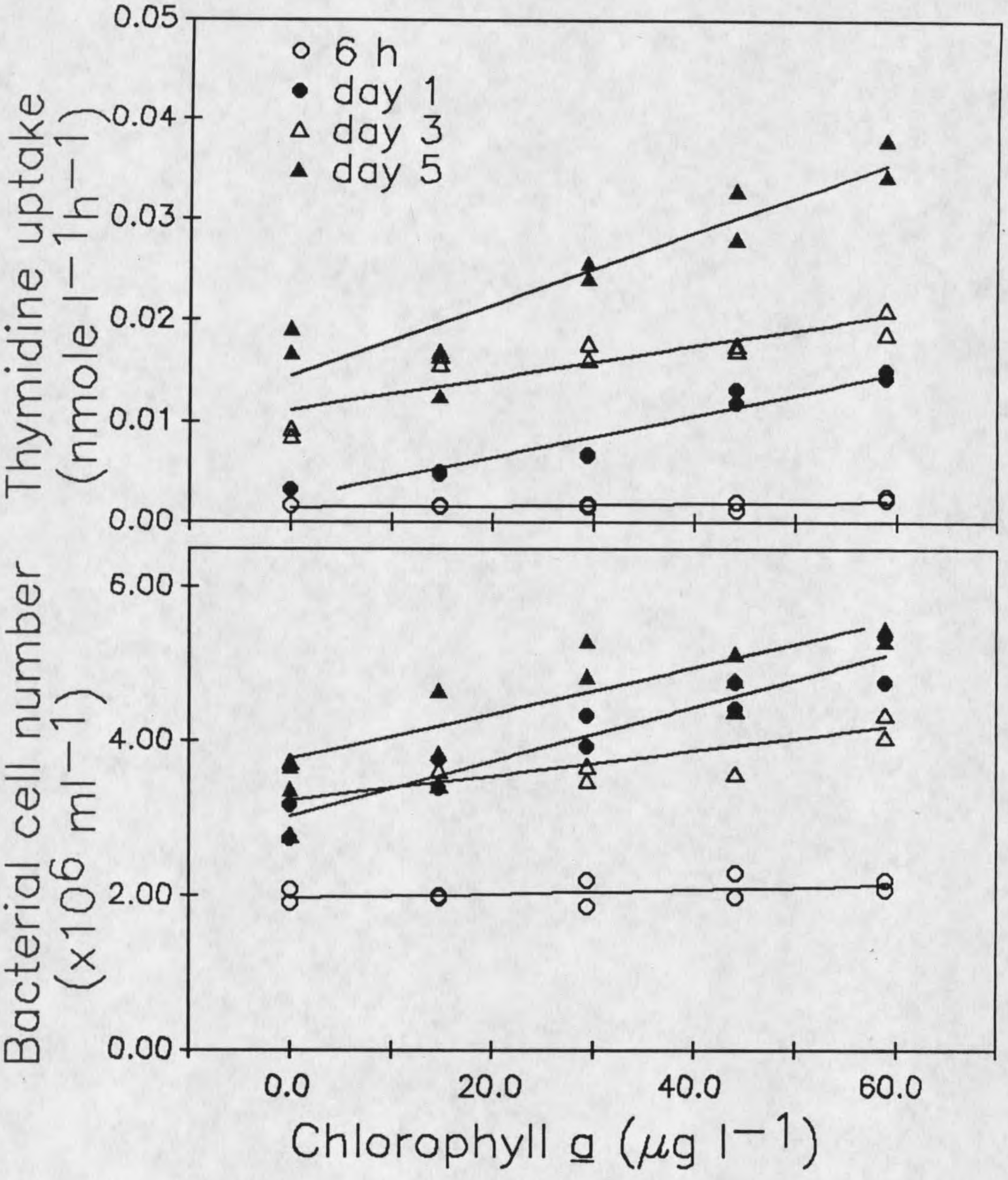

Figure 29. Bacterial thymidine incorporation and cell number at different densities of L. birgei at different length of incubation in experiment 1. The line is a least-squares fit through the data.

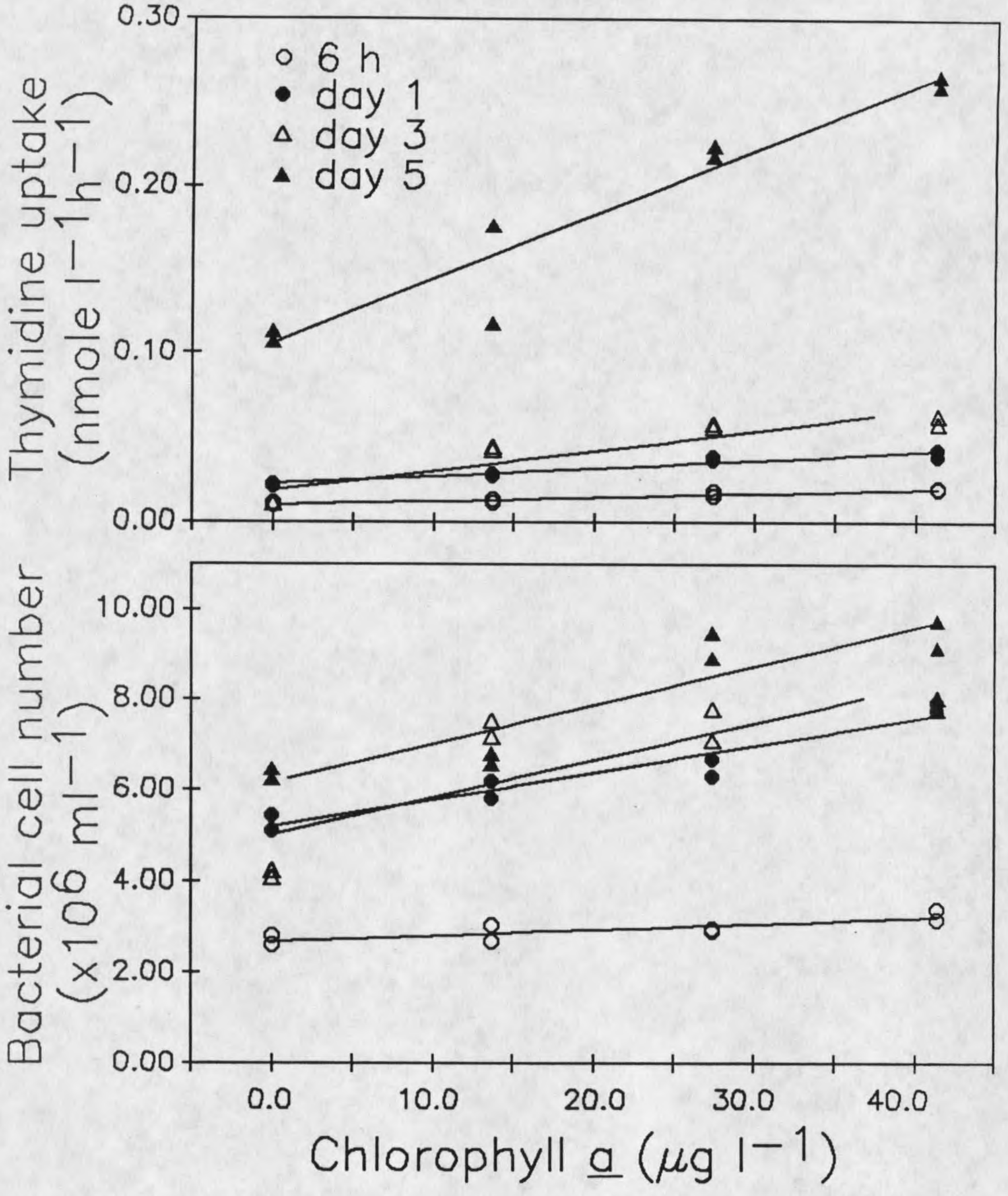

Figure 30. Bacterial thymidine incorporation and cell number in different densities of <u>A</u>. <u>flos-aquae</u> at different length of incubation in experiment 2. The line is a least-squares fit through the data.

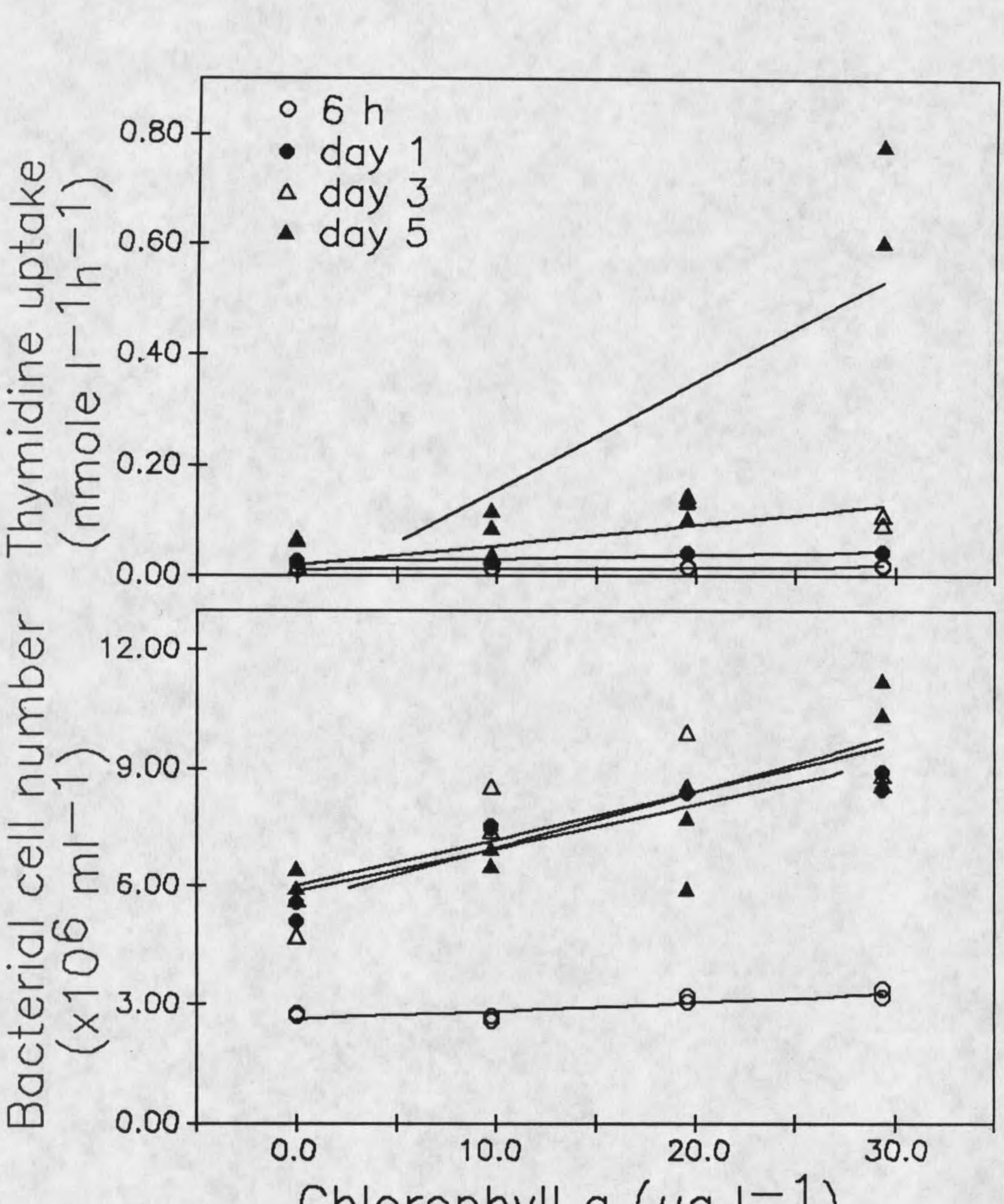

Figure 31. Bacterial thymidine incorporation and cell number in different densities of L. birgei at different length of incubation in experiment 2. The line is a least-squares fit through the data

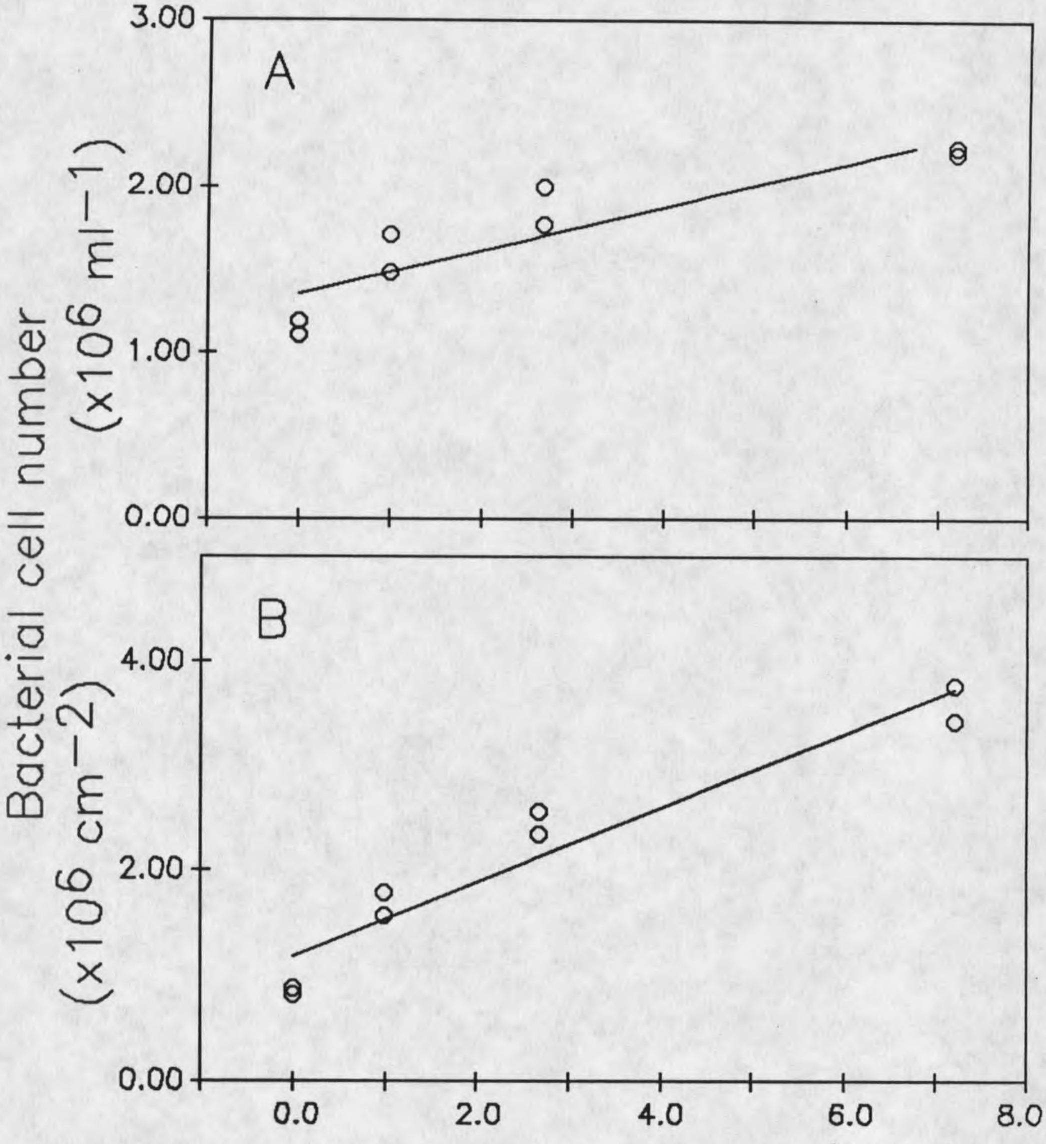

Figure 32. Bacterial cell number (A) free-living in the water outside of the tubing (B) attached to the outside surface of the tubing in different densities of A. flos-aquae after 5 days of incubation.

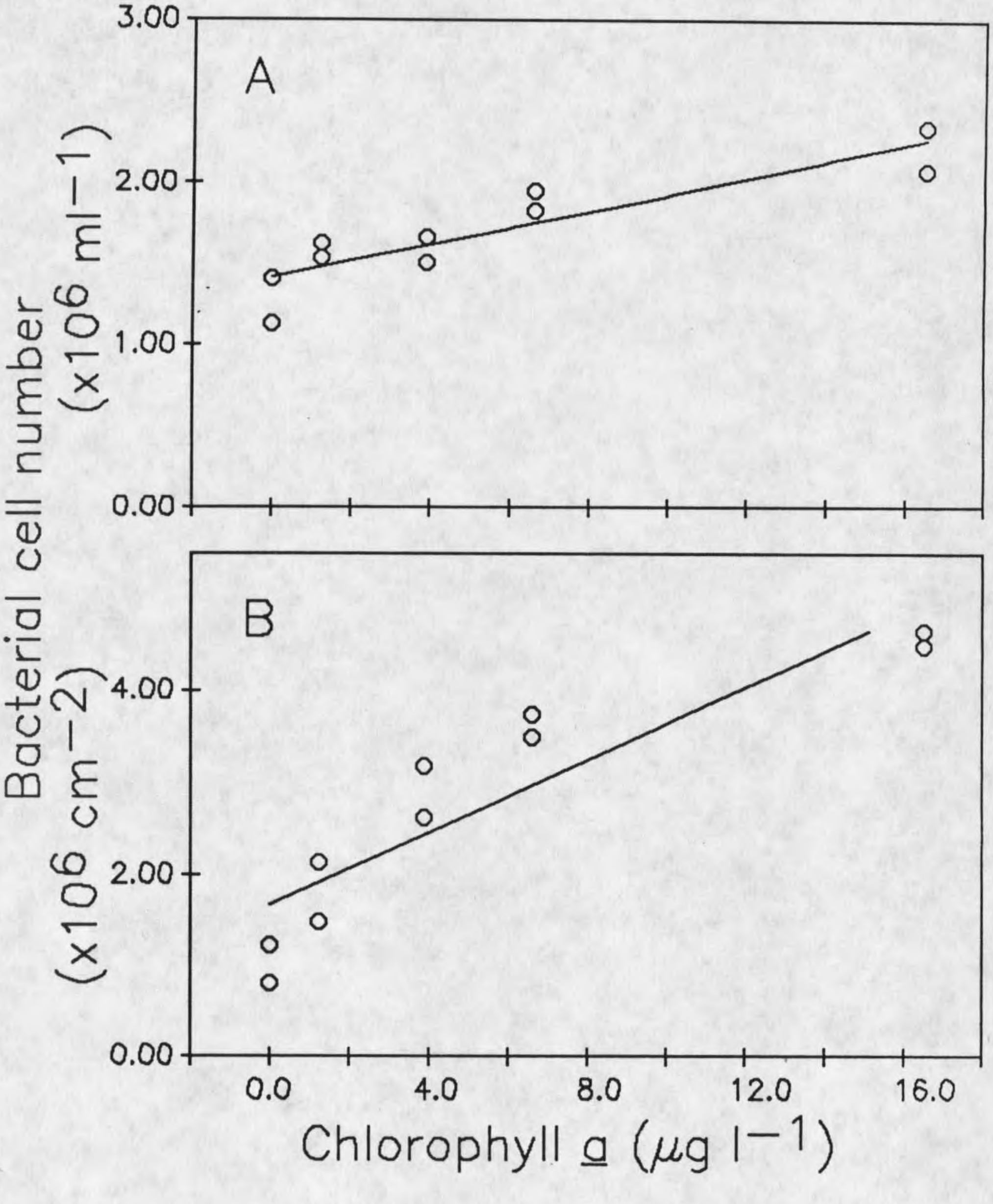

Figure 33. Bacterial cell number (A) free-living in the water outside of the tubing (B) attached to the outside surface of the tubing in different densities of L. birgei on day 5 after incubation.

Table 11. Correlation coefficients (r) for least-squares linear fits between cyanobacterial chlorophyll _a_, and bacterial thymidine uptake and cell number in the 3 experiments.

	Experiment 1				Experiment 2				Experiment 3	
Treatment	6 h	day 1	day 3	day 5	6 h	day 1	day 3	day 5	water	surface
Thymidine uptake										
A. flos-aquae	0.55^{**}	0.82^{***}	0.92^{***}	0.94^{***}	0.93^{***}	0.94^{***}	0.90^{***}	0.99^{***}	NA	NA
L. birgei	0.53^{**}	0.96^{***}	0.84^{***}	0.86^{***}	0.84^{***}	0.86^{***}	0.79^{**}	0.79^{**}	NA	NA
Cell number										
A. flos-aquae	0.71^{**}	0.92^{***}	0.93^{***}	0.94^{***}	0.84^{***}	0.96^{***}	0.84^{***}	0.92^{***}	0.90^{***}	0.96^{***}
L. birgei	0.48	0.96^{***}	0.75^{**}	0.82^{***}	0.87^{***}	0.92^{***}	0.81^{**}	0.81^{**}	0.85^{***}	0.91^{***}

** Significant at $p<0.05$

*** Significant at $p<0.01$

NA Treatment not included in the experiment

Table 12. Regression slopes of bacterial thymidine incorporation (10^{-4} nmole thymidine [μg chlorophyll^{-1} h^{-1}]$^{-1}$), bacterial cell number (10^{-2} cells [μg chl]$^{-1}$) versus cyanobacterial chlorophyll <u>a</u> for each time the experiments were sampled[a].

	<u>A. flos-aquae</u>				<u>L. birgei</u>			
	6 h	day 1	day 3	day 5	6 h	day 1	day 3	day 5
Thymidine uptake								
Expt-1	0.07	0.50	0.93	2.75	0.10	2.07	1.57	3.68
Expt-2	0.47	3.22	11.78	36.62	2.01	6.52	36.54	31.70
Cell number								
Expt-1	0.36	2.31	2.72	3.50	0.31	1.61	3.56	3.01
Expt-2	1.31	5.95	8.16	8.55	2.18	11.21	12.14	14.29
Experiment 3								
Free	NA	NA	NA	13.16	NA	NA	NA	7.94
Attached	NA	NA	NA	36.51	NA	NA	NA	19.70

[a] t-tests were used to compare slopes between experiment 1 (Expt-1) and experiment 2 (Expt-2), as well as between the bacteria of free living (Free) and bacteria attached on the surface of the tube (Attached). All the tests are significantly different at $p<0.01$.

NA Treatment not included in our experiment.

Time-course means (average of measurements at 6 h, days 1, 3, and 5 for experiment 1 and 2, and day 5 only for experiment 3) of bacterial thymidine incorporation and cell numbers at each density of cyanobacteria is listed in Table 3. Bacterial thymidine incorporation and cell numbers were usually significantly higher ($p<0.05$) at all densities of A. flos-aquae and L. birgei with respect to controls in both experiment 1 and 2 (Table 13), the exception being thymidine incorporation at the lowest density of L. birgei. In experiment 3, bacterial cell numbers in the bulk water outside the cyanobacterial tubes and on the outer surfaces of the tubes were generally significantly higher ($p<0.05$) than those in controls; free-living cells at the "low" and "mid-low" cyanobacterial densities and attached cells in the "low" density L. birgei treatment were exceptions (Table 13). The responses of bacteria to cyanobacteria were weaker at lower densities of both species of cyanobacteria in all the 3 experiments (Table 13).

Chlorophyll a concentrations of A. flos-aquae and L. birgei increased less than 18% during the 5-day incubation in both experiment 1 and 2 (Table 14). In experiment 3, chlorophyll a concentration on day 5 was below detection after the SDS wash owing to apparent chlorophyll degradation by SDS. Concentration of DOC consistently decreased from day 1 to day 5 at all cyanobacterial densities in both experiment 1 and 2 (Table 14). Concentrations of NH_4^+

Table 13. Bacterial thymidine uptake (nmol l^{-1} h^{-1}) and cell number (x10^{6} ml^{-1} or x10^{6} cm^{-2}) for each experiment[a]. Values represent the time-course means (6 h, days 1, 3, and 5) from 2 replicate flasks. See text for details.

Treatment	Chlorophyll *a* gradient				
	control	low	mid-low	mid-hig	high
Experiment 1					
A. flos-aquae					
Thymidine uptake	0.008	0.010**	0.011***	0.014***	0.017***
Cell number	2.937	3.305**	3.807***	4.180***	4.753***
L. birgei					
Thymidine uptake	0.008	0.088	0.013***	0.016***	0.018***
Cell number	2.937	3.340**	3.713***	3.931***	4.204***
Experiment 2					
A. flos-aquae					
Thymidine uptake	0.038	0.066***	0.083***	0.095***	NA
Cell number	4.627	5.728***	6.431***	7.121***	NA
L. birgei					
Thymidine uptake	0.030	0.044	0.079**	0.225***	NA
Cell number	4.859	6.228***	6.904***	7.880***	NA
Experiment 3					
A. flos-aquae					
Free-living cell	1.151	1.594**	1.875***	2.222***	NA
Attached cell	0.798	1.672***	2.451***	3.598***	NA
L. birgei					
Free-living cell	1.269	1.582	1.585	1.891**	2.212***
Attached cell	1.106	1.815	2.903***	3.613***	4.561***

a All the significant levels were tested by multifactor variance analysis.

** Significant compared with control at $p<0.05$

*** Significant compared with control at $p<0.01$

NA Treatment not included in the experiment

Table 14. Concentration of chlorophyll *a*, DOC, and NH_4^+ in treatments with different densities of *A. flos-aquae* and *L. birgei* for each experiment. NA=treatment not included in the experiment.

	Chlorophyll *a* (μg l^{-1})		DOC (mg l^{-1})		NH_4^+-N (μg l^{-1})	
Treatment	day 0	day 5	day 1	day 5	day 1	day 5
Experiment 1						
A. flos-aquae						
Control	0.00	0.00	71.1	76.2	25.0	23.9
Low	19.92	20.60	76.4	75.0	16.9	40.3
Mid-low	39.84	41.20	78.2	71.4	8.8	22.3
Mid-high	59.76	61.80	77.9	61.8	12.4	53.8
High	79.68	82.40	60.5	43.9	11.5	66.4
L. birgei						
Control	0.00	0.00	77.1	76.2	25.0	23.9
Low	14.82	14.72	71.7	66.5	23.2	11.5
Mid-low	29.62	29.44	65.1	57.6	25.0	16.9
Mid-high	44.46	44.16	65.2	55.4	22.3	10.6
High	59.28	58.88	63.5	48.8	18.9	13.3
Experiment 2						
A. flos-aquae						
Control	0.00	0.00	171.4	154.3	259.7	16.0
Low	13.07	14.27	137.4	128.9	244.4	305.6
Mid-low	26.13	28.53	112.5	97.2	205.8	615.9
Mid-high	41.12	42.81	90.0	78.4	186.0	739.9
L. birgei						
Control	0.00	0.00	107.8	94.0	121.2	13.3
Low	9.00	10.57	105.3	91.3	127.5	13.3
Mid-low	18.00	21.14	144.5	118.0	118.5	15.1
Mid-high	27.00	31.71	140.0	103.6	76.3	7.9
Experiment 3						
A. flos-aquae						
Control	0.00	NA	NA	5.8	NA	91.7
Low	0.99	NA	NA	26.3	NA	20.0
Mid-low	2.68	NA	NA	27.6	NA	23.2
Mid-high	7.20	NA	NA	26.1	NA	142.7
High	NA	NA	NA	NA	NA	NA
L. birgei						
Control	0.00	NA	NA	4.9	NA	50.2
Low	1.24	NA	NA	16.1	NA	5.2
Mid-low	3.88	NA	NA	11.8	NA	7.7
Mid-high	6.62	NA	NA	13.0	NA	2.8
High	16.51	NA	NA	14.9	NA	4.4

increased in A. flos-aquae and decreased in L. birgei treatments over the same period at all cyanobacterial densities in both experiments 1 and 2 (Table 14).

Discussion

Our experiments revealed a significant positive correlation between cyanobacterial density and bacterioplankton thymidine incorporation and cell numbers. These results corroborate the positive correlations observed between bacterioplankton abundance or production and phytoplankton biomass from other pelagic systems (e.g. Fuhrman et al. 1980; Larsson and Hagström 1982; Bird and Kalff 1984; Cole et al. 1988; Chrzanowski and Hubbard 1989, White et al. 1991). In an experimental laboratory study of model algal-bacterial biofilms, Murray et al. (1986) reported that bacterial thymidine incorporation was 4 to 16-fold greater in the presence of diatom algae than in their absence. Bacteria incubated with algae in an arctic river were also found to be more active during periods of high algal photosynthesis (Peterson et al. 1985). Our results, in concert with these correlative and experimental studies, imply that materials from eukaryotic algae and cyanobacteria are important substrates for bacterial growth in aquatic systems.

Although our experimental methodology has many advantages over previous studies of algal-bacterial interactions, several potential weaknesses should be noted. Firstly, the 1.0 μm filters used to isolate the bacterial inoculum in the experiments eliminated phytoplankton and bacterial grazers, but may also have excluded bacteria larger than 1.0 μm. Hence, the bacterial response we measured would reflect only those organisms smaller than 1.0 μm. Secondly, the molecular weight cut-off of the tubing allowed only compounds smaller than 12,000-14,000 molecular weight to pass through the membrane barrier. It has been reported that 95% of the dissolved organic carbon compounds released by marine phytoplankton have molecular weight smaller than 3,500 (Wiebe & Smith, 1977). Consequently, our selected molecular weight cut-off range will isolate bacteria from only a small (and presumably more recalcitrant) fraction of that released by the cyanobacteria. Thirdly, bacterial growth inside the tubes may contribute to the consumption of a portion of the cyanobacterial products, thereby underestimating the potential effect of cyanobacterial products on bacteria outside the tubes.

During the 5-day incubations, chlorophyll a concentration increased less than 18% in the experiments indicating that there was no substantial net growth of the cyanobacteria. Despite the physiological state of the cyanobacteria, higher densities of cyanobacteria did produce increasingly higher bacterial growth and activity. The consistent decrease in DOC concentration from day 1 to day 5 in experiments 1 and 2 at all densities of cyanobacteria indicates that DOC consumption exceeded production during the experiments. The decreases of DOC concentrations with the increase of cyanobacterial densities on day 5 for both A. flos-aquae and L. birgei in experiment 1 and for A. flos-aquae in experiment 2 indicates that bacterial activity can control DOC under our experimental condition, even with an apparently high DOC supply rate. Several other studies have indicated that bacterial use of phytoplankton products is rapid. Herbst and Overbeck (1978), using axenic algae and 2 different bacteria cultures to test release and consumption rates of phytoplankton products, found that algal products never accumulated in the growth medium because bacterial use balanced release. Bell and Sakshaug (1980) also reported that a 4-fold increase in bacterial activity was enough to prevent a large accumulation of dissolved extracellular products during an algal bloom in Trondheimsfjord, Norway.

Our experiments showed that the bacterial species in close association with the cyanobacteria (presumably attached) had a greater response to cyanobacterial products than free-living forms. Bell and Sakshaug (1980) also found that bacterial response was greater when bacteria were exposed to algal species to which they were in close association with in nature. We further observed that the responses of bacteria to cyanobacteria were greater for those forms attached to the surface of the cyanobacterial tubes than those that were free-living. The greater activity on the tube surface may have resulted from a number of physiological or genetic factors (e.g. Marshall 1992) which we did not investigate.

In conclusion, our experiments revealed a direct dependence of bacterioplankton abundance and activity on cyanobacterial density. This response was strongest in bacteria closely associated with cyanobacteria and those able to attach to surface near the source of the cyanobacterial products. The commensalism we observed in our experiments may play a major role in the regulation of bacterial abundance and succession in natural aquatic systems. Future studies should focus on the physiological and/or genetic mechanisms by which bacteria respond to cyanobacterial products.

CHAPTER 6

LABORATORY EVIDENCE OF BACTERIAL CHEMOTAXIS TO CYANOBACTERIA

Introduction

The materials released from living and senescent phytoplankton include carbohydrates, lipids, peptides, organic phosphates, volatile substances, growth inhibitors and stimulators, enzymes, phenolic substances, vitamins, and toxins (Jones and Cannon 1986), which are usually lumped under the term "extracellular products" (Fogg 1966). It has been well known that these extracellular products play an important role in supporting bacterioplankton production (Larsson and Hagström 1982; Burney et al. 1982), which in turn plays an important role in the microbial loop (Azam et al. 1983). However, dissolved organic matter (DOM) in the form directly utilized by bacterioplankton, is often at low concentrations in aquatic ecosystems (Azam and Ammerman 1984). How can such a diluted labile DOM pool support high bacterioplankton production?

Since all DOM sources are particulate, DOM production events would create microzones of high DOM concentration in the vicinity of the source. The non-random distribution of nutrient molecules has led Azam and Ammerman (1984) to propose that bacterioplankton might cluster in the vicinity of sources of sustained DOM production (e.g., exuding algal cells or detritus undergoing hydrolysis). The special coupling between bacterioplankton and DOM sources would increase the encounter frequency between bacterioplankton and the DOM emanating from the sources. A similar concept termed "phycosphere" has also been proposed by Bell and Mitchell (1972) to describe such spatial relationship between bacterioplankton and their DOM sources. The spatial relationship between bacterioplankton and the DOM concentration gradients in the vicinity of the sources has been indirectly demonstrated by a chemotactic attraction of bacteria to filtrates of old algal cultures (Bell and Mitchell 1972). Highly species-specific bacterial responses to certain carbohydrates, amino acids, and nucleotide bases, some of the possible components of DOM, have also been observed (Fogel et al. 1971; Gullucci and Paerl 1983). However, to my knowledge, there is no evidence that bacterioplankton are attracted directly by healthy phytoplankton, although healthy phytoplankton have been reported an important DOM source for bacterioplankton growth (Fogg 1983; Jones and Cannon 1986; Gomes et al. 1991).

The purpose of this study was to investigate the validity of the phycosphere concept by measuring chemotactic attraction of bacterioplankton to different concentrations of phytoplankton. In the current chapter, motile bacterioplankton cells isolated from Hebgen Lake were used in laboratory experiments to show the chemotactic response of bacterioplankton to chambers containing intact filaments of N_2-fixing and non-N_2-fixing cyanobacteria species isolated from the same lake. Such a design will provide evidence for bacterioplankton-phytoplankton phycosphere relationship, which would provide information on the role of phytoplankton extracellular production in regulating bacterioplankton production.

Materials and Methods

Isolation of Bacteria and Cyanobacteria

The motile bacterium Aeromonas hydrophila (identity confirmed by Microbial ID, Inc., Newark, Del.) was isolated from the surface water of Hebgen Lake during September 1989. This species was isolated by plating surface lake water on amended lake water agar containing 0.1% Bacto-Yeast Extract, 0.25% Bacto-Dextrose, 0.2% Bacto-Peptone, and 2.0% Bacto-Agar in lake water filtered through a 0.4 µm pore size filter. The medium was autoclaved at 1.1 kg cm^{-2} pressure for 15 minutes. After growing under room temperature (ca. 25 ^{o}C) for about 30 hours, the numerically dominant bacterial type according to Gram staining and cell and colony morphology among the isolates was transferred onto an amended lake water medium and sent out to be identified. This medium contained water filtered through a polycarbonate membrane filter (pore size, 0.4 µm) amended with 0.25% Bacto-Peptone and 1.5% Bacto-Agar. The cultured bacterium was then transferred onto and maintained on peptone agar (0.05% Bacto-Peptone with 1.5% Bacto-Arga) until experimentation.

The non-N_2-fixing cyanobacterium Lyngbya birgei, and the N_2-fixing cyanobacteria Aphanizomenon flos-aquae and Anabaena flos-aquae were also isolated from essential unialgal bloom at the surface water of Hebgen Lake. These cyanobacteria dominated the column of Hebgen Lake during July an August 1990 when the current experiments were conducted. The cyanobacteria were maintained under about 200 μE m^{-2} s^{-1} at lake temperature and used for experiments within three days after collection.

Preparation of the Bacterium and the Cyanobacteria for the Experiments.

Aeromonas hydrophila was grown on peptone agar (0.5% Bacto-Peptone with 2.0% Bacto-Agar) for 24 hours at room temperature (ca. 23 ^{o}C) and transferred into lake water (filtered through a polycarbonate membrane filter with pore size of 0.2 μm and autoclaved under 1.1 kg cm^{-2} pressure for 15 minutes [here after referred to as filter-sterilized water]), amended with 0.25% Bacto-Peptone, 50 μM EDTA and 3 mM methionine. The additions of EDTA and methionine were to ensure the motility of the bacteria (Adler 1973; Gallucci and Paerl 1983). This liquid culture was grown in a 125-ml Erlenmeyer flask at 23 ^{o}C on a G10 Gyrotory shaker at 60 rpm to a bacterial density of approximately 10^{10} cells ml^{-1}. The bacterial culture was again transferred (1 ml in 100 ml of medium) into filter-sterilized lake water (lacking Bacto-Peptone) containing 50 μM EDTA, 3 mM methionine, and 100 nM ^{3}H-thymidine (80.7 Ci mmol^{-1}) and grown at condition as described above for 2 hours. The labeled bacterial suspension was then filtered onto a sterile 0.2-μm pore size membrane filter under gentle vacuum and washed 5 times with filter-sterilized lake water. The bacterial cells on the filter were resuspended in filter-sterilized lake water with 50 μM EDTA and 0.005% (wt/vol) Tween 80 to bring the bacterial density to 8.0 x 10^8 cell ml^{-1}. The addition of Tween 80 was to prevent bacterial attachment to surfaces (Adler 1973).

The cyanobacterial samples from essentially unialgal blooms of Anabaena flos-aquae, Aphanizomenon flos-aquae, and Lyngbya birgei (confirmed by microscopic observation) were gently filtered onto polycarbonate membrane filters (pore size, 8 μm). The cyanobacteria on the filters were rinsed with filter-sterilized lake water three times and then gently passed through 18- and 20-gauge sterile hypodermic needles fitted on 20-ml syringes. The expressed sample was washed five more times on an 8-μm-pore-size membrane filter with filter-sterilized lake water. This procedure removed most of the bacteria and debris associated with the cyanobacteria while dispersing the aggregates without causing visible damage to the filaments. The dispersed cyanobacteria were resuspended in filter-sterilized lake water into different concentrations and preincubated at 25 °C and 200 $\mu E\ m^{-2}\ s^{-1}$ for three hours before the chemotaxis assay.

Chemotaxis Apparatus

The chemotaxis reservoir for the labeled bacteria was Tygon tubing (formulation R-3603; VWR Scientific) with an inner diameter of 0.8 cm and a wall thickness of 0.16 cm. Holes used to insert syringes were made along the tube by a heated steel wire. The distance between adjacent holes was 5 cm. Before each assay, the tube was autoclaved at a pressure of 1.1 kg cm^{-2} for 10 minutes and then positioned on a plywood board covered by absorbing paper by thin wires so that the holes were parallel to the plane of the board. One end of the tube with 5-cm extension from the last hole was closed with a metal clamp and the other end with the same extension length was raised slightly by a piece of cardboard (Figure 34).

Chemotaxis Assay Procedures

The preincubated cyanobacterial suspensions were degassed using a vacuum of 120 mm Hg for about 5 minutes to remove existing bubbles and prevent their formation in the medium. After gentle mixing to ensure homogeneous suspensions, 0.5 ml of the cyanobacteria from each density were drawn into a sterile, disposable glass syringe (Glaspak B-D; Becton Dickinson, Rutherford, N.J.) of 2.5 ml capacity.

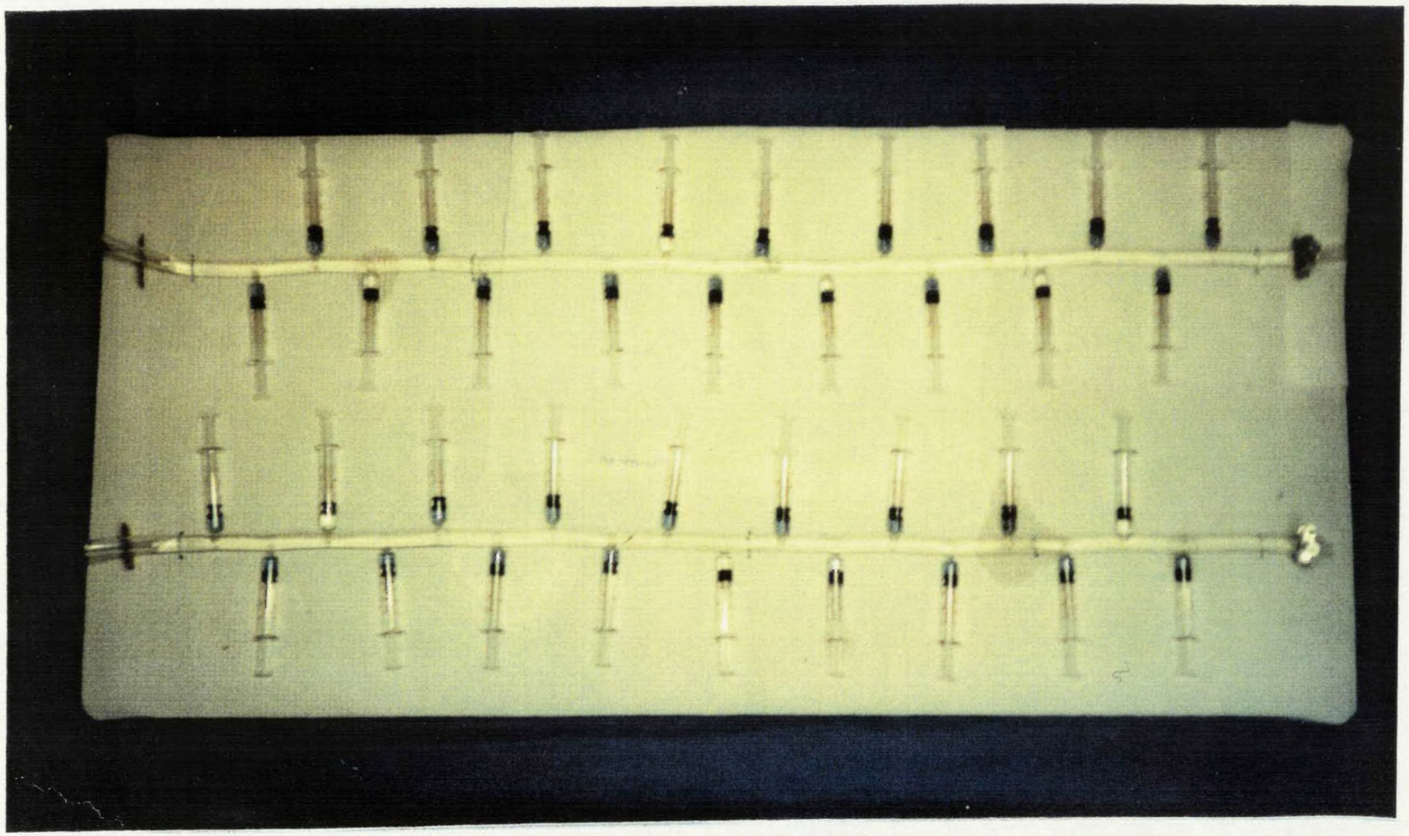

Figure 34. Apparatus used to assess the chemotactic attraction of bacteria to cyanobacteria.

Care was taken to ensure there was no bubble formation inside of the syringe. The tips of two or three replicate syringes of each cyanobacterial density were randomly inserted into the holes on the tubing. Two or three syringes filled with 0.5 ml degassed filter-sterilized lake water were inserted into the same tubing and used as controls.

The syringes were mounted horizontally on the plywood board following adjustment to ensure that the syringe contents completely filled to the tips of the syringes. The freshly prepared radiolabeled bacterial cell suspension was then slowly introduced into the tubing through the open end to fill all but the last 2 cm of the tubing. Care was taken to ensure that no air bubbles which could trap bacteria were introduced into the tubing. The open end of the tubing was then sealed using a metal clamp. The whole apparatus was incubated horizontally at 23 ± 1 ^{o}C for three hours in the dark.

Two initial cyanobacterial samples from each of the densities were taken to determine chlorophyll a concentration using an ethanol extraction method (Sartory and Grobbelaar 1984).

Two initial radiolabeled bacterial samples were taken and immediately fixed using 5% formaldehyde to determine the initial radioactivity. Bacterial cell numbers on the same samples were also determined using acridine orange direct counts (Hobbie et al. 1977).

After 3 hours of incubation, the syringes were removed from the Tygon tubing and their tips were carefully rinsed with deionized water. The contents of the syringes were transferred to separate scintillation vials with 3% formaldehyde (final concentration). Ten ml of CytoScint scintillation cocktail were added to each of the vials. The radioactivity of the samples was determined by standard liquid scintillation spectrometry using a Beckman LS-100C liquid scintillation counter. The counting efficiency was determined by the external standard ratio method using ^{3}H-toluene as a reference and acetone as the quench agent.

The experiments were conducted using freshly collected Anabaena flos-aquae, Aphanizomenon flos-aquae, and Lyngbya birgei separately during July 1990. The same procedures were repeated using all the 3 freshly collected cyanobacterial species in August 1990.

Results

The effects of increasing chlorophyll a concentrations of Anabaena flos-aquae, Aphanizomenon flos-aquae and Lyngbya birgei on the attraction of A. hydrophila are shown in Figures 35 and 36. The responses of the A. hydrophila to the cyanobacteria were measured in terms of disintegrations of ^{3}H per minute (dpm) accumulating in the syringes. Simple linear regressions on the mean of the replicates of dpm with

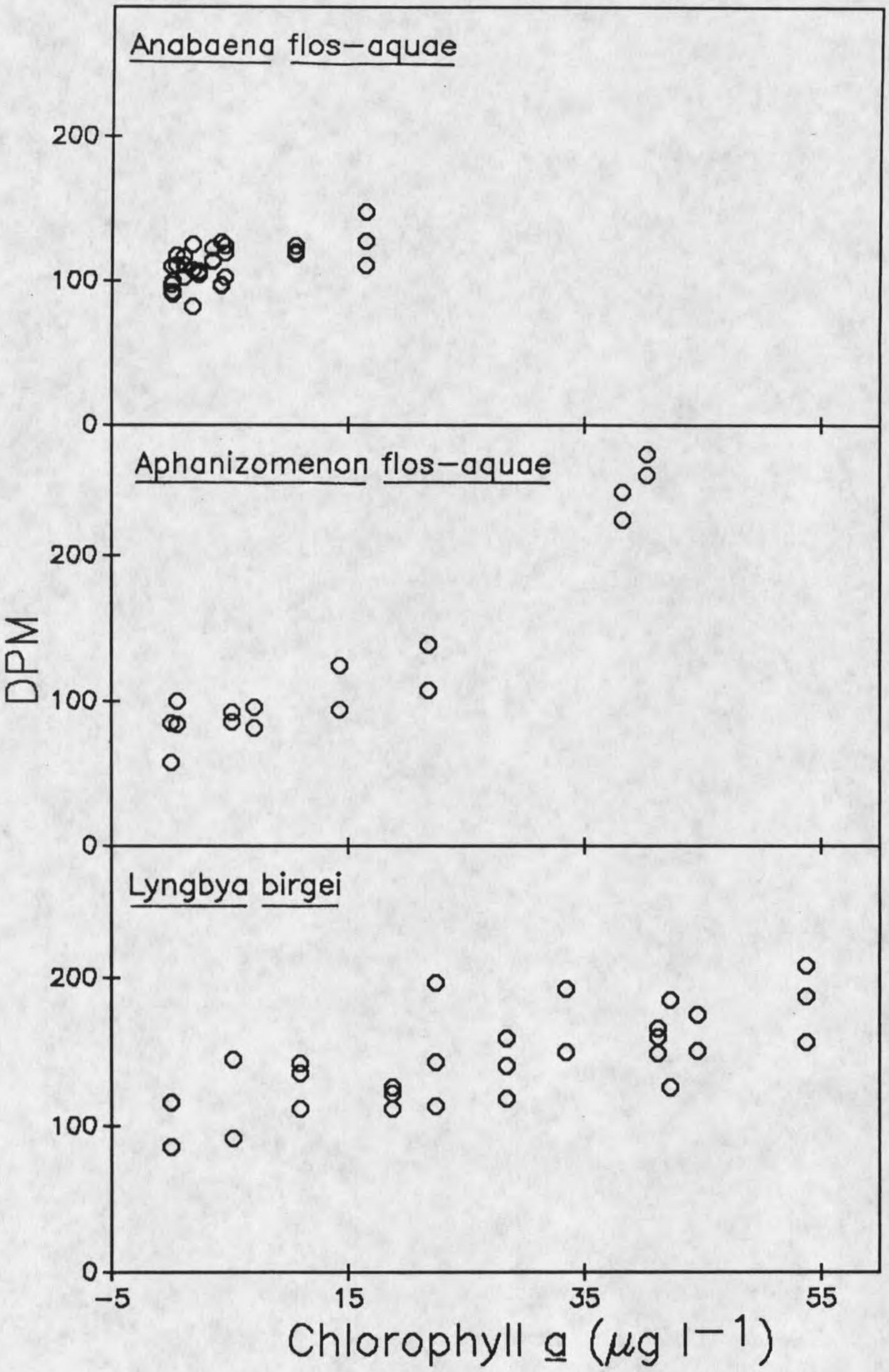

Figure 35. Chemotactic response of A. hydrophila to various concentrations of Anabaena flos-aquae, Aphanizomenon flos-aquae, and Lyngbya birgei (expressed by chlorophyll a) in the July experiment.

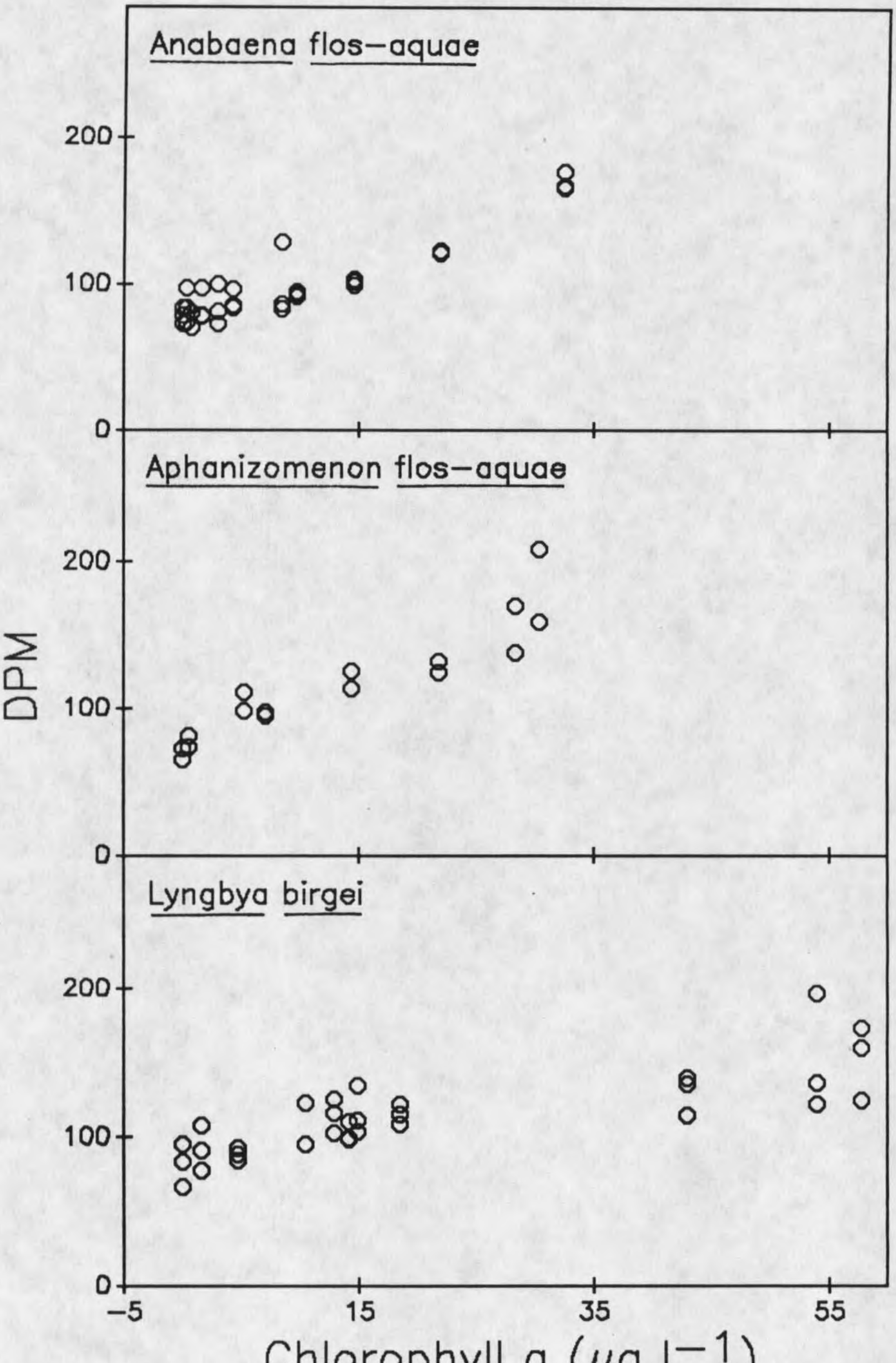

Figure 36. Chemotactic response of A. hydrophila to various concentrations of Anabaena flos-aquae, Aphanizomenon flos-aquae, and Lyngbya birgei (expressed by chlorophyll a) in the August experiment.

chlorophyll showed that the accumulations of radiolabeled bacteria in the syringes were significantly correlated with the cyanobacterial concentrations for all the 3 tested species (Table 15). The regression slopes for Anabaena flos-aquae were 2.41 and 3.75, for Aphanizomenon flos-aquae were 2.94 and 4.29, and for Lyngbya birgei were 1.07 and 1.33 dpm (μg chlorophyll a)$^{-1}$ at the 2 different month experiments (Table 16).

The chemotactic attractions of A. hydrophila by different cyanobacterial species were compared by comparison of linear regression slopes (Dixon and Massey 1983). Radiolabeled A. hydrophila were attracted more strongly ($p<0.01$) to Anabaena flos-aquae and Aphanizomenon flos-aquae than by Lyngbya birgei (Table 16). The attraction difference between Anabaena flos-aquae and Aphanizomenon flos-aquae was not statistically significant ($p>0.1$, Table 16).

Discussion

The chemotactic apparatus has been tested before the actual experiments. Neutral red solution was placed in the syringes and water in the Tygon tubing and vice versa to verify that there was no significant bulk transport of the liquid from the syringes into the tubing or from the tubing into the syringes. The chemotactic apparatus used in this study allowed the use of a relatively large quantity of test materials compared with the amounts used in the capillary

Table 15. Simple linear correlation coefficients (r) between chlorophyll *a* concentration in the syringes and the mean dpm of two or three replicates of radiolabeled bacteria attracted into the syringe for a given chlorophyll concentration in July and August experiments.

	July experiment			August experiment		
Cyanobacteria	n	r	p-value	n	r	p-value
Anabaena flos-aquae	11	0.772	0.005	11	0.967	0.000
Aphanizomenon flos-aquae	8	0.936	0.000	8	0.948	0.000
Lyngbya birgei	11	0.824	0.000	11	0.952	0.000

Table 16. Simple linear regression slopes of chlorophyll _a_ concentrations in the syringes and dpm of the radiolabeled bacteria attracted to the syringes in the July and August experiments.

	Ana* v. Aph*	Ana v. Lyn*	Aph v. Lyn
July experiment			
Slope	2.41 v. 2.94	2.41 v. 1.07	2.94 v. 1.07
p-value	>0.10	<0.01	<0.01
August experiment			
Slope	3.75 v. 4.28	3.75 v. 1.33	4.28 v. 1.33
p-value	>0.10	<0.01	<0.01

* Ana = _Anabaena flos-aquae_, Aph = _Aphanizomenon flos-aquae_, Lyn = _Lyngbya birgei_.

tube method (Adler 1973; Wellman and Paerl 1981). The inner diameter of syringe tip used in this assay apparatus was 0.9 mm, which is comparable with that of capillary tubes used in the previous studies (Adler 1973; Wellman and Paerl 1981; Gallucci and Paerl 1983). This small diameter prevents bulk flow of liquid in and out of the syringe. However, the syringe capacity is large, which allows a homogeneous large cyanobacterial sample to be used.

Microscopic observation on the same bacterial sample kept in a petri dish during the chemotactic assay has shown that active motility of the bacteria was maintained in the suspension for at least four hours. Degassing the cyanobacterial suspensions before starting the experiment avoided gas bubble formation in the syringe contents eliminating the possible impediment on bacterial movement. The EDTA addition was to chelate certain heavy metals to eliminate their possible impacts on the bacterial motility. Tween 80 was to prevent bacterial attachment to surfaces (Gallucci and Paerl 1983). Methionine was added because it has been known that chemotaxis of some strains of bacteria are stimulated in the presence of methionine (Adler 1973). The reason for carrying out this experiment in the dark was because preliminary experiments showed that gas bubbles were produced in cyanobacterial suspensions in the syringes when incubated under light (presumably through photosynthesis).

The significant correlation between cyanobacterial chlorophyll concentrations and the amounts of radiolabeled bacteria attracted to them implies that cyanobacterial extracellular products establish a chemoattractive gradient (phycosphere) to which the bacteria respond. This result is consistent with results obtained from alanine, glycine, serine, and threonine, the constituents in the extracellular products of blue-green algae (Gallucci and Paerl 1983). My result also conforms to the result obtained from filtrates of old algal cultures (Bell and Mitchell 1972). The results reported here, in conjunction with those reported in the literature, imply that the phycosphere produced by the phytoplankton influences the abundance and composition of the bacterial population by releasing extracellular products. The growth and location of the bacterial population may also be influenced by the presence of phytoplankton.

The highly significant differences between the cyanobacterial species from comparing linear regression slopes imply that A. hydrophila responses to different species of cyanobacteria are different. The two N_2-fixing cyanobacterial species, Anabaena flos-aquae and Aphanizomenon flos-aquae, showed stronger response by A. hydrophila than the non-N_2-fixing species Lyngbya birgei does. The responses of A. hydrophila to the two N_2-fixing species, Anabaena flos-aquae and Aphanizomenon flos-aquae, were essentially the same. These differences may have been caused by the additional products of N_2 fixation; bacterioplankton have been shown to be strongly N-deficient in Hebgen Lake (see Chapter 3).

This study provides evidence of chemotactic attraction of A. hydrophila to three species of cyanobacteria isolated from Hebgen Lake. The higher the cyanobacterial densities, the more radiolabeled bacterial cells were attracted to them. Anabaena flos-aquae and Aphanizomenon flos-aquae attracted more bacterial cells than Lyngbya birgei. These results confirmed the phycosphere theory, in which bacterioplankton utilize organic matter in close metabolic and spatial coupling with sources of organic matter (Azam and Ammerman 1984). This bacterioplankton-phytoplankton relationship within the phycosphere may serve to support bacterial production and to maintain phytoplankton bloom in freshwater ecosystems.

CHAPTER 7

LABORATORY EVIDENCE OF TEMPERATURE INFLUENCE ON BACTERIOPLANKTON

Introduction

The relationship between bacterial growth and temperature has been a subject of considerable interest among microbial ecologists. In early studies, many efforts have been made to characterize temperature properties of different groups of bacteria (e.g., Ingraham 1958; Baig and Hopton 1969). Most studies of temperature-bacterial relationship have been done on cultured organisms (e.g., Christophersen 1973; Van Uden 1984). Since the recognition of the importance of bacteria as sources of organic carbon in the microbial loop, water temperature has been recognized as a major factor regulating the abundance and production of bacterioplankton in aquatic ecosystems (Coffin and Sharp 1987; Joint and Pomeroy 1987; Robarts and Wicks 1990). However, the role of water temperature as a factor regulating the growth of microbial population has received relatively little attention (Lovell and Konopka 1985) and demands further investigation. In particular, information is scarce on the temperature regulation of interactions among phytoplankton biomass, production and the supply of nutrients to bacterial populations (Pomeroy and Wiebe 1988; Robarts and Wicks 1990).

The aim of this chapter is to 1) estimate optimum temperature of summer and winter bacterioplankton population; 2) evaluate temperature influence on maximum density of bacterioplankton; 3) appraise the interacting influence of temperature and nutrient supply on bacterioplankton density and growth.

Materials and Methods

Abundance Response

Aeromonas hydrophila (identity confirmed by Microbial ID, Inc, Newark, Del.) was isolated from the surface water of Hebgen Lake in August 1989. This species was the numerically dominant type (on the basis of Gram staining and cell and colony morphology during dilution plating) among those isolates. A. hydrophila was maintained on peptone agar (0.05% Bacto-Peptone with 1.5% Bacto-Agar) under room temperature (ca. 18-30 °C) in the dark until experimentation.

Experiments were carried out during August 1991. A. hydrophila was grown in 0.5% Bacto-Peptone in filtered (pore size, 0.2 μm) and autoclaved lake water, at 22 °C on a shaker in the dark, to a bacterial density of approximately 10^8 cell ml^{-1}. The culture was transferred into six 250-ml Erlenmeyer flasks filled with 200 ml filtered (pore size, 0.2 μm) and autoclaved lake water to get a final bacterial density of $0.1x10^6$ cell ml^{-1}. Two of the flasks were enriched with a combination of 150 μg NH_4^+-N, 50 μg PO_4^{3-}-P, and 4 mg glucose-C l^{-1} in addition to background nutrient concentration (NPC treatment). Another two of the flasks were enriched with 0.01% Bacto-Peptone (bacto-peptone treatment). The remaining two flasks filled with lake water were used as lake water treatment. The six flasks were incubated in a temperature controlled incubator at 4 ± 1 °C on a variable speed rotator at 50 rpm in the dark. Bacterial cell number from each flask was determined every 20-24 hours until a maximum density was reached. The above procedures were repeated at 10, 16, 22, 28, 31, and 34 °C.

Activity Response

A polythermostat, modified from Romanenko (1982), consisted of a long trough made by transparent plastic. The trough was divided into six equal-sized chambers labeled from I to VI. An ice-cold water inlet (using tap water mixed with ice and stored in a cooler as a water source) was connected to chamber I. A hot water inlet (using hot tap water [ca. 55 °C] stored in a cooler as a hot water source) was led to each chamber from II to VI. There were two small holes on each of the chamber divisions, which allowed a small portion of the water to flow from chamber I to VI. An outlet was connected on chamber VI. A stirrer located underneath each chamber allowed the water to be well mixed and a uniform water temperature was obtained in each of the chambers. By adjusting the amount of water from the hot and cold inlets, a desired water temperature (±1.5 °C) was obtained in a given chamber (Figure 37).

Twenty liters of Hebgen Lake water were collected in August 1991 at 0.5 m depth from the surface of the Grayling Arm and returned to the laboratory at the temperature of collection. The lake water was filtered through 1.0 μm filters to remove most organisms larger than bacteria. Ten ml of the filtered lake water were added to each of 36 20-ml glass scintillation vials. A combination of 150 μg NH_4^+-N, 50 μg PO_4^{3-}, and 4 mg glucose-C l^{-1} in final concentration in addition to the background nutrient level was added to half of the vials and referred to as NPC treatment. The other half of the vials contained unenriched lake water and were referred to as lake water treatment. Three vials from the NPC treatment and three vials from the lake water treatment were preincubated in one of the six chambers with water temperature of 4, 10, 16, 22, 28, 34 °C for two hours. After the preincubation, bacteria in two (one NPC treatment, one lake water treatment) of the six vials in each of the chambers were killed by adding 0.3 ml formaldehyde into each vial. The sample in each of the 36 vials was inoculated with high activity (55 Ci mmole^{-1}) methyl-^{3}H thymidine (ICN Radiochemical INC.) to a final concentration of 20 nM. ^{3}H-thymidine was evaporated to dryness and rehydrated with deionized water before use to eliminate products of self radiolysis and to remove ethanol. The inoculated sample was

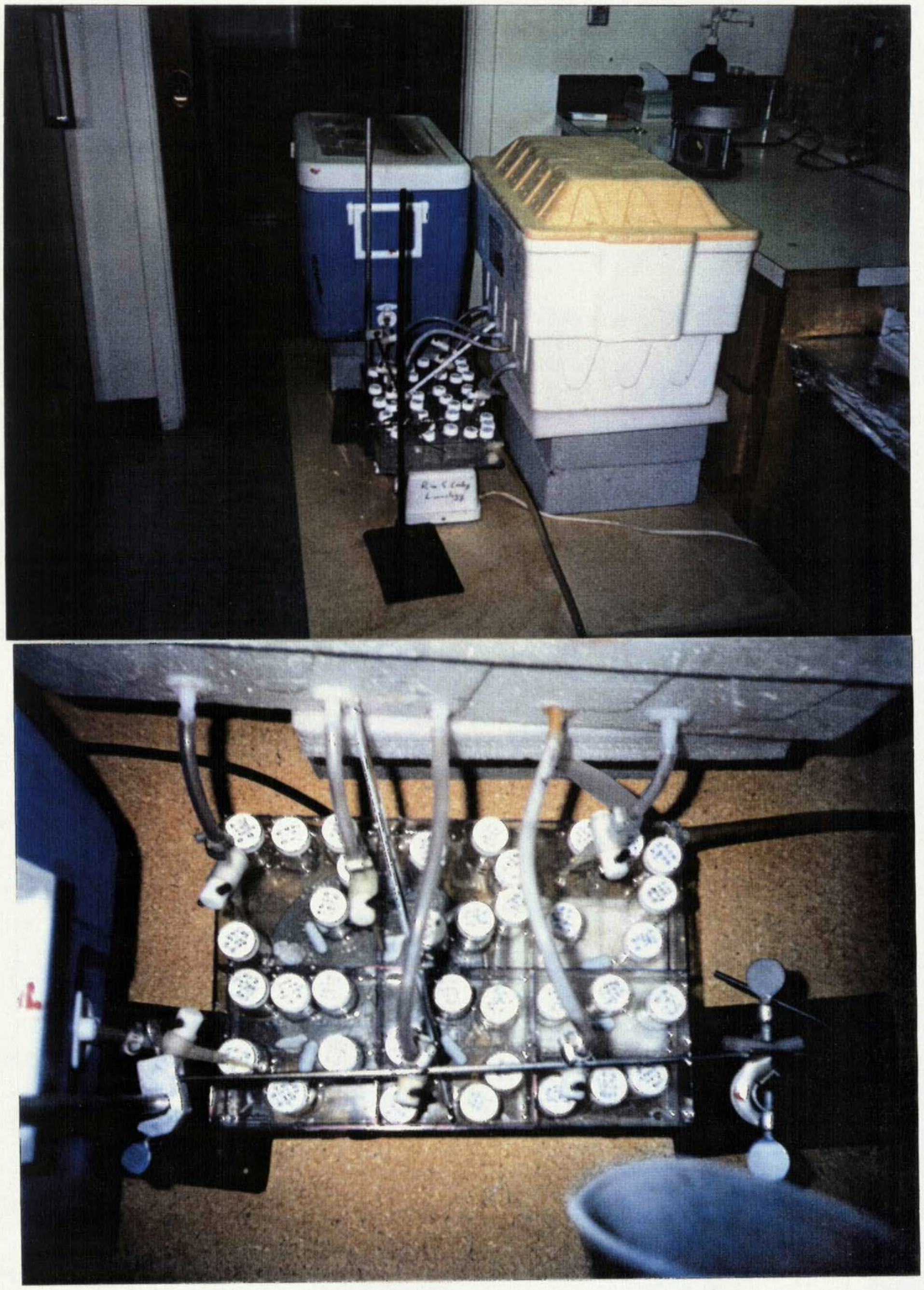

Figure 37. Polythermostat used to assess the temperature response of bacterioplankton.

incubated in the dark for 60 minutes. Activity was terminated by adding 10 ml ice-cold 10% trichloroacetic acid (TCA) to each vial. Following overnight extraction at 4 °C, samples were filtered onto 0.2 μm membrane filters (Poretics Corporation). After rinsing 5 times (2 ml each rinse) with ice-cold 5% TCA, the filter was transferred to a 20 ml polyethylene scintillation vial with 7.0 ml Cytoscint scintillation cocktail (ICN Radiochemical, Irvine, CA). Radioactivity in each sample was determined by standard scintillation spectrometry using a Beckman LS-100C liquid scintillation counter. Counting efficiency was determined by the external standard ratio method using ^{3}H-toluene as reference and acetone as the quenching agent. The formaldehyde killed vials were used to correct for non-biosynthetic ^{3}H-thymidine assimilation. This procedure was repeated at 7, 13, 19, 25, 31, and 37 °C.

The above experiment was repeated using a water sample collected in November 1991 and the cultured bacteria, _A. hydrophila_. The basic media for _A. hydrophila_ experiment was filtered (0.2 μm) and autoclaved lake water from the November sampling.

Biological and Chemical Measurements

Concentrations of total nitrogen (TN), total phosphorus (TP), dissolved organic carbon, and bacterial cell numbers for the filtered lake water were measured before the experiments. TN concentrations after persulfate digestion were determined as nitrate using the Cd reduction method (Eppley 1978). TP concentrations after persulfate digestion were measured by an orthophosphate method (Stainton et al. 1977). Dissolved organic carbon (DOC) concentrations were analyzed with a Dorhmann carbon Analizer using glucose as standard. Bacterial cell concentrations were determined on samples filtered onto 0.2 µm polycarbonate filters, stained with acridine orange, and counted using epifluorescence microscope (Hobbie 1977).

Calculation of the Temperature Characteristics

The energy activation (E) of bacterial biosynthesis was calculated in each experiment by the Arrhenius equation:

$$v=Ae^{-E/RT} \tag{1}$$

where v is the metabolic activity. For the abundance response experiment, v (equivalent to k) was calculated by the following equation during the exponential growing phase:

$$N_t=N_oe^{kt} \tag{2}$$

where N_o and N_t are the initial and final bacterial density (cell ml^{-1}), t is time (h), K is growth rate (cell ml^{-1} h^{-1}).

For the activity response experiment, v was the ^{3}H-thymidine incorporation rate (nmol l^{-1} h^{-1}). A is a constant (same unit as v), E is the temperature characteristic or activation energy of the reaction (cal mol^{-1}), R is the gas constant (1.987 cal $^oK^{-1}$ mol^{-1}), and T is the absolute temperature (^{o}K) of the reaction. The slope of a linear regression of ln v against 1/T, for the linear part of the curve leading up to the optimal temperature, was used to determine E. The Q_{10} values were calculated from E value by equation (3):

$$\ln Q_{10} = \frac{E(T_2 - T_1)}{RT_1T_2} \qquad (3)$$

where Q_{10} is the factor by which the rate changes upon varying the temperature 10 °C, and T_1 and T_2 are the temperature limits (°K) from which Q_{10} is desired.

Results

Temperature Optima of Different Bacterioplankton Populations

The optima growth temperature of summer bacterioplankton population (23 °C origination) was about 29 °C. The optima growth temperature of winter bacterioplankton population (2.1 °C origination) was 25 °C and that of room temperature (20 °C) cultured bacterial population was 27 °C (Figures 38 and 39, Table 17). The temperature optima differences among the tested bacterial populations reflected the ambient water temperature where they were originated. The temperature optima of A. hydrophila obtained from ^{3}H-thymidine incorporation and cell change were essentially the same. Nutrient additions had no effect on optimum growth temperature of bacterioplankton regardless of their temperature originations (Figures 38 and 39).

The bacterioplankton optimal growth temperatures in all my experimental treatments were greater than the ambient water temperature from which the bacteria were originated. The difference between optimal growth temperature for different bacterioplankton origination was smaller than the difference between the ambient water temperature from which they were originated (Table 17).

The energy of activations of bacterioplankton growth (E) from winter populations were greater than those from summer populations. Such differences were not significantly affected by nutrient additions. The Q_{10} values for bacterioplankton growth of winter population were higher than those of summer populations. The trends for E values were paralleled by Q_{10} values because of the proportional relationship between E and Q_{10} (Table 17).

Temperature Effect on Bacterioplankton Maximum Density

The maximum *A. hydrophila* density under different temperatures and nutrient conditions are given in Table 18. Under ambient nutrient conditions (Table 19), incubation temperature (4-34 ^{o}C) had no significant effects on bacterioplankton maximum density. However, in the NPC treatment or bacto-peptone treatment, incubation temperature showed significant effect on bacterioplankton maximum density. The maximum bacterioplankton density in NPC

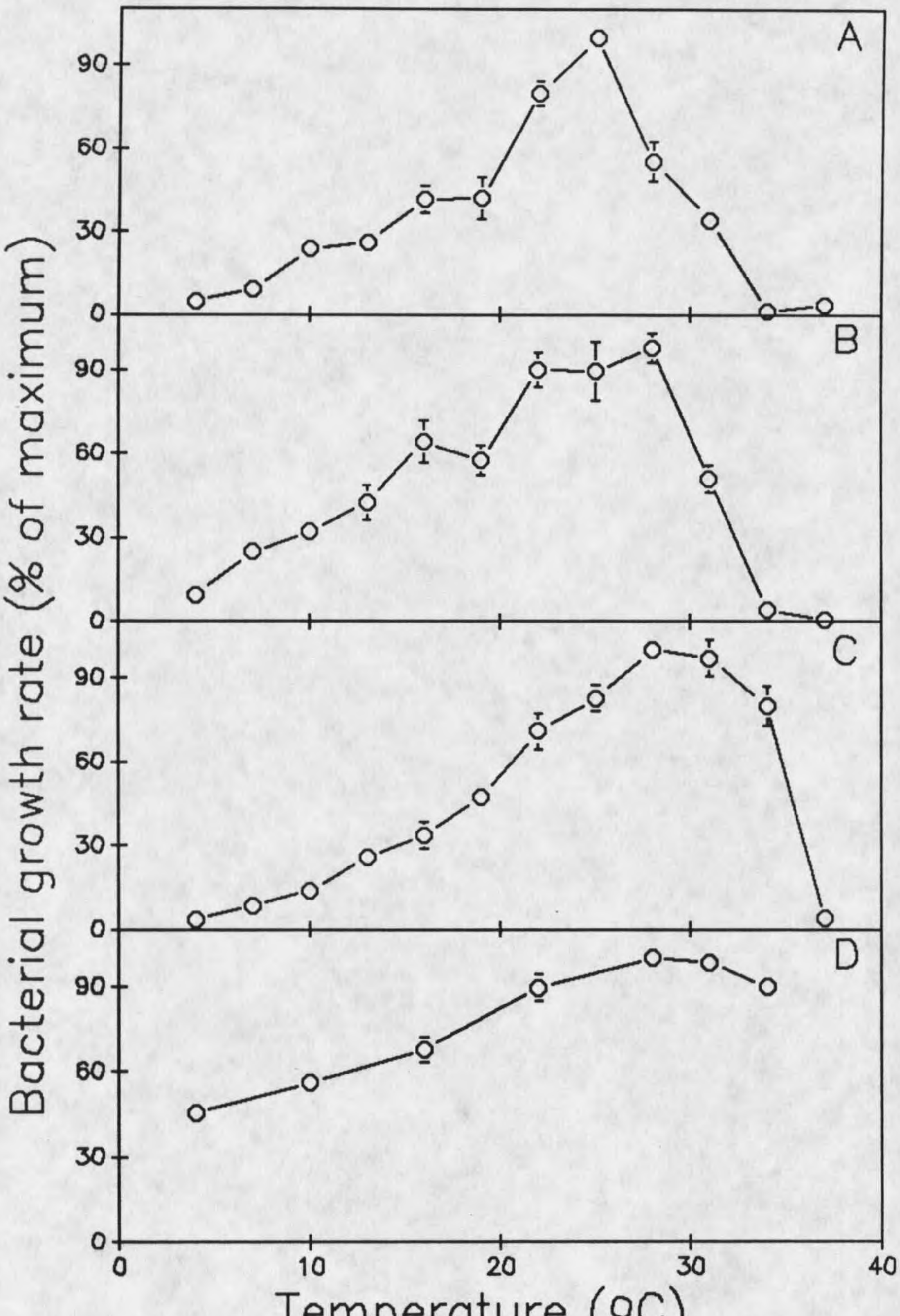

Figure 38. Optimal growth temperature of different bacterioplankton populations from Hebgen Lake in the lake water treatment experiment. (A) winter population (thymidine incorporation), (B) summer population (thymidine incorporation), (C) cultured population (thymidine incorporation), and (D) cultured population (cell increments).

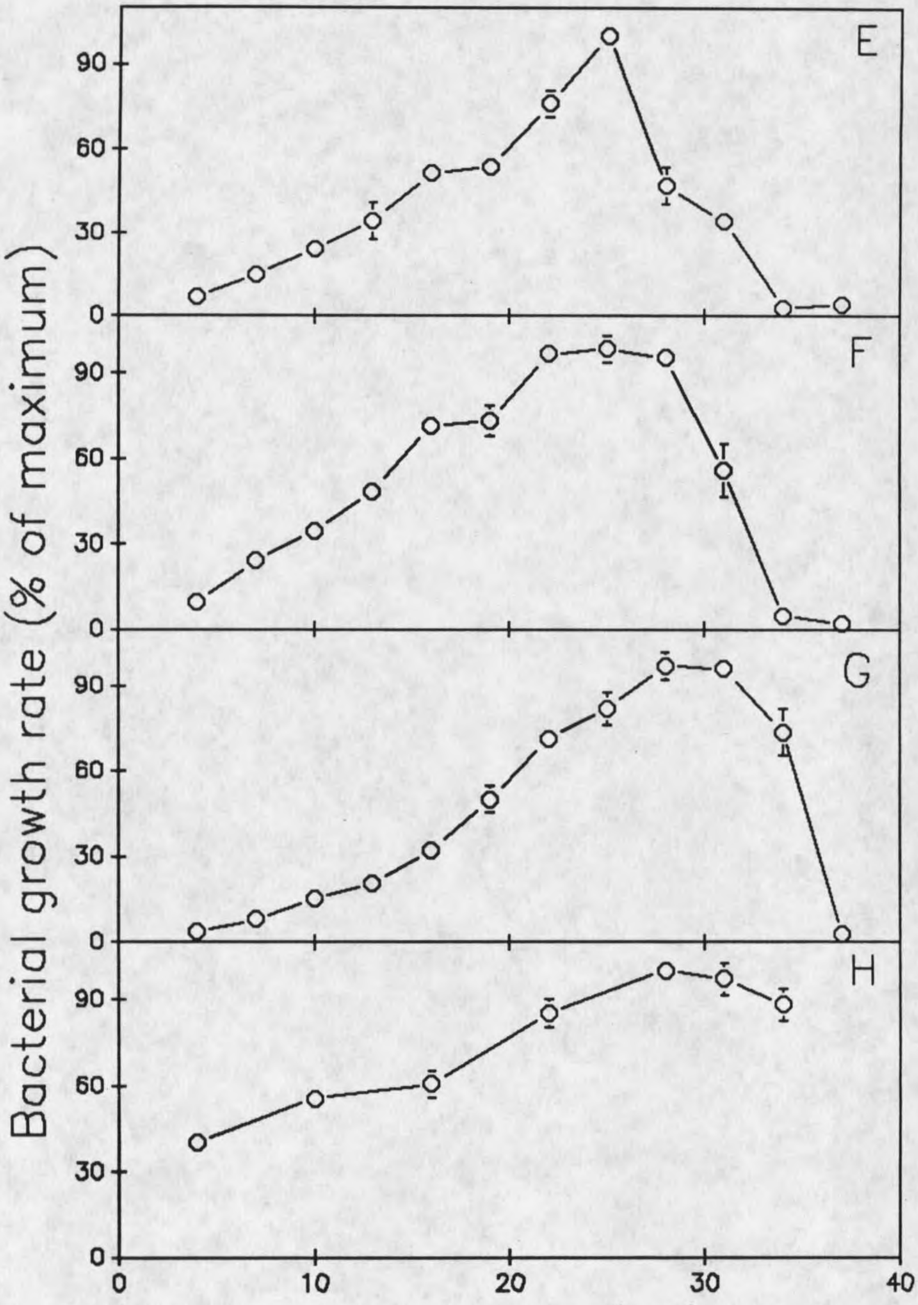

Figure 39. Optimal growth temperature of different bacterioplankton populations from Hebgen Lake in the nutrient enrichment experiment. (E) winter population (thymidine incorporation), (F) summer population (thymidine incorporation), (G) cultured population (thymidine incorporation), and (H) cultured population (cell increments).

Table 17. _In situ_ temperature (^{o}C), optimal temperature of growth (^{o}C), the energy of activation of growth (E in cal mol^{-1}), and Q_{10} values of growth for bacterioplankton populations from Hebgen Lake. The temperature bounds from which E and Q_{10} were dirived range from 4 ^{o}C to the optimum temperature.

Treatment	Origin temperature	Optimal temperature	E	Q_{10}
Summer lake water[1]	19.8	27	3668	49.2
Summer NPC addition[1]	19.8	27	3721	49.9
Winter lake water[1]	2.1	25	5618	75.4
Winter NPC addition[1]	2.1	25	4922	66.1
A. hydrophila lake water I[1]	22.0	28	5113	68.6
A. hydrophila NPC addition I[1]	22.0	28	5189	69.6
A. hydrophila lake water II[2]	22.0	28	3616	48.5
A. hydrophila NPC addition II[2]	22.0	28	1590	21.3
A. hydrophila bacto-peptone addition[2]	22.0	28	2280	30.6

1 E and Q_{10} values were calculated from ^{3}H-thymidine incorporations.
2 E and Q_{10} values were calculated from cell increments.
I Experiment for activity response.
II Experiment for abundance response.

Table 18. The maximum density and the hours taken to reach the maximum density for A. hydrophila incubated at different temperatures and nutrient conditions.

	Lake water		NPC addition		Bacto-peptone addition	
Temperature (°C)	(x10^6 ml^{-1} ± 1SE)	(h)	(x10^6 ml^{-1} ± 1SE)	(h)	(x10^6 ml^{-1} ± 1SE)	(h)
4	2.9±1.3	144	3.2±1.7	144	46.6± 9.8	192
10	2.6±0.2	72	4.2±0.5	72	177.8± 0.6	96
16	3.1±0.9	48	4.4±0.2	48	255.8±15.2	96
22	2.8±0.3	52	18.2±1.4	60	258.7±39.8	72
28	2.8±0.5	20	13.3±2.8	20	429.7±28.0	72
31	2.8±0.4	20	11.1±0.8	20	428.6±13.4	54
34	2.5±0.4	20	8.0±1.1	20	197.9±40.6	54

Table 19. Background nutrient concentrations ($\mu g\ l^{-1}$) and bacterioplankton abundances (10^6 cell ml^{-1}) for the bacterioplankton abundance response experiment and activity response experiment.

Experiment	TN	TP	DOC	Bacterioplankton
Summer activity response	236	27.5	2760	2.31
Winter activity response	303	39.4	5029	0.45
<u>A. hydrophila</u> activity response	303	39.4	5029	NA
<u>A. hydrophila</u> abundance response	198	24.2	2231	NA

NA=not applicable.

treatment at 22 °C was 5.7 times higher than that of 4 °C and 2.3 times higher than that at 34 °C. The maximum bacterioplankton density in bacto-peptone treatment at 28 °C was 9.2 times greater than that at 4 °C and 2.2 times greater than that at 34 ° C (Table 18).

The higher the incubation temperature, the fewer hours it took to reach maximum bacterioplankton density. The hours taken to reach maximum density at 4 °C was 7.2 times longer than at 34 °C in both lake water and NPC treatments. It took 3.6 times longer at 4 °C than at 34 °C to reach maximum bacterioplankton density in the bacto-peptone treatment (Table 18).

The higher the maximum bacterioplankton density, the longer it took to reach the maximum density when the bacteria were incubated in the same temperature. Such result was obvious when comparisons were made between bacto-peptone treatment and lake water or NPC treatments. The difference of in time taken to reach maximum bacterioplankton density between lake water and NPC treatments is difficult to resolve because the observation interval was too long to allow the detection of relatively small differences (Table 18).

Interacting Influence of Temperature and Nutrient on Bacterioplankton Growth and Maximum Density

The optimum temperature for maximum bacterioplankton density increased with an increase of nutrient level. A.

hydrophila temperature optima were about 16 ^{o}C in lake water, 22 ^{o}C in NPC treatment, and 28 ^{o}C in bacto-peptone treatment (Table 18).

The NPC and bacto-peptone treatments significantly increased A. hydrophila maximum density compared with lake water treatment. The mean maximum density was 3.2 times greater in NPC treatment and 92.9 times greater in bacto-peptone treatment than that in lake water treatment (Table 20).

Nutrient addition significantly increased ^{3}H-thymidine incorporation of the summer bacterioplankton population but not winter population or cultured A. hydrophila (Table 20). The nutrient stimulation of ^{3}H-thymidine incorporation for the summer population occurred only in the temperature range of 10-28 ^{o}C (Figure 40).

Discussion

The optimal growth temperatures of the tested bacterioplankton were greater than the ambient water temperature when they were collected. Romanenko (1982), Joint and Pomeroy (1987), and Servais and Billen (1989) have reported a similar difference between optimal growth temperature and temperature in situ for both natural bacterial population and laboratory cultured bacteria. No explanations about such temperature difference have been made by these authors. The optimal temperature of

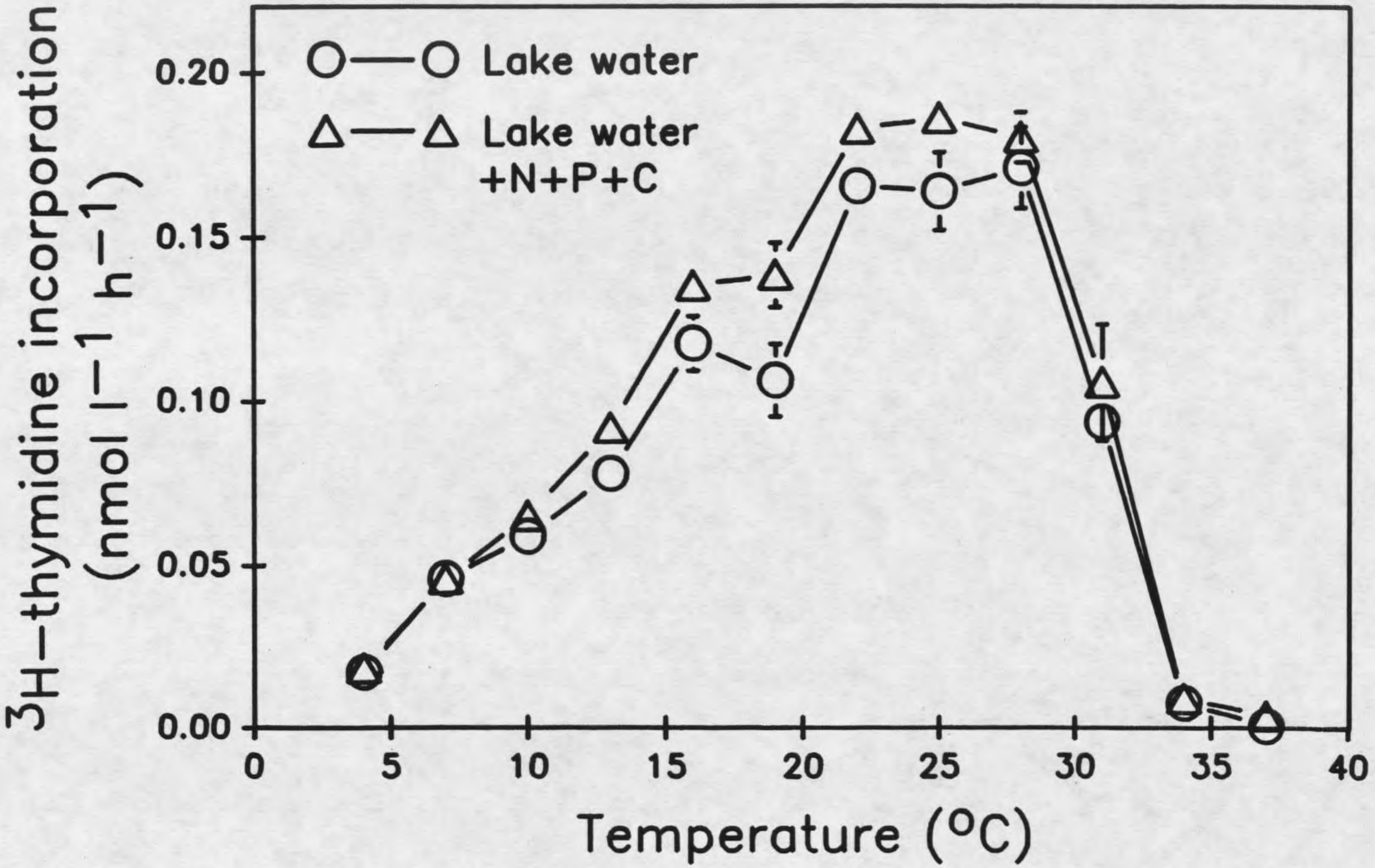

Figure 40. ^{3}H-thymidine incorporation of the summer bacterioplankton population from Hebgen Lake.

Table 20. Mean bacterioplankton ^{3}H-thymidine incorporation (nmol l^{-1} h^{-1}) and cell abundance (x10^{6} ml^{-1}) in different nutrient treatment experiments. NPC = lake water enriched with inorganic nitrogen, inorganic phosphorus, and organic carbon.

Experiment	Nutrient treatment	Mean±1SD	P-value*
Summer activity response	Lake water	0.104±0.058	
	NPC addition	0.116±0.063	0.0122
Winter activity response	Lake water	0.021±0.017	
	NPC addition	0.022±0.015	0.9337
<u>A. hydrophila</u> activity response	Lake water	0.068±0.055	
	NPC addition	0.070±0.057	0.2466
<u>A. hydrophila</u> abundance response	Lake water	2.786±0.195	
	NPC addition	8.914±5.573	0.0270
<u>A. hydrophila</u> abundance response	Lake water	2.786±0.195	
	bacto-peptone	137.4±137.4	0.0028

* Paired t-test on the mean of two replicates at each tested temperature.

phytoplankton photosynthesis has been shown to be related to the mid-summer maximum water temperature, which was interpreted as an adaptation for the organisms to be able to survive this temperature to remain in their habitat (Boylen and Brock 1974; Konopka and Brock 1978). Such a physiological strategy to maintain a temperature optimum higher than in situ to provide protection to phytoplankton from transient heating seems plausible for the studied bacterial populations. In a study in the English Channel, Joint and Pomeroy (1987) found that bacterioplankton populations from maximum temperature of 9-18 ^{o}C had a temperature optimum of 22 ^{o}C. Romanenko (1982) found that a bacterioplankton population from a maximum water temperature of 20 ^{o}C had a optimal temperature of 29 ^{o}C. The bacterioplankton populations from Hebgen Lake with a maximum summer water temperature of 20.6 ^{o}C had an average optimal temperature of 27 ^{o}C.

Ingram (1958) and Baig and Hopton (1969) reported that "warm-water" organisms tend to have higher E values than "cold-water" microorganisms. This trend is not clear in Hebgen Lake where winter population with a lower growth optimal temperature had a higher E value than a summer population with a higher growth optimal temperature. The cultured bacteria isolated during summer from Hebgen Lake appeared to have different E values under different nutrient conditions. Shaw (1967) and Hanus and Morita (1968), in studying yeast and bacteria, reported that there was no consistent difference in the E value of mesophiles and psychrophiles. These investigations together with my results, indicate that the absolute values of E, as Q_{10}, provide little information on determining the thermal characteristics of microorganisms.

Effects of temperature on bacterial growth are well known. As temperature increases, the growth rate rises from an undetectable level to an optimum and then falls back to an undetectable level. In some cases, there may be more than one optimum. The study of temperature influence on bacterial abundance has been done in a similar manner as that on bacterial growth. Pure cultured bacteria are incubated in a nutrient enriched medium for a given length of period at different temperatures. A temperature-density curve similar to growth can be obtained. As temperature increases, bacterial density rises to an optimum and then falls back to a minimum level. Although the optimal temperature obtained from measuring bacterial density was lower than that obtained from measuring growth rate (Christophersen 1973), both methods are essentially the same in measuring optimal temperature. Such measurements do not provide information on whether bacterioplankton abundance in a given ecosystem is limited by temperature or factors other than temperature.

My study used a density criterion that measures maximum bacterioplankton density in a given medium regardless of the incubation length, which is not commonly used in literature. The measured maximum density is presumably the maximum density that can be obtained in a given water with the absence of grazers' influence. Such information can only be obtained from pure bacterial culture experiment because of the difficulty in removing the grazing effects during such a long incubation period. When summer Hebgen Lake water was used as medium, the maximum bacterial densities obtained from different temperatures were not significantly different (no optimal temperature was found). This similarity of maximum bacterioplankton densities indicates that the maximum density was regulated by factors other than temperature. When the same lake water enriched with NPC or bacto-peptone was used as medium, an optimal temperature was found for maximum bacterioplankton density. This result indicates that the maximum bacterioplankton densities obtained from below or above the optimal temperature were temperature limited or inhibited. The results from lake water and nutrient enrichment treatments indicate that bacterioplankton maximum density in lake water treatment was nutrient limited.

The influence of temperature on bacterioplankton population size is complex (Christophersen 1973). Temperature affects not only the rate of cellular reaction involved in bacterioplankton growth, but also the environmental conditions where they live in (Sinclair and

Stokes 1963). The maximum bacterioplankton density in my experiment was the balance of growth and death. For a given temperature, the growth was over balanced death before the population reaches its maximum and the growth was off balanced death after the population reached its maximum. The bacterioplankton maximum density is the carrying capacity at that temperature, which was determined by nutrient and other environmental factors. When the same bacterial population was incubated in different nutrient levels but under the same temperature condition, the bacteria growing in the high nutrient medium had a higher maximum density and a longer incubation time to reach the maximum density than the bacteria growth in the low nutrient medium. For the population growing at different temperatures but the same nutrient condition, if sufficient nutrient is provided, a highest maximum density will be expected at the optimal temperature as in the case of my NPC and bacto-peptone treatments. However, if insufficient nutrient is provided, the growth advantage at the optimal temperature is overwhelmed by the nutrient limitation. As in the case of my lake water treatment, the maximum density showed no difference under the tested temperature range. These results imply that bacterial abundance is regulated by both temperature and nutrient. The suboptimal bacterial growth rate at suboptimal and superoptimal temperature may be explained by enzyme denaturation. It has been reported that the values of the activation energies in the normal range of growth were in the order of 3-20 kcal mol^{-1}. The activation energies calculated from the data in the range of temperature minimum were in the order of 30-100 kcal mol^{-1} and those at temperature maximum were in the order of 100-300 kcal mol^{-1} (Christophersen 1973).

Bacterioplankton abundance has been reported to decrease from estuarine interiors to coastal regions to ocean waters (Van Es and Meyer-Reil 1977). The reported bacterial numbers in eutrophic lakes (e.g., Hobbie and Wright 1979; Riemann et al. 1982) were also higher than those in oligotrophic lakes (e.g., Pedrós-Alió and Brock 1982; Scavia and Laird 1987). These reported results support the conclusion presented here on that higher nutrient level supports higher bacterioplankton density. The NPC enrichment stimulated ^3H-thymidine incorporation of the summer bacterial population only at 10-28 ^{o}C. At temperature below 10 ^{o}C the enrichment did not show any significant effect on bacterioplankton growth. A study conducted by Scavid and Laird (1987) showed a similar result that bacterioplankton growth rates were strongly related to temperature below 10 ^{o}C. Above 10 ^{o}C, the relationship with temperature was weak, and it was likely that substrate supply controlled growth in Lake Michigan. The NPC enrichment in Hebgen Lake winter water did not show any significant effect on winter bacterioplankton and the cultured bacterial populations at any of the tested temperature range. This may be caused by the high nutrient levels in the incubation medium (winter water, see Table 7.3).

Laboratory experiments conducted with summer and winter bacterioplankton populations and cultured isolate from Hebgen Lake showed that water temperature, in conjunction with nutrient level, has significant effects on bacterioplankton density and growth. All the bacterioplankton populations used in the experiments appear to be mesophilic according to their optimal growth temperatures (Morita 1975). All the tested bacterioplankton populations had higher optimal growth temperature (25-28 °C) than the temperature <u>in situ</u> (2.1-22.6 °C), which indicates the activity of these bacterioplankton populations are temperature limited, especially the winter population. However, NPC enrichment in the summer water significantly increased bacterioplankton growth only between 10-28 °C indicating the important influence of nutrient in this temperature range. My results indicate that bacterioplankton maximum density is determined by nutrient level at a given temperature if the impact of grazers is eliminated. Temperature showed significant effect on the maximum bacterial density of the isolate from Hebgen Lake only after the lake water was enriched with NPC. This result implies that the influence of temperature on maximum density is dominant at high nutrient level whereas the influence of nutrient is dominant at low nutrient level. Because the nutrient level and temperature in Hebgen Lake vary seasonally, the dominant factor controlling bacterioplankton density and growth is also a subject of variation.

CHAPTER 8

GENERAL CONCLUSIONS AND MANAGEMENT IMPLICATIONS

General Conclusions

Relative to the monitoring studies on the epilimnetic water column and river inflows of Hebgen Lake, the following major points could be drawn from the preceding chapters:

1). During the ice-free seasons of 1988 and 1989, Hebgen Lake bacterioplankton cell number ranged from 0.6×10^6 to 2.9×10^6 cell ml^{-1} (average = 1.7×10^6), which is in the range reported from eutrophic lakes (cf. Chapter 1). Bacterioplankton production varied between 0.09 and 0.82 μg C l^{-1} h^{-1} (average = 0.28), which is in the lower range reported from freshwater systems (cf. Chapter 1).

2). Water stratification had a significant effect on epilimnetic bacterioplankton production but not on bacterioplankton cell numbers (Chapter 2).

3). Regression analysis on data from the entire study period showed that the bacterioplankton variables (production, activity/cell, biomass, cell number) were most strongly correlated to water temperature, chlorophyll _a_ and PON (Chapter 2).

4). Regression analysis on data from different seasons showed that factors significantly correlated to bacterioplankton activity and biomass were water temperature in spring, TP and TN in summer, and TN and TOC in the fall

(Chapter 2).

5). River water had higher proportions of TP and TN than TOC, compared with epilimnetic lake water. Bacterioplankton activity and cell number were stimulated when lake water was enriched with river water (Chapter 2). Such results suggest that the supply of TP and TN from river water plays an important role in regulating bacterioplankton growth.

The monitoring results have provided general information on most objectives of this report. However, the conclusions are not robust and need to be further verified. The relationship between bacterioplankton parameters and environmental parameters obtained from regressions do not prove causality. Factors correlated to bacterioplankton growth are not necessarily factors regulating bacterioplankton growth and factors without correlation to bacterioplankton may not be necessarily unimportant for bacterioplankton. Such discrepancies became obvious when relationships between bacterioplankton parameters and NH_4^+, SRP, and DOC, the most preferred forms of nitrogen, phosphorus, and organic carbon by bacterioplankton, were examined. The lack of correlation between bacterioplankton parameters and NH_4^+, SRP, and DOC do not mean these nutrients are not important for bacterial growth, but imply that nutrients supply rates are far more important than their concentrations (Chapter 2). Therefore, several <u>in situ</u> and laboratory experiments were conducted to verify these uncertainties.

Regarding regulation of bacterioplankton growth by inorganic and organic nutrients, the following findings are worthy of reiteration:

1). Inorganic nitrogen and phosphorus are as important as organic carbon in the regulation of bacterioplankton growth. This result does not support the common view that the growth rates of bacterioplankton are limited by the availability of reduced carbon substrates (see Chapter 3 for discussion). This conclusion is consistent with the very recent findings from oligotrophic and meso-eutrophic lakes (Toolan et al. 1991; Coveney and Wetzel 1992).

2). The dominant nutrient regulating bacterioplankton growth varied among different seasons of the same year and the same season of different years. In four of my eight experiments, organic carbon alone significantly stimulated bacterioplankton growth. The dominant nutrient stimulating bacterioplankton growth was inorganic phosphorus alone, inorganic nitrogen and phosphorus, organic carbon and inorganic nitrogen, and inorganic nitrogen and phosphorus and organic carbon in one of the eight experiments, respectively (Chapters 3 and 4).

With respect to the relationship between bacterioplankton and phytoplankton, the following conclusions are inferred from the present study:

1). Bacterioplankton growth was enhanced in the presence of phytoplankton photosynthesis regardless of inorganic nitrogen and phosphorus additions (Chapter 4).

2). Bacterioplankton growth was increasingly stimulated by the increase of phytoplankton density (Chapter 5).

3). The movement of bacterioplankton toward phytoplankton was stimulated by the increase of phytoplankton density (Chapter 6).

4). Inorganic nutrient enrichments stimulated bacterioplankton growth both directly and indirectly via phytoplankton products (Chapters 3 and 4).

Finally, considering the influence of temperature on bacterioplankton growth, the following summarization can be made:

1). Bacterioplankton summer populations, winter populations and an isolate from Hebgen Lake had higher optimal growth temperature than the temperature <u>in situ</u>. Such a result indicates bacterioplankton growth is potentially limited by water temperature (Chapter 7).

2). The effect of nutrient on bacterioplankton growth was influenced by temperature. A combination of inorganic nitrogen and phosphorus and organic carbon enrichments in the summer lake water significantly increased bacterioplankton growth only at 10-28 ^{o}C. The same nutrient enrichment in winter lake water showed no significant effect on bacterioplankton growth, which was presumably caused by higher nutrient content in the winter water (Chapter 7).

3). The maximum bacterioplankton density was determined by nutrient level at a given temperature if the impact of grazing was eliminated. The influence of temperature on bacterioplankton maximum density was dominant at high nutrient level whereas the influence of nutrient was dominant at low nutrient level (Chapter 7).

Management Implications

There are three general management interests in studying bacterioplankton. Firstly, bacterioplankton play an important role in nutrient recycling, which is one of the crucial factors controlling primary productivity in aquatic systems. Secondly, bacterioplankton production and abundance are closely associated with phytoplankton production and abundance. Precise management of phytoplankton requires information of bacterioplankton. Thirdly, bacterioplankton biomass production plays an important role in the microbial loop, which determines how energy is transferred from organic products to higher levels of the food chain.

My results, in conjunction with previous studies (see discussion in Chapters 3 and 4), showed that bacterioplankton are not only remineralizers but also net inorganic nitrogen and phosphorus consumers. Bacterioplankton growth in Hebgen Lake is both temperature and substrates controlled and their maximum density is substrate controlled if there are no grazers. River inflows play an important role in the supply of substrates for bacterioplankton. Therefore, quantitatively and qualitatively controls of nutrient input from river inflows are essential for control of bacterioplankton population in Hebgen Lake.

My results showed that bacterioplankton growth is limited by either organic carbon or inorganic phosphorus and nitrogen or all three. It is well established that phytoplankton growth can be limited directly by phosphorus or nitrogen. Direct inorganic nutrient limitation of phytoplankton could lead to indirect inorganic nutrients limitation of bacterioplankton where exuded phytoplankton carbon is necessary for bacterioplankton growth. Alternatively, inorganic nutrients could directly limit bacterioplankton where insufficient inorganic nutrients are present to maintain bacterioplankton growth. The consequence of the latter could result in competition between bacterioplankton and phytoplankton for inorganic nutrients. A common ecological problem in aquatic systems is a cyanobacterial bloom. The tight relationship between bacterioplankton and cyanobacteria may be one of the reasons for blooms of the latter. Algal-bacterial exchange within the phycosphere (discussed in Chapter 6) may serve to maintain the nuisance blooms on the surface of freshwater bodies, even when bulk nutrient are low. Therefore, one of the ways to control cyanobacterial blooms may be to control bacterioplankton.

The term microbial loop has been used to describe carbon flow through a dissolved organic carbon-bacterium-protozoan food chain rather than through the classical phytoplankton-zooplankton-predator food chain. Evidence from both marine and freshwater systems has shown that carbon flow through the microbial loop is relatively inefficient because of the additional steps in the food chain (Ducklow et al. 1986, Tranvik 1989). However, it has also been demonstrated when bacterioplankton biomass is grazed by macrozooplankton (>140 μm) the energy transportation is much more efficient than through microbial loop (Riemann 1985; Christoffersen et al. 1990). Studies from both eutrophic lakes and experimental enclosures have shown that planktivorous fish have significant impact on the dominance of bacterioplankton grazers (Riemann 1985; Christoffersen et al. 1990). It may be feasible to manage plankton communities in Hebgen Lake, as well as in other similar eutrophic lakes, to prosper macrozooplankton by control of planktivorous fish. The dominated macrozooplankton may reduce bacterioplankton and phytoplankton blooming by grazing, which can also increase the efficiency of carbon transportation from bacterioplankton to fish.

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