ISBN 0-612-00508-9

Canada

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

This work is dedicated to my husband, George and my parents, Gregory and Elefteria Tzaras.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Frances Pick for her guidance, understanding and constant encouragement during the past few years. I would also like to thank the members of my committee, Dr. David Currie and Dr. Larry Flanagan for their time and interest. Special thanks to Dr. David Lean and Dr. Asit Muzumder whose enthusiasm and insight contributed greatly to the success of this project. Many thanks to Dr. Ellen Bentzen, Dr. Bill Taylor, Jane Almond and Marc Proulx for collecting samples and providing water chemistry data. Fran and David, the time spent at Jack's Lake will always be remembered as will Jane's card playing and canoeing abilities. Financial support for this study was provided through an NSERC grant to Frances Pick.

To all my friends Jill, Mike, Mario, Claudia, Mark, Paul, Anneke, Sofia, Ben, and Andrew your friendship and moral support kept me almost sane. I would also like to thank my surrogate parents, Pam and Iain, "for just about, well...everything". Thanks for not letting me sleep in the snowbank. OPA!!

To my family and friends in Nobleton and New York, your continuous support and belief in me were indispensable throughout my academic and everyday life. I would also like to express my deepest gratitude to my parents, Gregory and Elefteria, who never had many opportunities but always gave me plenty. Most importantly, I would like to thank my husband, George, whose patience, love and understanding kept things in perspective and gave me the determination to succeed. This is just the beginning.

In loving memory of Heather J. McMurter (1960 -1993) who always shared my fascination with the microbial world.

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ABSTRACT

The "microbial loop" of aquatic systems consists of picoplankton (heterotrophic and photosynthetic), nanoflagellates (heterotrophic and mixotrophic forms), and ciliates. Heterotrophic flagellates (HNAN) are considered to be the principal grazers of both photosynthetic picoplankton (PICO) and heterotrophic bacteria (HBAC). The factors controlling PICO and HBAC abundances have been studied extensively. However, the factors which regulate HNAN remain less clear.

An experimental study was conducted to examine the effects of nutrients and planktivorous fish additions on HNAN in freshwater systems using large enclosures (8m in diameter) installed at both 4 m and 11 m depths in a small oligotrophic lake. In addition to obligate heterotrophs, mixotrophic flagellates (MIXO) were also examined as were their prey, HBAC and PICO.

HBAC levels increased throughout the sampling period and were significantly higher in the nutrient enriched enclosures. While HBAC did not respond in a consistent manner to the presence of fish, the effect of nutrients was greater when fish were present. HBAC levels did not differ between the shallow and deep enclosures.

PICO abundance was significantly affected by all three treatment variables. PICO levels decreased with nutrients, increased with fish and were more abundant in the shallow enclosures. Furthermore, the strong nutrient effect on PICO was greater when fish were absent and in the deep enclosures.

Nutrient additions alone or in combination with fish resulted in significantly lower HNAN levels relative to the control and fish only enclosures. HNAN were not affected in any way by the presence of fish nor in a consistent way by depth of the water column. However, the nutrient effect observed was significantly greater in the deep enclosures.

MIXO were most abundant early in the season (exceeding HNAN but declined significantly by mid summer and were generally not as abundant as HNAN. MIXO levels were significantly lower in the nutrient enriched enclosures. Depth of the water column had a significant effect on MIXO, but this effect was not clear in the seasonal trends. MIXO levels were not affected in a consistent manner by the presence of fish, however, there were significant nutrient and fish interaction effects.

Experimental results did not demonstrate that increases in nutrient levels would lead to increases in HNAN as current empirical models imply. The relationship between HBAC and HNAN was therefore examined within the range of trophy manipulated in the enclosures. A lake survey was conducted in dimictic lakes in Ontario and Western Quebec. The 26 lakes ranged in total phosphorus from 2.4 to 42.7 $\mu g\ L^{-1}$ and differed in food chain length. HBAC was positively correlated with lake productivity, as estimated by total phosphorus ($r^2 = 0.70$, $p < 0.001$), chlorophyll a ($r^2 = 0.31$, $p = 0.003$) and dissolved organic carbon ($r^2 = 0.48$, $p = 0.001$), HNAN was slightly correlated with total phosphorus ($r^2 = 0.22$, $p < 0.015$). However, there was no significant

correlation between HNAN and HBAC ($r = 0.23$, $p = 0.255$) as previous empirical relationships based on much wider ranges in bacterial abundance have suggested. Within this oligotrophic to mesotrophic range of temperate lakes, HNAN abundance cannot be predicted from HBAC. Presumably, there are other factors regulating HNAN (i.e predatory control) which do not allow for an increase in their abundance with increasing water productivity.

GENERAL INTRODUCTION

Pelagic Food Chains

Pelagic food chains in aquatic systems have traditionally been described by a linear model (Fig. 1). Phytoplankton greater than 20 μm in diameter, such as diatoms, are at the base of the chain. These algal cells are consumed by zooplankton such as cladocerans and copepods, which are grazed upon by small planktivorous fish, which in turn serve as prey for larger piscivorous fish. Bacteria were initially allocated the role of degrading dead organic matter, an essential but presumably minor role compared to the predator-prey processes of larger organisms (Pomeroy, 1974). In addition, bacteria were thought to exist mainly in littoral and benthic sediments where organic degradation was most active. However, the discovery of new staining techniques, and the use of epifluorescence microscopy, demonstrated free living bacteria to be an extremely abundant component in plankton communities (Hobbie *et al.* 1977). Shortly after came the discovery of a significant assemblage of autotrophic cells less than 2 μm in the oceans (Waterbury *et al.* 1979) and lakes (Caron *et al.* 1985). The high abundance of microbes less than 20 μm has led to a re-evaluation of their roles in aquatic systems.

Picoplankton, which includes heterotrophic bacteria and autotrophic cells measuring between 0.2 - 2 μm in diameter, and nanoplankton between 2-20 μm are now considered important contributors to energy flow and nutrient cycling in marine and freshwater systems (Azam *et al.* 1983, Goldman and

Caron 1985, Porter *et al.*, 1985, Caron *et al.* 1988, Cole *et al.*, 1988). Heterotrophic picoplankton (bacteria) can reach densities of 10^5 to 10^6 cells mL^{-1} and may constitute more than 50% of the combined pico-nanoplankton biomass in lakes (Caron *et al.*, 1985). Analyses of carbon flow through food webs have estimated that up to 60% of the carbon fixed by phytoplankton during primary production is utilized by bacteria (Azam *et al.* 1983, Pace *et al.* 1984, Cole *et al.* 1988). In turn, particulate organic material returned to the system by bacteria corresponds to 20-30% of phytoplankton production (Azam *et al.* 1983).

Photosynthetic picoplankton are comprised of cyanobacteria, prochlorophytes, and eukaryotic algae. In oligotrophic systems they may reach densities of 10^4 to 10^5 cells mL^{-1}. They can comprise as much as 80% of the total phytoplankton biomass and can account for an equivalent amount of primary production (e.g. Olson *et al.* 1990).

Both heterotrophic bacteria and autotrophic picoplankton exhibit rapid growth rates and short generation times. The standing stocks of bacteria and picoplankton in most waters do not seem to fluctuate greatly (Watson *et al.* 1977, Porter and Feig 1980, Güde *et al.* 1985, Pick and Caron 1987). Yet there is evidence that their production rate is substantial (e.g. Riemann 1983). This suggests that heterotrophic and autotrophic cells are consumed down to some refuge concentration at the same rate that they grow.

Nanoplankton sized cells also include both obligate heterotrophic forms

as well as photosynthetic forms. The typical concentration of nanoplankton in surface waters is around 10^3 cells mL^{-1}. Heterotrophic nanoflagellates, include a very diverse group of non pigmented, eukaryotic, unicellular organisms. They range in size from 2 - 10 μm, and usually possess one or more flagella. As heterotrophic organisms, they require preformed organic substances for growth. Organic materials are obtained either by absorption across their cell membrane (osmotrophy) or by ingestion of particulate material (phagotrophy). In general, heterotrophic flagellates appear to be the major bacterivores in most aquatic systems (Fenchel 1982, Güde 1986, Sherr *et al.* 1986; Sanders and Porter 1988). For example, Sanders *et al.* (1989) showed that nanoflagellate grazing can account for 55-99% of the total bacterivory in a eutrophic lake.

In addition to obligate heterotrophic flagellates, mixotrophic flagellates can also consume bacteria. Mixotrophic flagellates depending on their growth requirements, and environmental conditions can be phagocytic, feeding on bacteria and pico-algae, or photosynthetic. While they are similar in shape and size to obligate heterotrophs, mixotrophic flagellates can be distinguished by the presence of chloroplasts and by prey items in their food vacuoles. The advantages of combining photosynthetic and phagotrophic modes of nutrition are quite obvious. When photosynthesis is limited under low light conditions, phagotrophy could be important for the acquisition of carbon. Conversely, photosynthesis can maintain a cell during periods of reduced particulate food (Sanders 1991). The importance of mixotrophs as grazers in planktonic

communities was virtually unknown until the 1980's and the factors that determine their abundance are still largely unknown. At times, mixotrophs can be as abundant as heterotrophs and can exhibit a significant grazing impact on picoplankton populations, as high as 80% in some systems (Bird and Kalff 1986, Sanders *et al.* 1989).

The Microbial "Loop"

The classical model of a linear planktonic food chain did not account for cells less than 20 μm and hence did not incorporate any of the microbial production. This gave rise to a new concept termed the microbial "loop" (Azam et al., 1983). Azam et al. (1983) proposed that bacteria which grow on non-living dissolved organic matter are consumed by small protozoan flagellates. These flagellates remineralize much of the organic nitrogen and phosphorus to inorganic compounds and return these nutrients back into the system to be utilized by phytoplankton for further production. The dissolved organic matter (DOM) derived from the excretions of phytoplankton and zooplankton is returned to the classical food chain (for larger metazoan consumption) in the form of bacterial and bacterivore biomass (Azam *et al.*, 1983) (Fig. 1). Without this loop, very little of the microbial production could be incorporated into the linear food chain. The reason being that most of the metazoan grazers cannot feed effectively on cells smaller than 5 μm (Sherr and Sherr, 1991). Thus to the conventional linear food chain a microbial "loop" can be added transferring much of the energy of pelagic systems through a DOM - bacterial-protozoan

pathway (Azam *et al.* 1983).

The microbial loop may be linked to higher trophic levels by microzooplankton such as rotifers and ciliates (Pace and Orcutt 1981), as well cladocerans and copepods. In turn, these larger metazoans can then serve as prey for larger invertebrates and vertebrates. However, there is controversy as to whether or not this source of microbial energy is passed on to higher trophic levels through predation, thereby serving as a link, or if it is lost through respiration, creating a sink or dead end pathway. Some studies have suggested that bacterial production was efficiently funnelled to higher consumers (Pace *et al.* 1984, Fasham 1985). Experimental tests conducted by Ducklow *et al.* (1986) revealed that very little of the organic carbon incorporated by bacteria is passed up the food web. A recent study conducted by Wylie and Currie (1991) suggests that both bacteria and autotrophic cells may contribute similar amounts of carbon to higher trophic levels, although the efficiency of transfer may be quite low in both cases.

The conditions that regulate the microbial loop are not well understood. It has not been established when or in what systems the microbial loop prevails over the classical food chain. It is has been suggested that in marine systems strongly stratified waters may be dominated by the microbial loop whereas weakly stratified waters may be dominated by the classical diatom- zooplankton - fish food chain (Cushing 1989). This may be because small photosynthetic flagellates and cyanobacteria tend to dominate primary production in low

nutrient (oligotrophic), stratified waters, whereas larger phytoplankton (diatoms) are more important in turbulent, nutrient rich environments. A similar argument has been proposed for seasonal changes within lakes. Porter *et al.* (1988) suggested that in low nutrient (oligotrophic) lakes, before the onset of stratification, large increases in phytoplankton production are primarily due to diatoms and not picoplankton. At this time, some primary production passes through the traditional food chain. With the onset of stratification, this new production usually sinks to the hypolimnion, leaving elevated levels of dissolved organic matter (DOM) (a major source of phosphorus and nitrogen) in the epilimnion. This is followed by an increase in activity of picoplankton and their predators, the nanoflagellates. Owing to the negligible sedimentation rate of picoplankton sized cells, the carbon which otherwise would have been lost to the sediments remains in the epilimnion and is available to the larger metazoans. Microbial production is therefore significant during summer stratification, and organic carbon is provided to the epilimnetic zooplankton at the critical time when phytoplankton production is most nutrient limited. With the breakdown of stratification during autumn, larger phytoplankton production increase and lower temperatures slow microbial activity. In more nutrient rich (eutrophic) systems where nutrient loading may prevail throughout the summer, new production (i.e larger phytoplankton derived primary production) can be sustained throughout the summer season. Picoplankton production is smaller and contributes significantly less to the total carbon production.

In the aquatic systems studied to date, heterotrophic flagellates appear to be the main organisms which consume picoplankton directly; they represent the key link to higher trophic levels. However, the actual grazing impact of heterotrophic flagellates appears to vary among habitats, *e.g.* from off-shore to coastal waters in oceans (Wright and Coffin, 1984), or in lakes with different trophic structures (Riemann, 1985). Determining the factors that regulate the abundance and activity of heterotrophic flagellates appears central to establishing the overall importance of the microbial loop in aquatic systems. The aim of this study was to investigate the factors which regulate the abundance of heterotrophic flagellates in temperate lakes.

Research Objectives and Hypotheses

One main factor which may regulate heterotrophic flagellate abundances is the abundance of their prey, i.e the picoplankton. The second factor could be predation. The third factor could be the mixing regime. Two separate approaches were taken to examine these factors. Firstly, an experimental study was conducted to determine the effects of nutrients and food web alterations on the microbial community. Specifically, the effects of nutrient and planktivorous fish additions on picoplankton (heterotrophic and photosynthetic cells) and nanoflagellates (mixotrophic and heterotrophic) were examined using large enclosures (Chap. 1).

The main objectives of the experimental study were: 1) To test whether an increase in bacterial abundance corresponds to an increase in the number

of their natural predators, the heterotrophic flagellates. 2) To determine if the heterotrophic flagellate abundance was affected by depth of the water column. This would suggest that the microbial loop is more prevalent in stratified systems as opposed to continuously mixing systems.

The following hypotheses were tested:

1) H_A: Increased nutrient levels support higher bacterial levels with no change in the number of nanoflagellates (heterotrophic and mixotrophic).

   H_B: Increased nutrient levels support higher bacterial levels thereby increasing the number of nanoflagellates.

2) H_A: Presence of fish leads to increased protozoan abundance through reduction of large zooplankton but leads to no changes in bacterial levels.

   H_B: Presence of fish leads to increased protozoan numbers resulting in a decrease in bacterial levels. grazing.

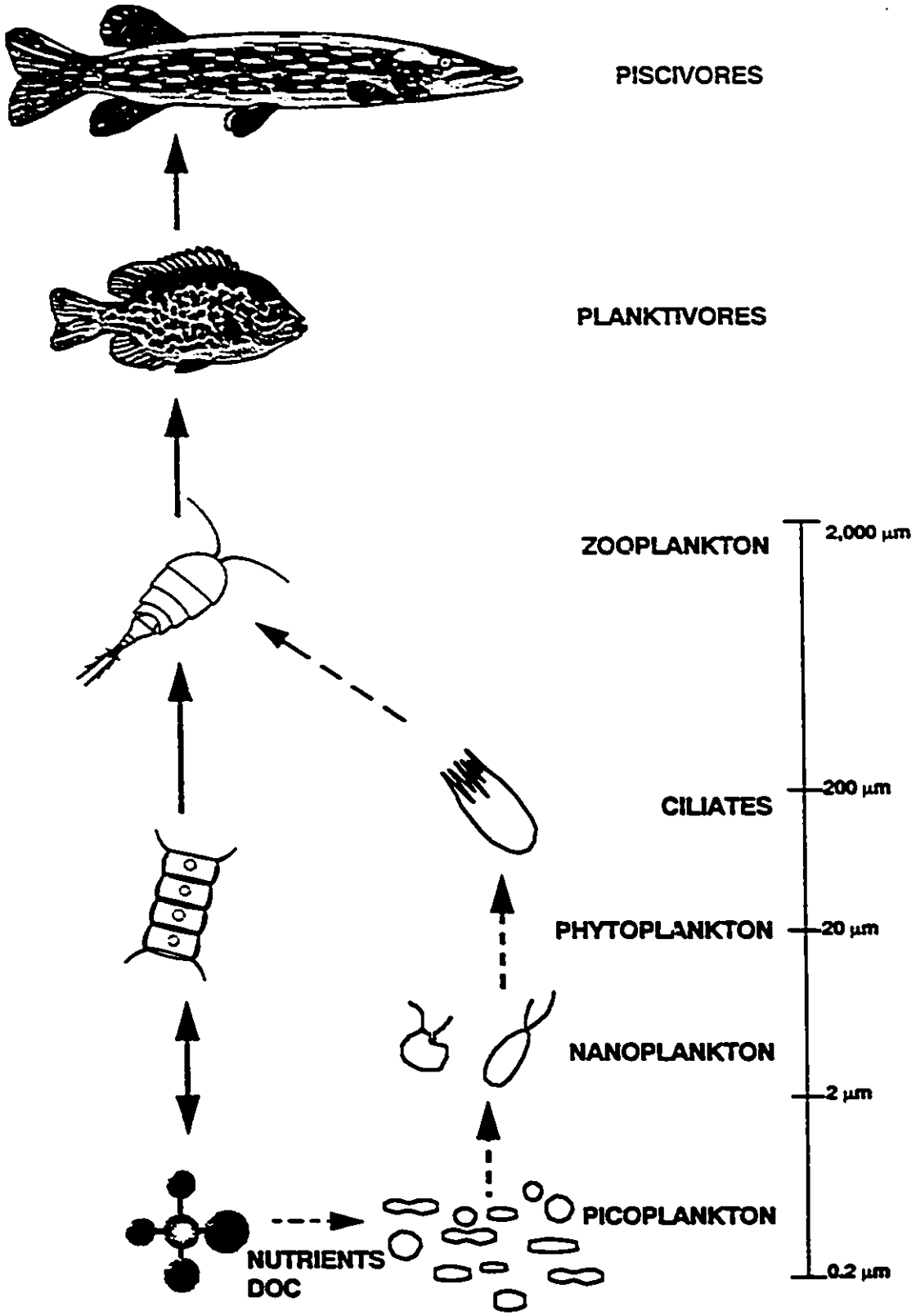
3) H_A: There is no difference between the shallow and deep enclosures with respect to bacterial and flagellate abundance.

   H_B: Deep enclosures support higher bacterial levels and thus include higher flagellate levels in comparison to the shallow enclosures.

In the second part of this research (Chapter 2), a survey of 26 Ontario and Eastern Quebec lakes was conducted in order to determine whether enclosure findings were representative of natural unperturbed lake systems.

The relationship between bacterial and heterotrophic flagellate abundance was examined with respect to lake trophy and food web structure.

Figure 1. A simplified diagram of the pelagic food chain including the microbiai loop. The compartments to the left joined by the solid lines represent the classical food chain. Dashed lines represent the microbial loop which return dissolved organic matter (DOM) via bacteria and protozoan grazing to the classical food chain. Redrawn from Fenchel (1987).


PISCIVORES
PLANKTIVORES
ZOOPLANKTON
2,000 µm
CILIATES
200 µm
PHYTOPLANKTON
20 µm
NANOPLANKTON
2 µm
PICOPLANKTON
0.2 µm
NUTRIENTS
DOC

INTRODUCTION

During the past decade, the ecological role of protozoa in freshwater systems has become the focus of extensive research (Fenchel 1987). Heterotrophic nanoflagellates (HNAN) are generally the most abundant protozoa in the euphotic zone of aquatic systems and are now considered an important component in pelagic food webs (Pomeroy 1974, Azam *et al.* 1983, Sherr and Sherr 1984). Several studies have recognized the importance of these protozoa as the principal consumers of bacteria. They control bacterial populations through grazing and convert bacterial production into larger particles, which can then be utiiized as food by larger metazoans (Sherr and Sherr, 1984, Andersen and Fenchel, 1985; Sanders *et al.* 1989). Furthermore, they play a key role in regulating nutrient flow through the microbial loop of aquatic systems. Substantial amounts of organic carbon, nitrogen, and phosphorus contained in their prey (picoplankton) are released into the environment as regenerated minerals (Caron *et al.* 1985, Goldman and Caron 1985).

Several factors may regulate the abundance of HNAN, but the relative importance of these factors is not known. The abundance of prey may be the most important factor regulating HNAN seasonal levels (Fenchel 1982). A close coupling between HNAN and their food, the picoplankton, has been observed in different aquatic systems (e.g. Rassoulzagedan and Sheldon 1986). In turn, bacterial abundance or productivity have been correlated with algal biomass or primary production over a wide range of marine and freshwater systems (Bird

and Kalff 1984, Cole *et al.* 1988). Recently, Berninger *et al.* 1991, described a significant correlation between bacterial abundance and HNAN abundance over a wide range of freshwater systems, suggesting that system productivity or levels of dissolved organic carbon may be the most significant factor regulating HNAN.

Secondly, the abundance of predators on HNAN may also be important. Zooplankton and ciliates are both potential consumers of HNAN (Sanders and Porter 1990). Seasonal declines in HNAN have been related to increases in large cladoceran zooplankton (e.g. Jürgens and Güde 1991) or ciliates (Weisse *et al.* 1990). Experimental additions of *Daphnia* partially regulated HNAN abundance in lake container experiments (Pace and Funke 1991).

Because zooplankton community structure can be altered by fish planktivory (e.g. McQueen *et al.* 1986), "top down" control of HNAN is possible. Riemann (1985) first showed experimentally that increases in planktivorous fish caused significant increases in HNAN. He attributed the increase to reduced predation from two large cladoceran species (*Daphnia cucullata* and *D. galeata*). In comparing two lakes with contrasting food web structure, Vaqué and Pace (1991) found that the lake dominated by planktivorous fish (and containing small cladoceran species) also contained the highest flagellate abundance compared to the lake dominated by piscivorous fish and large *Daphnia* sp.

A third factor is thermal stratification which may have profound effects on

the relative importance of the classical versus microbial food web (Cushing 1989). In stratified water small cells tend to dominate biomass and primary production and energy flow through the microbial loop is theoretically more significant (Porter *et al.* 1988, Kiørboe et al. 1990).

In general, experimental tests of the role of nutrients *versus* predation in regulating HNAN are few and have been conducted in small containers or enclosures over short time periods (Riemann 1985, Pace and Funke 1991). Furthermore the effects of depth and lake stratification on the microbial loop in general have yet to be tested. In the following study, large enclosures were used to investigate the response of the microbial loop to altered nutrients and overall food web structure. To address the relative significance of nutrient loading (bottom-up) or predatory (top-down) control on heterotrophic flagellate abundance, we examined fertilized and unfertilized enclosures that either contained or excluded planktivorous fish. In addition, these manipulations were examined in stratified (deep) versus continuously mixing (shallow) systems.

MATERIALS AND METHODS

Study Site

The enclosures were installed in Lac Croche (74° 00' N, 45° 59' W), a small lake located at the Université de Montréal field station approximately 70 km north of Montréal. Lac Croche is situated on the Precambrian Canadian Shield and is typical of the many lakes of this region. Lac Croche is a soft water, slightly dystrophic lake with a pH of 6.9 and dissolved organic carbon (DOC) levels between 4-5 mg L^{-1}. Spring total phosphorus levels average 5 µg L^{-1}.

The surface area of the lake spans 4.82 ha and has a total volume of 4.1 X 10^5 m^3. There is approximately 1.09 km of shoreline and the dominant vegetation bordering the shoreline is leather leaf and white birch (*Betula papyrifera)*. The lake is a dimictic with a maximum depth of 11.5 m and a mean depth of 8.5 m. The thermocline is found at 3 m in early June and the lake remains stratified until vertical mixing which begins early in October. The photic zone or depth at which 1% of surface radiation penetrates is approximately 7 m during the summer months. Epilimnion summer temperatures range between 20°C to 25°C while hypolimnetic water temperatures range from 5°C to 6°C.

Additional information concerning morphometric and physical characteristics of Lac Croche can be found in Lafond *et al.* (1990).

Enclosure Design

Sixteen large enclosures were used to investigate the effects of nutrient loading, presence of planktivorous fish, and depth of the water column on the microbial community. The enclosures measured 8m in diameter and were installed at two different depths. Eight enclosures were installed in the littoral zone of the lake at a depth of 4m, resulting in a total volume of 2.0×10^5 L per enclosure. These will be referred to as the shallow enclosures. The second set of eight enclosures were installed in the deepest portion of the western basin of the lake at 12 m depth. The deep enclosures each had a total volume of 6.0×10^5 L. The volume of the epilimnion in the deep enclosures was the same as the total volume of the shallow enclosures.

The enclosures were constructed out of nylon reinforced polyethylene sheeting suspended in the water column by an 8 inch polyurethane floating collar supported by a 2" X 4" wood frame. A metal chain anchored the bottom of each enclosure in the sediment and prevented mixing of enclosure water with the surrounding lake water. Enclosures remained uncovered at both ends to allow for interaction with the underlying sediments and the atmosphere. A similar enclosure design was used in the experiments conducted at Lake St. George by McQueen *et al.* (1986) and Mazumder *et al.* (1988).

Experimental Design

The enclosures were arranged in a 3X2 factorial design. The three treatment variables under investigation were nutrients, presence of planktivorous fish and depth of the water column. Each variable was manipulated at two levels presence versus absence, shallow versus deep accordingly. The resulting treatment combinations included: 1) nutrient addition and fishless (N), (2) nutrient addition and fish present (N+F), (3) no nutrient addition and fishless (control) (C) and (4) no nutrient addition and fish present (F). Each treatment combination had two replicates at each depth.

Treatment combinations requiring nutrients received nitrogen and phosphorus additions on a weekly basis at a N to P ratio of 13:1. Nitrogen was added as sodium nitrate ($NaNO_3$) at a level of 47.97 mg N m^{-2} d^{-1}. Phosphorus was added in a liquid state as phosphoric acid (H_3PO_4) at a level of 3.69 mg P m^{-2} d^{-1}. This fertilization rate represents a typical loading for a temperate eutrophic system (Mazumder *et al.* 1988). Fishless enclosures were seined to remove any fish. Enclosures containing fish were stocked with redbelly dace (*Phoxinus eos* Cope), an indigenous planktivorous species naturally present in Lac Croche. Approximately 160-170 minnows, 6-8 cm in length and weighing 2.6 g per fish, were added to give a final stocking of 5 g m^{-2}. To ensure that fish remained in the designated enclosures, fish fences measuring 0.6 m high were erected to prevent fish from jumping into adjacent enclosures or escaping. In addition, fish traps were installed in the fishless enclosures to ensure that the

fishless treatments contained no fish.

Sampling Procedure

Enclosures were sampled from June 19, 1991 (Julian day 173) until September 14, 1991 (Julian day 254) resulting in a total of 7 sampling dates. Picocyanobacteria samples were collected until August 28, 1991 for a total of 6 sampling dates. Deep enclosures were not sampled during week 1. Thus in the statistical analysis data collected from week 1 were not included. The enclosures were fertilized on a weekly basis and sampling was conducted every other week. The time interval between the sampling dates and the enrichment dates were constant throughout the sampling period.

Epilimnetic water samples were collected using a plexiglass integrated tube sampler (6.5 cm in diameter) over a 0-3 m depth. All samples were collected from the midpoint of each enclosure between 8:00 and 10:30 am. A total 16 litres of lake water were collected from each enclosure and stored in 20 litre opaque polyethylene containers which were pre-rinsed with surface whole lake water prior to each use. Sub-samples of whole lake water (90 mL) were preserved immediately with 10 mL of 10% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7). Samples were stored at 4°C in the dark to be used for picoplankton and nanoflagellate slide preparations, which were made within 10 days of the collection date.

Sample Preparation

Preserved lake water samples were filtered through 0.2 μm (for picoplankton) and 1.0 μm (for nanoflagellate counts) Poretic filters prestained in a 0.2% solution of Irgalan black in 2% acetic acid. Sartorius filters (0.45 μm) were used as backing under the Poretic filter to create an even vacuum over the filter surface.

Picocyanobacteria slides were prepared by drawing 12 mL of water sample onto the filter using approximately 7 kPa of vacuum pressure. Filters were mounted on slides with one drop of low fluorescing immersion oil added to the centre of the filter and covered with a glass coverslip. Picocyanobacteria slides were counted immediately to minimize cell autofluorescence fading.

Direct counts of heterotrophic bacteria and nanoflagellates were determined using DAPI (4'6 diamidino-2-phenylindole) staining and epifluorescence microscopy following the procedure used by Porter and Feig (1980). DAPI is a fluorescent stain which binds to any DNA present in the cell. The DAPI working solution (final concentration 0.001%) was added in a 3:1 ratio of sample to stain. DAPI was added using a 0.2 μm Acropore filter fitted to a 5 cc syringe prior to each use. Depending on cell concentration, sample volume varied between 2-3 mL for bacteria, and 12-15 mL for nanoflagellates. The samples were left to stain for approximately 6 minutes. A foil tent placed over the tower prevented illumination breakdown of the DAPI stain. The samples were then drawn down onto the filter with 7 kPa of vacuum pressure.

The sides of the filter tower were rinsed with 2 mL of 0.2 µm filtered distilled water. The filters were mounted on slides and a drop of low fluorescing immersion oil was placed between slide and coverslip. Bacteria slides were kept frozen (-20°C) until enumeration at a later date. Nanoflagellate slides were also stored at -20°C and counted immediately so that chlorophyll *a* autofluorescence could be used to distinguish heterotrophic from autotrophic flagellates.

Picoplankton and Nanoflagellate Enumeration

Cell enumeration was conducted using a 100X oil immersion objective on a Jenamed 2 epifluorescence microscope fitted with a 50W HBO mercury lamp for a final magnification 1200X. Picocyanobacteria counts were performed using blue excitation (450- 490 nm) and green excitation (510-570 nm) (Pick and Agbeti 1991). Under blue excitation yellow fluorescing cells were picocyanobacteria with phycoeyrthrin pigmentation. Under green excitation, the red fluorescing cells included all picocyanobacteria cells, due to the presence of phycocyanin. A minimum of 500 cells were counted per sample from randomly selected whole field views.

Heterotrophic bacteria counts were performed using ultra violet light (UV), with a peak excitation at 365 nm. Heterotrophic bacteria cells appeared blueish white under UV. A minimum of 500 bacteria cells were randomly counted per sample with the aid of an ocular grid.

Nanoflagellate counts were conducted under blue and UV light. Heterotrophic flagellates could be distinguished from phototrophic flagellates based on the absence or presence of photosynthetic pigments. Heterotrophic flagellates fluoresced weakly and appeared green under blue light and whitish under UV. Conversely, mixotrophic flagellates fluoresced red to orange under blue excitation owing to the presence of chlorophyll *a* and other photosynthetic accessory pigments and yellowish under UV. Between 60-100 nanoflagellate cells were counted from each sample or until 45 random whole field views had been examined.

Statistical Analysis

A repeated measures analysis of variance (RMANOVA) model was selected to evaluate the treatment effects, any temporal effects, as well as their interaction effects on the seasonal abundance of nanoflagellates and picoplankton. Repeated measures analysis of variance allows the testing of hypotheses involving any number of treatments whose effects are measured repeatedly in the same subjects. In this study the response variables (i.e. nanoflagellate and picoplankton abundance) were examined for different treatment effects over a 4 month period (June-September).

When several measurements are taken on the same experimental unit, and these measurements are a response to levels of an experimental treatment, the measurements are not independent (SAS, 1988). Unlike

ordinary analysis of variance models, a repeated-measures design takes the correlation factor into account and allows the use of variables which are interdependent through time. Furthermore, using a single overall univariate analysis of variance model would increase the probability of committing a Type I error (i.e rejection of the null hypothesis when it is in fact true and should be accepted (Zar 1984)).

The overall statistical model used was a Model I (fixed effects), three-way repeated measures analysis of variance. The data were analyzed using SAS software version 6.03 (SAS 1988). A General Linear Model (GLM) procedure was used to conduct the analyses. The overall model was constructed using three class variables (nutrients, fish, and depth) arranged in two levels (presence or absence, and shallow vs. deep), and a time trial factor consisting of seven sampling dates for heterotrophic bacteria, six sampling dates for nanoflagellates and five sampling dates for picocyanobacteria.

RMANOVA examines specifically, the between-subject effects (in this case the different treatments), the within-subject effects (temporal effects), and the within-subject by between-subject interactions. A total of four RMANOVAs were conducted, one for each dependent variable.

The between-subject effects are those due to the main treatment effects, that is, the addition of nutrients, presence of fish, and depth of the water column. The response of the dependent variable to the treatment effects

is averaged over the entire sampling period. The error term is calculated from the number of enclosures.

Repeated measures analysis accounts for the between-subjects effects, then focuses the analysis on the treatment effects within each subject, that is, how the subject's response varies with time. Therefore, the tested null hypothesis is whether the response of the dependent variable to the different treatments is the same over time. Specifically, the within-subjects analysis tests determines whether the slope of the best fit line through the observations plotted against time is similar for the different treatment combinations (Gurevitch and Chester 1986).

The GLM procedure in SAS computes within subject effects and within subject-by between subject interaction effects using multivariate and univariate tests. GLM uses the multivariate method of specifying the model even if only a univariate analyses is required. This approach is considered more efficient in dealing with the correlation of repeated measures. However, when multivariate and univariate results differ, univariate results are more accurate for smaller sample sizes (SAS 1988). In our study, multivariate and univariate tests yielded similar results, therefore only multivariate tests for within subject effects were included in the results section.

GLM conducts four separate tests (Wilk's Lambda, the Pillai's Trace, the Hotelling-Lawley Trace, and Roy's Greatest Root) of the multivariate statistic which tests for any temporal effects. Even though all four tests give basically

the same results, the Pillai's trace is considered to be the most robust of the four (Chatfield and Collins 1980) and thus was the preferred choice in the analysis. The error terms associated with the within-subject analyses are derived from the number of repeated measures.

Nanoflagellate and heterotrophic bacterial abundance were all log transformed to satisfy the assumptions of normality and homogeneity of the residuals. The only assumption which pertains to the multivariate tests for within subject effects is that the dependent variables have a normal distribution with a common covariance matrix across the between subject effects (SAS 1988).

RESULTS

Heterotrophic Bacteria

The seasonal distribution of heterotrophic bacteria (HBAC) abundance for the replicate shallow enclosures is shown in Figure 2. HBAC levels ranged from 3.1 X 10^5 to 5.7 X 10^6 cells mL^{-1} throughout the sampling period. There was a general increase in HBAC throughout the summer months, with the highest cell abundance observed at the end of the sampling period (Sept. 14, 1991). A slight decline in numbers was observed during midsummer between July 30 and August 13 in all enclosures. The nutrient enriched enclosures (alone or in combination with fish) supported greater HBAC levels than was observed in the control and fish only enclosures.

HBAC trends in the deep enclosures were similar to those observed in the shallow enclosures. Mean cell abundance varied between 2.3 X 10^5 to 9.5 X 10^6 during the sampling period, and maximum cell abundance was observed at the end of the sampling period on September 14, 1991 (Fig. 3). There was a slight drop in HBAC abundance during midsummer around July 30, 1991. HBAC levels in the nutrient enriched enclosures (with and without fish) were slightly higher than the control and fish only enclosures. Fish only enclosures had the lowest HBAC levels, but the levels were similar to those found in the control enclosures.

The overall effects of the three treatment variables, i.e. nutrient additions (N), presence of planktivorous fish (F), and depth of the water column (D), as

well as their interaction effects on HBAC are summarized in the RMANOVA between-subject section in Table 1. Mean seasonal HBAC levels were significantly higher in the enclosures with added nutrients ($p < 0.001$) (Fig.4). While HBAC did not respond in a consistent manner to the presence of fish ($p = 0.632$), a significant nutrient and fish interaction effect was observed (N X F; $p = 0.031$). The effect of nutrients was greater in the presence of fish. HBAC levels were significantly higher in the enriched enclosures containing fish in comparison to those without fish (Fig.4). There was no significant difference in HBAC abundance between the shallow and deep enclosures ($p = 0.071$).

Examination of the overall temporal effects (Table 2) indicated that HBAC abundance varied throughout the sampling period ($p = 0.001$). The seasonal changes in HBAC abundance were influenced by nutrients ($p = 0.001$). Seasonal changes in HBAC were not dependent in a consistent manner on the presence of fish ($p = 0.123$). HBAC seasonal trends were significantly different in the shallow versus deep enclosures ($P = 0.006$) and depended further on whether planktivorous fish were present (W X F X D; $p = 0.001$) and whether nutrients were added (W X N X D; $p = 0.010$).

Photosynthetic Picoplankton

Throughout the entire study, the picoplankton was dominated by high levels of picocyanobacteria containing the accessory pigment phycocyanin. In the shallow enclosures, picocyanobacteria (PICO) levels were highest in the nutrient enriched enclosures (with or without fish) early in the sampling season

(Figure 5). Cell abundance reached a maximum of 1.8 X 10^6 cells mL^{-1} in the nutrient only enclosures during mid summer (July 16) but dropped three orders of magnitude to 2.5 X 10^3 cells mL^{-1} on the following sampling date (July 30). PICO levels in the nutrient only enclosures remained considerably lower (<10^4 cells mL^{-1}) in comparison to the other enclosures for the remainder of the sampling period. N + F enclosures exhibited high PICO levels in early July, but PICO levels declined rapidly from 1.3 X 10^6 to 7.0 X 10^4 during week 3 and remained relatively constant around 2.0 X 10^5 cells mL^{-1} for the remainder of the summer months. PICO levels in the control and fish only enclosures remained at 10^5 cells mL^{-1} throughout the entire sampling period.

A greater range in PICO abundance was observed in the deep enclosures in comparison to the shallow enclosures (Fig. 6). Maximum cell abundance was observed in the N + F enclosures during week 2 of sampling (July 4). However, PICO levels declined steadily and by the fourth sampling week PICO abundance in the N + F enclosures varied between 10^2 to 10^3 cells mL^{-1} for the remainder of the summer months. Similarly, PICO abundance in the nutrient only enclosures was initially higher than the control and fish only enclosures during early summer. PICO abundance declined 3 orders of magnitude following the third sampling date. PICO levels remained relatively stable at 10^3 cells mL^{-1} until the end of the sampling period when densities reached a minimum of 10^2 cells mL^{-1}.

Control and fish only enclosures exhibited virtually identical trends

throughout the sampling period (Fig. 6). In both cases, PICO abundance did not deviate greatly from 10^5 cells mL^{-1}.

The RMANOVA between subject analysis indicated that PICO abundance was significantly affected by all three treatment variables (Table 3). Mean PICO levels decreased with nutrient additions ($p < 0.001$); increased in the presence of fish ($p < 0.001$); and were more abundant in the shallow enclosures compared to the deep ($p < 0.001$) (Fig. 7).

The effects of nutrients are greater when fish are absent (N X F; $p = 0.005$; Fig. 7). Mean PICO levels were lower in the nutrient enriched enclosures with no fish in comparison to those with fish. Similarly, the effects of nutrients are greater in the deep enclosures (N X D; $p < 0.001$; Fig. 7). PICO levels were significantly lower in deep enclosures with added nutrients in comparison to the shallow.

RMANOVA within subject tests (Table 4) revealed that seasonal variation in PICO levels depends on nutrient additions ($p = 0.003$) and the depth of the water column ($p = 0.002$) but does not depend consistently on the presence of fish ($p = 0.371$).

Heterotrophic Flagellates

The seasonal distribution of HNAN abundance for replicate shallow enclosures is shown in Figure 8. HNAN abundance ranged from 0.09 to 7.6 X 10^3 cells mL^{-1}. Maximum HNAN was found in the control enclosures at the end of the sampling period on Sept. 14. For most of the summer, excluding the

second sampling week, HNAN abundance was highest in the control and fish only enclosures and lowest in the nutrient enriched enclosures. In the nutrient enriched enclosures (with or without fish) HNAN levels generally remained well below 2.0 X 10^3 cells mL^{-1}. A sharp increase in HNAN abundance was observed in the nutrient enriched enclosures on the last sampling date, but HNAN levels still remained lower than what was found in the control and fish only enclosures.

Seasonal HNAN data for the deep enclosures indicated similar trends to what was observed in the shallow enclosures (Figure 9). HNAN abundance ranged from 0.1 to 3.7 X 10^3 cells mL^{-1}. Highest HNAN abundance was observed in the fish enclosures. Fish only and control enclosures followed very similar patterns with HNAN increasing after the second sampling week (July 16) for the duration of the summer. The nutrient enriched enclosures, alone or in combination with fish, exhibited much lower HNAN than in the control and fish only enclosures. Fertilized enclosures maintained HNAN levels generally less than 1.5 X 10^3 cells mL^{-1} except in the N only enclosures where there was a slight increase to 1.8 X 10^3 cells mL^{-1} in September.

The overall effects of the various treatment combinations on HNAN abundance are summarized in Table 5. RMANOVA between-subject analysis revealed that nutrient additions had a highly significant effect on HNAN ($p < 0.001$). HNAN levels were reduced when nutrients were added (Fig. 10). HNAN were not affected in any way by the presence of fish ($p = 0.130$) nor in a

consistent way by the depth of the water column ($p = 0.851$). There was however, a significant N X D interaction effect ($p = 0.019$). The nutrient effect observed was greater in the deep enclosures (Fig. 10). HNAN levels in the deep fertilized enclosures were considerably lower in comparison to the fertilized shallow.

RMANOVA within-subject analyses reported seasonal variation in HNAN abundance was due to all three treatment variables (Table 6). The HNAN response to the addition of nutrients ($p = 0.009$), presence of fish ($p = 0.019$), and depth of the water column ($p = 0.002$), including all the interaction effects of these treatment combinations, varied considerably (refer to Table 4) during the 4 month sampling period.

Mixotrophic Flagellates

Mixotrophic flagellate (MIXO) abundance in the shallow enclosures reached a maximum early in the sampling season but declined steadily throughout the remainder of the summer (Fig. 11). The highest MIXO levels were observed in the control enclosures during early July (4.8×10^3 cells mL^{-1}). MIXO levels in the fish only (F) enclosures were generally lower and followed a similar pattern to what was observed in the control enclosures. The fertilized enclosures exhibited the lowest number of MIXO, usually less than 1.0×10^3 cells mL^{-1}.

Seasonal MIXO data are presented in Figure 12. MIXO levels peaked in early July (6.4×10^3 cells mL^{-1}) in the N+F enclosures. However, MIXO

numbers sharply declined to less than 0.01 X 10^3 cells mL^{-1} by early August and close to zero until the completion of the study. The MIXO levels in the control and nutrient enclosures also peaked in early July (between 1.5 to 2.5 X 10^3 cells mL^{-1}) and remained below 0.4 X 10^3 cells mL^{-1} for the remainder of the season. The fish only enclosures exhibited a more gradual decline and supported higher MIXO levels at the end of the season in comparison to the other treatments.

The effects of the various treatment combinations on MIXO abundance are summarized in Table 7. MIXO levels were significantly lower in the fertilized enclosures ($p < 0.001$; Fig. 13). There was also a significant depth effect ($p < 0.001$). MIXO levels were generally lower in the deep enclosures in comparison to the shallow. However, this depth effect was not clear in the MIXO seasonal trends. MIXO levels were not affected in a consistent manner by the presence of planktivorous fish. However, there was a significant N X F interaction effect ($p = 0.006$). Seasonal mean MIXO data indicates that the effect of nutrients was greater in the enclosures without fish (Fig. 13). Nutrient enclosures with no fish exhibited lower MIXO levels than enriched enclosures with fish.

The within-subject effects and their interaction effects are summarized in Table 8. Seasonal variation of MIXO abundance depended on the addition of nutrients, presence of fish, and depth of the water column. Seasonal changes were different in each enclosure.

Table 1. Between Subject Effects: Summary of the repeated measures Three-Way ANOVA procedure examining the treatment effects of nutrient additions (N), presence of planktivorous fish (F) and depth of the water column (D) on the log transformed dependent variable heterotrophic bacterial abundance.

Source	SS	DF	MS	F Ratio	P Value
N	11.22	1	11.22	117.64	0.001
F	0.02	1	0.02	0.25	n.s.
N X F	0.69	1	0.69	7.27	0.031
D	0.43	1	0.43	4.54	n.s.
N X D	0.06	1	0.06	0.62	n.s.
F X D	0.05	1	0.05	0.50	n.s.
N X F X D	0.01	1	0.01	0.09	n.s.
Error	0.67	8	0.10		

n.s. p>0.07

Table 2. Within Subject Effects: Summary of the repeated measures Three-Way ANOVA repeated procedure examining the response of log transformed heterotrophic bacterial abundance to nutrient additions (N), presence of planktivorous fish (F) and depth of the water column (D) during the sampling period. (W) represents sampling week. A total of seven sampling dates were included in the anaiyses.

Source	DF	F Ratio	P Value
W	6	26.03	< 0.001
W X N	6	6.46	< 0.001
W X F	6	1.80	n.s.
W X N X F	6	1.01	n.s.
W X D	6	3.56	0.006
W X N X D	6	3.26	0.010
W X F X D	6	5.99	< 0.001
W X N X F X D	6	0.07	n.s.

n.s. p>0.14

Table 3. Between Subject Effects: Summary of the repeated measures Three-Way ANOVA procedure examining the treatment effects of nutrient additions (N), presence of fish (F), and depth of the water column (D) on the log transformed dependent variable picocyanobacteria abundance.

Source	SS	DF	MS	F Value	P Value
N	63.05	1	63.05	78.58	< 0.001
F	22.12	1	22.12	27.57	< 0.001
N X F	12.27	1	12.27	15.29	0.005
D	62.54	1	62.54	77.96	< 0.001
N X D	34.16	1	34.16	42.58	< 0.001
F X D	0.37	1	0.37	0.46	n.s.
N X F X D	0.86	1	0.86	1.07	n.s.
Error	6.42	8	0.80		

n.s. p>0.33

Table 4. Within Subject Effects: Summary of the repeated measures Three-Way ANOVA procedure examining the response of log transformed picocyanobacteria abundance to the addition of nutrients (N), presence of fish (F), and depth of the water column (D) during the sampling period. W represents sampling week. A total of five sampling dates were included in the analyses.

Source	F Value	DF	P Value
W	51.62	4	< 0.001
W X N	20.41	4	0.003
W X F	1.34	4	0.371
W X N X F	1.11	4	0.444
W X D	23.79	4	0.002
W X N XD	47.49	4	< 0.001
W X F X D	111.25	4	< 0.001
W X N X F X D	134.34	4	< 0.001

Table 5. Between Subject Effects: Summary of the repeated measures Three-Way ANOVA procedures examining the treatment effects of nutrients additions (N), presence of fish (F), and depth of the water column (D) on the log transformed dependent variable heterotrophic flagellate abundance.

Source	SS	DF	MS	F Value	P Value
N	16.50	1	16.50	154.39	< 0.001
F	0.30	1	0.30	2.84	n.s.
N X F	0.21	1	0.21	1.96	n.s.
D	0.004	1	0.004	0.04	n.s.
N X D	0.91	1	0.91	8.55	0.019
F X D	0.04	1	0.04	0.38	n.s.
N X F X D	0.002	1	0.002	0.02	n.s.
Error	0.11	8	0.10		

n.s. p>0.130

Table 6. Within Subject Effects: Summary of the repeated measures Three-Way ANOVA procedures examining the response of log transformed heterotrophic flagellate abundance to the addition of nutrients (N), presence of fish (F), and depth of the water column (D) during the sampling period. W represents sampling week. A total of six sampling dates were included in the analyses.

Source	F Value	DF	P Value
W	92.19	5	< 0.001
W X N	16.71	5	0.009
W X F	11.05	5	0.019
W X N X F	17.17	5	0.008
W X D	41.58	5	0.002
W X N X D	8.18	5	0.032
W X F X D	36.18	5	0.002
W X N X F X D	43.44	5	0.001

Table 7. Between Subject Effects: Summary of the repeated measures Three-way ANOVA procedure examining the treatment effects of nutrient additions (N), presence of fish (F), and depth of the water column (D) on the log transformed dependent variable mixotrophic flagellate abundance.

Source	SS	DF	MS	F Value	P Value
N	33.95	1	33.95	83.06	<0.001
F	0.009	1	0.009	0.02	n.s.
N X F	5.58	1	5.58	13.65	0.006
D	14.45	1	14.45	35.35	<0.001
N X D	1.65	1	1.65	4.04	n.s.
F X D	1.77	1	1.77	4.33	n.s.
N X F X D	11.91	1	11.91	29.15	<0.001
Error	3.27	8	0.41		

n.s. p>0.071

Table 8. Within Subject Effects: Summary of the repeated measures Three-Way ANOVA examining the response of log transformed mixotrophic flagellate abundance to nutrient additions (N), presence of fish (F), and depth of the water column (D) during the sampling period. W represents sampling week. A total of six sampling dates were included in the analyses.

Source	F Value	DF	P Value
W	67.98	5	< 0.001
W X N	32.37	5	0.003
W X F	50.18	5	0.001
W X N X F	39.54	5	0.002
W X D	87.79	5	< 0.001
W X N X D	67.25	5	< 0.001
W X F X D	12.34	5	0.015
W X N X F X D	3.33	5	0.013

Figure 2. Log transformed mean heterotrophic bacterial abundance for shallow enclosures from June 19 to September 14. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions; N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

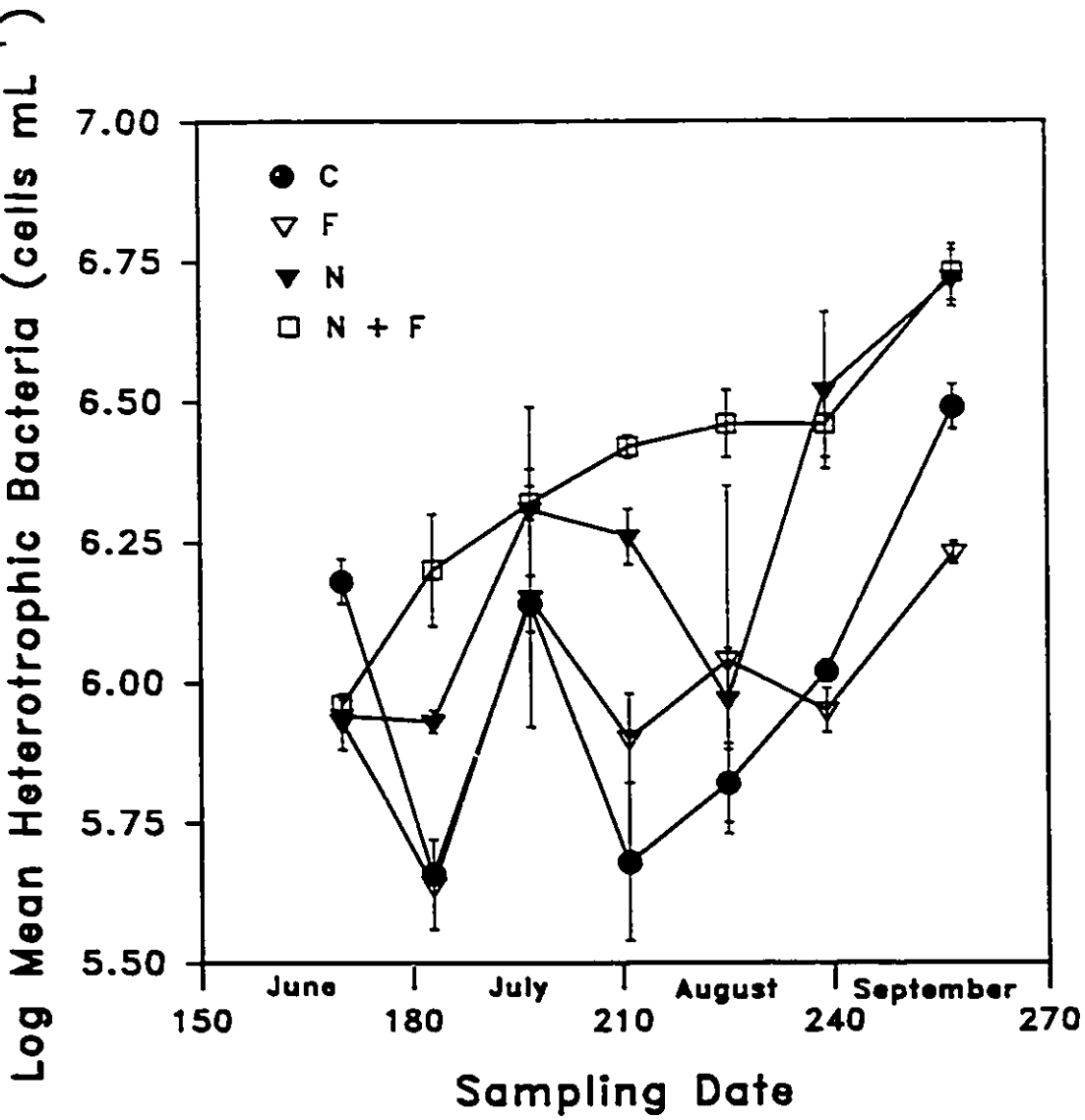

Log Mean Heterotrophic Bacteria (cells mL^{-1})
7.00
6.75
6.50
6.25
6.00
5.75
5.50
C
F
N
N + F
June
July
August
September
150
180
210
240
270
Sampling Date

Figure 3. Log transformed mean heterotrophic bacterial abundance for deep enclosures from July 4 to September 14. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions; N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

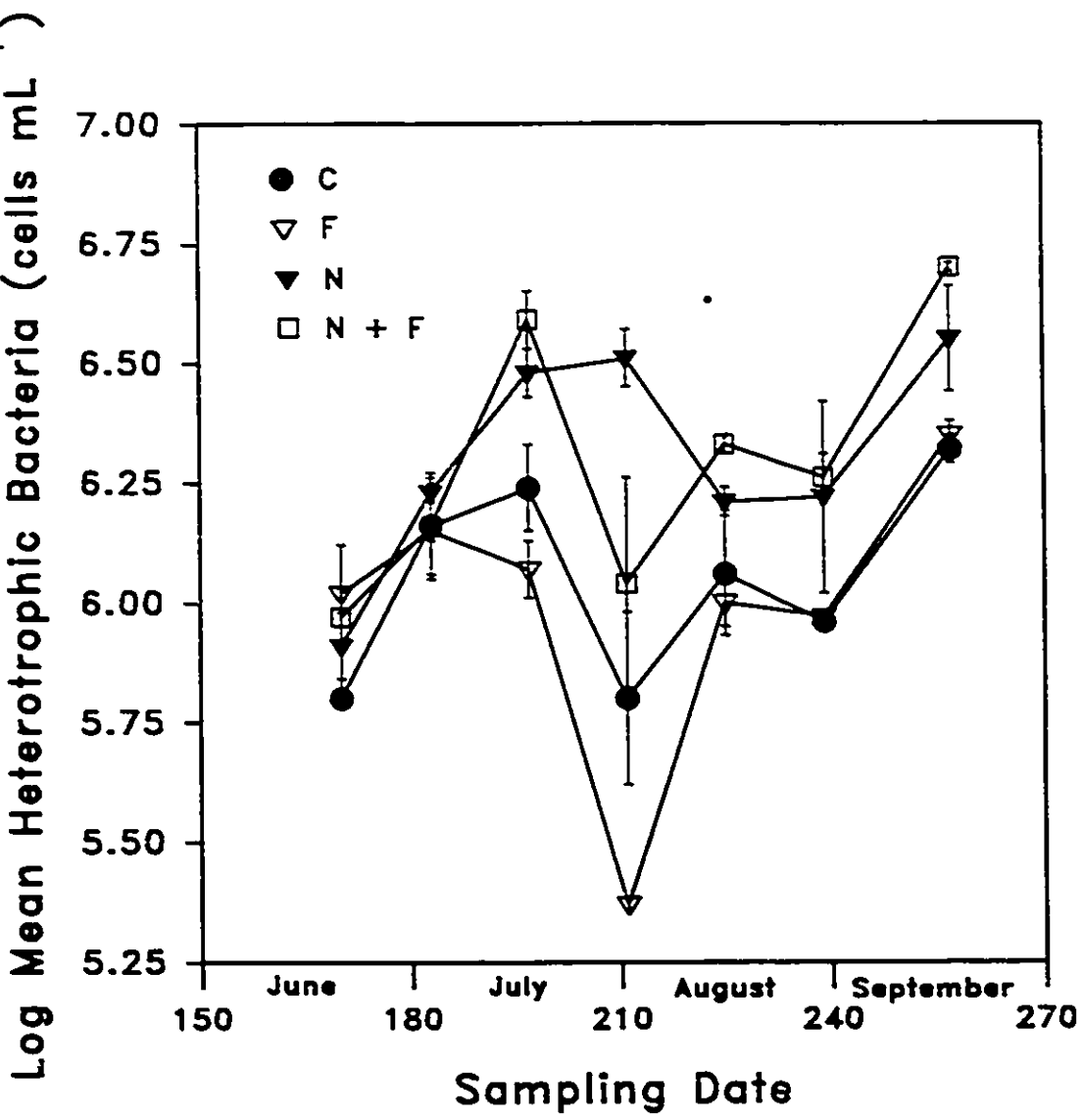

Log Mean Heterotrophic Bacteria (cells mL^{-1})
7.00
6.75
6.50
6.25
6.00
5.75
5.50
5.25
C
F
N
N + F
June
July
August
September
150
180
210
240
270
Sampling Date

Figure 4. Overall effects of the treatment variables on seasonal mean heterotrophic bacterial abundance. Treatment variables examined included: Nutrient additions, presence of planktivorous fish and depth of the water column. Heterotrophic bacterial abundance has been log transformed. Seven sampling dates were included in the analyses. Bars represent standard errors.

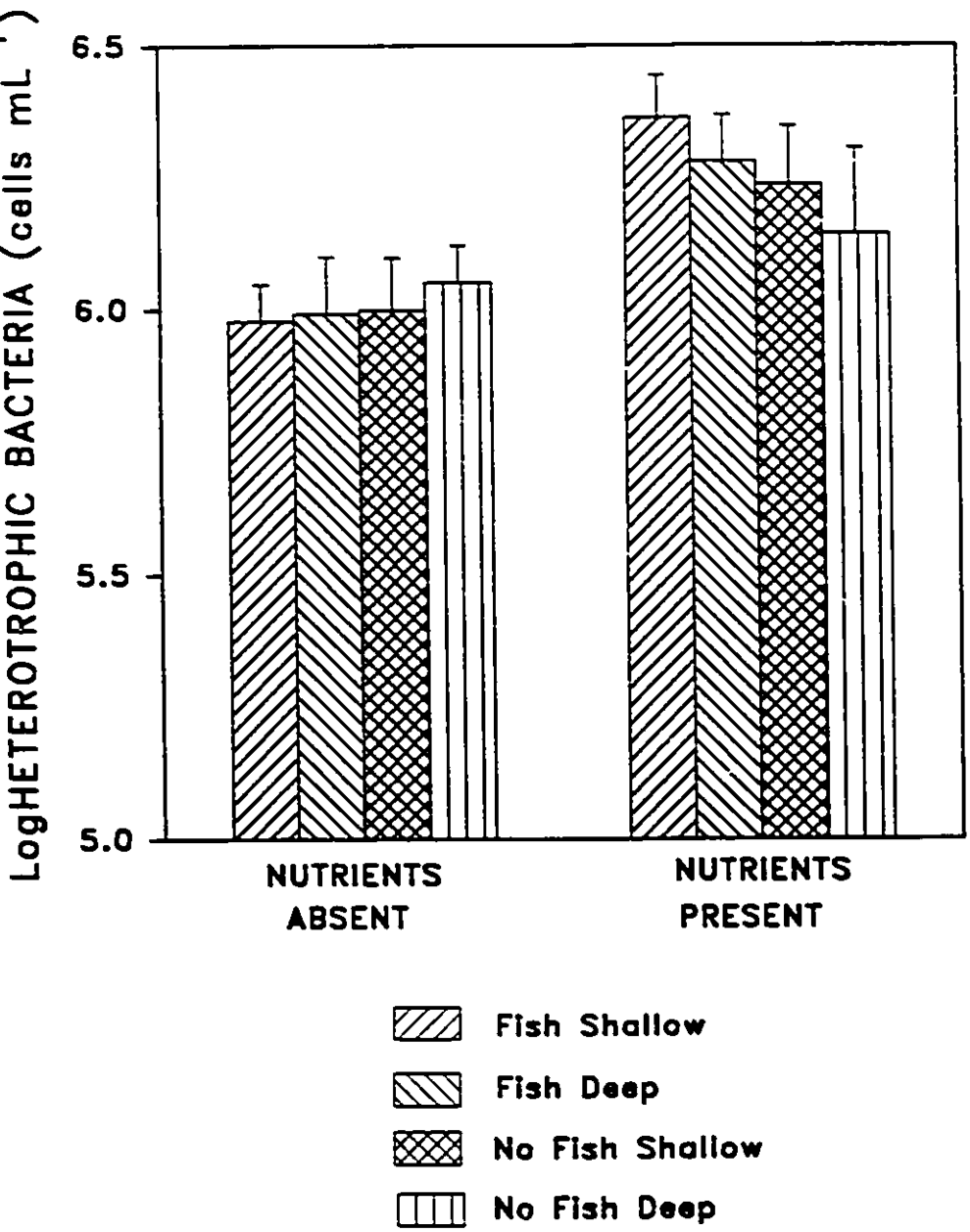

LogHETEROTROPHIC BACTERIA (cells mL^{-1})
6.5
6.0
5.5
5.0
NUTRIENTS ABSENT
NUTRIENTS PRESENT
Fish Shallow
Fish Deep
No Fish Shallow
No Fish Deep

Figure 5. Mean picocyanobacterial abundance for the shallow enclosures from June 19 to August 27. Data has been log transformed. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions, N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

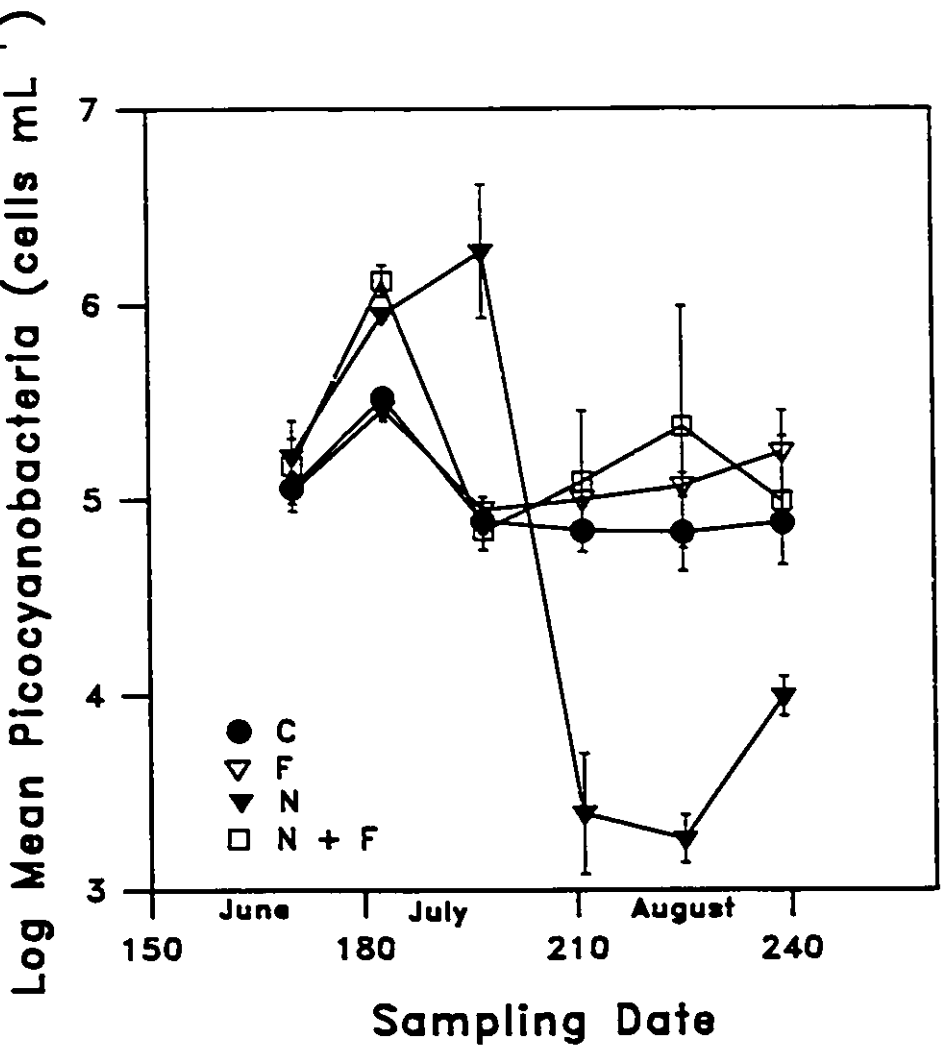

Log Mean Picocyanobacteria (cells mL^{-1})
7
6
5
4
3
C
F
N
N + F
June
July
August
150
180
210
240
Sampling Date

Figure 6. Mean picocyanobacterial abundance for the deep enclosures during the sampling period July 4 to August 27. Data has been log transformed. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions, N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

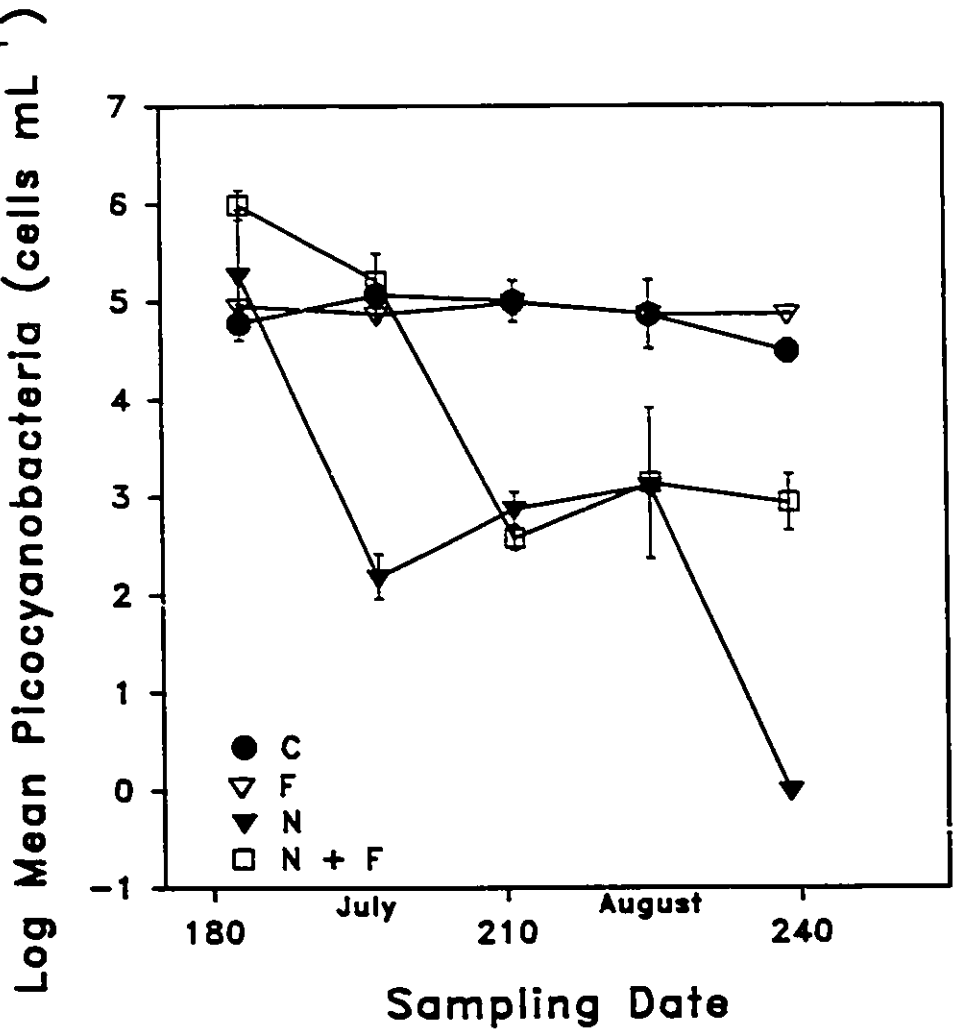

Log Mean Picocyanobacteria (cells mL^{-1})
7
6
5
4
3
2
1
0
−1
C
F
N
N + F
July
August
180
210
240
Sampling Date

Figure 7. Overall effects of the treatment variables on seasonal mean picocyanobacterial abundance. Treatment variables examined included: nutrient additions, presence of planktivorous fish and depth of the water column. Picocyanobacterial abundance has been log transformed. Five sampling dates were included in the analyses. Bars represent standard errors.

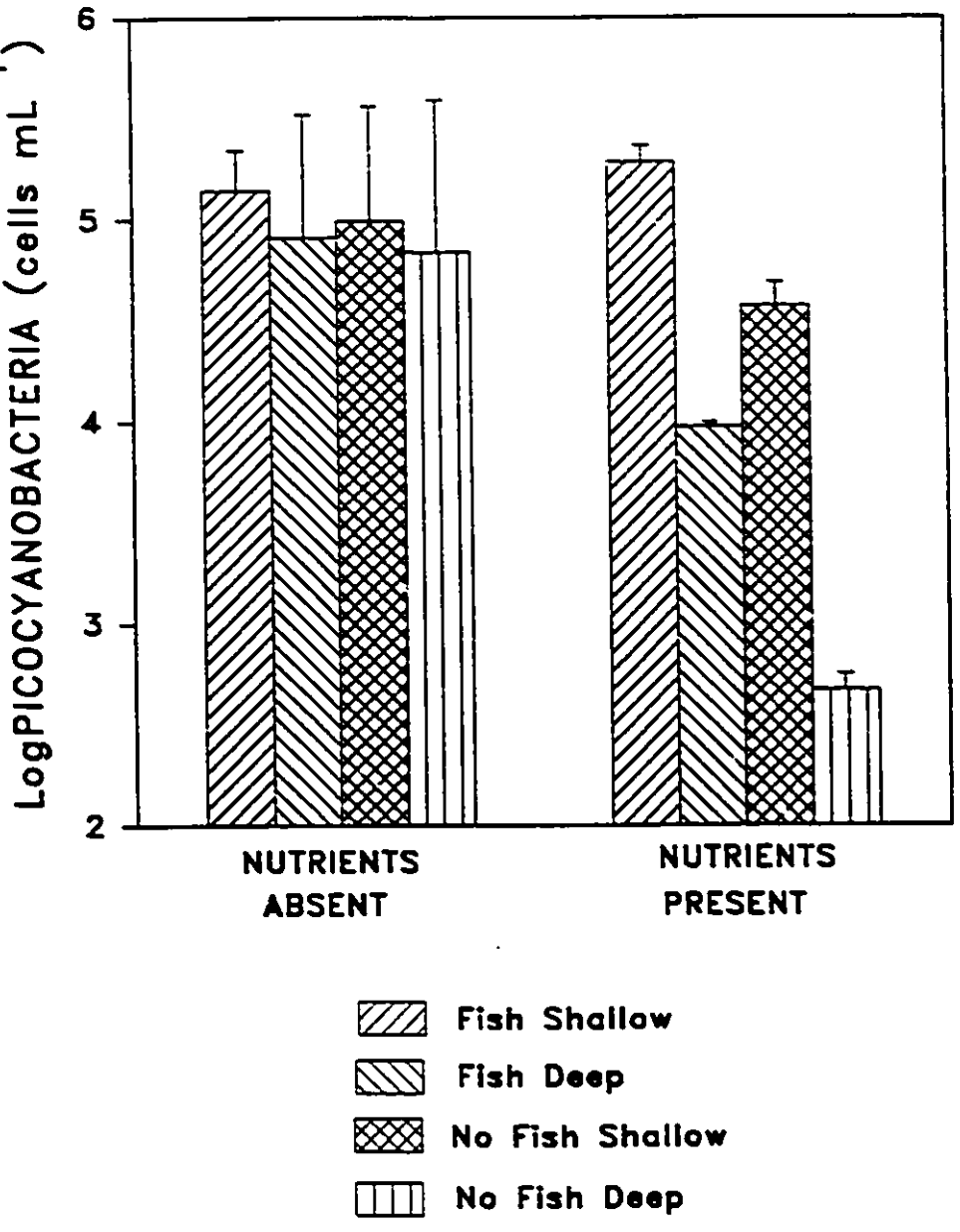

LogPICOCYANOBACTERIA (cells mL^{-1})
6
5
4
3
2
NUTRIENTS ABSENT
NUTRIENTS PRESENT
Fish Shallow
Fish Deep
No Fish Shallow
No Fish Deep

Figure 8. Mean heterotrophic flagellate abundance for the shallow enclosures during the sampling period June 19 to September 14. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions, N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

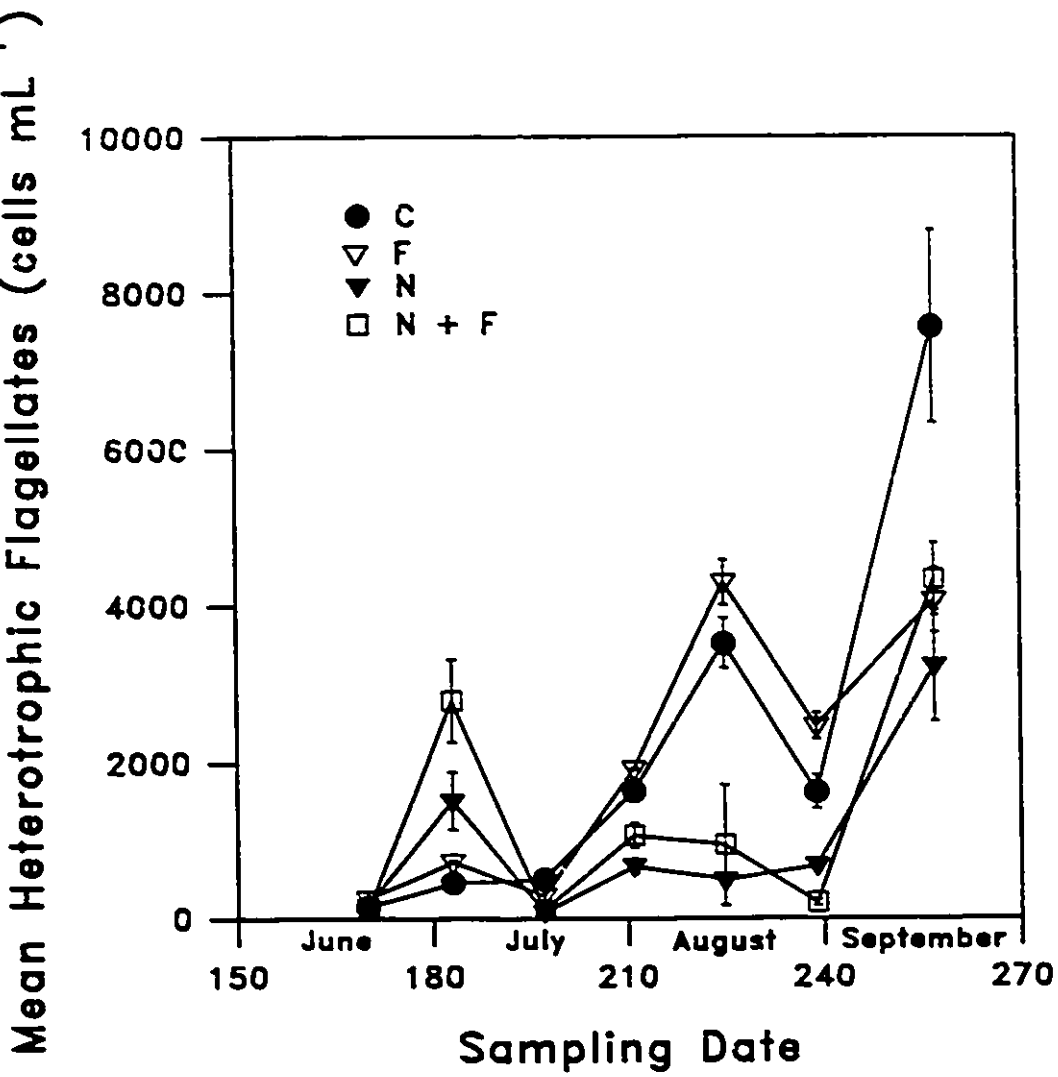

Mean Heterotrophic Flagellates (cells mL^{-1})
10000
8000
6000
4000
2000
0
C
F
N
N + F
June
July
August
September
150
180
210
240
270
Sampling Date

Figure 9. Mean heterotrophic flagellate abundance for the deep enclosures during the sampling period July 4 to September 14. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions, N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

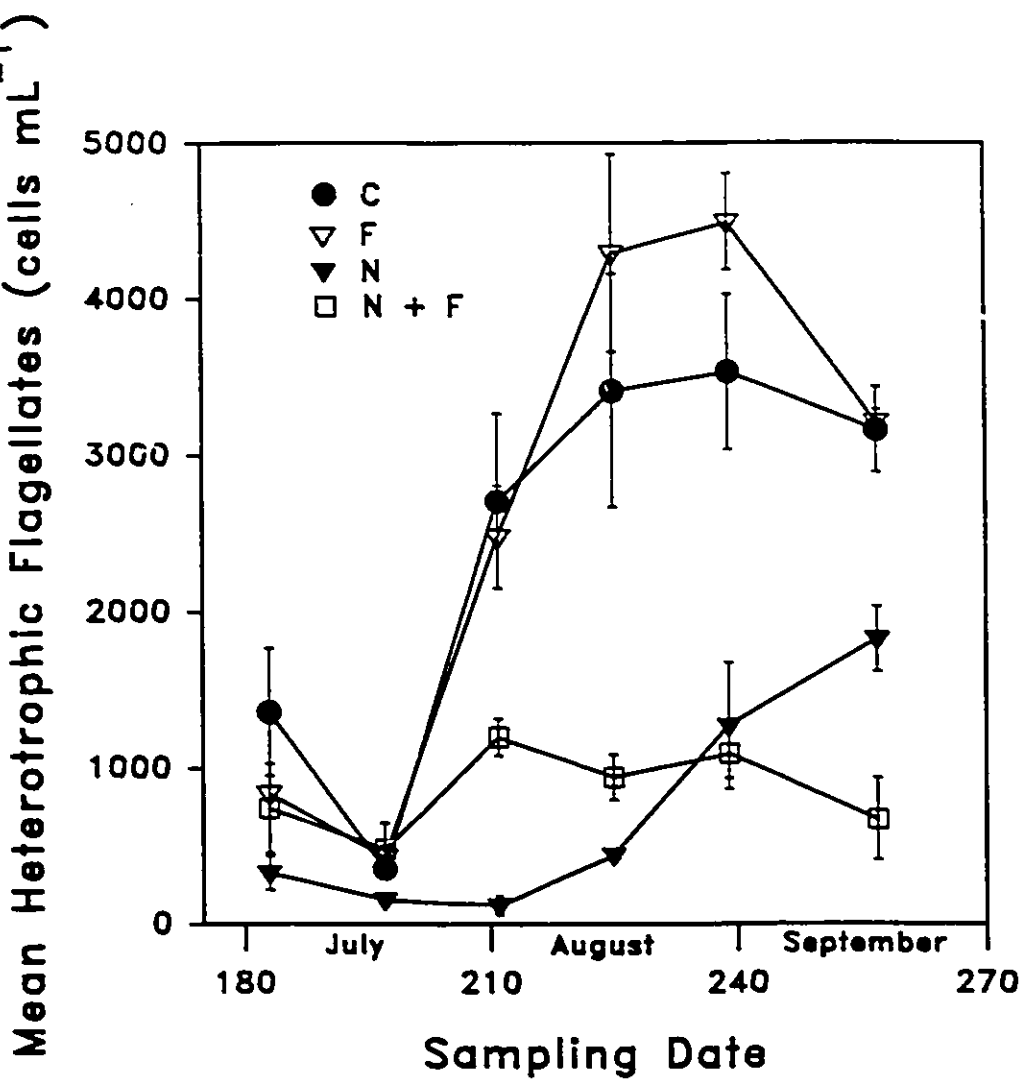

Mean Heterotrophic Flagellates (cells mL^{-1})
5000
4000
3000
2000
1000
0
C
F
N
N + F
July
August
September
180
210
240
270
Sampling Date

Figure 10. Overall effects of the treatment variables on seasonal mean heterotrophic flagellate abundance. Treatment variables examined included: nutrient additions, presence of planktivorous fish and depth of the water column. Heterotrophic flagellate abundance has been log transformed. Six sampling dates were included in the analyses. Bars represent standard errors.

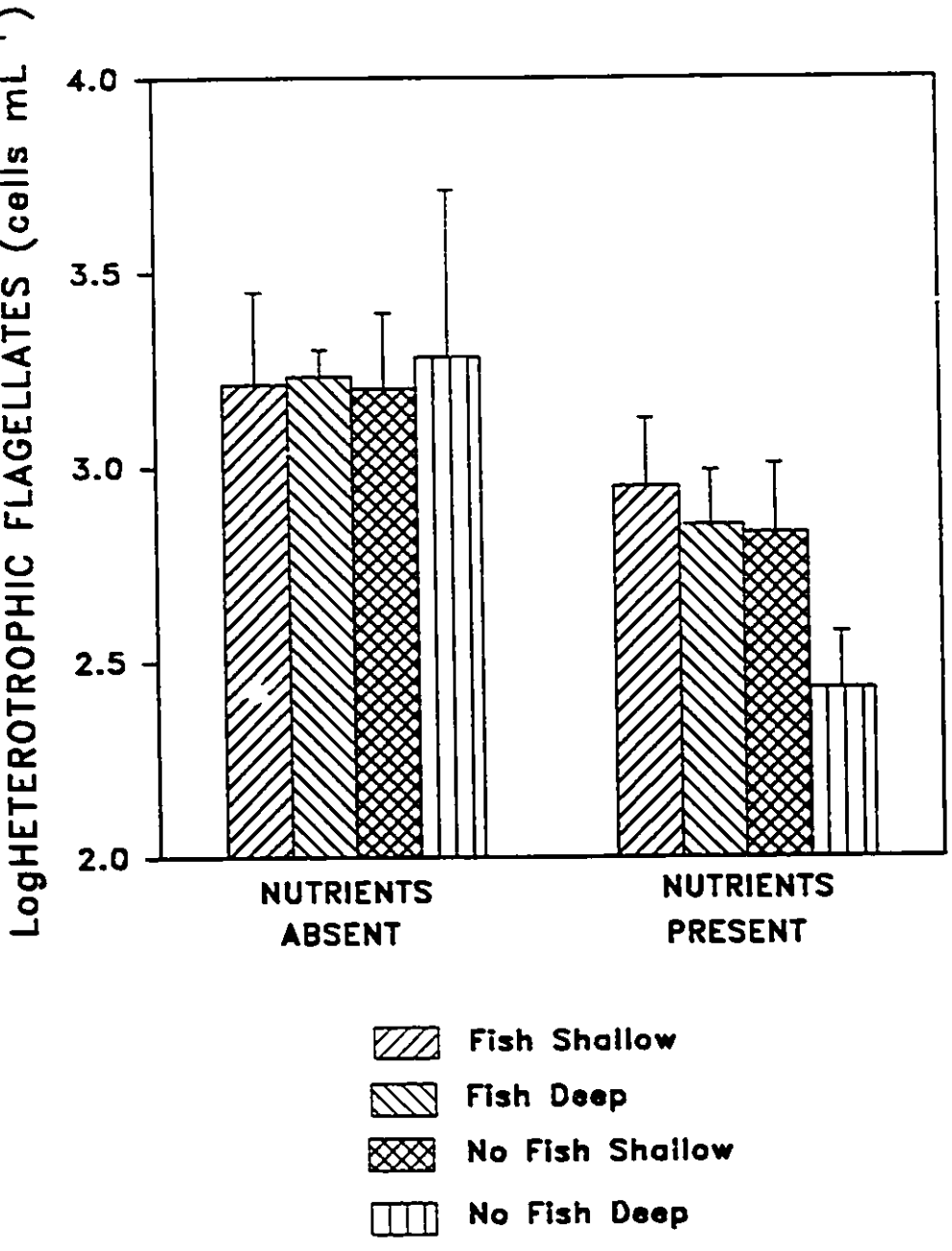

LogHETEROTROPHIC FLAGELLATES (cells mL^{-1})
4.0
3.5
3.0
2.5
2.0
NUTRIENTS ABSENT
NUTRIENTS PRESENT
Fish Shallow
Fish Deep
No Fish Shallow
No Fish Deep

Figure 11. Mean mixotrophic flagellate abundance for the shallow enclosures during the sampling period June 19 to September 14. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions, N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

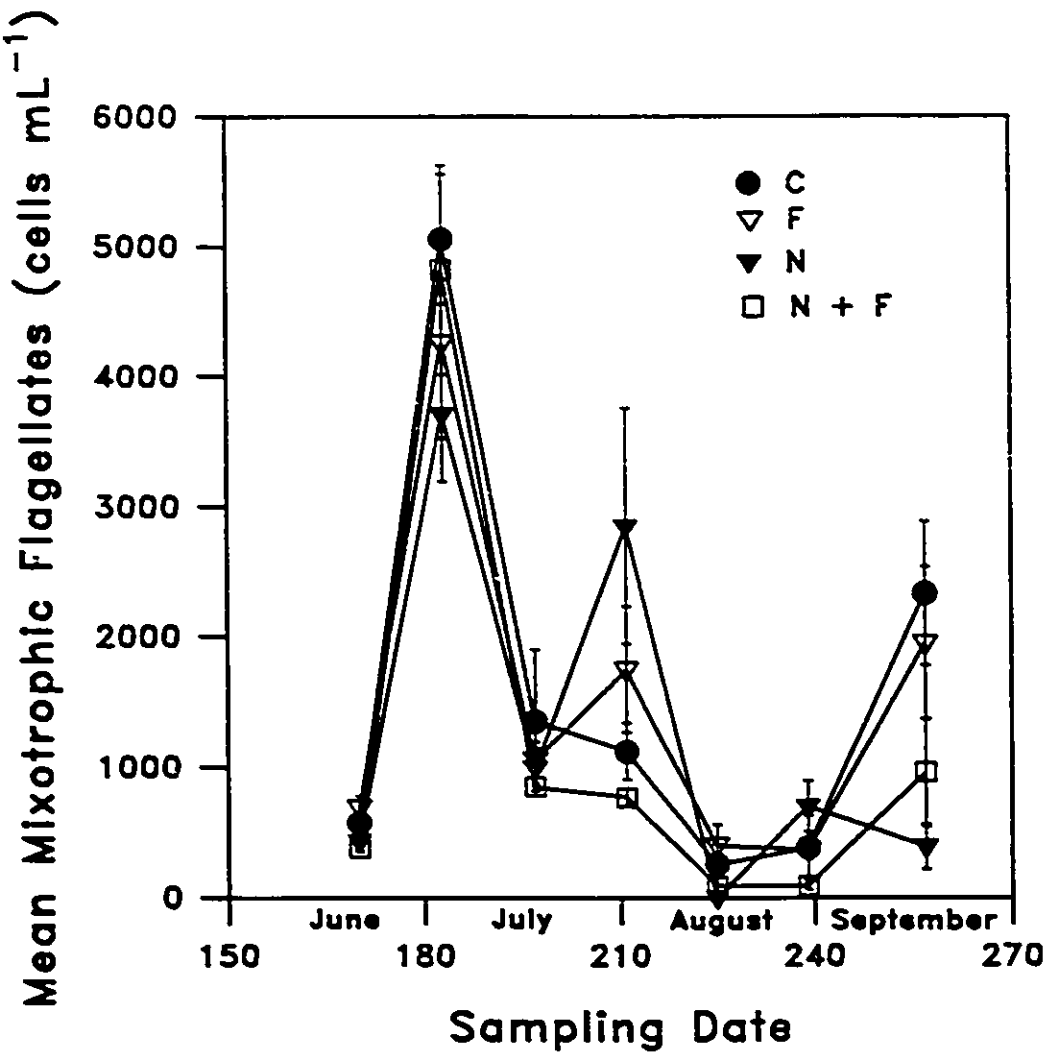

Mean Mixotrophic Flagellates (cells mL^{-1})
6000
5000
4000
3000
2000
1000
0
C
F
N
N + F
June
July
August
September
150
180
210
240
270
Sampling Date

Figure 12. Mean mixotrophic flagellate abundance for the deep enclosures during the sampling period June 19 to September 14. Error bars represent the standard deviation between replicate enclosures. The treatment effects include: C - Control (no fish and no nutrient additions), F - planktivorous fish additions, N - nutrient additions, N + F - nutrient and fish additions. Numbers on the x axis represent Julian days.

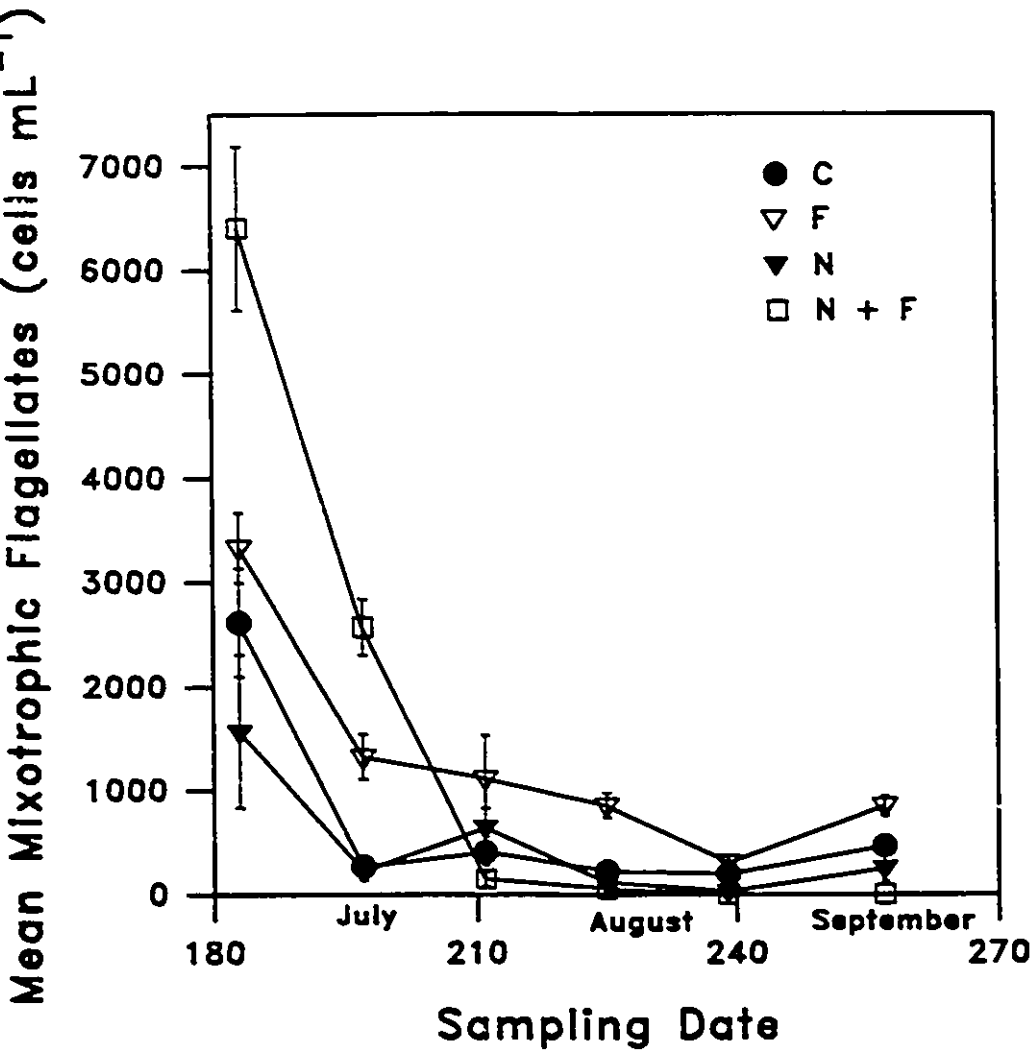
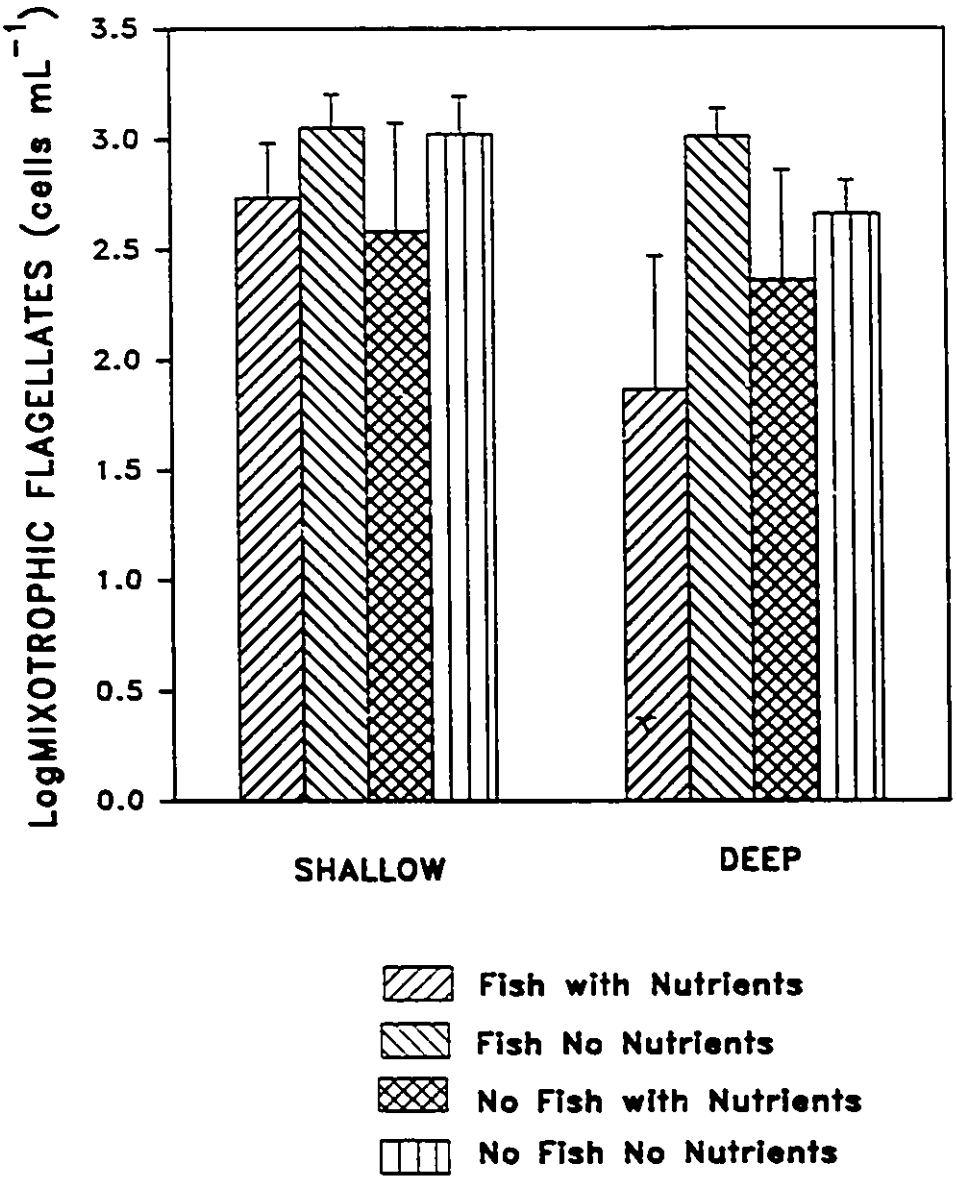

Mean Mixotrophic Flagellates (cells mL^{-1})
7000
6000
5000
4000
3000
2000
1000
0
C
F
N
N + F
July
August
September
180
210
240
270
Sampling Date

Figure 13. Overall effects of the treatment variables on seasonal mean mixotrophic flagellate abundance. Treatment variables examined included: nutrient additions, presence of planktivorous fish and depth of the water column. Mixotrophic flagellate abundance has been log transformed. Six sampling dates were included in the analyses. Bars represent standard errors.


LogMIXOTROPHIC FLAGELLATES (cells mL^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SHALLOW
DEEP
Fish with Nutrients
Fish No Nutrients
No Fish with Nutrients
No Fish No Nutrients

DISCUSSION

Of the three independent variables examined, nutrients clearly had the most significant effect on the abundance of heterotrophic flagellates (HNAN). In the fertilized enclosures HNAN concentrations were always significantly lower than in the non-fertilized enclosures. These results are contrary to the initial hypothesis that the addition of nutrients would lead to an increase in HNAN abundance.

The significant reduction in HNAN abundance observed in the fertilized enclosures cannot be explained by a lack of prey because there was a significant increase in bacterial abundance (HBAC) in the fertilized enclosures. Presumably, the increase in HBAC was triggered by the observed increases in algal biomass (Proulx et al. 1993) and production (Perin et al. unpublished) in the enriched enclosures.

In contrast to this study, Riemann (1985) found that nutrient additions had no significant effect on HNAN within smaller experimental enclosures, for two dates in early summer, for which data was provided. However, it should be noted that his lake was highly eutrophic and nutrients may not have been limiting the system and HBAC biomass was not stimulated by nutrient additions. In the short term (4 day), bag experiments of Pace and Funke (1991) nutrient additions also lead to no change in HNAN in one lake (Peter), but a significant increase was observed in the other lake studied (Paul). The two lakes in this study were not very different in their trophic status and the authors suggested

that the differences were due to differences in predation pressure.

The seasonal and between treatment patterns in HNAN abundance followed more closely the abundance of picocyanobacteria (PICO) than that of the HBAC in the enclosures. PICO levels were significantly reduced under nutrient enriched conditions. This pattern has been found empirically both within and among lakes (Pick and Agbeti 1991). PICO have been found to serve as additional food sources for HNAN in many pelagic environments (Pick and Caron 1987, Nagata 1988). However, it must be noted that, in general, HNAN and PICO levels were not significantly correlated with the exception of one sampling date (July 30) when a positive relationship was observed (r^2= 0.432, p = 0.03).

Mixotrophic flagellates (MIXO) were also examined in the enclosures, because at times they may be significant consumers of bacteria (Bird and Kalff 1987). These MIXO consisted of small chrysophytes and some cryptomonads, which were presumed to be bacterivores, but direct feeding experiments are required to confirm their potential impact on bacteria. In the enclosures, MIXO were important numerically only at the beginning of the experiment (the end of June) when they reached higher levels of abundance than the HNAN in both the shallow and deep enclosures. For the rest of the summer, MIXO were not as important numerically as HNAN. In particular, the nutrient and fish treatments in particular lead to a significant increase in MIXO in the deep enclosures. A similar peak in MIXO levels at the end of June was found in all

the shallow enclosures. The effect of nutrients on MIXO was not clear in the seasonal trends.

Spring blooms of mixotrophic flagellates are typical of many temperate lakes and as a result strong seasonal patterns of bacterivory by MIXO have been reported (Sanders *et al.*, 1989). In Lake Oglethorpe, Georgia, spring blooms of mixotrophic flagellates were responsible for 79% of the bacterial grazing. Mixotrophs were less abundant during summer stratification and could only account for 2% of the bacterial grazing during this period.

Interestingly, while there was a depth effect on MIXO, there was no depth effect on the abundance of HNAN. This depth effect was probably not due to any significant differences in nutrient concentrations since both the shallow and deep enclosures maintained similar nutrient levels in the epilimnion during the summer (Mazumder pers. comm.).

Originally, the enclosures were installed at two different depths to mimic stratified (deep) and continuously mixing (shallow) systems. When a deep lake stratifies, nutrient levels tend to decline in the surface waters and accumulate below the thermocline. Because small cells can outcompete larger cells for available nutrients under low nutrient conditions, the microbial loop may prevail (Porter et al. 1989). In contrast, in shallow lakes which continuously mix from top to bottom, nutrients get recirculated and remain in the epilimnion. Under these conditions, picoplankton production may be lower in comparison to the larger phytoplankton.

Therefore, a larger increase in HBAC and HNAN levels in the deep enclosures relative to the shallow enclosures, was anticipated, presuming that the shallow enclosures would accumulate higher nutrient levels due to the continuous nutrient loading and continuous mixing. However, Lac Croche has extremely nutrient poor sediments which appeared to adsorb nutrients and the shallow enclosures did not accumulate more nutrients when compared to the deep enclosures. The results do not support the initial hypothesis that the deep enclosures would contain higher levels of HBAC and HNAN.

In the fertilized enclosures the standing stocks of HBAC and HNAN levels ranged between 3.6×10^6 cells mL^{-1} to 9.7×10^6 cells mL^{-1} and $.05 \times 10^3$ cells mL^{-1} to 4.6×10^3 cells mL^{-1} respectively. In laboratory growth experiments certain HNAN populations have been known to reach maximal growth and ingestion rates at bacterial densities of about 5×10^6 cells mL^{-1} (Jürgens 1992). Yet in natural systems, HNAN are unable to reach abundances their resources could potentially support. One possible explanation for this phenomenon is predator control on HNAN. Several studies have reported a significant feeding impact of large zooplankton invertebrates within the microbial loop. Certain cladoceran and ciliate species have been found to graze extensively on HNAN as well as on bacteria (Sanders and Porter 1990, Weisse *et al.* 1990, Jürgens 1992, Vaquè and Pace 1992) thereby controlling all components of the microbial food web.

Following this argument, the addition of planktivorous fish would reduce

zooplankton grazing and allow for increased HNAN (or increased phytoplankton and bacterioplankton levels which could then support greater numbers of HNAN). In the enclosures without fish therefore zooplankton grazing would be expected to lead to a direct decrease in HNAN or a decrease in phytoplankton and bacterioplankton, thereby limiting HNAN abundance. However, the presence of fish did not change the overall abundances of either HBAC or HNAN. HBAC levels were slightly lower in the fish enclosures but this effect was not significant.

The lack of change at the HNAN level was not due to a lack of change at the level of the zooplankton community. In the enclosures containing planktivorous fish the biomass and size distribution of zooplankton were significantly altered (Mazumder et al. in prep) as were phytoplankton biomass and size distribution (Proulx *et al.* 1993).

In the enclosures containing fish, the zooplankton community was dominated by rotifers, small microzooplankton measuring between 41-200 um. Increased microzooplankton levels with the addition of planktivorous fish is consistent with other experimental studies (Mazumder *et al.* 1988, Lazzaro *et al.*, 1992).

Many planktonic rotifers are relatively unselective, feeding on particles in the size range of 0.5 to 20 μm (Rothhaupt 1990). Although, this size range includes heterotrophic flagellates and bacteria as well as picocyanobacteria, the impact of rotifers is generally considered to be much less significant than that of

large cladocerans (Arndt 1993). This is supported by the fact that the increase in rotifers clearly did not have a negative impact on HNAN.

The zooplankton community of the deep fishless enclosures was dominated by the large cladocera *Daphnia middendorffiana* (Mazumder pers. comm.). No large *Daphnia* sp. developed in any of the shallow enclosures. The presence of *Daphnia* in the deep enclosures appeared to have no significant effect on HNAN or HBAC. This result is in contrast to several descriptive and experimental studies, which have indicated that populations of large *Daphnia* have negative impacts on HNAN. Jürgens (1992) found that the lowest HNAN numbers were found in German lakes where *Daphnia* species dominated the zooplankton. In the experiments of Riemann (1985), enclosures containing fish had elevated levels of HNAN presumably because of reduced predation from large *Daphnia*. In his fishless enclosures, zooplankton invertebrates (particularly cladocerans) increased in numbers whereas heterotrophic flagellate numbers decreased and could not fully account for the bacterial turnover. Furthermore, Riemann (1985) concluded that in enclosures without fish, the cladocerans were effective grazers on bacteria (consumed all bacterial production). In the bag experiments of Pace and Funke (1991), the "top-down" effect of *Daphnia* was usually stronger than the nutrient effect in determining final densities of HNAN.

The underlying cause for the strong negative impact of nutrients on HNAN is not known. The results support the general theory that the microbial

loop is weaker in eutrophic conditions than in oligotrophic systems (Porter *et al.* 1988). It is clear that predation by *Daphnia* is not the explanation, but it is still possible that predation by other grazers not impacted by *Daphnia* was occurring. For example, ciliates are common in eutrophic water where bacterial densities are high and some species may graze on small heterotrophic flagellates and pico- and nanophytoplankton, thus providing another link to higher trophic levels. In mesotrophic to eutrophic lakes where bacterial densities are high, ciliates have been shown to play a key role in the energy transfer to higher trophic levels (Porter *et al.* 1979, Pace 1982, Weiss et al. 1990). Beaver and Crisman (1982) found that ciliate concentrations varied from less than 10 to around 200 cells mL^{-1} in Florida lakes and that there was a significant correlation between ciliate numbers and chlorophyll a. If ciliates were responsible for the decline of HNAN in the enclosures, then one would expect ciliate abundance to increase with nutrient additions and not be affected by the presence of planktivorous fish. At present ciliate abundance and biomass are being calculated so that their role in these systems (enclosures) may be determined.

An alternative explanation is that competition for bacteria with other bacterivores (possibly ciliates) or competition for dissolved nutrients with phytoplankton, suppresses HNAN in the nutrient enclosures. Although HNAN are generally considered to obtain their mineral nutrients through feeding on bacteria, there is no evidence to suggest that they cannot obtain dissolved

inorganic nutrients across the cell membrane as do phytoplankton. It is possible that under the conditions of continuous nutrient additions larger algae may have outcompeted the HNAN for nutrients.

INTRODUCTION

The distribution and functional role of protozoan communities in marine systems have been well documented. However, less is known about the *in situ* dynamics of freshwater protozoa. As in marine systems, freshwater protozoan densities are potentially regulated by the interaction of nutrient supply and grazing pressure.

An existing model predicts that heterotrophic flagellate abundance is regulated by resource availability ("bottom-up"). Beminger *et al.* (1991) analyzed a large number of freshwater systems and found a significant positive correlation between heterotrophic flagellates and bacteria. This relationship is observed over four orders of magnitude in bacterial abundance and includes extreme environments (glacially fed ponds and the sediment water interface), as well as temperate lake systems.

Large variations in heterotrophic flagellate densities have also been observed in lakes with similar bacterial densities. This may suggest that factors other than resource availability may be important in controlling heterotrophic flagellate abundance. Predation or "top-down" control on heterotrophic flagellate abundance can be very significant at certain times of the year (Güde 1988, Güde 1989, Weisse 1991). For example, Jürgens and Güde (1991) examined the seasonal variation in the intensity of heterotrophic flagellate grazing on bacteria and postulated that the most probable reason for the decline in flagellates early in the summer was the increase of daphnids which

are effective grazers on heterotrophic flagellates. Similarly, ciliates have been found to graze extensively on heterotrophic flagellates, especially in eutrophic environments (Weisse 1991).

The enclosure study (Chapter One) does not support the findings of Berninger *et al.* (1991). There was no increase in heterotrophic flagellate abundance related to increased bacterial levels in the nutrient enriched enclosures. Additionally, dense populations of *Daphnia* (in the fishless enclosures) had no significant effect on heterotrophic flagellate levels.

In order to determine if the enclosure results are representative of other dimictic lakes in our region, a lake survey was conducted to examine the abundance of heterotrophic flagellates and bacteria in relation to lake trophic status. Lakes were chosen to represent a range of productivities (estimated from total phosphorus, cholorphyll *a* and dissolved organic carbon concentrations) and food web structure as determined by a qualitative index of planktivory.

MATERIALS AND METHODS

Study Area and Lake Classification

A total of 26 lakes were examined during the summer of 1992. The main study area was in the Kawartha-Haliburton area of eastern Ontario, and Gatineau Park in western Quebec, Canada. In addition, samples from Lake Ontario and Lake Superior were included (Table 9).

The lakes were categorized into three classes following the Rasmussen *et al.* (1990) classification for lake trout lakes, which centers on food chain lengths. Class I lakes exhibit a relatively short food chain with no intermediate trophic levels between zooplankton and the top pelagic predator, lake trout. Class II lakes contain lake trout which feed mainly on pelagic forage fish (primarily coregonids), which in turn feed on zooplankton and small invertebrates. Lastly, Class III lakes, possessing the longest food chain, contain lake trout which feed on coregonids. However, coregonids in turn feed upon a larger invertebrate species *Mysis relicta*, which are major predators of zooplankton.

Sampling Procedure

One sample per lake was collected during summer stratification between July 16 and July 28, 1992, using a Van Dorn bottle at a midpoint in the epilimnion (as determined from temperature profiles taken on the same day). At the time of sampling, all lakes were stratified (with the exception of Lac

Renaud which is too shallow). Epilimnetic temperatures were between 22°C and 24°C in all the lakes with the exception of Lake Superior which rarely exceeds 14°C in the summer. Sub-samples of whole lake water were preserved immediately with 10% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7). Lake samples were stored at 4°C in the dark until enumeration, which was usually within ten days of the collection date.

Total phosphorus measurements were performed following the procedure used by Strickland and Parsons (1972). Dissolved organic carbon and chlorophyll *a* concentrations were analyzed using the methods in the Analytical Methods Manual (Environment Canada 1979).

Sample Preparation and Enumeration

Refer to the materials and methods section of chapter 1.

Statistical Methodology

The variables used throughout the statistical analysis include: total phosphorus (TP), total chlorophyll *a* (Chl *a*), dissolved organic carbon, (DOC), heterotrophic bacteria abundance (HBAC), heterotrophic flagellate abundance (HNAN), mixotrophic flagellate abundance (MIXO), and an independent categorical variable denoting lake class (CLASS I, II, III). These variables were log transformed in order to stabilize the variances and satisfy the assumptions of normality and homoscedasticity. The categorial class variables were not

transformed.

Equations relating nanoflagellate or bacterial abundance to measures of water productivity were established with simple linear regressions and correlations using SAS (version 6.03, 1988). The independent variables, log TP, log Chl *a* and log DOC, were each regressed against the dependent variables, log HBAC, log HNAN, log MIXO respectively. Simple Pearson Correlations were performed between log HNAN, log MIXO, log HBAC, log TP, log Chl *a*, and log DOC. A Pearson correlation matrix was used to test for multicollinearity among the independent variables.

Lastly, the relationship between heterotrophic flagellate and bacterial abundance with respect to lake CLASS was examined. An analysis of covariance (ANCOVA) was performed to compare the regression equations between the dependent variable log HNAN and the independent variables log HBAC and log HBAC*CLASS (interaction term).

RESULTS

Bacteria And Lake Trophic Status

A wide range of trophic conditions were represented by the 26 study sites (Table 9). Of the 26 lake sampled, 5 lakes were categorized as CLASS I; 7 lakes as CLASS II; and 9 lakes as CLASS III. The CLASS category for five of the lakes was not known or not appropriate in our analysis. Total phosphorus concentration ranged from 2.4 to 42.7 μg litre^{-1}. DOC and Chl *a* values ranged from 2.4 to 7.6 μg litre^{-1} and 1.0 to 32.6 μg litre^{-1} respectively. Maximum TP value corresponded to the highest DOC and Chl *a* values all of which were found in the Bay of Quinte of Lake Ontario. HBAC abundance ranged from 6.2 X 10^5 to 6.7 X 10^6 cells mL^{-1} (Table 10).

In this survey, HBAC abundance was significantly and positively related to phosphorus, chlorophyll *a* and dissolved organic carbon levels and may be predicted reasonably well from any of these variables (Table 11). The best relationsnip was between HBAC and log TP (Fig.14). The overall equation for this relation is:

$$\log HBAC = 0.59 \log TP + 5.75$$

(r^2= 0.70; p = 0.0001; n=26)

A strong positive relationship between HBAC and logTP was also found by Currie (1990) in his survey of 23 temperate lakes.

The relationship between heterotrophic bacteria and log DOC was weaker than the bacteria-phosphorus relationship. The resulting equation is:

$$\log HBAC = 0.35 \log DOC - 3.37$$

(r^2=0.46; p = 0.0001; n=26)

A similar relationship was found between bacteria and DOC in humic lakes (Tranvik 1989).

A positive relationship was also found between HBAC and log Chl *a* concentration among our study sites. This relationship is presented in the following equation:

$$\log HBAC = 0.24 \log Chl\ a - 3.11$$

$$(r^2 = 0.31;\ p = 0.003;\ n=26)$$

This significant relationship between heterotrophic bacterial abundance and chlorophyll *a* concentration is consistent with several previous studies (Bird and Kalff, 1984; and Cole *et al.*, 1988).

Chl *a*, and DOC and TP explained 31, 46 and 70% respectively of the variability in bacterial abundance. A closer examination of the association between these significantly correlated variables revealed multicollinearity of the independent variables (Table 12). The addition of log Chl *a* and log DOC in the overall model did not account for any more of the variability in HBAC then was explained by log TP alone. After eliminating collinear variables, the best predictor of HBAC was log TP (adjusted $r^2 = 0.67$).

Heterotrophic Flagellates And Lake Trophic Status

HNAN abundance was approximately 3 orders of magnitude lower than HBAC and levels ranged between 7.8×10^2 to 5.63×10^3 cells mL^{-1} (Table 10). Simple Pearson correlations found no significant relationships between HNAN and DOC or

Chl *a* measurements. HNAN abundance was slightly correlated with log TP concentration (Table 11) (Fig.15):

$$\log HNAN = 0.18 \log TP - 0.82$$

(r^2=0.22, p = 0.015, n = 26)

Mixotrophic Flagellates and Lake Trophic Status

MIXO abundance ranged between 6.75 X 10^2 to 3.75 X 10^3 cells mL^{-1} (Table 10). Pearson correlation analyses indicated no significant relationships between logMIXO and any of the measured parameters (i.e logTP, logDOC, logChl *a*) among our chosen lakes (see Appendix I).

Heterotrophic Flagellates, Bacteria and Lake Class

HNAN and HBAC were not correlated in this study (r = 0.23, p = 0.255, n=26) (Table 11)(Fig.16). In contrast, Berninger *et al.*(1991) found a significant positive correlation between HNAN and HBAC over a wide range of freshwater systems ($r^2$0.82, n=212). Sanders *et al.* (1992) found a similar but much weaker relationship in marine, estuarine, and freshwater systems (r^2= 0.50, n=600).

Furthermore, HNAN and HBAC were not related among our different CLASS lakes (p = 0.72; Table 13). The overall ANCOVA model found no significant differences between the regression equations for each lake CLASS (p = 0.161).

Table 9. Study lake, class, location, total phosphorus (TP µg L^{-1}), dissolved organic carbon (DOC mg L^{-1}) and chlorophyll *a* (Chl *a* µg L^{-1}) concentrations, for 26 Canadian lakes.

LAKE	LOCATION	TP	DOC	Chl *a*
CLASS I				
Silent	44°55'N,78°04'W	4.6	4.2	1.5
Eels	44°54'N,78°08'W	6.7	4.5	2.1
Anstruther	44°45'N,78°12'W	3.4	4.2	1.8
Kawagama	45°18'N,78°45'W	2.4	2.4	1.8
Kenesis	45°13'N,78°38'W	2.4	2.6	2.6
CLASS II				
Haliburton	45°12'N,78°24'W	5.1	3.7	1.4
Halis	45°06'N,78°45'W	3.1	2.9	1.0
Oxtongue	45°22'N,78°55'W	6.3	4.3	4.7
St. Nora	45°09'N,78°50'W	3.9	2.9	3.0
Little Hawk	45°32'N,78°40'W	4.0	2.4	3.6
Koshlong	44°58'N,78°29'W	4.6	4.1	1.1
Brown	45°36'N,75°55'W	26.0	6.2	5.6
CLASS III				
Gull	44°54'N,78°47'W	5.6	3.7	6.2
Crystal	44°45'N,78°29'W	6.6	6.3	2.0
12 Mile	45°01'N,78°44'W	5.1	3.4	1.1
Boskung	45°04'N,78°44'W	3.9	3.3	1.7
Fairy	45°20'N,79°11'W	5.6	5.5	3.8
L. of Bays	45°15'N,79°00'W	4.1	2.8	1.7
Superior	45°43'N,78°49'W	3.0	3.1	1.2
Ontario (Stn.403)	43°35'N,78°13'W	16.2	3.5	6.7
Ontario (Stn.401)	43°53'N,78°15'W	11.7	2.9	3.0
UNCLASSED				
Bay of Quinte	44°20'N,78°52'W	42.7	7.6	32.6
Rice-East	44°10'N,77°05'W	23.0	5.7	7.8
Rice-West	44°20'N,78°35'W	35.0	5.6	16.0
Mulvihill	45°29'N,75°51'W	19.6	6.2	5.5
Renaud	45°36'N,76°00'W	21.4	6.9	4.5

Table 10. Ranges of the variables measured in 26 Canadian lakes. Minimum and maximum values are included for TP (μg P L^{-1}), Chl *a* (μg L^{-1}), DOC (mg L^{-1}) concentrations, HBAC (10e+6 cells mL^{-1}), HNAN (10e+3 cells mL^{-1}) and MIXO (10e+3 cells mL^{-1}) abundance.

Variables	Minimum	Maximum
TP	2.4	42.7
Chl *a*	1.0	32.6
DOC	2.4	7.6
HBAC	0.62	6.70
HNAN	0.78	5.63
MIXO	0.68	3.75

Table 11. Simple linear regression analyses on the effects of log total phosphorous (LOGTP), log chlorophyll *a* concentration (LOGChl *a*) and log dissolved organic carbon (LOGDOC) on heterotrophic bacterial (HBAC), heterotrophic flagellate (HNAN) and mixotrophic flagellate (MIXO) abundance in 26 Canadian lakes. ** Asterix indicate significant relationtionship where $p < 0.05$

Dependent Variable	Independent Variable	n	r^2	p-value
logHBAC	logTP	26	0.70	0.0001**
logHBAC	logChl *a*	26	0.31	0.003**
logHBAC	logDOC	26	0.46	0.001**
logHNAN	logTP	26	0.22	0.015**
logHNAN	logHBAC	26	0.05	0.255

Table 12. Summary of Pearson correlation analyses examining the relationship between the independent variables total phosphorous (logTP), chlorophyll *a* (logCHL *a*) and dissolved organic carbon (logDOC) concentrations. Independent variables have been log transformed. ** Asterix indicate significant relationtionships and multicollinearity between variables ($p < 0.05$).

Variable A	Variable B	n	r	P value
logTP	logCHL*a*	26	0.86	<0.001**
logTP	logDOC	26	0.75	<0.001**
logCHL*a*	logDOC	26	0.59	<0.002**

Table 13. Summary of the ANCOVA examining the effect of lake class (CLASS) and log transformed heterotrophic bacterial abundance (logBAC) on the dependent variable, log heterotrophic flagellate abundance.

Source	SS	F Value	DF	P Value
CLASS	0.19	2.20	2	0.81
LogBAC	0.01	0.30	1	0.13
LogBAC * CLASS	0.19	2.22	2	0.72
Error	0.74		17	

Figure 14. The relationship between log transformed heterotrophic bacterial abundance and log transformed total phosphorus concentrations in 26 Canadian lakes during mid-summer. The solid line represents the regression model fitted to these data. The resulting equation is log HBAC = 0.59 logTP + 5.75 (r^2= 0.70, p = 0.0001). Dashed lines represent 95% confidence intervals.

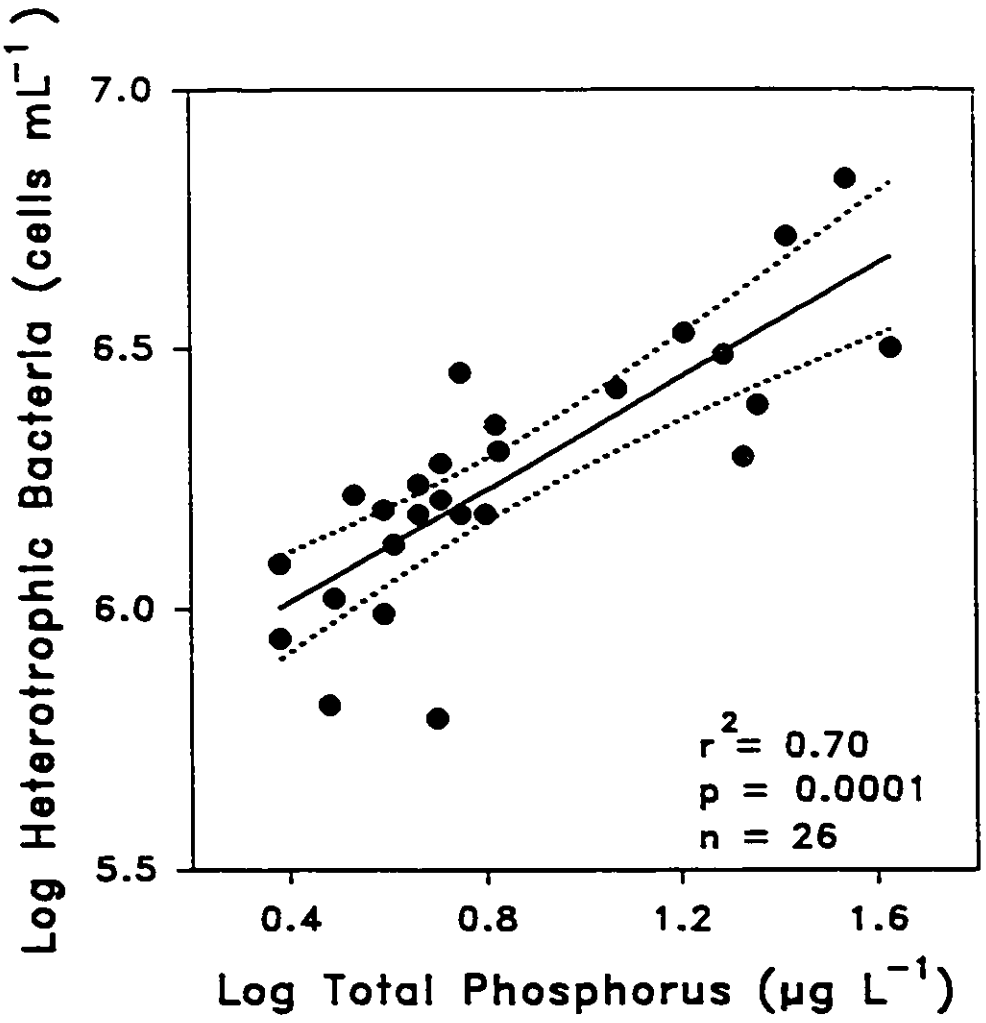

Log Heterotrophic Bacteria (cells mL^{-1})
7.0
6.5
6.0
5.5
0.4
0.8
1.2
1.6
Log Total Phosphorus (µg L^{-1})
r^2 = 0.70
p = 0.0001
n = 26

Figure 15. The relationship between log transformed heterotrophic flagellate abundance and log transformed total phosphorus concentrations in 26 Canadian lakes during mid summer. The resulting equation is log HNAN = 0.18 log TP - 0.82 (r^2 = 0.22, p = 0.015). The dashed lines represent 95% confidence limits.

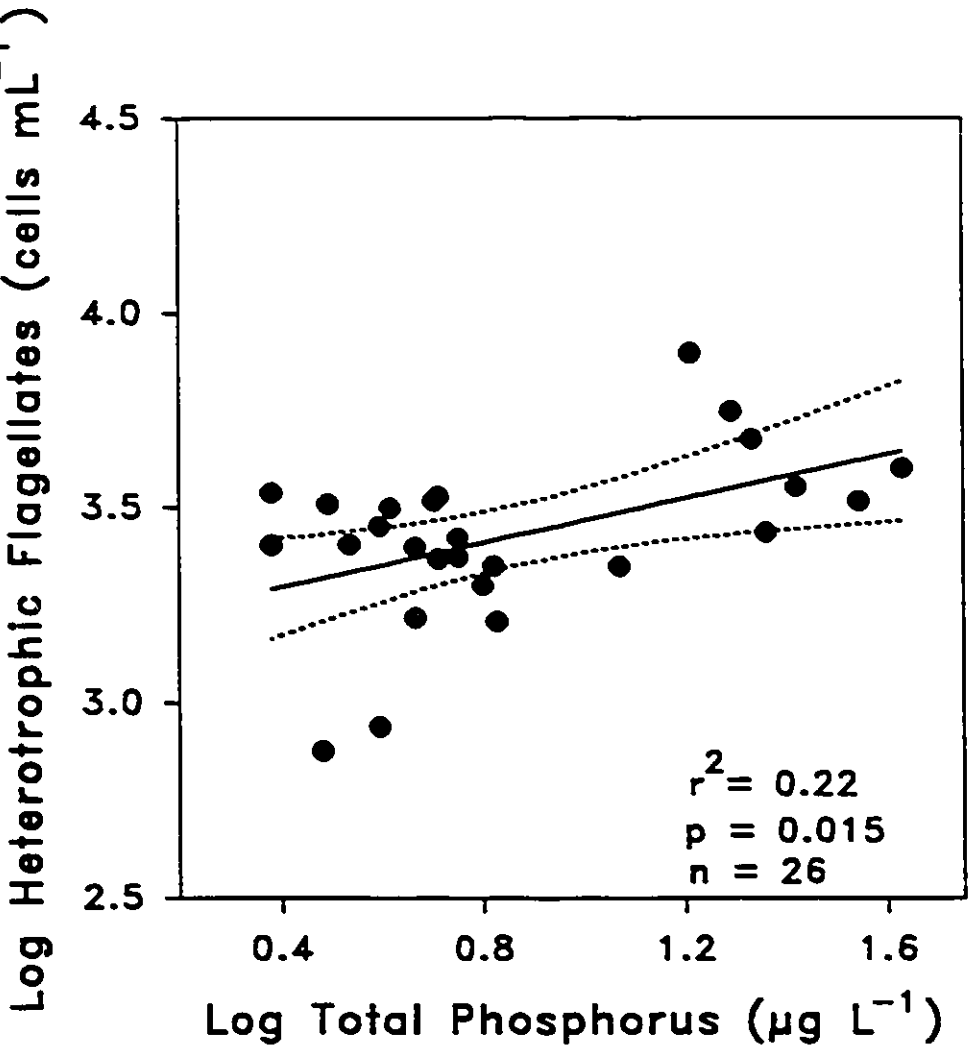
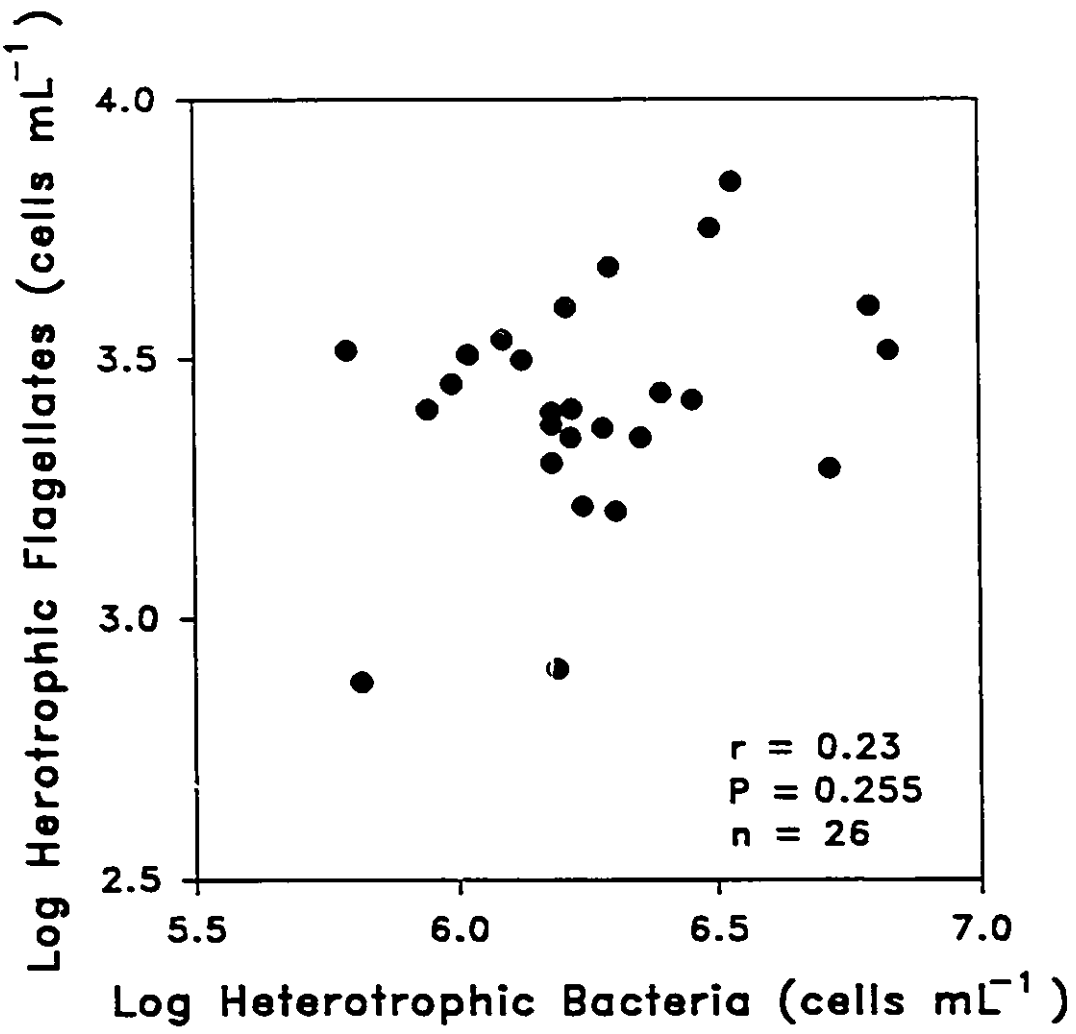

Log Heterotrophic Flagellates (cells mL^{-1})
4.5
4.0
3.5
3.0
2.5
0.4
0.8
1.2
1.6
Log Total Phosphorus (µg L^{-1})
r^2= 0.22
p = 0.015
n = 26

Figure 16. The relationship between log transformed heterotrophic flagellate and log transformed heterotrophic bacterial abundance in 26 Canadian lakes during mid summer. Pearson correlation analysis indicated no significant relationship ($r = 0.23$, $p = 0.255$).


Log Herotrophic Flagellates (cells mL^{-1})
4.0
3.5
3.0
2.5
5.5
6.0
6.5
7.0
r = 0.23
P = 0.255
n = 26
Log Heterotrophic Bacteria (cells mL^{-1})

DISCUSSION

This study presents data on heterotrophic flagellate (HNAN) and bacterial (HBAC) abundances in temperate dimictic lakes with differing productivities. Most work examining HNAN abundance has not taken TP, Chl *a* concentrations or primary productivity estimates into account. Although we did not measure primary productivity directly, TP, Chl *a* and DOC measurements are highly indicative of bacterial abundance and production. Bacterial abundance was positively correlated with logTP, and logChl *a*. In our study, HBAC abundance was more highly correlated to logTP than logChl *a* (Table 11). This was also observed by Currie (1990).

Bacterial productivity has been correlated with primary production over a wide range of marine and freshwater systems (Bird and Kalff 1984, Cole *et al.* 1988). Furthermore, the flux of photosynthate into the DOC pool increases with increasing primary productivity of systems. Since DOC is the substrate required for bacterial growth, higher levels of DOC in a system should lead to an increase in HBAC which in turn would lead to an increase in the number of HNAN, their primary predators. Berninger *et al.* (1991) demonstrated that the most productive waters have the highest DOC and also support the highest numbers of HBAC. In this study, the abundance of HBAC was also significantly correlated to both Chl *a* ($r^2 = 0.31$; $p = 0.003$) and DOC ($r^2 = 0.46$; $p = 0.0001$) concentrations (Table 11).

However, the same did not hold true for HNAN; there was no

relationship between HNAN abundance and log Chl *a* or log DOC concentrations (Appendix I). A slight positive relationship was observed between HNAN and logTP (Table 11). Total phosphorus explained approximately 22% of the variation in HNAN (Fig. 15). There are a few studies which have examined the relationship between HNAN abundance and water productivity: the majority have dealt with the relationship of HNAN to their immediate prey HBAC. However, HBAC turn over very rapidly in lakes, and standing stock is a poor indication of actual supply rates to HNAN. Supply rates of HBAC are likely related to primary production since bacterial production is highly correlated to primary production (Cole *et al.* 1988). It may be simply because TP is highly correlated with primary production in lakes (e.g. Smith 1979) that a statistical relationship between TP and HNAN was observed.

HNAN and HBAC were not correlated among the lakes in our survey (Fig. 16). Our results are consistent with most seasonal within lake studies (Pick and Caron 1987, Nagata 1988, Bennett *et al.* 1990) and with the results obtained from the enclosure study (Chapter One). Enclosures with nutrient additions alone or in combination with planktivorous fish exhibited higher primary productivity with respect to the control and fish only enclosures (Perin *et al.* in prep). However, HNAN abundance was not higher in the enriched enclosures. In fact the enclosure study indicated a strong negative correlation between HNAN and HBAC during mid summer despite increasing water productivity (r^2= - 0.64, $P \leq 0.001$) (Fig. 17). Such a negative relationship was

also observed by Psenner and Sommaruga (1992) in Mondsee, an Austrian lake (r^2= -0.90, P < 0.001) and Weisse (1991) in Lake Constance (r^2 = -0.23, P < 0.05).

There are a number of factors which could account for the difference in results between previous models and our lake survey results. One of the reasons may be attributed to methodological discrepancies. Due to their extremely small size and delicate structure, proper sampling and preservation techniques are crucial in maintaining nanoflagellate cell structure. Distinguishing between heterotrophic and mixotrophic flagellates can be very difficult since the only characteristic which separates them is the presence of photosynthetic accessory pigments in the mixotrophs. Bird and Kalff (1987) have shown that mixotrophic flagellates can be as abundant as heterotrophic flagellates and can exhibit equal grazing pressure on bacterioplankton. Mixotrophic flagellates were not included in the study conducted by Berninger *et al.* (1991). Samples were provided from various sources by mail and any delay from the time of sampling to counting could have caused fading of Chl *a* autofluorescence of the mixotrophic cells. Therefore it is possible that mixotrophic cells may have been included in their study. If this was the case, one would expect a stronger correlation. When lake survey MIXO and HNAN levels were combined, the relationship between total nanoflagellate abundance and bacteria was not significant (r=0.36, p=0.08). The main difference between the present study and that of Berninger *et al.* (1991) is one of scale.

The Berninger *et al.* (1991) study included freshwater systems ranging from ultraoligotrophic to hypereutrophic lakes and rivers. In these systems HBAC explained 82% of the variance associated with HNAN abundance. HBAC levels ranged from less than 10^6 to more than 10^9 cells mL^{-1}. The lakes in this study represent a narrower range in HBAC abundance (one order of magnitude as opposed to four orders). An analysis of the subset of Berninger *et al.* (1991) data set that falls within the same range of HBAC abundance (10^5-10^6 cells mL^{-1}) as found in this study indicates a weaker correlation (Fig. 18) than the overall model although this relationship remains significant ($r^2 = 0.10$, $p = 0.009$, $n = 66$).

Most temperate lakes have a fairly narrow range of HBAC between 10^5 to 10^6 cells mL^{-1}, where the average HBAC abundance is of the order of 10^6 cells mL^{-1}. Within this range, the variability in the data is too large to achieve any predictive value and there appears to be no relationship between HNAN and HBAC.

From previous studies and the data presented in this study, it seems that bacteria seem to be closely linked to their substrate supply. But the absence of a relationship between HNAN and "bottom-up" forces (i.e resource supply) suggests some other variable must exist which would explain the pattern observed in these oligo to mesotrophic lakes. This variable could be predatory regulation or "top-down" control of HNAN.

We examined the relationship between HNAN and HBAC with respect to

lake CLASS which relates to level of planktivory and we did not find a difference in the relationship between HNAN and HBAC among our CLASS lakes. However, this is only a general classification scheme based on the fish composition structure and the presence of the large, planktivorous, *Mysis relicta*. In general, CLASS I lakes have relatively higher cladoceran levels than CLASS III lakes (Pick and Hamilton in press), but the abundance of other potentially significant predators on HNAN such as ciliates remains to be examined. Vaqué and Pace (1992) in a comparison of two lakes with contrasting food webs (a piscivorous *vs* planktivore dominate system) also found no difference in HNAN abundance.

The role of ciliates and cladocerans and their predatory control of HNAN may be an important factor regulating HNAN abundance in freshwater systems. Weisse (1991) conducted a long-term study on the abundance and biomass of heterotrophic flagellates in Lake Constance, Germany. He concluded that HNAN abundance fluctuates seasonally with the relative importance of "top-down" versus "bottom-up" control varying at different times of the year. However, throughout most of the year grazing by ciliates was more important in regulating HNAN than control by bacterial food supply.

Similarly, Güde (1988, 1989) suggested that during certain periods of the seasonal cycle of lakes, cladocerans can effectively control HNAN abundance. Several other studies have suggested the importance of *Daphnia* species as significant grazers of HNAN (Sanders and Porter 1990, Psenner and

Scmmaruga 1992). Experiments conducted by Jürgens (1992) concluded that HNAN abundance is not food-limited but that they are kept below their carrying capacity due to increased predation pressure. HNAN abundance was lowest during times when *Daphnia* sp. dominated the zooplankton. Furthermore, Gasol and Vaqué (1993) discovered that in lakes where *Daphnia* sp. dominated the zooplankton community, the only significant variable predicting HNAN was the abundance of large cladocerans. However, in experimental studies HNAN are not always affected by large populations of *Daphnia* (Chapter One, Pace and Funke 1991).

There tends to be a shift in protozoan community composition with increasing lake trophy; heterotrophic flagellates may dominate in low productive systems (Davis *et al.* 1985), while ciliates and microzooplankton, mainly cladocera, become dominant components in more productive systems (Fenchel, 1987). We cannot make the generalization that heterotrophic flagellate abundance increases with increasing water productivity nor that heterotrophic flagellate abundance is significantly correlated to bacteria levels in temperate lakes. Fenchel (1987) suggested that bacteria availability regulates heterotrophic flagellate abundance in oligotrophic systems whereas grazer control suppresses heterotrophic flagellate abundance in eutrophic systems.

Resource control of HNAN among lakes and in the enclosures was not observed. Predation or competition might be regulating HNAN in these temperate lakes. Yet, evidence of "top-down" control is not conclusive. Future

research examining the relative importance of ciliate and cladoceran grazing on HNAN is required to determine the importance of "top-down" control in these lake systems.

Figure 17. The relationship between log heterotrophic flagellate and log bacterial abundance in the 16 enclosures installed in Lac Croche. Each data point represents a separate enclosure sampled during mid summer (July 30). Enclosures were manipulated to test for the following treatment effects: planktivorous fish additions, nutrient additions, planktivores and nutrients , and no planktivores and no nutrients (control).

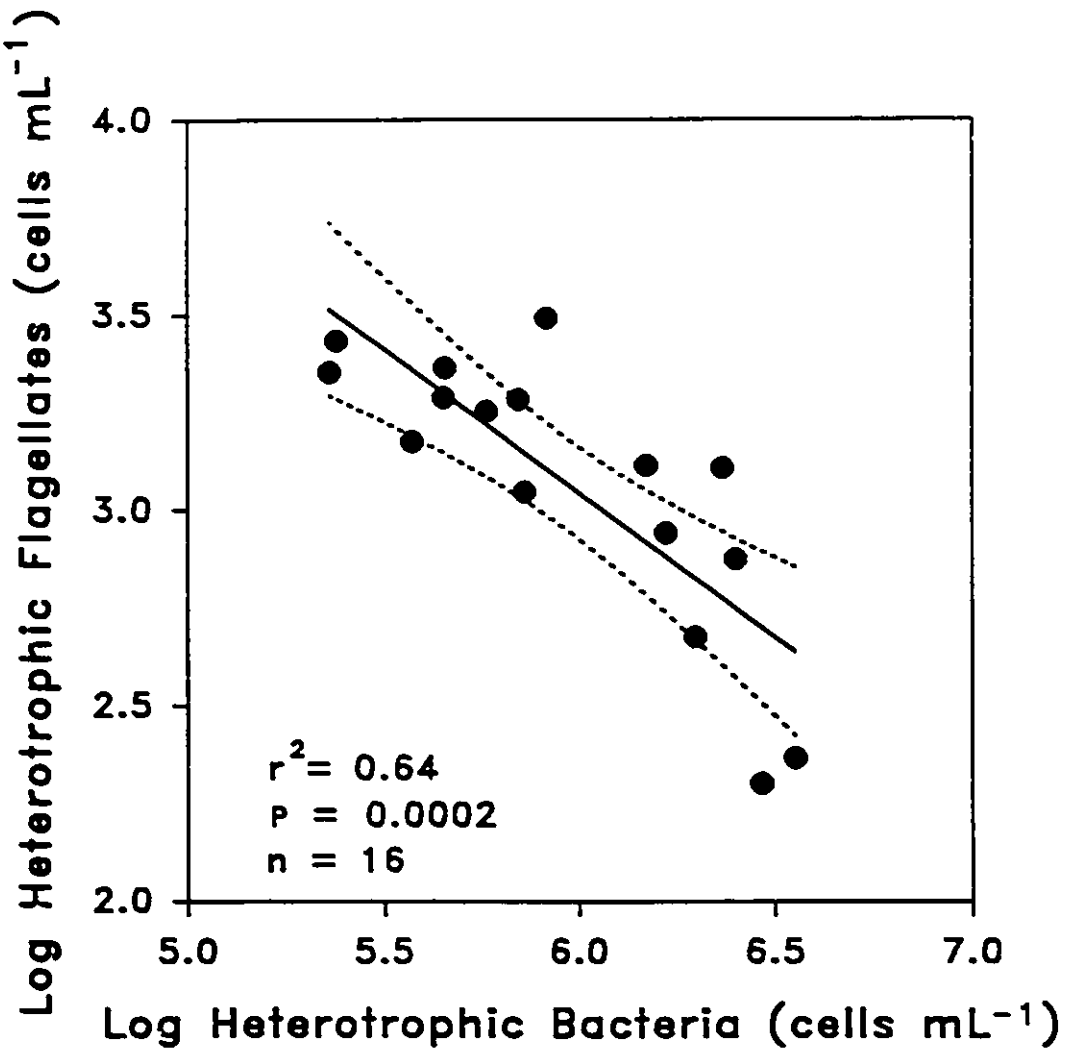
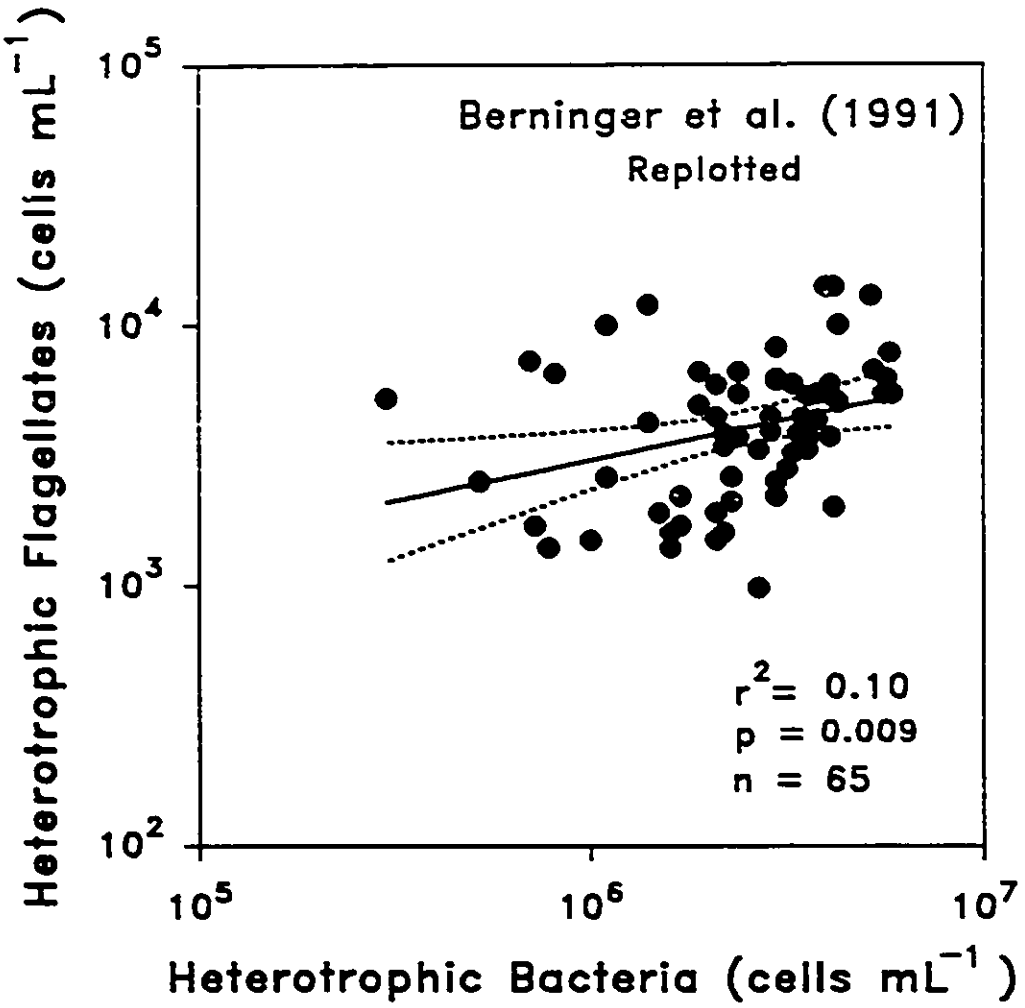

Log Heterotrophic Flagellates (cells mL^{-1})
4.0
3.5
3.0
2.5
2.0
r^2= 0.64
P = 0.0002
n = 16
5.0
5.5
6.0
6.5
7.0
Log Heterotrophic Bacteria (cells mL^{-1})

Figure 18. The relationship between heterotrophic flagellate and bacterial abundance from a subset of the data examined by Berninger *et al.*, (1991). Data points include lakes within the range of our survey (10^2 - 10^3 for HNAN and 10^5-10^6 for HBAC).


Berninger et al. (1991)
Replotted
r^2= 0.10
p = 0.009
n = 65
Heterotrophic Flagellates (cells mL^{-1})
Heterotrophic Bacteria (cells mL^{-1})
10^2
10^3
10^4
10^5
10^5
10^6
10^7

CONCLUDING REMARKS

This study demonstrated that heterotrophic flagellates and their food resource (picoplankton) are not strongly coupled in temperate oligotrophic to mesotrophic lakes. Manipulated enclosures with increased nutrient levels supported higher bacterial levels but did not show a corresponding increase in HNAN. HNAN levels were significantly reduced with the addition of nutrients.

The presence of planktivorous fish altered zooplankton community structure but did not affect heterotrophic flagellate abundance. Fishless enclosures were dominated by dense *Daphnia* populations, yet there was no indication of predatory control by *Daphnia* on the heterotrophic flagellates.

The deep enclosures did not support higher HNAN levels. This effect was not due to differences in lake trophy since nutrient availability was similar in both the deep and shallow enclosures.

Overall, heterotrophic flagellate abundance did not increase with increasing water productivity. This suggests that the microbial loop may be more important in oligotrophic systems than eutrophic systems.

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Appendix 1- Summary of Pearson correlation analyses examining the relationship between log total phosphorous (logTP), log chlorophyll *a* (logCHL *a*), log dissolved organic carbon (logDOC) concentrations, heterotrophic flagellates (HNAN) and mixotrophic flagellates (MIXO) in temperate lakes.

Variable A	Variable B	n	r	P value
logHNAN	logCHL *a*	26	0.22	0.332
logHNAN	logDOC	26	0.24	0.238
logMIXO	logChl *a*	23	0.27	0.217
logMIXO	logTP	23	0.28	0.191
logMIX0	logDOC	23	0.28	0.203

Bacteria Counts

WEEK 1
June 19 , 91

ENCLOSURE	No. Cells	Cells/ml	Mean	S.D
SHALLOW				
#1) NUT.	532	8.49E+05	8.61E+05	1.24E+04
#2) NUT.	528	8.73E+05		
#3) NUT.+ FISH	509	9.09E+05	9.20E+05	1.07E+04
#4) NUT.+ FISH	521	9.31E+05		
#5) CONTROL	570	1.59E+06	1.50E+06	8.75E+04
#6) CONTROL	539	1.42E+06		
#7) FISH	534	9.17E+05	8.48E+05	6.93E+04
#8) FISH	523	7.79E+05		
DEEP				
#9) NUT.	517	7.22E+05	8.16E+05	9.48E+04
#10) NUT.	510	9.11E+05		
#11) NUT. + FISH	509	7.10E+05	9.25E+05	2.15E+05
#12) NUT. + FISH	638	1.14E+06		
#13) CONTROL	513	6.37E+05	6.34E+05	3.50E+03
#14) CONTROL	508	6.30E+05		
#15) FISH	567	1.01E+06	1.04E+06	2.41E+04
#16) FISH	594	1.06E+06		

WEEK 2
July 2, 91

ENCLOSURE	No. Cells	Cells/ml	Mean	S.D
SHALLOW				
#1) NUT.	560	8.34E+05	8.554E+05	2.16E+04
#2) NUT.	589	8.77E+05		
#3) NUT.+ FISH	674	1.82E+06	1.574E+06	2.51E+05
#4) NUT.+ FISH	622	1.32E+06		
#5) CONTROL	500	5.32E+05	4.590E+05	7.27E+04
#6) CONTROL	506	3.86E+05		
#7) FISH	508	4.88E+05	4.349E+05	5.30E+04
#8) FISH	513	3.82E+05		
DEEP				
#9) NUT.	550	1.64E+06	1.691E+06	5.29E+04
#10) NUT.	527	1.74E+06		
#11) NUT. + FISH	366	1.09E+06	1.136E+06	4.60E+04
#12) NUT. + FISH	516	1.18E+06		
#13) CONTROL	530	1.21E+06	1.451E+06	2.37E+05
#14) CONTROL	529	1.69E+06		
#15) FISH	526	1.38E+06	1.424E+06	4.18E+04
#16) FISH	525	1.47E+06		

Bacteria Counts

WEEK 3
14-Jul-91

ENCLOSURE	No. Cells	Cells/ml	Mean	S.D
		SHALLOW		
#1) N	642.00	1.47E+06	2.06E+06	5.90E+05
#2) N	534.00	2.65E+06		
#3) N+F	589.00	2.19E+06	2.09E+06	1.04E+05
#4) N+F	533.00	1.98E+06		
#5) C	557.00	1.51E+06	1.39E+06	1.19E+05
#6) C	512.00	1.27E+06		
#7) F	579.00	1.92E+06	1.41E+06	5.06E+05
#8) F	520.00	9.04E+05		
		DEEP		
#9) N	543.00	3.23E+06	2.99E+06	2.47E+05
#10) N	525.00	2.74E+06		
#11) N+F	513.00	3.55E+06	3.92E+06	3.65E+05
#12) N+F	575.00	4.28E+06		
#13) C	555.00	1.50E+06	1.76E+06	2.54E+05
#14) C	540.00	2.01E+06		
#15) F	537.00	1.07E+06	1.17E+06	1.05E+05
#16) F	514.00	1.28E+06		

WEEK #4
July 30/91

ENCLOSURE	No. Cells	Cells/ml	Mean	S.D
		SHALLOW		
#1) N	690.00	1.58E+06	1.61E+06	3.44E+04
#2) N	720.00	1.65E+06		
#3) N+F	625.00	2.33E+06	2.42E+06	9.68E+04
#4) N+F	638.00	2.52E+06		
#5) C	386.00	3.71E+05	3.43E+05	2.82E+04
#6) C	380.00	3.14E+05		
#7) F	526.00	3.48E+05	5.23E+05	1.75E+05
#8) F	370.00	6.97E+05		
		DEEP		
#9) N	543.00	2.93E+06	3.25E+06	3.15E+05
#10) N	516.00	3.56E+06		
#11) N+F	530.00	8.29E+05	7.77E+05	5.20E+04
#12) N+F	510.00	7.25E+05		
#13) C	507.00	4.57E+05	3.92E+05	6.53E+04
#14) C	505.00	3.27E+05		
#15) F	562.00	2.29E+05	2.34E+05	4.44E+03
#16) F	524.00	2.38E+05		

Bacteria Counts

WEEK #5
14-Aug-91

ENCLOSURE	SHALLOW			
	No. Cells	Cells/ml	Mean	S.D
#1) NUT.	504.00	7.90E+05	9.33E+05	1.43E+05
#2) NUT.	542.00	1.08E+06		
#3) NUT.+ FISH	571.00	1.06E+06	1.32E+06	2.60E+05
#4) NUT.+ FISH	585.00	1.58E+06		
#5) CONTROL	562.00	7.28E+05	6.54E+05	7.38E+04
#6) CONTROL	516.00	5.80E+05		
#7) FISH	516.00	1.71E+06	1.10E+06	6.09E+05
#8) FISH	525.00	4.89E+05		
DEEP				
#9) NUT.	515.00	5.29E+05	6.54E+05	1.26E+05
#10) NUT.	524.00	7.80E+05		
#11) NUT. + FISH	512.00	7.26E+05	8.32E+05	1.06E+05
#12) NUT. + FISH	535.00	9.37E+05		
#13) CONTROL	523.00	9.16E+05	1.16E+06	2.39E+05
#14) CONTROL	515.00	1.39E+06		
#15) FISH	517.00	1.10E+06	1.01E+06	8.83E+04
#16) FISH	527.00	9.23E+05		

WEEK #6
28-Aug-91

ENCLOSURE	SHALLOW			
	No. Cells	Cells/ml	Mean	S.D
#1) NUT.	516.00	2.56E+06	3.32E+06	7.56E+05
#2) NUT.	547.00	4.07E+06		
#3) NUT.+ FISH	518.00	2.57E+06	2.67E+06	3.02E+05
#4) NUT.+ FISH	533.00	3.17E+06		
#5) CONTROL	515.00	1.10E+06	1.05E+06	4.15E+04
#6) CONTROL	510.00	1.01E+06		
#7) FISH	508.00	9.45E+05	8.88E+05	5.75E+04
#8) FISH	502.00	8.30E+05		
DEEP				
#9) NUT.	525.00	1.12E+06	1.65E+06	5.33E+05
#10) NUT.	513.00	2.18E+06		
#11) NUT. + FISH	527.00	1.96E+06	1.82E+06	1.37E+05
#12) NUT. + FISH	510.00	1.69E+06		
#13) CONTROL	513.00	8.99E+05	8.93E+05	5.25E+03
#14) CONTROL	507.00	8.88E+05		
#15) FISH	510.00	9.49E+05	9.43E+05	6.51E+03
#16) FISH	503.00	9.36E+05		

Bacteria Counts

WEEK 7
14-Sep-91

ENCLOSURE	# CELLS	CELLS/ml	MEAN	S.D
		SHALLOW		
#1) N	884.00	5.64E+06	5.22E+06	4.20E+05
#2) N	847.00	4.80E+06		
#3) N+F	891.00	5.69E+06	5.29E+06	3.93E+05
#4) N+F	874.00	4.90E+06		
#5) C	809.00	3.29E+06	3.09E+06	1.91E+05
#6) C	845.00	2.90E+06		
#7) F	589.00	1.75E+06	1.69E+06	6.85E+04
#8) F	543.00	1.62E+06		
DEEP				
#9 N	506.00	2.33E+06	2.82E+06	4.92E+05
#10) N	816.00	3.31E+06		
#11) N+F	559.00	1.66E+06	1.48E+06	1.87E+05
#12) N+F	520.00	1.29E+06		
#13) C	725.00	2.16E+06	2.07E+06	9.23E+04
#14) C	663.00	1.97E+06		
#15) F	780.00	2.32E+06	2.22E+06	9.98E+04
#16) F	713.00	2.12E+06		

Picocyanobacteria Counts

Week 3, July 16

ENCLOSURE	Shallow CELLS/mL	MEAN	S.D
#1) N	2.76E+06	1.75E+06	1033500
#2) N	7.13E+05		
#3) N+F	5.81E+04	6.99E+04	11800
#4) N+F	8.17E+04		
#5) C	8.63E+04	7.80E+04	8350
#6) C	6.96E+04		
#7) F	7.90E+04	8.83E+04	9300
#8) F	9.76E+04		
Deep			
#9) N	6.97E+01	1.39E+02	70
#10) N	2.09E+02		
#11) N+F	9.30E+04	1.64E+05	70500
#12) N+F	2.34E+05		
#13) C	1.10E+05	1.10E+05	0
#14) C	1.10E+05		
#15) F	8.32E+04	7.42E+04	9000
#16) F	6.52E+04		

Week 4, July 30

ENCLOSURE	Shallow CELLS/mL	MEAN	S.D
#1) N	3.62E+03	2.48E+03	1145
#2) N	1.33E+03		
#3) N+F	5.81E+04	1.23E+05	64450
#4) N+F	1.87E+05		
#5) C	7.46E+04	6.98E+04	4800
#6) C	6.50E+04		
#7) F	1.01E+05	9.98E+04	1250
#8) F	9.85E+04		
Deep			
#9) N	9.65E+02	7.61E+02	204
#10) N	5.57E+02		
#11) N+F	4.57E+02	3.82E+02	75
#12) N+F	3.07E+02		
#13) C	1.33E+05	1.01E+05	32400
#14) C	6.82E+04		
#15) F	1.17E+05	9.76E+04	19450
#16) F	7.81E+04		

Picocyanobacteria Counts

Week 5, Aug. 13

ENCLOSURE	Shallow CELLS/mL	MEAN	S.D
#1) N	2.19E+03	1.83E+03	365
#2) N	1.46E+03		
#3) N+F	2.28E+04	2.36E+05	213100
#4) N+F	4.49E+05		
#5) C	8.84E+04	6.70E+04	21400
#6) C	4.56E+04		
#7) F	1.05E+05	1.17E+05	11500
#8) F	1.28E+05		
Deep			
#9) N	1.20E+03	1.28E+03	80
#10) N	1.36E+03		
#11) N+F	2.09E+02	1.36E+03	1176
#12) N+F	2.56E+03		
#13) C	1.10E+05	7.24E+04	37600
#14) C	3.48E+04		
#15) F	8.84E+04	7.31E+04	15300
#16) F	5.78E+04		

Week 6, Aug. 28

ENCLOSURE	Shallow CELLS/mL	MEAN	S.D
#1) N	8.08E+03	9.69E+03	1610
#2) N	1.13E+04		
#3) N+F	4.96E+04	9.83E+04	48700
#4) N+F	1.47E+05		
#5) C	8.33E+04	7.65E+04	6850
#6) C	6.96E+04		
#7) F	1.16E+05	1.72E+05	56000
#8) F	2.28E+05		
Deep			
#9) N	0.00E+00	0.00E+00	0
#10) N	0.00E+00		
#11) N+F	1.25E+03	8.69E+02	381
#12) N+F	4.88E+02		
#13) C	3.68E+04	3.07E+04	6100
#14) C	2.46E+04		
#15) F	7.29E+04	7.42E+04	1300
#16) F	7.55E+04		

Shallow		Week1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
		Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros
control	1	569.90	141.06	6735.16	580.62	1737.93	439.98	1275.95	1495.94	362.89	3846.56	552.97	1839.49	2884.94	9797.92
	2	575.54	169.28	2556.59	337.13	967.96	571.98	967.96	1781.93	145.15	3182.23	214.42	1410.65	1178.31	5328.02
Mean		572.72	155.17	4645.88	458.88	1352.95	505.98	1121.96	1638.94	254.02	3514.4	383.7	1625.07	2031.63	7562.97
St.Dev.		3.99	19.95	2954.70	172.17	544.45	93.34	217.78	202.23	153.965	469.752	239.39	469.752	1206.77	3160.70
N	1	435.26	220.06	4136.90	1741.84	1099.95	153.99	4196.28	475.05	0	444.73	598.11	662.07	22.00	4377.82
	2	372.41	124.14	2277.81	1138.90	890.96	22.00	1499.93	600.00	0	600.32	1794.3	668.4	549.98	2067.98
Mean		428.84	172.10	3207.36	1440.37	995.46	88.00	2848.11	537.53	0	488.725	1196.2	675.235	285.99	3222.87
St.Dev.		79.80	67.83	1314.58	426.34	147.78	93.33	1906.61	279.93	0	591.827	845.86	591.827	373.34	1633.38
F	1	626.33	315.99	2176.62	707.40	956.96	230.99	2089.91	1935.92	653.19	3835.17	325.3	2294.31	2363.04	4479.08
	2	750.46	191.85	5341.68	754.56	1154.95	351.99	1407.94	1913.92	145.15	4953.39	402.2	2622.46	1534.49	3849.59
Mean		688.40	253.92	3759.15	730.98	1055.96	291.49	1748.93	1924.92	399.17	4294.28	363.75	2458.39	1948.77	4064.32
St.Dev.		87.77	87.78	2238.04	33.35	140.00	85.56	482.23	15.56	359.239	932.122	54.377	932.122	585.87	586.52
N+F	1	381.13	118.49	3541.77	2264.41	868.96	66.00	716.09	1277.36	48.38	679.32	67.71	236.99	661.90	3866.91
	2	402.18	84.64	6096.48	3309.52	824.97	153.99	813.97	857.96	130.64	1201.88	112.85	163.64	1251.96	4790.09
Mean		381.66	101.57	4819.13	2786.97	846.97	110.00	765.03	1067.66	89.51	940.6	90.28	200.315	956.93	4328.50
St.Dev.		29.03	23.94	1806.45	739.00	31.11	62.22	69.21	296.56	58.1666	369.506	31.919	369.506	417.24	652.79

Deep	Week1	Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
		Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros	Mixos	Heteros
control	1	1799.91	928.99	219.99	344.65	175.99	2309.90	174.19	3846.56	254.02	3882.88	451.60	2987.60
	2	3135.33	1785.40	322.65	366.65	549.98	3101.87	277.11	3182.23	145.15	3182.23	483.90	3354.70
Mean		2467.62	1357.20	271.32	355.65	362.99	2705.89	225.65	3514.4	199.59	3532.56	467.75	3161.15
St.Dev.		944.28	605.57	72.59	15.56	264.45	560.01	72.7754	469.752	76.983	495.434	22.84	273.72
N	1	2090.22	217.66	163.30	163.30	8760.00	0.00	145.15	444.73	0	983.17	193.50	1612.80
	2	1045.11	435.32	290.31	145.15	522.56	232.25	37.06	-	62.21	1555.22	725.80	2028.79
Mean		1567.67	326.49	226.81	154.23	4641.28	116.13	91.105	-	31.105	1269.2	459.65	1820.80
St.Dev.		739.00	307.82	89.81	12.83	5824.75	164.23	76.4312	591.827	43.989	404.5	378.39	2486.19
F	1	3570.80	870.93	1473.94	483.98	1407.94	2243.91	535.95	3635.17	285.08	4035.17	725.80	3286.00
	2	3091.79	801.25	1165.95	373.98	813.97	2705.89	967.7	4953.39	349.84	4953.39	1016.10	3157.10
Mean		3331.30	836.09	1319.95	428.98	1110.96	2474.90	751.825	4294.28	307.45	4494.28	870.95	3211.55
St.Dev.		338.71	49.27	217.78	77.78	420.00	326.67	305.293	932.122	59.949	459.12	205.27	77.00
N+F	1	4257.86	1258.00	3425.64	158.06	197.99	1275.95	63.73	679.32	0	979.32	0.00	1006.40
	2	7548.02	225.70	2757.93	798.35	99.00	1110.95	43.55	1201.88	0	1201.88	0.00	335.00
Mean		5902.94	741.85	3091.79	478.21	148.50	1193.45	53.64	940.6	0	1090.6	0.00	670.70
St.Dev.		2326.49	729.95	472.14	452.75	70.00	116.67	14.2694	369.506	0	157.374	0.00	474.75

Appendix 5

Lake Survey Data

LAKE #	BACTERIA Cells mL-1	HNAN Cells mL-1	MIXOS Cells mL-1	PICOCYAN Cells mL-1
CLASS 1				
#31) Silent	1.740E+06	1648.54	1747.90	5.649E+05
#32) EELS	2.020E+06	1612.46	1552.90	6.002E+05
#33) ANSTRUTHE	1.660E+06	2538.13	2276.90	4.720E+04
#48) KAWAGAMA	1.220E+06	3442.23	1161.20	5.644E+04
#51) KENESIS	8.780E+05	2528.49	870.93	8.256E+04
CLASS 2				
#39) HALIBURTO	1.910E+06	2334.08	846.40	9.212E+04
#42) HALLS	1.050E+06	3221.49	561.90	4.243E+04
#46) OXTONGUE	1.520E+06	2003.13	3919.20	1.975E+04
#47) LITTLE HAW	6.170E+05	3285.77	870.90	3.546E+04
#50) ST. NORA	1.550E+06	870.93	855.10	1.150E+05
CLASS 3				
#36) GULL	2.840E+06	2635.50	848.20	5.299E+04
#37) CRYSTAL	2.260E+06	2235.38	725.80	8.949E+04
#40) TWELVEMIL	1.620E+06	3368.86	995.30	7.838E+04
#41) BOSKUNG	9.770E+05	2829.84	675.30	1.047E+05
#45) FAIRY	1.520E+06	2368.92	1358.60	5.487E+04
#49) L. OF BAYS	1.330E+06	3151.92	1078.30	6.090E+04
#34) RICE-EAST	2.470E+06	2724.75	1194.40	5.231E+05
#35) RICE-WEST	6.700E+06	3285.77	1187.60	6.008E+05
#38) KOSHLONG	1.520E+06	2493.60	1906.90	5.644E+04
LAC MULVIHILL	3.082E+06	5627.52	2009.80	
LAC RENAUD	1.970E+06	4756.60	3751.70	
BROWN	5.190E+06	3577.50	1045.10	
BAY QUINTE	3.170E+06	3996.01		
L.Ont #403	3.390E+06	7913.00		
L.Ont.#401	1.650E+06	2240.00		
L.Superior	6.540E+05	757.00		