INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Université d'Ottawa · University of Ottawa

Picocyanobacteria in oligotrophic to mesotrophic lakes: variables affecting their abundance and analysis by flow cytometry

by
Brigitte Lavallée

Thèse soumise à la
Faculté des études supérieures et post-doctorales
Université d'Ottawa
en vue de l'obtention de la maîtrise ès sciences

l'Institut de Biologie d'Ottawa-Carleton

Thesis submitted to the
Facutly of Graduate and Postdoctoral Studies
University of Ottawa
in partial fulfilment of the requirements for the
M.Sc. degree in the

Ottawa-Carleton Institute of Biology

December 2001

REMERCIEMENTS / ACKNOWLEDGEMENTS

Je voudrais remercier particulièrement mon superviseur, Dr Frances R. Pick, qui a su au cours de ces deux années d'études partager ses connaissances sur le phytoplancton et les milieux aquatiques; elle sera toujours pour moi une très grande référence. Je la remercie aussi pour ces grandes conversations lors de nos randonnées en 4Runner vers Montréal ou Rimouski. Je voudrais aussi remercier les membres de mon comité, Dr David J. Currie et Dr Campbell R. Wyndham pour leurs commentaires constructifs. Un merci à tous les étudiants du laboratoire qui m'ont tous donné un coup de pouce à un moment ou un autre.

Je tiens à remercier Serge Demers et son équipe de l'Institut des Sciences de la Mer (ISMER) à Rimouski pour nous avoir initié à la cytométrie en flux. Un grand merci à Yves Prairie et Denis Flipo de l'Université du Québec à Montréal (UQAM) pour nous avoir permis d'utiliser le cytomètre en flux FACScan. Un remerciement tout particulier à Denis qui a toujours su trouver du temps dans son horaire très chargé pour m'aider avec le cytomètre et répondre à mes questions.

À ma grande famille qui m'appuie, c'est toujours très rassurant de vous savoir près de moi... Une attention particulière à mon amour Christian; il a su m'appuyer et m'encourager à tout moment. Son expertise et sa critique scientifique m'ont été d'un grand recours.

TABLE OF CONTENTS

Acknowledgements ii

Table of Contents iii

Résumé iv

Abstract vi

List of Tables viii

List of Figures ix

General Introduction 1

Chapter 1 Picocyanobacteria abundance in relation to growth and loss rates in oligotrophic to mesotrophic lakes 18

Abstract 19

Introduction 20

Material and Methods 22

Results 26

Discussion 30

Chapter 2 The use of flow cytometry to study picocyanobacteria in lakes 50

Abstract 51

Introduction 52

Material and Methods 57

Results 61

Discussion 68

Summary and Conclusion 99

References 100

Appendix I 106

Appendix II 110

RÉSUMÉ

Les picocyanobactéries (PC) sont des algues bleues de taille microscopique responsables du dixième de la production primaire totale des systèmes marins et dulcicoles. L'écologie des populations des PC dans les lacs tempérés ainsi que leurs signatures cytométriques ont été étudiées.

Au premier chapitre, l'importance relative de la croissance des PC en comparaison avec leur taux de pertes ainsi que l'influence des variables physiques et chimiques sur l'abondance *in situ* des PC dans 48 lacs furent étudiées. Les taux de croissance et de pertes furent estimés à partir d'une incubation avec un antibiotique qui inhibe la division cellulaire; l'abondance des PC fut déterminée à l'aide d'un microscope à épifluorescence. Les taux de croissance et de pertes des PC furent fortement corrélées. Le taux de pertes fut la variable qui a expliqué le mieux le taux de croissance intrinsèque. Le taux de pertes en combinaison avec le pH de l'épilimnion furent les meilleures variables pour expliquer l'abondance des PC dans les lacs échantillonnés. Le pH fut une meilleure variable que le phosphore total ou l'azote total pour estimer l'abondance *in situ* des PC.

Au second chapitre, l'utilisation de la cytométrie en flux fut examinée afin de déterminer la facilité à détecter les populations de PC et d'estimer leur abondance dans les lacs d'eau douce. La cytométrie en flux est très utilisée en océanographie mais elle reste une technique précaire en limnologie où son utilisation se restreint à l'identification et l'estimation de populations de bactéries hétérotrophes. L'identification des PC s'est révélée être relativement simple où les PC contenant de la phycoérythrine se distinguaient facilement des PC ne contenant pas ce pigment photosynthétique par une différence en intensité de fluorescence du pigment en question. L'estimation de l'abondance des PC par cytométrie en flux en comparaison avec la microscopie par épifluorescence a démontré une très grande différence des résultats entre les deux techniques. Ainsi, l'identification de particules comme étant des PC est une tâche ardue et arbitraire où les données d'abondances risquent d'être très différentes selon la personne qui analyse les résultats de

cytométrie. Un facteur important qui joue un rôle critique quant à l'utilisation de cette technique en eau douce est la présence de bruit de fond qui semble plus importante qu'en milieu marin. Par ailleurs, la cytométrie en flux se révèle être une technique efficace pour l'étude de la distribution de pigments photosynthétiques et de fournir des informations de base sur la composition d'une communauté planctonique analysée. La microscopie par épifluorescence demeure la technique la plus appropriée pour déterminer l'abondance des PC dans les lacs tempérés.

ABSTRACT

Picocyanobacteria (PC) are microscopic blue green algae responsible for a tenth of the total primary production of marine and freshwater systems. In this research, the ecology of temperate lake PC populations and their flow cytometry signatures were studied.

In the first chapter, I examined the relative importance of growth versus losses of picocyanobacteria (PC) and the influence of physical and chemical variables on their *in situ* abundance in 48 temperate freshwater lakes. Growth and loss rates of PC were estimated from *in situ* incubations using a selective metabolic inhibitor technique and abundance of PC was determined by epifluorescence microscopy. Growth and loss rates of PC were highly correlated. The loss rate was the variable that best explained the variation among lakes in the intrinsic growth rates of PC. Loss rates in combination with pH were the variables that explained the most variation in PC abundance among lakes. pH was a better predictor than total phosphorus (TP) or total nitrogen (TKN) to estimate summer PC abundance.

In the second chapter, I examined the use of flow cytometry to identify PC and determine their abundance in lake water samples. Flow cytometry is widely used in marine systems but has not been applied much to freshwater. Identification of dot clusters as PC was possible due to their high abundance in the water samples; phycocyanin-rich and phycoerythrin-rich PC could be distinguished by the difference in phycoerythrin fluorescence from bivariate dot plots of phycoerythrin fluorescence as a function of their chlorophyll *a* fluorescence. There were discrepancies between flow cytometry and epifluorescence counts of PC. This was mainly because the clustering of dots or "events" could be quite arbitrary since discrimination between clusters was often difficult.

In addition, the background noise appears higher in lake samples than marine ones. The source of this noise needs to be identified and corrected in future studies. However,

flow cytometry is a promising tool for examining pigment distributions and for rapid assessments of the phytoplanktonic community. Epifluorescence remains the most reliable technique to determine PC abundance and pigment composition in freshwater lakes.

LIST OF TABLES

Table 1.1 Location, morphometry and picocyanobacteria abundance by epifluorescence microscopy of 48 lakes sampled once in July or August 2000 ..... **35**

Table 1.2 Summary of estimated growth and loss rates from the literature and the present research using different techniques **37**

Table 2.1 Picocyanobacteria (PC) abundance determined by flow cytometry and epifluorescence microscopy for lakes of the Rideau Canal waterway **76**

LIST OF FIGURES

Figure A Schematic of a flow cytometer equipped with two lasers (type FACS Calibur) **15**

Figure B Flow cytometry data charts of Lake Opinicon, Ont., to demonstrate data presentation on charts (dot plots) **17**

Figure 1.1 Relationship between log transformed chlorophyll *a* (chl *a*) concentrations and the log transformed total phosphorus (TP) **39**

Figure 1.2 Relationship between log transformed PC abundance and concentration of **A)** total phosphorus and **B)** total nitrogen **41**

Figure 1.3 Relationship between log transformed abundance of PC and pH of the sampled sites **43**

Figure 1.4 **A)** Growth and **B)** loss rates of PC in comparison with the log transformed concentration of total phosphorus **45**

Figure 1.5 **A)** Growth and **B)** loss rates of PC in comparison with the log transformed PC abundance **47**

Figure 1.6 Relationship between growth and loss rates of PC **49**

Figure 2.1 Flow cytometric signatures of West Bay (left column) and Green Bay (right column) from Bob's lake, Ont. having distinct physical and chemical variables **78**

Figure 2.2 Flow cytometric signatures of four lakes of the Rideau Canal waterway. Signatures are similar except for the formation of a cluster of dots from Upper Rideau lake down to Opinicon lake **80**

Figure 2.3 Flow cytometric signatures of water systems having similar physical and chemical variables: **A)** lakes Simon and Barrière, two lakes connected one to the other; **B)** two bays from lake Memphrémagog **82**

Figure 2.4 Flow cytometric signature of Lake Onondaga, New York State. **A)** initial signature from FL2 (phycoerythrin fluorescence) vs FL3 (chl *a* fluorescence); **B)** gates are created around clusters of dots; **C)** same as in *B* although limits of the gates are invisible; **D)** FL2 vs FSC (cell relative size) dot plot showing size range of clustered cells **84**

Figure 2.5 Comparison of counts of picocyanobacteria in water samples of four lakes from flow cytometry and epifluorescence microscopy. For each lake, four samples were treatments with triplicates for each treatment **86**

Figure 2.6 Flow cytometric signature of *Synechococcus leopoliensis* cultures grown at different light regimes for 6 days with a light:dark cycle of 14:10 ..... **88**

Figure 2.7 Effect of preservation on the flow cytometric signature of *Synechococcus leopoliensis* culture grown for 6 days at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ **90**

Figure 2.8 Effect of preservation and filtration on the flow cytometric signature of Bob's lake (West Bay), Ont. (September 3rd 2001) **92**

Figure 2.9 Comparison of flow cytometric signatures by **A)** FACScan and **B)** FACS Vantage for lake water sample of Bob's lake (West Bay), Ont. (September 3rd 2001) **96**

Figure 2.10 Comparison of flow cytometric signatures of West Bay and Green Bay from Bob's lake (September 3rd 2001) with a FACS Vantage able to detect phycocyanin fluorescence (FL4) **98**

GENERAL INTRODUCTION

Picocyanobacteria and their role in aquatic systems

The importance of algae in aquatic systems is undisputed but many discoveries remain to be made, particularly with respect to their diversity. One remarkable discovery made over the last twenty years has been the detection of photosynthetic organisms less than 2 μm in diameter that could not be previously detected by conventional light microscopy (Waterbury et al. 1979; Johnson & Sieburth, 1979). Autotrophic picoplankton includes prokaryotic cyanobacteria (also called picocyanobacteria), as well as eukaryotic algae (chlorophytes, chrysophytes and a few diatoms) that are less than 2 μm in diameter. Both autotrophic picoplankton and heterotrophic bacteria are subject to predation pressure from heterotrophic organisms ranging in size from 2-20 μm (nanoplankton: flagellated protozoans), 20-200 μm (microplankton: ciliated protozoans, rotifers), 200-2000 μm (mesoplankton: ciliated protozoans) and finally from some zooplankton greater than 2 mm. Picoplankton forms the base of microbial food chains or loops (Azam et al. 1983).

The microbial loop consists of the assimilation of carbon from dissolved organic carbon (DOC) by heterotrophic bacteria and perhaps phytoplankton and incorporation of this carbon into their tissues; then predators, particularly flagellates ingest these microorganisms. The predators incorporate some of the particulate carbon but will also release carbon as DOC and DIC (dissolved inorganic carbon) for re-assimilation (Azam et al. 1983). Autotrophic picoplankton contributes new organic carbon into the loop through direct assimilation of DIC. Collectively, these organisms comprise a microbial food chain

or loop depending on how much energy is actually transferred up to the top of aquatic food chains.

The abundance of picocyanobacteria (PC) in aquatic systems is impressive. Raven (1998) estimated the global abundance of PC to be about 10^{26} individuals and to contribute to one tenth of the total primary production of aquatic systems. They were first discovered in marine environments but their presence in freshwater habitats is as important (Stockner and Antia 1986). They can be found in concentrations of up to a million per ml in lakes and oceans, especially in oligotrophic systems. They exhibit the highest growth rates among autotrophic organisms, reaching 2.0 d^{-1} (Reynolds 1984) meaning that the populations can double in less than 9 hours.

The relatively small size of PC is an advantage for growth and survival in aquatic systems. PC have the highest surface to volume ratio of all phytoplankton; this property confers a high efficiency for acquiring nutrients and light. In addition, by virtue of their small size, PC also have small needs, i.e. they will survive better than bigger algae in oligotrophic systems (Gervais et al. 1997; Raven 1998). Lastly, PC have a low sedimentation rate which can be significant for larger phytoplankton, especially those lacking flagella.

Taxonomy and pigmentation

PC consist mostly of two genera based on the plane of their cell division and on their morphology: a coccoid shape and division in one or more planes (*Synechocystis*) or rod shape with one plane of division as crosswise (*Synechococcus*) (Rippka 1988). Most studies do not make the distinction between the genera and only use the genus *Synechococcus* or the term "picocyanobacteria" as used in this present thesis. Reproduction is by simple binary fission, the mother cell producing two identical daughter cells.

Like all cyanobacteria, picocyanobacteria possess biliprotein accessory pigments along with chlorophyll a for light absorption. Biliproteins have high concentrations of nitrogen which may serve as a reserve of nutrient when nitrogen becomes scarce in the aquatic environment (Postius and Böger 1998). The various biliproteins have absorption wavelengths different from chlorophyll *a* which help PC to acquire light energy at depth or in coloured waters where light quality is more restricted (Gervais et al. 1997).

Phycocyanin is present in all cyanobacteria and is blue in colour. Phycoerythrin is a red coloured pigment found only in some cyanobacteria taxa, including PC. Most of the marine PC contain phycoerythrin while in freshwater systems, both types appear abundant. PC lacking phycoerythrin tend to be present in more coloured (brown) or turbid waters (Pick 1991).

The importance of PC in aquatic systems has lead to numerous studies from an ecological perspective as well as physiological and molecular perspectives. Physiological and molecular studies on either *Synechococcus/Synechocystis* or *Prochlorococcus* are mostly done with cultures. The cell cycle of the PC has been well described and studied with the application of fluorochromes to stain DNA content of cells for epifluorescence microscopy or flow cytometry (e.g. Liu et al. 1999). Ecological research in marine environments consists of analysis of population abundance or bulk pigmentation patterns (e.g. Olson et al. 1990; Bautista and Jiménez-Gómez 1996; Landry et al. 1996).

Population dynamics of PC in the water column: gains and losses

To survive in aquatic systems, all phytoplankton need light and nutrients. However, species differ in their relative requirements and competitive abilities (Kalff and Knoechel 1978; Tilman et al. 1982; Pick and Lean 1987). Survival is the result of a balance between gains and losses: the net growth of algae is affected by the availability of physical and chemical variables, as well as by predation from heterotrophic organisms.

Phytoplankton with a high buoyancy can have better access to light than others. Availability of nutrients is also an important factor in algal growth especially when algae are in systems where nutrients are not abundant; some species have better uptake rates which lead them to dominate over others. Inversely, in highly eutrophic systems, where nutrients are not limiting algal growth, then another factor will become restrictive and this is usually light. When the amount of algae in the water column or at the surface of the

water becomes too high, the penetration of light is reduced and phytoplankton with less buoyancy are the first to decline.

Loss of autotrophic picoplankton is mainly due to grazing by heterotrophs protozoans and ciliates (Hall et al. 1993; Gaedke and Weisse 1998). Other factors such as virus lysis and possibly natural cell death can be important. Viruses are common and very abundant, up to 10^8 ml^{-1} in aquatic environments and their abundance is related to infection rates (Suttle and Chan 1994).

Epifluorescence microscopy

The use of epifluorescence microscopy led to the initial discovery of photosynthetic picoplankton. When algal pigments are excited by specific wavelengths of light, they produce fluorescence at longer wavelengths.

In epifluorescence microscopy, excitation and emission filter sets for the observation of fluorescent structures and a mercury lamp producing a high energy light are added to a light microscope. The excitation filter allows only specific wavelengths to reach the specimen on the slide. In response to the energy absorption, the specimen will reject a part of the energy by emitting light of wavelengths longer than the absorbed ones. This process of emitting light to reject energy is called fluorescence. For example phycocyanin absorbs light energy between 510 and 560 nm (corresponding to the colour green of the light spectrum) and energy is emitted back in the range of 590 nm

(corresponding to red light). Everything else that does not contain fluorescent molecules is not visible on the microscope slide and the background is black. For the visualisation of phycocyanin, a green excitation filter (510-560 nm) with a barrier filter that allows for emission at 590 nm (in the red light) is appropriate. For the visualisation of both phycoerythrin and chl *a*, a blue excitation filter (450-490 nm) with a barrier filter (520 nm) leads to fluorescence of chl *a* in the red spectrum while phycoerythrin emits in the yellow.

The green filter set allows for the discrimination between cyanobacteria and other phytoplankton. Cryptophyceae are visible as well because they contain phycocyanin, but because of their shape, greater size and high fluorescence are easily distinguished from PC. The blue filter set, which excites chl *a* and phycoerythrin, enables the observation of almost all eukaryotic phytoplanktonic organisms at the same time because chl *a* emits a red fluorescence and phycoerythrin emits a yellow-orange fluorescence. PC containing phycoerythrin will appear yellow-orange under blue excitation, however PC lacking phycoerythrin will not be visible because PC have relatively low concentrations of chl *a* which make phycoerythrin fluorescence dominant under blue excitation.

Flow cytometry

Flow cytometry is a more recent technology than epifluorescence microscopy and can provide more qualitative and quantitative information. With flow cytometry, water samples are passed through a capillary tube in front of a laser beam. Every particle passing

through the beam is counted and analysed for certain parameters: size, cellular complexity and fluorescence emission from specific wavelengths of excitation. Particles detected by the laser beam are called events. Data for each event are saved to a computer with a specific software for further analyses.

Many applications are found for flow cytometry in human and animal physiology, microbiology, virology, molecular biology and oceanography. For example, enumeration of viruses in oceanography can now be accomplished by staining with specific fluorochromes (Marie et al. 1999). The discovery of a new photosynthetic picocyanobacterium in marine systems, *Prochlorococcus* spp., a prochlorophyte, was possible due to the power of detection of a weak chl *a* fluorescence by the flow cytometer (Chrisholm *et al.*, 1988). In limnology, the study of phytoplankton by flow cytometry is very limited (Fahnenstiel et al. 1991; Corzo et al. 1999) but methods to enumerate heterotrophic bacteria by flow cytometry have been successfully developed (Porter et al. 1995; del Giorgio et al. 1996).

Figure A is a schematic of a FACS Calibur (Becton Dickinson) flow cytometer. It is composed of two lasers, most often a 488 nm argon laser and a 630 nm helium-neon laser. This flow cytometer differs from a FACScan (Becton Dickinson) by the fact that it only has the 488 nm argon laser. Both lasers pass through the flow chamber where the water sample flows. A water sample is sucked inside the flow cytometer where it is first diluted with a filtered bacterial-free liquid such as Hematall (Fisher Scientific) or FACSFlow (Becton Dickinson) and then passes into a capillary in order to allow particles to pass separately one at a time through the lasers beams.

A laser beam only travels in a straight forward direction. As the light beam intercepts a particle, a part of the beam will be scattered from its forward direction. In a flow cytometer, a photodiode is placed in front of the laser beam to detect the scattered beam in comparison with its initial straight forward direction. As a result, a particle of greater size will scatter the laser beam at a greater angle from its initial point than a smaller size particle. The photodiode collects the forward scattered light beam for each particle and transfer this information signal as an electrical output, or voltage, to the computer for post-analysis as the forward light scatter signal (FSC). The photodiode collects light beams that scatter up to a certain degree from the initial point, meaning that there is a limit to the size of particle that can be analysed (~ 40 μm).

Other detectors in the flow cytometer are the photomultiplier tubes (PMTs). A PMT is more sensitive than a photodiode because the former is placed in front of the beams while the latter receive deviated beams which make the signals weaker. The first PMT is called side light scatter (SSC). It collects the light beam that is scattered at right angle (90°) more or less. The quantification of these high light deviations is not possible although the greater the scattering, the greater the cellular complexity or granularity of the particle (Olson et al. 1993).

The other PMTs are used for the detection of fluorescence at specific wavelengths. The 488 nm argon laser excites photosynthetic pigments chl *a*, and phycoerythrin but also all kinds of fluorochrome dyes added to samples for other types of analyses. In response to excitation, photosynthetic pigments will emit a fluorescing light of longer wavelengths around 585 nm for phycoerythrin and 670 nm for chl *a*. Phycocyanin requires excitation

by a longer wavelength; a helium-neon laser is then necessary. This laser of 630 nm excites phycocyanin which will emit back a fluorescence around 660 nm. Depending on the type of flow cytometer, there will be a PMT for each different fluorochrome that requires analysis. The flow cytometer mainly used in this thesis has three PMTs: FL1 for specific fluorochrome dyes (e.g. FITC); FL2 for phycoerythrin fluorescence and FL3 for chl *a* fluorescence. Dichroic mirrors are set in the flow cytometer in order to decompose and intercept different wavelengths depending on the disposition of PMTs. Optical filters are also set in front of each PMT to allow the latter to collect the proper wavelengths associated with a specific pigment or dye.

Interpretation of flow cytometry data

When a sample is analysed, each particle present is detected by laser and quantified by its size, cellular complexity, fluorescence of chl *a*, phycoerythrin and sometimes phycocyanin depending on the flow cytometer used (Fig. B). Abbreviations used for these parameters are:

- **FSC**: forward light scatter, giving the relative size of each cell;
- **SSC**: side scatter, giving an idea of the cellular complexity of each cell;
- **FL1**: green fluorescence at 515-545 nm which does not correspond to any pigment in picocyanobacteria but can detect the fluorescence of fluorescently labelled microspheres (FLB) or other stains used to colour microorganisms, such as FITC. It is often used as a threshold to remove background noise.

- **FL2**: phycoerythrin orange fluorescence (564-606 nm);
- **FL3**: chl *a* red fluorescence (> 650 nm);
- **FL4**: phycocyanin red-orange fluorescence (653-669 nm). This parameter is only found in some flow cytometers, such as the FACSvantage or FACScalibur (Becton Dickinson) equipped with a laser of 620-635 nm that is able to excite phycocyanin and allophycocyanin.

Depending on the flow cytometer used, the range of emission wavelengths detected can vary. All axes of the parameters are recorded on a log scale, from 10^0 to 10^4. The flow cytometer cannot determine the actual intensity of fluorescence emitted by the particles. Since the values are simply relative, fluorescently labelled beads (FLB) of known diameter are added at a specific concentration to the water samples in order to calibrate the size scale (FSC) as well as to determine the cell abundance of any group of cells in the water sample because the flow rate of cells passing through the cytometer is known to be unprecise.

As an example, Figure B is a water sample collected in summer 2000 for Lake Opinicon, Ont. Five thousand events were analysed by the flow cytometer. From those 5000 events, FLB are easily identified because of their high fluorescence in FL1 but also because of their high abundance (heavy peak in the dot plot). FLB used for freshwater analyses have pigments that will fluoresce differently from phytoplankton in order to distinguish them clearly from the latter. FLB normally will have a high fluorescence in FL1 and a precise diameter with a low standard deviation that will create a heavy events groupment on a plot with FL1 on Y-axe and FSC on X-axe (Fig. B *A*: dots inside the gate

are coloured in red). When a "gate" is created around the FLB in the dot plot, a colour can be given to all events clustered in the gate; also, the software is able to provide the number of events inside the gate. By knowing the amount of FLB gated in the dot plot and the total FLB concentration within the water sample, the total concentration of a specific group of algae gated elsewhere can be calculated. By combining the different parameters in 2 or even 3 dimension dot plots, one can differentiate groupings of cells having common characteristics. A threshold was set on the chl *a* fluorescence (FL3) in order to reduce the background noise that had a very weak fluorescence and was sometimes more abundant than phytoplankton so that only cells of interest could be visualised.

Thesis objectives

Previous studies suggest significant effects of light, temperature, nitrogen and/or phosphorus and grazing on PC (Pick 1991; Stockner and Shortreed 1991; Wehr 1991; Agawin et al. 1998). Stockner (1991) suggested that autotrophic picoplankton abundance increases with the trophic status in freshwater lakes but that their contribution to total phytoplankton biomass and productivity would decrease. Pick (2000) showed that PC abundance in Canadian and New Zealand lakes increased with trophic status as measured by total phosphorus (TP) in the water, from oligotrophy to mesotrophy (0-20 $\mu g \cdot l^{-1}$), where the abundance reached a plateau (20-100 $\mu g \cdot l^{-1}$) and then decreased in hyper-eutrophic systems (>100 $\mu g \cdot l^{-1}$). However, literature data of Stockner and Shortreed (1991) on British Columbia (Canada) and Søndergaard (1991) on Europe lakes taken together did not show a significant trend between PC abundance and TP (Pick 2000).

The observed abundance of PC is the result of the balance between growth and loss processes. But the importance of each of those processes is unclear. Logically, growth of PC should be higher in nutrient-rich systems, but at some high nutrient level, growth rates should be constant. In contrast, grazing pressure should increase as the overall nutrient and productivity levels increase, although the composition of the grazers will likely change.

Since not all physical or chemical variables affect phytoplankton to the same extent, the importance of each of these effects separately and in combination is not clear.

The aim of this thesis was to determine the relative importance of physical and chemical factors that affect growth and loss processes of PC.

In order to enumerate samples rapidly and with more precision, I explored the use of flow cytometry to study PC ecology. Flow cytometry has been well developed and used in oceanography but its main use in freshwater has been restricted to bacteria. Flow cytometry signals of PC from a series of lakes were analysed and compared with epifluorescence microscopy techniques.

Figure A Schematic of a flow cytometer equipped with two lasers (type FACS Calibur). A combination of dichroic mirrors and optical filters allows the detection of specific fluorescence for each photomultiplier tube and the photodiode in FSC.

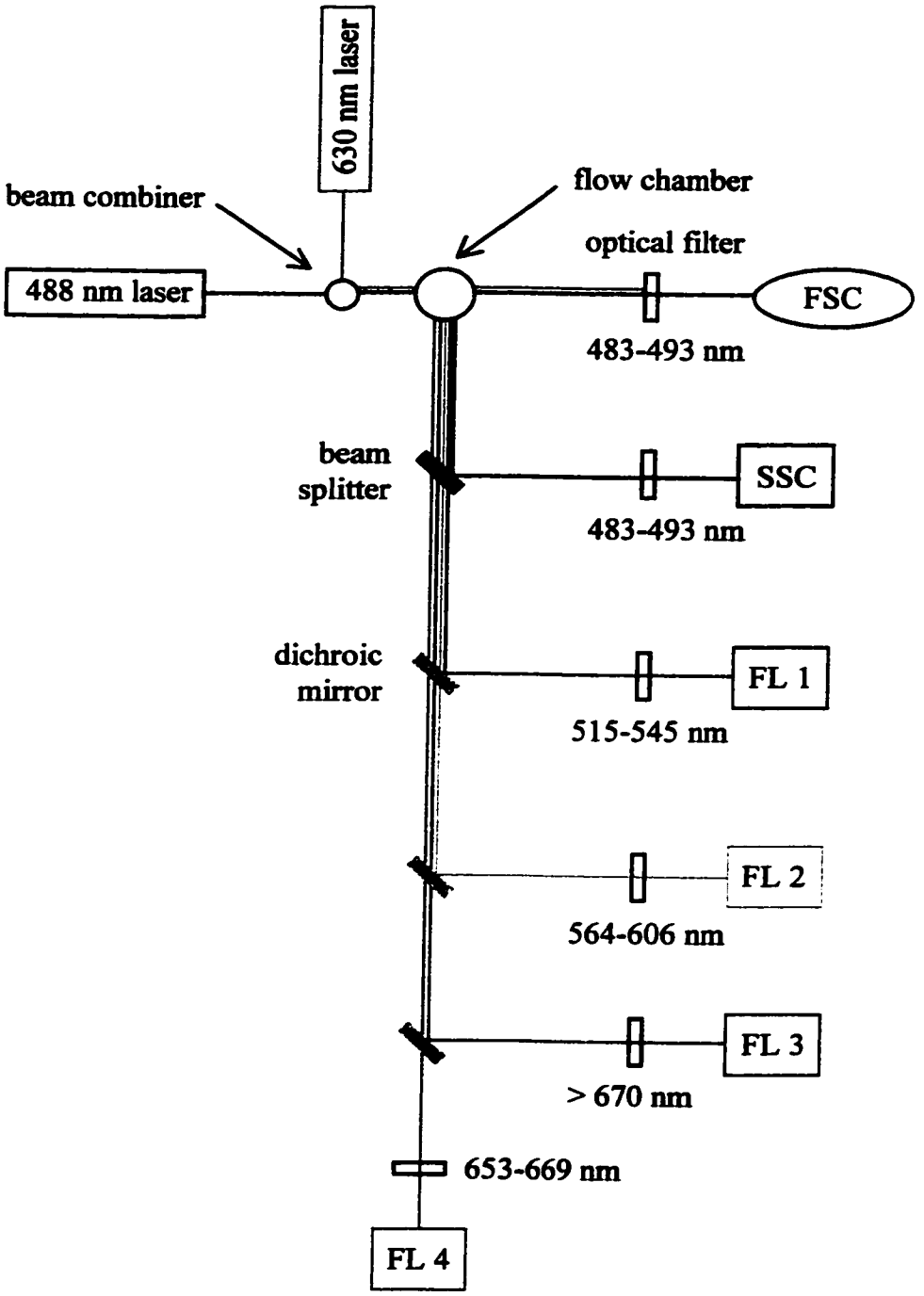
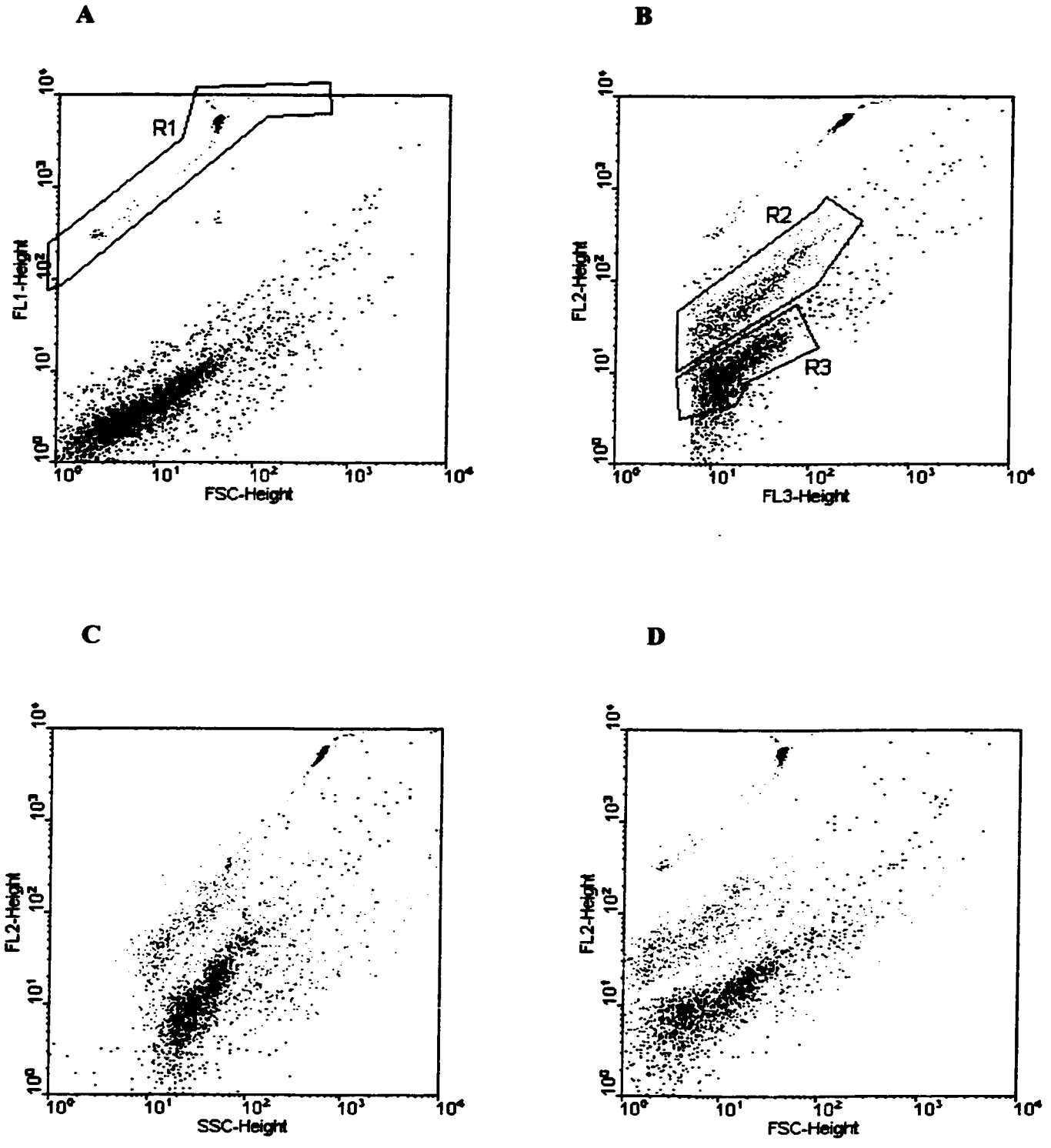

630 nm laser
beam combiner
flow chamber
optical filter
488 nm laser
FSC
483-493 nm
beam
splitter
SSC
483-493 nm
dichroic
mirror
FL 1
515-545 nm
FL 2
564-606 nm
FL 3
> 670 nm
653-669 nm
FL 4

Figure B Example of a flow cytometry signature from Opinicon Lake, Ont. **A)** a gate is created on the basis of FL1 fluorescence of the fluorescently labelled beads; all events gated (the beads) are coloured in red. **B)** the dot plot of phycoerythrin fluorescence (FL2) as a function of chlorophyll *a* fluorescence (FL3). Two populations of picoplankton have been gated with different colours. **C) and D)** FL2 fluorescence as a function of SSC and FSC in order to observe the relative values for both populations. As observable in *B)*, a threshold was set on the FL3 parameter in order to suppress a part of background noise.


A
R1
FL1-Height
FSC-Height
B
R2
R3
FL2-Height
FL3-Height
C
FL2-Height
SSC-Height
D
FL2-Height
FSC-Height

CHAPTER 1

Picocyanobacteria abundance in relation to growth and loss rates in oligotrophic to mesotrophic lakes

ABSTRACT

The relative importance of growth versus losses of picocyanobacteria (PC) and the influence of physical and chemical variables on their *in situ* abundance were examined during summer in 48 lakes in Quebec, Ontario and New York State. The lakes were selected based on their trophic state, resulting in a range of total phosphorus (TP) from 1 to 42 $\mu g \cdot l^{-1}$. Growth and loss rates of PC were estimated from *in situ* incubations using a selective metabolic inhibitor technique and the abundance was determined by epifluorescence microscopy. Growth and loss rates of PC were highly correlated. The growth rates generally exceeded loss rates and ranged from negative values to a maximum of 1.93 d^{-1} for growth and from negative values to a maximum of 1.25 d^{-1} for losses. The loss rate was the main variable that best explained the variation among lakes in the intrinsic growth rates. pH was a better predictor, over the range of 6.4 to 9.1, than TP or total nitrogen (TKN) to estimate summer PC abundance. Also, loss rates in combination with pH were the variables that explained the most variation in PC abundance among lakes. Temperature, TKN and the TKN:TP ratio variables were also significant but to a much lesser extent.

INTRODUCTION

Autotrophic picoplankton (APP) contribute significantly to the primary productivity of lakes and *oceans* (Johnson and Sieburth, 1979; Fahnenstiel et al. 1986; Stockner and Antia 1986). APP comprises phytoplankton smaller than 2 μm in diameter and includes both prokaryotic algal groups (cyanobacteria), and eukaryotic groups (chlorophytes and chrysophytes). Cyanobacteria of picoplanktonic size (picocyanobacteria) usually dominate APP, with some peaks of others groups depending on the season (Fahnenstiel and Carrick 1992; Gaedke and Weisse 1998). Picocyanobacteria (PC) can be rod shaped, represented by the genus *Synechococcus* or coccoid, represented by *Synechocystis*. Because of their high surface to volume ratio, PC are efficient at nutrient uptake under low nutrient concentration. This property likely provides PC with a competitive advantage in oligotrophic systems relative to larger phytoplankton including bloom forming cyanobacteria (Wehr 1990; Agawin et al. 2000).

APP is an important link in the microbial loop (Azam et al. 1983). APP is subject to intense grazing pressure by heterotrophic organisms of the plankton. Grazing of PC is primarily due to heterotrophic nanoflagellates (2-20 μm), rotifers and ciliates (20-200 μm) (Weisse 1988; Vanni and Temte 1990; Hall et al. 1993). Other losses for APP include viral lysis (Suttle and Chan 1994) and possibly cell death.

Not all physical or chemical variables affect phytoplankton to the same extent. Previous studies suggest effects of light (Pick 1991; Vörös et al. 1998), temperature (Pick and Caron 1987; Ning and Vaulot 1992; Agawin et al. 1998), nitrogen and/or phosphorus

(Stockner and Shortreed 1991; Wehr1989; Wehr 1990; Vörös et al. 1998; Agawin et al. 2000; Rhew and Ochs 2000) and grazing (Wehr 1991; Müller 1996; Gaedke and Weisse 1998) on PC. The importance of each of these effects separately and in combination is not clear. Stockner (1991) proposed that as the autotrophic picoplankton abundance increases with trophic status, their productivity as well as their contribution to total phytoplankton biomass would decrease. But Pick (2000), who obtained PC abundance pattern along a trophic gradient different from what Stockner (1991) had found, showed that PC abundance in Canadian and New Zealand lakes increased with trophic status as measured by total phosphorus (TP) in the water, from oligotrophy to mesotrophy (0-20 $\mu g \cdot l^{-1}$), where the abundance reached a plateau and then decreased in hypereutrophic systems (>100 $\mu g \cdot l^{-1}$). However, literature data of Stockner and Shortreed (1991) on British Columbia (Canada) and Søndergaard (1991) on Europe lakes taken together did not show a significant trend between PC abundance and TP (Pick 2000).

The purpose of this study was to determine which variables affect growth and loss rates of PC in lakes, in particular if the trophic status could explain better those rates than explain PC abundance. Since the observed abundance of organisms is a balance between their intrinsic growth and losses, we examined the factors controlling both these rates. We also attempted to determine if PC abundance in freshwater lakes could be predicted by other physical or chemical variables of these systems than the trophic status of lakes.

MATERIAL AND METHODS

Sampling. Forty-eight lakes were sampled once from July 17th to August 29th 2000; two lakes, Bob's and Memphremagog were sampled in two bays, which had differences in physical and chemical variables for a total of 50 sites (Table 1.1). The lakes were located in the provinces of Ontario and Quebec (3 regions: Estrie, Laurentians and Outaouais) and in New York State. Lakes were chosen on the basis of their concentration of total phosphorus (TP), in order to obtain as wide a range of values as possible. An integrated sample of the epilimnion was taken in the middle of the lake or in the deepest area with a 2.5 cm diameter Tygon tube with a weight attached at the end. Water was kept in a 9 L polyethylene carboy in a cooler until processed. Water transparency was measured with a 20 cm diameter Secchi disk. Photosynthetically active radiation (PAR) measurements were taken every 0.5 meter with a LICOR LI-185 quatum/radiometer. For lakes sampled in Ontario, the Laurentians and Outaouais (except lakes Constance, Forgeron, Mackay and Vert), temperature and conductivity were measured with a YSI model 33 S-C-T probe and pH with a Oakton Waterproof pHTestr2. For lakes in New York, Estrie and four in Outaouais, the three variables were measured with a Hydrolab Minisonde 4*a*. Calibrations between equipment were done at the beginning of the sampling period.

Chlorophyll *a* and nutrient analyses. Duplicate water samples at each site were analysed for TP, TKN and nitrite/nitrate by the Regional Municipality of Ottawa-Carleton, Surface Water Quality Branch, Ottawa, Ontario. The analysis of TP was performed by acid digestion followed by the molybdate and colorimetric analysis, total Kjeldahl nitrogen was determined by Kjeldahl acid digestion and nitrite/nitrate were determined by EPA method

300.0 ion chromatography (Regional Municipality of Ottawa-Carleton, 1993). Samples were filtered for chlorophyll *a* (chl *a*) on Nuclepore polycarbonate filters (4.7 cm diameter) of 0.2 μm or 2 μm pore size in duplicate and kept frozen until extraction. Total chl *a* corresponds to the 0.2 μm fraction chlorophyll while chl *a* associated with APP corresponds to the total chlorophyll 0.2 μm fraction minus the 2 μm fraction. Thawed filters were extracted with 4 ml of dimethyl sulfoxide (DMSO) and incubated at 60°C for 10 minutes, then refiltered with 21 ml of 90% acetone (Burnison 1980). Chl *a* fluorescence was measured with a Turner Designs fluorometer and calibrated by spectrophotometry using the trichromatic equation of Jeffrey & Humphrey (1975).

Growth and loss rate experiments. The growth rate of a population of microorganisms can be modelled by the equation:

$$N_t = N_0 e^{rt} \qquad (1)$$

where N_t and N_0 represent the abundance of organisms at time t and 0 and r is rate of increase. The rate of increase of microorganisms *in situ* (r) is the result of the balance between the intrinsic growth (k) and various loss processes such as natural senescence, viral lysis and grazing by protozoans and zooplankton. Measurements of growth and loss rates were estimated by the following equation:

$$r = k - g = \ln (N_t / N_0) / t \qquad (2)$$

where r is the apparent rate of increase or decrease of the organism; k is their intrinsic growth rate; g is their loss rate; N_t and N_0 represent the abundance of organisms at time t (24 hours) and zero. At each site, the loss rate was measured from the variation of abundance of PC after 24 hours of incubation in the presence of ampicillin; this assumes

that PC growth (k) was zero during the incubation when the antibiotic was present (r = -g). Apparent growth rates (r) were determined by using the apparent increase of PC over 24 hours in control treatments.

Ampicillin is an antibiotic from the group of ß-lactamines which can inhibit the synthesis of peptidoglycane, a major component of the cell wall in bacteria. Ampicillin is only effective, i.e. will cause death of bacteria, when synthesis of the cell wall is occurring. The picocyanobacterial strains studied to date are all sensitive to ampicillin (Campbell & Carpenter 1986), but it is conceivable that other as yet untested strains are resistant to this specific antibiotic. The concentrations used do not inhibit eukaryotes (Campbell and Carpenter 1986; Ning and Vaulot 1992).

Water samples from each site were pre-screened with a 250 µm mesh size net if large cladocerans (*Daphnia* spp.) were observed in the samples; then they were incubated in triplicate for 24 hours in 300 ml polycarbonate Erlenmeyer flasks. The flasks were placed in a plastic cage settled in a water tank in the greenhouse or immersed *in situ* near shore at a depth where the amount of light penetrating the cage would approximate the intensity required for maximal algal growth (100-300 $\mu E \cdot m^{-2} \cdot s^{-1}$). Treatment flasks consisted of adding 5 $mg \cdot l^{-1}$ of ampicillin (Sigma Chemical Co.); the control flasks consisted of lake water only. Subsamples of 18 ml were taken at time 0 and 24 hours for each Erlenmeyer and 2 ml of 10% glutaraldehyde buffered with sodium cacodylate was added for a total concentration of 1% glutaraldehyde samples were kept in dark at 4°C until enumeration a few weeks later.

Enumeration of PC. Between 2 and 10ml of preserved water were vacuum filtered at low pressure (less than 200 mm Hg) on a Nuclepore 0.2 μm pore size filter with a Millipore 0.45 μm pore size backing filter under the former to ensure an even distribution of the cells. Identification and enumeration of PC were done with a Zeiss Jenamed 2 epifluorescence microscope equipped with a blue excitation filter (450-490 nm, barrier filter 520 nm) and a green excitation filter (510-560 nm, barrier filter 590 nm). The green filter was used to count the total abundance of PC while the blue filter was used in for a qualitative assessment of those PC containing phycoerythrin as well as eukaryotic picoplankton (Pick 1991). Enumeration was done at 1250x magnification where 15 randomly chosen fields were counted to obtain a minimum of 300 cells.

Statistical analysis. PC abundance, chlorophyll *a*, physical and chemical variables (except TKN:TP ratio) required log transformation in order to satisfy homogeneity and normality of the residuals. Pearson correlations and multiple regression analyses of interactions and effects of variables on growth and loss rates and *in situ* PC abundance were performed with Systat 10 (Wilkinson 2000).

RESULTS

PC abundance *in situ* and physical and chemical variability among lakes

APP was dominated by PC in all lakes sampled. Abundance of PC *in situ* varied from traces (less than 100 ml^{-1}) to 4.68 x 10^5 ml^{-1} (Table 1.1). Over the 50 sites, TP values ranged from 1 to 42 $\mu g \cdot l^{-1}$ and TKN from 149 to 900 $\mu g \cdot l^{-1}$. The correlation between log transformed TP and TKN was high (Pearson's $r = 0.88$, $p < 0.001$). Only two lakes were sampled in New York State compared to Estrie (n = 14), Outaouais (n = 13), Laurentians (n = 10) and Ontario (n = 11). The New York State lakes had TKN, conductivity and Secchi disk values and *in situ* PC abundance that differed from those in the other regions.

Total chl *a* ranged from 1.83 in Lake Simon to 68.98 $\mu g \cdot l^{-1}$ in Oneida and was correlated with TP ($r = 0.65$, $p < 0.001$). The chlorophyll in the picoplankton fraction (< 2 μm) ranged from 0.06 in Indian to 11.40 $\mu g\ l^{-1}$ in Oneida and was less correlated to TP than total chl *a* ($r = 0.41$, $p = 0.003$). The correlation between total chl *a* and the fraction of chl *a* < 2.0 μm (attributed to APP) was high ($r = 0.77$). The contribution of APP to total chl *a* decreased with trophic status, although the difference between the slopes was not significant ($p = 0.083$, Fig. 1.1). No significant relationship was observed between *in situ* PC abundance and total chl *a* or APP chl *a* concentrations.

Abundance of PC decreased slightly with TP and TKN although the relationships were not significant (TP: $r = 0.10$, $p = 0.470$; TKN: $r = 0.10$, $p = 0.480$, Fig. 1.2). When Oneida Lake was removed from the data set, only the relationship with TKN was

significant ($r = 0.36$, $p = 0.012$). The relationship of abundance of PC *in situ* and TP or TKN did not vary between regions except for New York State. This region should be considered separately since only two lakes were sampled and a bloom of filamentous cyanobacteria *Aphanizomenon* was occurring in Oneida Lake at time of sampling. Above all physical and chemical variables, pH of the epilimnion was the most highly correlated with PC abundance over the observed range of 6.4 to 9.1 ($r = 0.49$, $n = 47$, $p = 0.0005$, Fig. 1.3). Removal of Oneida Lake data was done due to extreme values that were affecting significantly any relationship of PC abundance with physical and chemical variables. Also, because of extremely low concentration of PC in the lake water sample, growth and loss rates of PC could not be appropriately estimated.

Factors affecting growth and loss rates

Among the 50 sites, the estimated intrinsic growth rates of PC ranged from –0.31 d^{-1} to 1.93 d^{-1} and averages 0.41 d^{-1} whereas loss rates ranged from –0.33 d^{-1} to 1.25 d^{-1} and averaged 0.26 d^{-1} across the lakes. Growth and loss rates of PC both increased with TP although only the relationship of growth rate with TP was significant (growth: $r = 0.32$, $p = 0.027$; loss: $r = 0.26$, $p = 0.062$; Fig. 1.4). Neither growth or loss rates showed a significant trend with TKN (growth : $r = 0.26$, $p = 0.061$; loss : $r = 0.22$, $p = 0.112$) When compared to the *in situ* PC abundance, only loss rate of PC was found to increase significantly while PC growth rate was found to be independent of the *in situ* abundance (loss: $r = 0.44$, $p < 0.002$; growth: $r = 0.03$, $p = 0.814$; Fig. 1.5). Since only traces of PC were found in Oneida Lake, growth or loss rates could not be estimated for that lake.

Growth and loss rates of PC were highly correlated among the lakes: loss rate increased with growth rate ($r = 0.75$, $p < 0.0001$; Fig. 1.6). The 1:1 line in Figure 1.6 shows that growth rates are on average higher than the loss rates (slope = 0.665, $p = 0.0002$).

Multiple regressions were performed to determine the effect of physical or chemical variables (pH, TKN:TP ratio; log transformed variables: TP, TKN, epilimnion temperature and conductivity, Secchi disk depth, light attenuation coefficient) on the growth and loss rates. The model that best explained the estimated growth rates based on r^2 values was:

$$\text{growth rate} = 1.59 + 0.96\text{LOSS} - 0.18\text{pH} \qquad r^2 = 0.62$$

where LOSS is the loss rate and explains 56% of the variation alone. TKN:TP ratio was also a significant variable however when included in the former model, it explained only slightly more of the observed variation in growth rates ($r^2 = 0.64$). The best model to explain the estimated loss rates was:

$$\text{loss rate} = -1.60 + 0.65\text{GROWTH} + 0.20\text{pH} \qquad r^2 = 0.68$$

where GROWTH is the growth rate. In both models, conductivity could replace pH as the two variables were highly correlated. However, the models with pH had a slightly better fit.

Factors affecting PC abundance *in situ*

No relationship was found between PC abundance at the time of sampling and the concentration of TP in the water samples; TKN was a better predictor of *in situ* PC ($r = 0.36$, $p = 0.012$). The best multiple regression model that could predict *in situ* PC abundance in these lakes was:

$$\log \text{PC abundance} = -9.280 + 0.029\text{pH} + 0.394\text{LOSS} + 1.527\log\text{TKN} + 0.006\text{TKN:TP} + 6.035\log\text{TEMP} \qquad r^2 = 0.53$$

where LOSS is the loss rate and logTEMP is the log transformed temperature. pH accounted alone for 21% of the variability. If conductivity was considered in the model instead of pH, the explained variability was lower ($r^2 = 0.48$).

DISCUSSION

The goals of this study were to identify the main variables related to the *in situ* picocyanobacteria abundance across a trophic gradient of lakes. The results obtained on growth and loss rates suggest a strong positive relationship between those two rates. This correlation is indicative of the interaction between microbial predators and their preys. Laboratory experiments have demonstrated that flagellate grazing can positively influence bacterial growth rates (e.g. Hahn et al. 1999). Grazing leads to the release of nutrients in water which are then rapidly reassimilated by microorganisms, thereby increasing the growth rate of those organisms, as a part of the microbial loop (Sherr et al. 1986).

We anticipated an effect of nutrients on growth rates from both culture work and previous field studies. Liu et al. (1998) observed an increase in the growth rate of marine *Synechococcus* spp. with nitrate concentration and Rhew and Ochs (2000) also showed nitrogen limitation of growth rate of APP (prokaryotes and eukaryotes) in a reservoir. When considered alone, there was in fact a weak positive correlation between growth rates and TP. However, when all variables were considered in a multiple regression, only loss rates and pH of the epilimnion best explained the intrinsic growth rates of APP.

We also considered temperature as an important variable, since Ning and Vaulot (1992) observed a positive relationship of *Synechococcus* spp. with temperature ranging from 9°C to 20°C in marine environment. Agawin et al. (1998) obtained a significant positive relationship between growth and temperature over time, with maximum growth rates from dilution experiments of 1.5 d^{-1} in summer and minimum growth rates of 0.2 d^{-1}

in winter in a Mediterranean bay. However, in the present study there was no effect of water temperature on growth rates, most likely because epilimnetic temperature varied only from 19°C to 25°C among the lakes in summer. However, there was a slight effect of temperature on *in situ* PC abundance.

For a couple of lakes, the estimated growth or loss rates had negative values, meaning that for some experiments, cell division did occur in samples treated with ampicillin (negative loss rates) or that losses of PC occurred in control samples where those losses were higher than those estimated in samples treated with ampicillin (negative growth rates). Since the experimental method was applied in the same way for each lake, we cannot explain these unrealistic rates even if we used the proper concentration of ampicillin which normally should not affect eukaryotes (Campbell and Carpenter 1986; Ning and Vaulot 1992). If the negative growth and loss rates are not taken in consideration, the estimated rates are quite representative of those in the literature (Table 1.2).

Several techniques have been developed for the measurement of growth and loss rates. Landry (1994) described and criticised these techniques that he grouped in three categories: techniques that infer the rates from natural populations in situ (e.g. frequency of dividing cells), from tracer studies (e.g. fluorescently labelled prey) and community manipulations (e.g. dilution and selective metabolic inhibitor techniques). Each technique has advantages and disadvantages that can limit the effectiveness or precision of the measurement. The selective metabolic inhibitor technique is among the simplest technique

in that it requires fewer samples and entails perhaps the fewest assumptions. The results obtained by this technique are comparable with literature data (Table 1.2).

The selective inhibitor technique does not allow for identification of the actual loss processes. These would include grazing, viral infection, autolysis of cells due to aging and/or apoptosis. All of these would tend to increase with population size. Suttle and Chan (1994) estimated *Synechococcus* spp. lysis by cyanophages, to be of 5 to 14% of the PC population per day in the marine environment. It is generally believed that grazing is the main factor responsible for PC losses in freshwater and marine environments. However, future studies should take into consideration all these losses together and not conclude that the observed rates are only the result of grazing by predators.

The observed *in situ* abundance of PC did not show a trend with the trophic status of lakes as defined by TP. This result is in agreement with Vörös et al. (1998) who used total chl *a* as the indicator of trophy and covered a wider range of trophic states including European hypereutrophic lakes (0.2 to 390 μg chl $a \cdot l^{-1}$). In contrast, Burns and Stockner (1991) found a negative relationship of PC abundance with total chl *a* over a similar range as Vörös et al. (1998). An interesting result came from Pick (2000) who noticed a polynomial relationship of PC abundance with TP where the abundance increased up to 15 $\mu g \cdot l^{-1}$ to attain a plateau to then decreased in very eutrophic lakes. We found a positive relationship of PC abundance with nitrogen (TKN) when Oneida Lake was removed from the data set; this result is in agreement with the results of Wehr (1989; 1991) for APP biomass. Agawin et al. (2000) found a negative trend of APP biomass with nitrogen in the

marine environment over a range of 0.1 to 10 μM. Wehr (1989; 1990; 1991; 1993) also did not find a trend with TP, suggesting that PC are not limited by phosphorus compared to algae of larger size.

Light was not a significant variable alone or in the model to explain *in situ* PC abundance which goes with the findings of Wehr (1990; 1993) while Pick (1991) and Wehr (1991) both found a negative effect of light attenuation on PC and APP abundance respectively. The range of light attenuation coefficients of the 50 sites may have been too small to statistically detect an effect of the light penetration.

Concentrations of soluble reactive phosphorus (SRP), ammonium (NH_3) and nitrate/nitrite (NO_3/ NO_2) were not included as variables tested in the analysis because many of the values were close to detection limits (detection limit of 1 $\mu g \cdot l^{-1}$ for SRP, 3 $\mu g \cdot l^{-1}$ for ammonium and nitrate/nitrite). SRP in particular is difficult to estimate accurately in oligotrophic lakes and chemical analyses are known to overestimate biologically available phosphate (Hudson et al. 2000).

Concentrations of soluble reactive phosphorus (SRP), ammonium (NH_3) and nitrate/nitrite (NO_3/ NO_2) could not be added to the chemical variables tested in the present study due to null values or values close to the detection limits of the apparatus (detection limit of 3 $\mu g \cdot l^{-1}$). This limitation problem leads to questioning on the possible omission of these nutrients as variables affecting growth and abundance of PC. High precision of the analyzing equipment should be a factor to consider and priories when planning nutrient analyses of water samples.

Above all, the loss rate of PC, as well as pH, was an important variable explaining the *in situ* PC abundance. Even though growth rate was highly correlated with the loss rate, only the latter was significantly correlated with *in situ* PC abundance. This means that as the abundance of PC increases, higher grazing pressure by predators likely increases proportionally. The predation pressure can stimulate a microbial population to increase its productivity in order to compensate for the increasing losses; this is possible through the rapid recycling of nutrients by the microbial loop. Sherr et al. (1986) found a positive relationship between abundance of heterotrophic bacteria and the grazing rate by heterotrophic nanoflagellates in estuarine waters.

In conclusion, the abundance of PC was more closely related to loss rates in freshwater lakes instead of physical and chemical variables such as TP. The biotic control of PC appears to supercede any physical or chemical factor, implying an important top-down control of picoplanktonic organisms in freshwater systems.

Table 1.1 Location, morphometry and picocyanobacteria abundance by epifluorescence microscopy in 48 lakes sampled once in July or August 2000. **SA**: surface area; **TP**: total phosphorus; **TKN**: total Kjeldahl nitrogen; **PC**: picocyanobacteria. PC abundance are the mean of 2 or 3 replicates.

Region / Lake	Latitude	Longitude	SA (km^2)	TP ($\mu g \cdot l^{-1}$)	TKN ($\mu g \cdot l^{-1}$)	PC abundance +/- SD ($10^4 \cdot l^{-1}$)
Outaouais, Que.						
Argile	45° 52'	75° 34'	4,51	11,5	290	3,52 (0,44)
Barrière	45° 53'	75° 06'	3,11	2,5	189	4,11 (0,10)
Black	45° 29'	75° 51'	0,01	11,5	398	0,53 (0,13)
Forgeron	46° 03'	75° 37'	0,55	15,5	550	0,99 (0,38)
Mulvihill	45° 29'	75° 51'	0,01	10,5	342	4,74 (0,06)
Orignal	45° 52'	75° 35'	0,37	8,5	290	3,88 (0,76)
Philippe	45° 36'	76° 01'	1,76	6,5	248	17,30 (1,05)
Renaud	45° 36'	76° 02'	0,10	24,0	535	1,04 (0,24)
Simon	45° 55'	75° 04'	30,73	1,0	179	3,56 (0,18)
Taylor	45° 36'	76° 03'	0,60	4,5	318	6,73 (0,99)
Vert	45° 54'	75° 36'	0,24	5,0	273	8,90 (0,36)
Ottawa, Ont.						
Constance	45° 24'	75° 58'	1,45	40,0	860	46,80 (6,23)
MacKay	45° 27'	75° 40'	0,79	19,0	585	17,10 (7,20)
Rideau lakes, Ont.						
Big Rideau	44° 42'	76° 13'	5,8	14,0	428	13,70 (0,51)
Bob's			9,27			
(Green Bay)	44° 38'	76° 35'		7,0	315	8,30 (0,09)
(West Bay)	44° 39'	76° 37'		9,5	372	20,60 (5,19)
Indian	44° 35'	76° 19'	2,66	15,0	400	14,60 (2,77)
Jack's	44° 41'	78° 04'	5,10	7,0	434	12,00 (1,09)
Opinicon	44° 33'	76° 20'	7,85	14,0	415	10,80 (0,59)
Otter	44° 46'	76° 07'	5,71	10,5	445	22,70 (2,91)
Upper Rideau	44° 40'	76° 21'	13,62	20,5	455	8,56 (0,87)
Upper Rock	44° 29'	76° 24'	0,76	10,0	470	9,75 (1,68)
Westport Sand	44° 40'	76° 25'	2,08	19,0	416	15,50 (0,90)
Wolfe	44° 40'	76° 29'	7,50	9,5	340	18,10 (1,87)
Laurentians, Que.						
Achigan	45° 56'	73° 57'	5,32	4,5	250	6,99 (1,23)
Anne	45° 49'	74° 18'	1,18	6,0	265	13,30 (3,50)
Connelly	45° 53'	73° 57'	1,21	16,0	350	2,54 (1,82)
Croche	45° 59'	74° 00'	0,18	4,5	275	8,31 (1,20)
En coeur	45° 58'	74° 00'	0,47	5,0	285	1,54 (0,41)
Hugues	45° 48'	74° 15'	n/a	7,0	320	6,86 (1,72)
Morency	45° 55'	74° 02'	0,26	5,0	304	9,36 (0,95)
Montaubois	45° 55'	74° 04'	0,16	7,0	235	1,81 (0,61)
Ours	45° 57'	74° 03'	0,14	20,5	525	8,21 (1,51)
Pin rouge	45° 57'	74° 02'	0,15	14,0	460	2,88 (0,38)

Table 1.1 (continued)

Region / Lake	Latitude	Longitude	SA (km^2)	TP ($\mu g \cdot l^{-1}$)	TKN ($\mu g \cdot l^{-1}$)	PC abundance +/- SD ($10^4 \cdot l^{-1}$)
Estrie, Que.						
d'Argent	45° 18'	72° 18'	1,0	5,0	311	14,10 (0,88)
Bowker	45° 42'	72° 21'	2,5	1,0	149	2,12 (0,42)
Brome	45° 14'	72° 30'	14,39	31,0	655	45,60 (2,85)
Brompton	45° 42'	72° 14'	11,90	3,0	245	0,99 (0,05)
Choinière	45° 25'	72° 37'	4,75	28,0	503	5,09 (1,18)
Fraser	45° 38'	72° 17'	1,6	4,5	260	0,46 (0,01)
Lovering	45° 17'	72° 15'	4,94	7,5	358	3,27 (0,47)
Magog	45° 30'	72° 04'	10,95	15,0	345	3,98 (1,24)
Massawippi	45° 21'	72° 00'	17,90	7,5	308	3,58 (0,82)
Memphrémagog			97,9			
(Magog Bay)	45° 16'	72° 11'		4,0	233	1,33 (0,22)
(Sargent Bay)	45° 10'	72° 17'		8,0	238	3,33 (0,51)
Montjoie	45° 40'	72° 10'	3,3	12,0	275	1,14 (0,16)
Orford	45° 18'	72° 16'	1,29	1,5	247	29,40 (4,76)
Stukely	45° 22'	72° 15'	4,0	1,0	222	6,76 (0,62)
New York State, U.S.						
Onondaga	43° 06'	76° 13'	12	27,5	655	15,10 (2,33)
Oneida	43° 14'	75° 48'	210	42,0	900	< 0.01

Table 1.2 Summary of estimated growth/loss rates from literature and the present research with different techniques. Values from the present research are the maximum estimated rates obtained. **FDC**: frequency of dividing cells

Location	Growth rate (d^{-1})	Loss rate (d^{-1})	Estimation method	Reference
Freshwater				
Superior (Canada)	1.5		carbon fixation	Fahnenstiel et al. 1986
Huron (Canada)	0.11 - 0.39	0.19 - 0.89	dilution	Fahnenstiel et al. 1991a
Huron (Canada)	0.32 - 0.37		FDC	Fahnenstiel et al. 1991b
Michigan (USA)	0.05 -0.39	0.11 - 0.42	dilution	Fahnenstiel et al. 1991a
Michigan (USA)	0.30 - 0.42		FDC	Fahnenstiel et al. 1991b
Jack's (Canada)	0.6 - 0.8	0.48 - 0.64	dilution	Pick and Agbeti 1991
Lakes	-0.31 – 1.93	-0.33 – 1.25	selective inhibitor	present research
Marine				
Pacific ocean (Japan)	1.20 – 2.47	0.55 – 1.44	FDC/ dilution	Kudoh et al. 1990
English Channel	0.25 - 0.72	0.21 - 0.64	selective inhibitor	Ning and Vaulot 1992
Central Pacific ocean	0.54 - 1.06	0.22 - 0.73	selective inhibitor	Liu et al. 1995
Mediterranean sea	0.2 - 1.5		FDC	Agawin et al. 1998

Figure 1.1 Relationship between log transformed chlorophyll *a* concentrations and the log transformed total phosphorus of 50 freshwater lakes sites. The chlorophyll *a* concentration of particles smaller than 2 μm was determined by subtracting the total chlorophyll *a* by the amount of chlorophyll *a* retained on a 2 μm pore size Nuclepore filter.

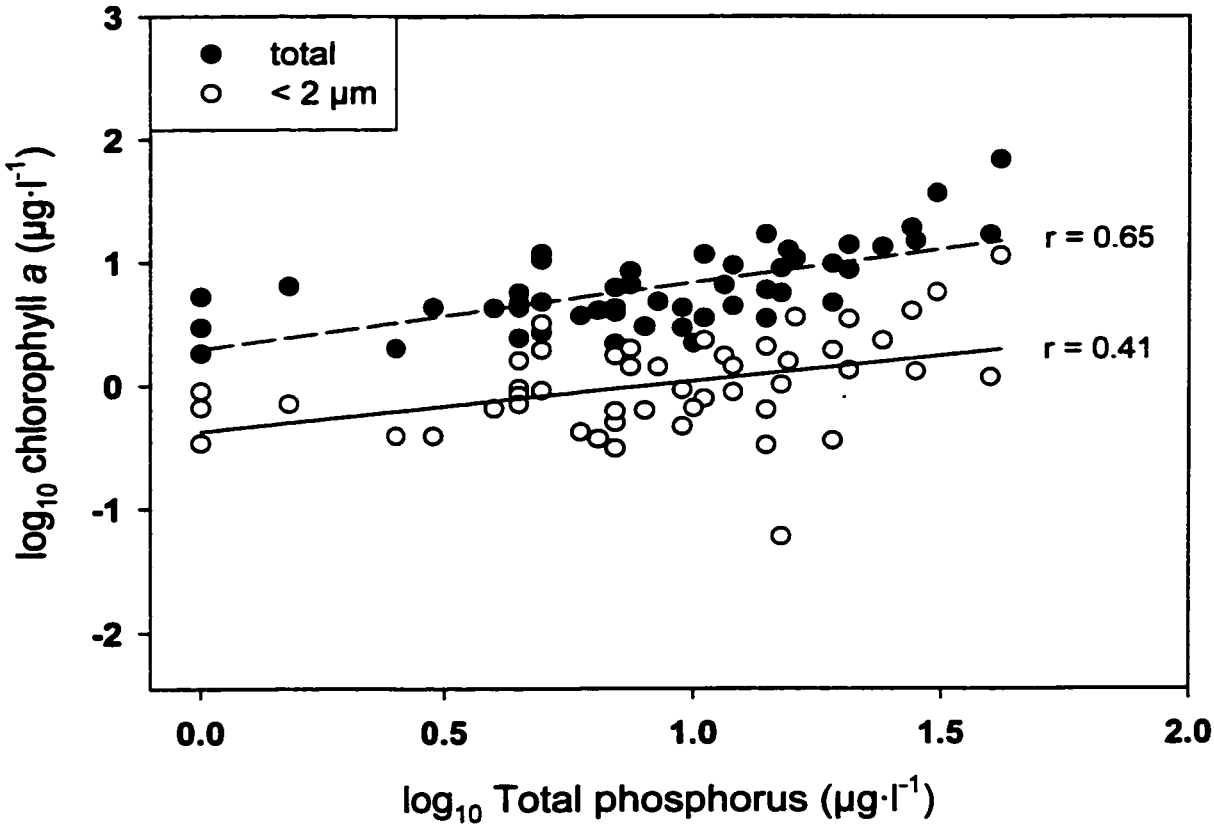
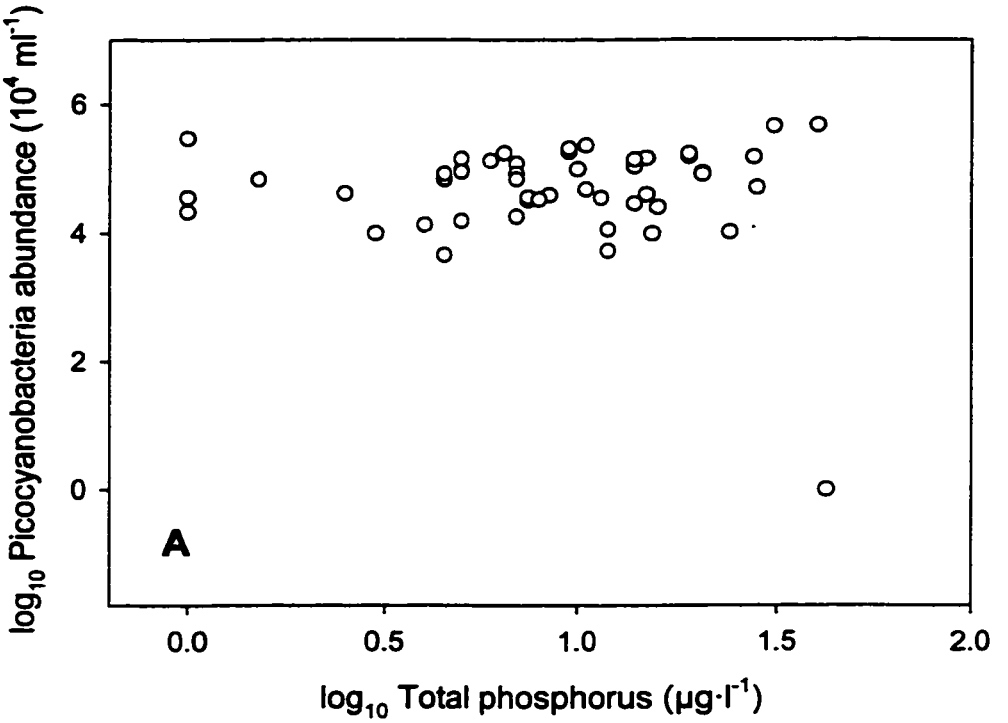

total
< 2 µm
r = 0.65
r = 0.41
log10 chlorophyll a (µg·l-1)
log10 Total phosphorus (µg·l-1)
3
2
1
0
-1
-2
0.0
0.5
1.0
1.5
2.0

Figure 1.2 Log transformed abundance of picocyanobacteria (ml^{-1}) and concentration of **A)** total phosphorus ($\mu g \cdot l^{-1}$) and **B)** total Kjeldahl nitrogen ($\mu g \cdot l^{-1}$) of temperate lakes. When the extreme point was removed from the data set (Oneida lake: point at the bottom of the figures), only the relationship with nitrogen became significant with picocyanobacteria abundance.


log10 Picocyanobacteria abundance (10^4 ml^-1)
6
4
2
0
A
0.0
0.5
1.0
1.5
2.0
log10 Total phosphorus (µg·l^-1)

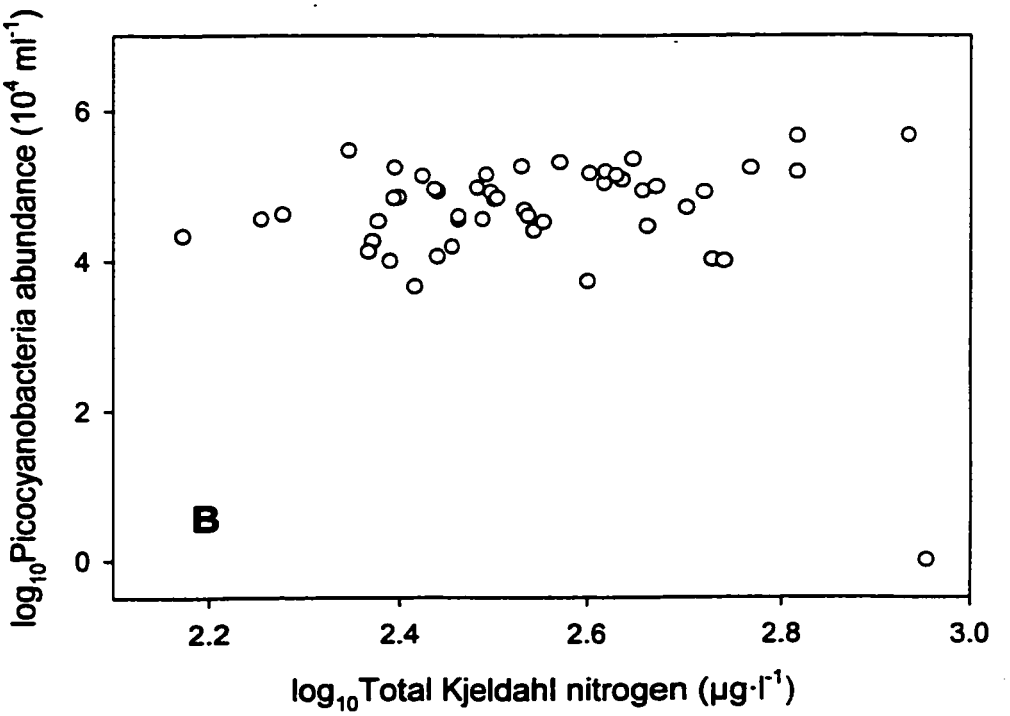

log10Picocyanobacteria abundance (10^4 ml^-1)
6
4
2
0
B
2.2
2.4
2.6
2.8
3.0
log10Total Kjeldahl nitrogen (µg·l^-1)

Figure 1.3 Log transformed abundance of picocyanobacteria (ml^{-1}) as a function of pH of the sampled sites ($r = 0.49$, $n = 47$, $p = 0.0005$). Lake Oneida was removed from the data set.

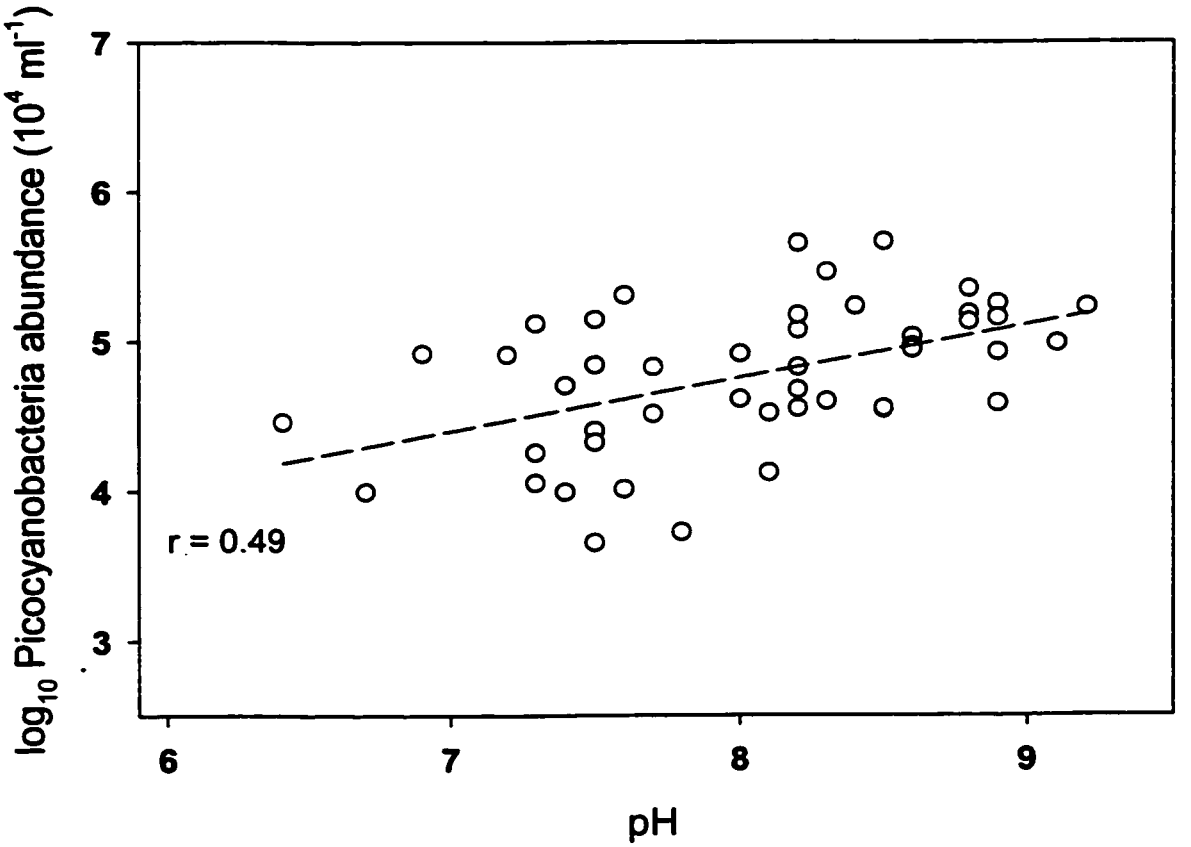
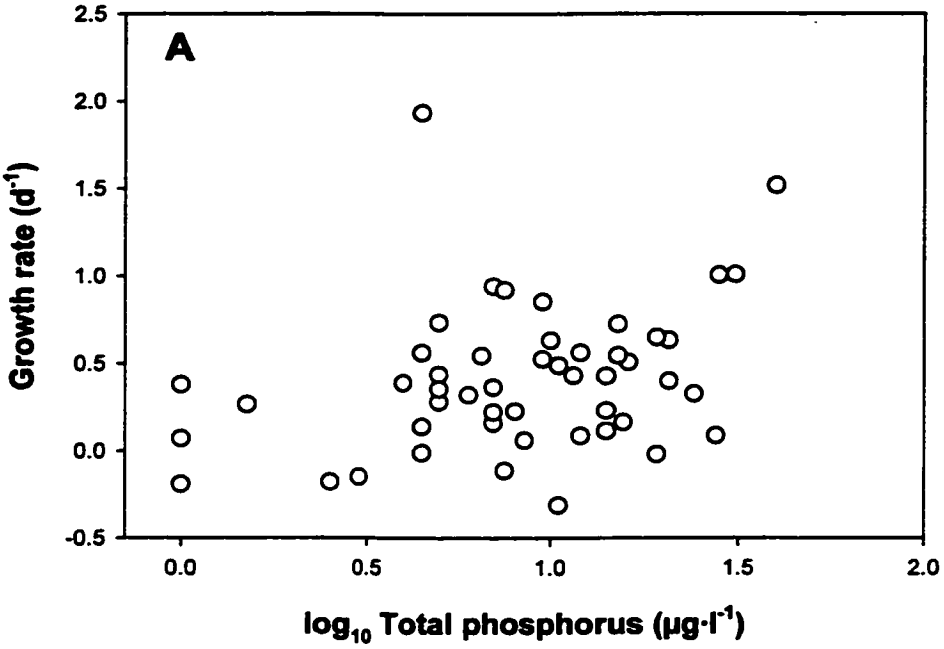

log10 Picocyanobacteria abundance (10^4 ml^-1)
7
6
5
4
3
r = 0.49
6
7
8
9
pH

Figure 1.4 **A)** Growth rate and **B)** loss rate (d^{-1}) of picocyanobacteria in comparison with the log transformed concentration of total phosphorus for each sampled site. Only growth rate had a significant relationship with total phosphorus (regression line) ($r = 0.32$, $n = 49$, $p = 0.027$).


A
Growth rate (d^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.0
0.5
1.0
1.5
2.0
$\log_{10}$ Total phosphorus ($\mu g \cdot l^{-1}$)

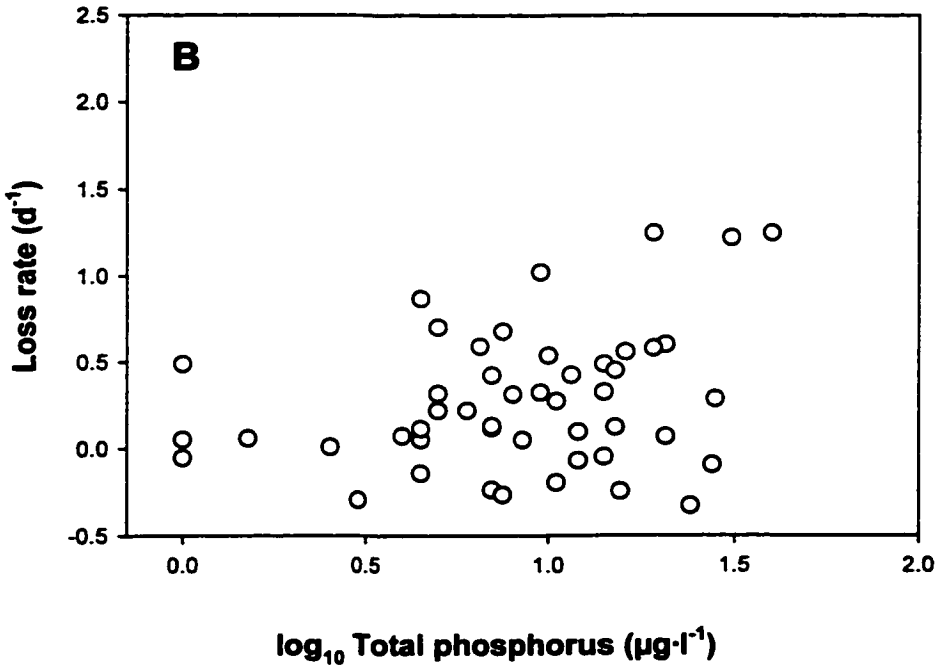
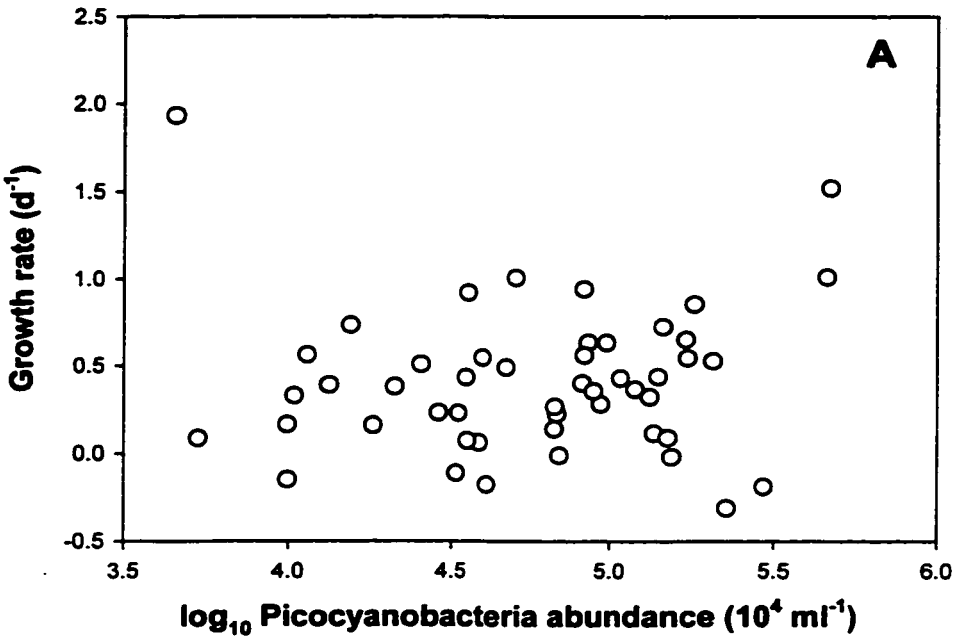

B
Loss rate (d^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.0
0.5
1.0
1.5
2.0
$\log_{10}$ Total phosphorus ($\mu g \cdot l^{-1}$)

Figure 1.5 **A)** Growth rate and **B)** loss rate (d^{-1}) of picocyanobacteria in comparison with their log transformed *in situ* abundance (ml^{-1}). Only loss rate has a significant relationship with picocyanobacteria abundance, although the variability is high ($r = 0.44$, $n = 49$, $p < 0.002$).


A
Growth rate (d^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
3.5
4.0
4.5
5.0
5.5
6.0
$\log_{10}$ Picocyanobacteria abundance (10^4 ml^{-1})

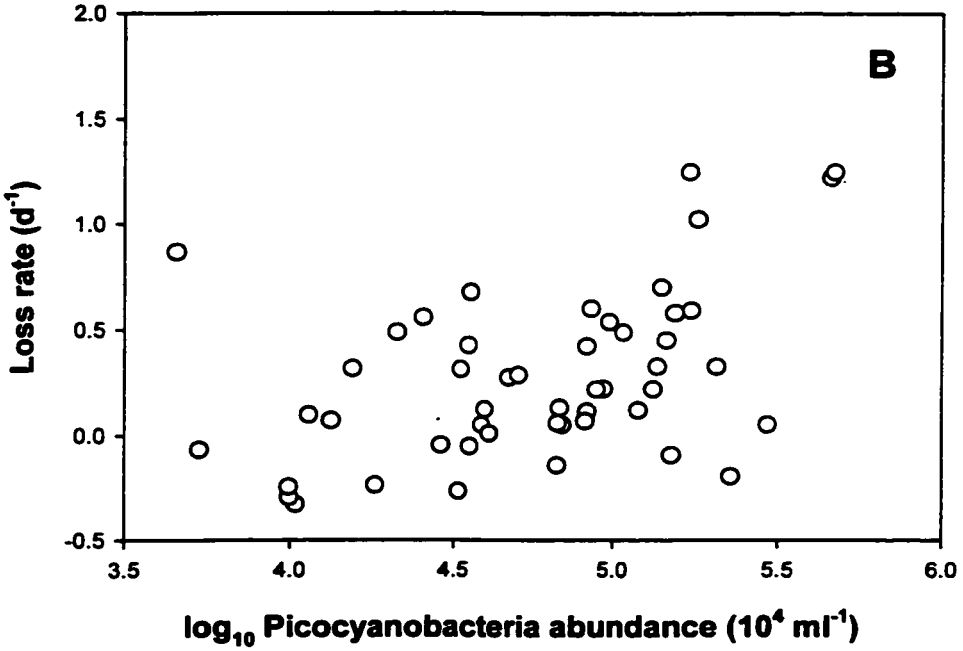
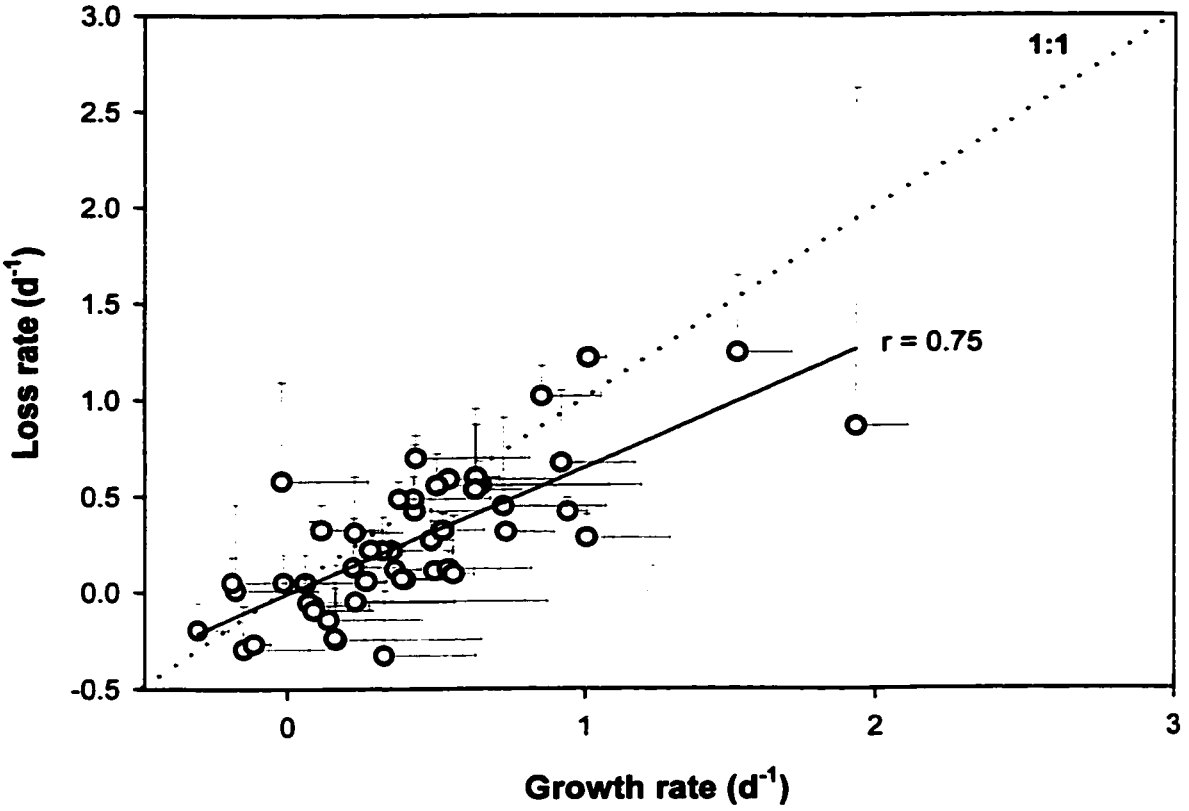

B
Loss rate (d^{-1})
2.0
1.5
1.0
0.5
0.0
-0.5
3.5
4.0
4.5
5.0
5.5
6.0
$\log_{10}$ Picocyanobacteria abundance (10^4 ml^{-1})

Figure 1.6 Relationship between growth and loss rates (d^{-1}) of picocyanobacteria ($r = 0.75$, $n = 49$, $p < 0.0001$). Each point represents an individual lake. Error bars represent the positive standard deviation of the mean ($n = 3$).


3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
1
2
3
Loss rate (d-1)
Growth rate (d-1)
1:1
r = 0.75

CHAPTER 2

The use of flow cytometry to study picocyanobacteria in lakes

ABSTRACT

Flow cytometry was used to examine freshwater picocyanobacteria (PC). The effect of two common preservatives on flow cytometry signals was examines with lake water samples and cultures of *Synechococcus leopoliensis*. An increase in phycoerythrin and chlorophyll *a* fluorescence was observed with specimens preserved with glutaraldehyde. The effect of light regime was examined with *S. leopoliensis* cultures. The three treatments gave different fluorescence and size signals where both size and fluorescence of chlorophyll *a* increased with decreasing light intensity. Flow cytometric signals from 48 lakes were analysed. PC lacking phycoerythrin could be easily differentiated from PC containing phycoerythrin by a difference in phycoerythrin fluorescence.

Counts of PC by flow cytometry were on average lower than epifluorescence microscopy counts, and in some cases up to four times lower than epifluorescence; occasionally, flow cytometry counts were higher than epifluorescence. Comparison of two flow cytometers, one equipped with a detector for phycocyanin fluorescence (FACS Vantage) and the other without (FACScan) showed a better discrimination of two PC types and background noise by the FACS Vantage. Even if flow cytometry analysis required less time than epifluorescence to determine PC abundance, the clustering of cells from dot plots of flow cytometry data was somewhat subjective, depending on the background noise in each lake. The lake to lake variation in signals was high and required adjustment of gates.

The use of flow cytometry in plankton research may be more limited in freshwater compared to marine environments because of higher background noise

INTRODUCTION

The importance of autotrophic picoplankton as part of the primary production of marine and freshwater systems is no longer in any doubt. Organisms less than 2 μm in diameter contribute to one tenth of the total primary production of aquatic systems and their relative importance is greatest in oligotrophic systems (Stockner and Antia 1986; Raven 1998).

Picocyanobacteria (PC), which include *Synechococcus* and *Synechocystis*, are among the most abundant phytoplankton: Raven (1998) estimated the *Synechococcus* global abundance to be about 10^{26} individuals. This genus is very important in freshwater lakes as well as in oceans. In addition, the abundance of *Prochlorococcus*, another prokaryotic picoplankton, is significant in many oceans. *Prochlorococcus* was discovered a few years after PC, with the application of flow cytometry to oceanography. Flow cytometry is now an essential step for any oceanographic study of picoplankton. The use of this equipment is obligatory for the study of *Prochlorococcus* because it cannot be observed by epifluorescence microscopy. *Prochlorococcus* is very small (< 0.7 μm) and its faint chl *a* fluorescence makes it unobservable by epifluorescence microscopy (Chrisholm et al. 1988).

Many studies use flow cytometry to describe phytoplankton abundance in marine environments and to estimate *in situ* biomass, biovolume, pigmentation patterns and to study cell cycles (e.g. Olson et al. 1990; Li 1994; Li 1995; Landry et al. 1996; Marie et al. 1997; Liu et al. 1999). Since heterotrophic microorganisms generally do not contain

fluorescing pigments, numerous fluorochrome dyes have been developed to stain heterotrophic cells before analysis by flow cytometry. Grazing studies have been performed with flow cytometry by estimating the disappearance over time of fluorescently labelled beads or stained live or dead bacteria due to predation by heterotrophic organisms (Monger and Landry 1992; Sherr and Sherr 1993).

The use of flow cytometry in phytoplankton ecology is pertinent because photosynthetic pigments produced by algae can emit fluorescence when excited by specific wavelengths of light. All PC contain phycocyanin, an accessory photosynthetic pigment. Some species and some of their strains will contain another photosynthetic pigment, phycoerythrin. PC containing only phycocyanin are designated as phycocyanin-rich while those PC containing both pigments are designated as phycoerythrin-rich.

Phycocyanin and phycoerythrin are biliproteins rich in nitrogen. Aside from their role in transmitting light energy to chlorophyll *a*, they are thought to serve as a reserve of nitrogen when nutrients become scarce in the environment (Glibert et al. 1986; Postius and Böger 1998). Because they can absorb a broad light spectrum, PC are found at depths down to 150 metres in marine environments (e.g. Olson et al. 1990; Crosbie and Furnas 2001). And the deeper PC are in the water column, the greater the amounts of biliproteins concentrated per cell (Olson et al. 1990; Bautista and Jiménez-Gómez 1996; Collier 2000).

The structure and fluorescence of biliproteins can be affected by chemical reagents. The reagents are used to preserve water samples when time has elapsed after

collection. Preservatives help to strength the cell wall and to minimise the degradation of photosynthetic pigments. Glutaraldehyde and paraformaldehyde are common preservatives used for water samples (del Giorgio et al. 1996; Landry et al. 1996; Crosbie and Furnas 2001).

Preservation of water samples for phytoplankton studies is as important as the techniques used to analyse samples. The use of flow cytometry to analyse phytoplankton populations in freshwater environments is still very limited, and has been mainly applied to the enumeration of pre-stained heterotrophic bacteria (Porter et al. 1995; del Giorgio et al. 1996; Porter et al. 1997). Early work by Fahnenstiel et al. (1991a) on the Great Lakes showed that the technique was promising in freshwater environments. Since Fahnenstiel et al. (1991a) there have been only 2 publications showing flow cytometry data freshwater picoplankton. The Corzo et al. (1999) study claims to have detected a *Prochlorococcus*-like organism in a reservoir in Spain, which would be the first discovery outside of a marine or estuarine environment. This microorganism, detected with a FACScan (Becton Dickinson) had a weak chlorophyll *a* (chl *a*) fluorescence and lacked phycoerythrin just like *Prochlorococcus* in oceans. However, *Synechococcus* can contain or lack phycoerythrin depending on the strain; also all *Synechococcus* spp. contain phycocyanin, a pigment present in cyanobacteria but not in prochlorophytes. The FACScan used by Corzo et al. (1999) was a flow cytometer equipped to detect phycoerythrin fluorescence (FL2) but not phycocyanin fluorescence (FL4). As a result, Corzo et al. (1999) probably could not definitively confirm the absence of phycocyanin in their *Prochlorococcus*-like picoplankton, which makes the results of their study questionable. It is possible that their microorganism was simply a small PC lacking phycoerythrin and not a prochlorophyte

(Collier 2000). Another flow cytometry study from freshwater (Collier 2000) did not reveal the presence of *Prochlorococcus*-like populations in Lake George and Hudson River in New York state; the flow cytometer used had the laser to detect phycocyanin fluorescence.

The goals of this study were to **1)** define the flow cytometric signals of PC populations in lakes; **2)** assess the accuracy and potential technical errors associated with this technique in comparison to epifluorescence microscopy.

From an ecological perspective, description and analysis of the flow cytometric signature of freshwater PC was assessed from many lakes. The changes in *S. leopoliensis* cultures signatures following growth at different light intensity regime were also studied. From a technical perspective, I compared flow cytometry versus epifluorescence microscopy counts of PC; I verified the relevance of using a flow cytometer with a phycocyanin fluorescence detector and finally, verified the effect of preserving water samples on the flow cytometric signals of PC.

For the first objective, water samples from all 50 lakes studied in the first chapter were analysed with a FACScan flow cytometer. For the second objective, *S. leopoliensis* cultures were grown at different light intensities and then analysed by flow cytometry to determine weather population characteristics would change significantly. To achieve the third objective, a water sample from Bob's Lake (West Bay), Ont. was analysed by two different flow cytometers, one of them lacking the proper laser and filter for the detection of phycocyanin. For the fourth objective, water samples from a couple of lakes were used

to determine picocyanobacteria abundance both by flow cytometry and epifluorescence microscopy. Finally, water samples from Bob's Lake (West Bay) were analysed by flow cytometry to verify which method of preservation (glutaraldehyde and paraformaldehyde) gives the best results in comparison with non-preserved water samples when analysing freshwater PC.

MATERIAL AND METHODS

Culture maintenance. A culture of the freshwater *Synechococcus leopoliensis* (UTCC 100) was obtained from the University of Toronto Culture Collection. The culture was grown in the laboratory at 23 °C in 250 ml glassware Erlenmeyer flasks with 100 ml of BG-11 medium (Sigma Chemical Co.) at approximately 28 $\mu E \cdot m^{-2} \cdot s^{-1}$ under white fluorescent light (Vita-lights) on a 14:10 hour light:dark cycle.

Light intensity experiment. It is well known that changes in the ambient light regime affect pigment concentrations and composition in algae. How the light regime changes flow cytometric signals is less well understood. Cultures were incubated in triplicate under three different light regimes: low green light (28 $\mu E \cdot m^{-2} \cdot s^{-1}$), normal white light (28 $\mu E \cdot m^{-2} \cdot s^{-1}$) and high white light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) for a period of 6 days on a 14:10 hour light:dark cycle in order to analyse PC in their exponential phase. Low green light is representative of the light quality and intensity available at depth in clear or non-coloured lakes (Hauschild et al. 1991). Subsamples were taken after 6 days and preserved with 10% glutaraldehyde buffered with sodium cacodylate for a final concentration of 1% glutaraldehyde. Samples were kept in the dark at 4°C until analysis for flow cytometry and epifluorescence microscopy.

Lake samples. A water sample was collected from West Bay of Bob's Lake, Ont. (Lat. 44° 39' Long. 76° 37') on July 12th 2000 and September 3rd 2001. For both samples, an integrated sample of the epilimnion was taken in the middle of the lake or in the deepest

area with a 2.5 cm diameter Tygon tube with a weight attached at the end. Water was kept in a 9 L polyethylene carboy in a cooler until processed. Glutaraldehyde buffered with sodium cacodylate was added to water samples for a total concentration of 1% glutaraldehyde and kept in the dark at 4°C until analysis by flow cytometry or observation under an epifluorescence microscope.

Incubation with inhibitors. Triplicate water samples from different lakes were incubated in a plastic cage settled in a water tank in the greenhouse at temperature corresponding to the lake's temperature; or *in situ* near shore for 24 hours with 5 $mg \cdot l^{-1}$ of ampicillin (Sigma Chemical Co.) as described in Chapter 1. Triplicate aliquots were subsampled four times during 24 hours and preserved with glutaraldehyde as described above. Preserved subsamples were observed by epifluorescence microscopy and analysed with a FACScan flow cytometer which could count populations of PC and discriminate between phycoerythrin-rich and phycocyanin-rich cell types.

Experiments with different preservation methods. Water samples from MacKay lake and *S. leopoliensis* culture grown at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ were either kept non-preserved; preserved with glutaraldehyde buffered with sodium cacodylate for a total concentration of 1% glutaraldehyde or preserved with paraformaldehyde for a final concentration of 1% (Ning et al. 2000). All preserved and non-preserved samples were kept in the dark at 4°C and analysed with a FACScan flow cytometer the following day. Water samples were either unfiltered or filtered through a 2 μm or 0.2 μm Nuclepore filter in order to estimate background noise. Since liquid nitrogen is rarely brought to the field for limnological sampling, this preservation technique was not considered in the present study.

Flow cytometry analysis. Observation and enumeration of PC were performed with two different flow cytometers. The first was a FACScan (Becton Dickinson) equipped with a 488 nm air-cooled argon-ion laser; further details about the cytometer and the software used are given in del Giorgio et al. (1996). Voltage settings used were the same as used by Corzo et al. (1999) with chl *a* as the threshold parameter. Sample analyses were performed at low (12 µl min^{-1}) or high flow rate (60 µl min^{-1}). Fluorescently labelled beads (FLB) used were yellow-green beads 0.99 µm diameter size (#17154 Polysciences Inc.) and added as a volume of 100 µl at 1 x 10^5 FLB·ml^{-1} (working solution of FLB) to 900 µl of water sample for a total concentration of FLB of 1 x 10^4 FLB·ml^{-1}. A total of 5 000 events were recorded depending on the speed of the flow cytometer at the moment; from 30 seconds to 3 minutes were needed to obtain all 5 000 events. Cultures of *S. leopoliensis* were diluted 10 times due to their high cell concentrations. A computer connected to the flow cytometer acquired the data with the software LYSIS II (Becton Dickinson). Further analyses of the data were processed with the software WinMDI version 2.8 available on the internet (http://pingu.salk.edu/software.html).

The second flow cytometer was a FACS Vantage (Becton Dickinson) equipped with 3 lasers: an argon-ion (365 nm), an argon-ion (488 nm) and an helium-neon (633 nm). The 365 nm laser was not used in this study, as it corresponds to UV excitation.

Epifluorescence microscopy. Glutaraldehyde-preserved water samples were vacuum filtered at low pressure (less than 200 mm Hg) on a Nuclepore 0.2 µm pore size filter with a Millipore 0.45 µm pore size backing filter under the former to ensure an even

distribution of the cells. PC were observed under an epifluorescence Zeiss Jenamed 2 microscope equipped with a blue excitation filter (450-490 nm, barrier filter 520 nm) and a green excitation filter (510-560 nm, barrier filter 590 nm). The green filter, which excites phycocyanin pigments, was used to count the total abundance of PC while the blue filter, which excites phycoerythrin as well as chl *a*, was used to distinguish PC containing phycoerythrin that fluoresce yellow-orange from eukaryotic picoplankton fluorescing a bright red (Pick 1991). Enumeration of PC was done at 1250X magnification where 15 randomly chosen fields were counted to obtain a minimum of 300 cells.

RESULTS

Flow cytometry signals among lakes

Water samples from each lake described in Chapter 1 were analysed with a FACScan (Becton Dickinson) flow cytometer. All 50 lakes are represented in Appendix IIA as dot plots of phycoerythrin fluorescence (FL2) in relation to chl *a* fluorescence (FL3) where each dot represents an event. This representation of data as FL2 versus FL3 allowed for the best discrimination of distinct phytoplankton populations, especially the PC containing phycoerythrin (phycoerythrin-rich).

For the majority of the lakes, the main pattern is a long linear distribution of events illustrating various ratios of phycoerythrin to chl *a* fluorescence (Fig. 2.1 *A*). Two groups of events are observed. What separates these two groups is a gap between phycoerythrin fluorescence (FL2) for a similar chl *a* fluorescence (FL3). The group that has the greatest phycoerythrin fluorescence (approximately 25 to 2 000 relative units) was identified as phycoerythrin-rich PC (green events in Fig. 2.1) while the group below (fluorescence of approximately 6 to 300 relative units) was identified as the phycocyanin-rich PC (blue events). PC abundance data from Pick (1991) indicated that the proportion of phycoerythrin-rich PC as the percentage of total PC abundance in summer water samples of West Bay and Green Bay averaged 66% and 93% respectively; in Figure 2.1 *A*, the percentage of phycoerythrin-rich PC were of 69% in West Bay and 95% in Green Bay. Dot plots of FSC vs FL3 (Fig. 2.1 *C*) demonstrate the similarity in size of the two groups of PC. Another cluster of events above the two PC groups represents the FLB that have been identified earlier from a dot plot of FITC fluorescence (FL1) as a function of particle

size (FSC). The FLB used have a mean diameter of 0.99 µm. In dot plots with FL2 the Y-axe and FSC the X-axe, the events found in the top and end right of the plot should be Cryptophyceae, which are flagellated microorganisms of 10 µm diameter in size that can be detected by flow cytometry as they contain chl *a* and phycoerythrin. Because of their large size compared to PC, their fluorescence would be higher than PC as well but their abundance would be lower as is observed by the small number of events (e.g. Fig. 2.1 *B*).

Great variation in flow cytometry patterns was found between lakes making direct comparisons difficult. However, lakes Opinicon, Indian, Upper Rideau and Big Rideau which are connected by locks as part of the Rideau Canal waterway, had similar signals (Fig. 2.2) even if they had distinct physical and chemical characteristics and chl *a* concentrations (Appendix IA). From the dot plots of phycoerythrin fluorescence as a function of chl *a* fluorescence, Big Rideau Lake had one population of PC; in Upper Rideau, that same population was present but a second population was apparent below the first population, *i.e.* a population containing less or no phycoerythrin and with fewer cells (Fig. 2.2). In Indian Lake, the latter population, probably of phycocyanin-rich PC, was more important until it becomes the dominant type in Opinicon Lake (Table 2.1). Similarly, Simon and Barrière lakes are connected by a channel 15 metres wide and have similar chemical characteristics, light attenuation coefficients and chl *a* concentrations (Appendix IA). Their flow cytometric signals were very similar (Fig. 2.3 *A*). This was also the case in Lake Memphrémagog where flow cytometric signals from Magog Bay and Sargent Bay were almost identical as were their physical and chemical variables (Fig. 2.3 *B*).

Of the 50 lakes sampled in summer 2000, the most diverse flow cytometric signal came from Onondaga Lake, New York State. The dot plot of phycoerythrin fluorescence versus chl *a* fluorescence revealed 6 distinct clusters of events (Fig. 2.4). Observation of Onondaga Lake water sample by epifluorescence microscopy lead to the identification of the following phytoplankton groups: phycoerythrin-rich PC; phycocyanin-rich PC; large cryptomonads containing phycocyanin and phycoerythrin (25-30 μm); small cryptomonads containing phycocyanin and phycoerythrin (5-7.5 μm); small eukaryotes containing only chl *a* (~ 5 μm); filamentous cyanobacteria with phycoerythrin as well as filamentous eukaryotes with only chl *a*. The various clusters could be reasonably assigned to these populations: Region 2 (R2: green events) correspond to phycoerythrin-rich PC and region R3 (dark blue) to phycocyanin-rich PC. R4 (pink) and R5 (light blue) have low values of phycoerythrin fluorescence (FL2) which would mean they should be phycoerythrin-free phytoplankton, probably eukaryotes. R6 (yellow) and R7 (dark red) show high phycoerythrin fluorescence and a much greater size than the other regions (Fig. 2.4 *D*); they could certainly be the phycoerythrin containing cryptomonads. Regions 4 and 5, even though they have a difference in chl *a* fluorescence, could be a cluster of a same phytoplanktonic group, such as the small eukaryotes with only chl *a*. Although epifluorescence microscopy revealed a size of approximately 5μm for the eukaryotes, their FSC data indicated a size similar to both types of PC.

Flow cytometry versus epifluorescence microscopy counts

The time required to identify and count PC was only slightly shorter via flow cytometry than epifluorescence microscopy. But when the lakes patterns were similar,

analysis by flow cytometry was much easier, especially for replicates. In general, counts of PC by epifluorescence microscopy were higher than the concentrations obtained from flow cytometry. Big Rideau and Indian Lakes had flow cytometry cell counts about half the cell counts from epifluorescence while Opinicon Lake had a fairly similar count for both techniques and Upper Rideau Lake had a higher count by flow cytometry (Table 2.1). The two techniques were compared for four other lakes; the lakes were chosen on the basis of a PC abundance higher than $10^4 \cdot ml^{-1}$ by epifluorescence (Fig. 2.5, Appendix IIB). Results of triplicate counts from antibiotic experiments showed a large difference between the techniques. For lakes Anne, MacKay and Vert, counts were lower with flow cytometry. Lake d'Argent inversely had higher flow cytometry counts although control samples at time 24 hours gave a fairly equal count by the two techniques. The difference in counts was up to four times higher with epifluorescence microscopy. Standard deviation of the mean counts was lower with flow cytometry for almost all samples.

Signal variations under different light intensities

Flow cytometric signals of PC cultures varied with the light regime. Cultures of *S. leopoliensis* grown at various intensities for 6 days and then preserved with 1% glutaraldehyde showed an increase in chl *a* fluorescence as the light intensity decreased (Fig. 2.6). Cells appeared on average smaller under high light relative to the low light regime. Likely, this would be the result of the rapid growth rate. In low light, especially in low green light, a group of events with low chl *a* fluorescence and a smaller size than other cells appeared.

Filtration and preservation of freshwater PC for flow cytometry

The different preservation techniques gave different flow cytometric signatures. *S. leopoliensis* cultures grown at high 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 6 days were analysed by flow cytometry either fresh, preserved with 1 % glutaraldehyde or preserved with 1 % paraformaldehyde (Fig. 2.7). Chlorophyll *a* fluorescence was similar for cultures treated with glutaraldehyde and paraformaldehyde, and lower for non-preserved sample. The non-preserved culture had slightly smaller FCS values than the preserved cultures.

Non-preserved Bob's Lake water (West Bay) gave flow cytometric signals distinct from water preserved with glutaraldehyde or paraformaldehyde (Fig. 2.8). The water sample preserved with glutaraldehyde had the highest values for relative cell size (FSC) and an extra cluster of cells (Fig. 2.8 *A1*, *A4*) in comparison to paraformaldehyde-preserved and non-preserved samples (Fig. 2.8 *B1*, *B4*, *C1*, *C4*). The latter two had more similar flow cytometric signals except for the phycoerythrin fluorescence which was weaker for the phycoerythrin-rich population in the non-preserved sample (Fig. 2.8 *C1*).

Lake water samples were filtered through 2 μm and 0.2 μm filters before preservation. All dot plots of filtrates < 2 μm had events of FSC values greater than the filter pore size. For example, glutaraldehyde-preserved whole water sample (Fig. 2.8 *A1*) had FSC relative values up to 10^4; in filtrate < 2μm (Fig. 2.8 *A2*), a decline in the number of events with high FSC values is observed near FCS value of 10^2. So all events with FSC values higher than 10^2 were considered as background noise. In the filtrate < 0.2 μm (Fig. 2.8 *A3*), the threshold value in FSC was more awkward to determine and FSC values were as high as in the filtrate < 2 μm (Fig. 2.8 *A2*). Regardless, all events detected by the flow

cytometer for that sample should be noise. Paraformaldehyde-preserved and non-preserved had also background noise but it seemed less important than with glutaraldehyde-preserved samples. However, filtration helped in the illustration of the difference in fluorescence intensity of the preservation reagents in comparison to fresh samples.

The filtrates < 0.2 μm should not contain any cells but viruses can pass through that pore size filter. Background noise was still present. The noise was more dispersed in the dot plots for glutaraldehyde-preserved samples where FSC relative values varied widely in comparison to paraformaldehyde-preserved and non-preserved samples (Fig. 2.8 *A3*, *B3*, *C3*). Figures of whole water and filtrate < 2 μm contain 5 000 events each while filtrate < 0.2μm contains 1 000 events. The time required to acquire 1 000 events was about 3 minutes and was less than a minute to acquire 5 000 events for whole water and filtrate < 2 μm.

FACScan versus FACS Vantage

The flow cytometric signatures of West Bay (whole water) varied between FACScan and FACS Vantage (Fig. 2.9). Dot plots of FL2 vs FL3 for both instruments showed the same long diagonal cluster of events but with differences at the lowest FL3 values. The R1 cluster with the highest FL2 values accounted for 21%(Fig. 2.8 *A3*) of the total 5 000 events by FACScan and 50% by FACS Vantage. The R2 cluster below with high FSC values accounted for 48% of the total 5 000 events via the FACScan and 29% via the FACS Vantage. The R3 cluster with low FL2 and low FSC values was considered

background noise and accounted for 9% on the FACScan and 4% on the FACS Vantage of the total events. Non-gated events are either background noise or phytoplanktonic cells with various fluorescence characteristics but abundant enough to generate a well defined cluster. Clustering of events as phycoerythrin-rich PC and phycocyanin-rich PC from dot plot FL2 vs FL3 was difficult to do with both flow cytometers as the clusters were not clearly defined.

Dot plots of FL2 vs FL4 for West Bay and Green Bay in Figure 2.10 show three main clusters of events with different fluorescence values. The first cluster has the highest phycoerythrin fluorescence, the second has a low phycoerythrin fluorescence but a high phycocyanin fluorescence while the last cluster has the lowest fluorescence values for both pigments. From the fluorescence differences, the first cluster would be phycoerythrin-rich PC, the second as phycocyanin-rich PC and the third would be background noise from the lake water. Confirmation of the PC clusters identification was done by comparing West Bay from Green Bay knowing from Figure 2.1 that Green Bay is dominanted by phycoerythrin-rich PC and that the phycocyanin-rich PC were much less abundant.

Background noise was more evident in West Bay than Green Bay of Bob's Lake. Dot plots of FL2 vs FSC (Fig. 2.10) show the difference in the amount of events clustered at low FL2 and low FSC values. This background noise accounted for 9.5% in West Bay and 4% in Green Bay of the total 5 000 events.

DISCUSSION

Flow cytometric signals among lakes

Most dot plots presented in the literature show phycoerythrin fluorescence (FL2) o on the y-axis versus either side scatter (SSC) or chl a fluorescence (FL3) on the x-axis (e.g. Corzo et al. 1999; Crosbie and Furnas 2001). Discrimination of phytoplanktonic populations seems to be the best with this display. However, in the present study, the side scatter parameter (SSC) was not very informative. The best dot plot that helped discriminate the PC types from all other phytoplankton was with that of phycoerythrin as a function of chl *a* fluorescence (FL2 vs FL3). This dot plot enabled the identification of phytoplanktonic groups from Onondaga Lake, although the identification of flow cytometric signals as specific groups was difficult.

The definition of a cluster of events in a gate was somewhat subjective. The limits of the clusters are hard to define when two or more clusters are close to each other, when clusters are widely dispersed, and when the level of background noise is high. As a result, gates inevitably miss or incorporate events considered in or out depending on the observer. Also, a comparison of events between lakes can not be done without moving, reducing or increasing the gates that limit the clusters. These changes are necessary because light intensity and nutrient conditions vary for each lake. Both light and nitrogen are known to affect pigment concentrations composition and hence fluorescence signals.

In general, the flow cytometric signatures of *S. leopoliensis* (e.g. Fig. 2.7), a phycocyanin-rich PC, were similar to those of the phycocyanin-rich PC from lakes (e.g.

Fig. 2.1). The signatures are analogous to those obtained by Collier (2000) who found a phycocyanin-rich PC population in the Hudson River, New York (Appendix IID). Since these phycocyanin-rich PC do not contain phycoerythrin, it would be expected that they would have FL2 values close to zero.

The dominance of phycocyanin-rich PC is typical of lakes having a high light attenuation coefficient (LAC) (Pick 1991; Vörös et al. 1998). This was demonstrated in Opinicon Lake which had a dominant population of phycocyanin-rich PC and a LAC of 0.75 while Indian Lake connected to Opinicon by locks had a LAC of 0.57 and its dominant population of PC was phycoerythrin-rich. Similarly, West Bay had a higher light attenuation coefficient (0.50) than Green Bay (0.35) and the percentage of phycocyanin-rich PC also dominated the PC assemblage as found by Pick (1991).

Flow cytometry and epifluorescence microscopy counts

Few studies have explicitly compared flow cytometry counts of PC with epifluorescence microscopy based counts. Hofstraat et al. (1994) found a good correlation between light microscopy and flow cytometry from marine samples for large phytoplanktonic groups but less effort was focused on PC. del Giorgio et al. (1996), stained freshwater heterotrophic bacteria with the fluorochrome dye SYTO 13 that fluoresces only in FL1 and obtained a good correlation between the two techniques and determined that the relationship was better when bacterial abundances ranged from 10^5 to 10^6 per ml.

In this study, counts obtained by flow cytometry were different from those obtained by epifluorescence microscopy although both were of the same order of magnitude. These differences raised the question of which technique gave the true abundance value. With epifluorescence, counts can be underestimated when PC are too abundant: the risk being that not all PC in each field observed will be counted, especially when colonial forms are present. Another problem with epifluorescence is that when populations have a very weak fluorescence, chances are that some cells will not be visible to the naked eye. This is the case for marine *Prochlorococcus* that has such weak chl *a* fluorescence that it is invisible under epifluorescence microscopy (Chisholm et al. 1988). Flow cytometry also has limitations. If the background noise is high, chances are that gating of events into clusters will be difficult and a part of the noise might fall into the gate. Also, the determination of abundance of phytoplanktonic populations can contain a source of error because the addition of a precise concentration of FLB to a water sample is difficult to control. Lastly, if a phytoplankton abundance is low, about less than $10^{-3} \cdot ml^{-1}$, the accuracy of the flow cytometer to detect this small population would be reduced as the contours of the cluster would be impossible to define.

Even though the counts with the two techniques gave different abundances of PC, the standard deviations of the mean were very small with flow cytometry compared to epifluorescence microscopy. This property of flow cytometry to be more reproducible can be an important factor depending on the study objectives.

In the case of the present study, slightly more time was afforded to epifluorescence microscopy counts than flow cytometry. As a neophyte, time spent on flow cytometry

could be considered high compared to a more experienced researcher. But as the level of knowledge and experience with flow cytometry increases, the precision of the internal standard FLB will improve and the time spent on analyses should get lower.

Signal variations from preservation methods

Differences in the flow cytometric signals between preserved and non-preserved water samples could lead to misinterpretations of phytoplankton populations because depending on the preservation method used, the number of clusters and their fluorescence characteristics would vary greatly. del Giorgio et al. (1996) found an effect of preservation by glutaraldehyde and formaldehyde on heterotrophic bacteria where FSC, SSC and fluorescence decreased, suggesting a shrinkage of the bacteria. This property of chemical reagents to modify cells structure and refraction indices is known (e.g. Allman et al. 1990) but the impacts on the PC signatures can lead to misinterpretations. For example, in Figure 2.8 *A*, FL2 vs FL3 dot plots demonstrated clusters of events in preserved samples, especially glutaraldehyde, that were not distinguishable in the non-preserved or paraformaldehyde-preserved samples. And contrarily to del Giorgio et al. (1996), the present study indicated that glutaraldehyde-preserved water samples appeared to have higher FSC values compared to non-preserved sample. This could be the result of an increased fluorescence of cells by the reagent as it stops the photon energy transfer to photosystems and traps the energy inside the cells. As a consequence, energy is evacuated by fluorescence of pigments, the main loss process used. The higher fluorescence might have disturbed the forward light scattered signals.

Background noise

Some of the variation in flow cytometric signals between lakes can be due to differences in background noise from non-living particles that can reflect a certain amount of the light beam. As the clusters of events spread out or are closer to the lowest values in both axes, more noise might be included into these clusters and result in an overestimation of cell abundance. Background noise was noticeable especially in brown coloured waters such as Oneida, Forgeron, Black, Renaud, Ours and Pin rouge (Appendix IIA). Also, in MacKay lake, which has a clear light green coloured water, had high background noise that could be due to the inputs of stormwater in this small urban lake. Also, low concentration of each phytoplankton group in a water sample would lead to poorly defined clusters of events.

Whole lake water samples, 2 μm or 0.2 μm filtrates were compared for background noise on the flow cytometer. Since both preservatives were filtered through 0.2 μm polycarbonate filters before addition to the whole water sample and the filtrates, presence of very weak fluorescence is the result of non-living particles. However, filtration can cause cell breakage of microorganisms lacking a tough protective cell wall; leakage of pigments or organelle fragments, could increase the background noise.

Events detected with a higher FSC value than the filtration pore size could be: cells that have leaked through the filter; non-phytoplanktonic cells or particles that acquired a fluorescence that made them appear larger upon preservation, or simply, cells that were present in the flow cytometer before the analysis of a sample and got mixed with filtered samples. The last possibility is realistic as we experienced this problem when a high

concentration of FLB was added to a first sample. As the following samples were analysed by the cytometer without adding FLB, some FLB were observed in the flow cytometric signature of those samples. To resolve the problem, backwashing of the cytometer was required to get rid of the extra FLB.

FACScan versus FACS Vantage

A flow cytometer equipped to detect phycocyanin (FACS Vantage) was used in comparison with one lacking this feature (FACScan). The use of the FACS Vantage was required in order to verify if the addition of the phycocyanin fluorescence (FL4) information would help in the identification of PC from freshwater samples and to assess the validity of the discovery of a *Prochlorococcus*-like population in freshwater (Corzo et al. 1999).

In oceanography, flow cytometric signatures appear simpler with two major clusters of *Synechococcus* and *Prochlorococcus* and less background noise. Freshwater contains more diverse autotrophic picoplankton, eukaryotes and prokaryotes, including PC with different pigmentation. Collier (2000) proposed that Corzo et al. (1999) might have misinterpreted *Prochlorococcus*-like signature as being phycocyanin-rich PC because they used a FACScan which has only one laser able to detect phycoerythrin (FL2) and chl *a* (FL3) fluorescence but not phycocyanin fluorescence. The misinterpretation of Corzo et al. (1999) could be explained by the fact that in the marine environment, PC are dominated by phycoerythrin-rich types while phycocyanin-rich type are almost absent. In freshwater, one type can dominate or both types can be present at the same time (Pick

1991; Maeda et al. 1992; Vörös et al. 1998). Not being aware of this piece of information, Corzo et al. (1999) would have identified the low FL2 and low FL3 signals as *Prochlorococcus*-like cells (Appendix IIC).

The cluster identified by Corzo et al. (1999) could be either truly *Prochlorococcus*-like cells or perhaps, background noise. The latter could be the case as the fluorescence was located very close to the threshold value in FL3 (Appendix IIC). Briefly, the debate regarding the conclusions of Corzo et al. (1999) is still not resolved but I agree with Collier (2000) who considers the *Prochlorococcus*-like population of Corzo et al. (1999) to be background noise. The use of a flow cytometry with phycocyanin fluorescence detector (FL4) is necessary to confirm the presence of *Prochlorococcus* in freshwater. New flow cytometers are currently being developed (e.g. OPA, EurOPA) with more parameters in order to obtain more data about each analysed particle and allow for better identification of phytoplankton groups (Collier 2000).

Flow cytometric signatures of FACScan and FACS Vantage were quite different. Clustering of PC types was difficult from FL2 vs FL3 dot plots while clustering from FL2 vs FSC gave great differences in the number of events in each cluster as well as the fluorescence values. The FACS Vantage had the advantage of clearly separating the PC clusters (R1 and R2) from the background noise cluster in FL2 vs FSC dot plots (Fig. 2.9).

An advantage in the analysis of West Bay and Green Bay by a FACS Vantage (Fig. 2.10) was that the dot plots of FL2 vs FL4 gave the possibility to clearly separate the two main pigment groups of PC.

Epifluorescence microscopy seems to be a more accuate technique but flow cytometry is more precise. Accuracy related to how well the technique approximates the true value; precision is the variability from repeating the samples. Epifluorescence microscopy will provide unambiguous information on the identification of an algal based on its form and pigment composition; results are clear: this particle is a phycoerythrin-rich picocyanobacterium or not. Flow cytometry will provide quantitative data on the characteristics of populations: size, shape, pigment fluorescence. A water sample can be analysed repeatedly and have very low standard deviation and coefficient of variation for each parameter. Flow cytometry is precise on the algal characteristics but this precision was not as relevant for this study.

Much work remains to be done to perfect the use of flow cytometry in freshwater to study phytoplankton, especially picoplanktonic organisms. Identification and elimination of the source of background noise would help to increase the accuracy of the flow cytometric signatures.

Flow cytometry can provide a first rapid analysis of a phytoplanktonic assemblage, but the use of epifluorescence in parallel remains necessary. Identification of phytoplankton from flow cytometry charts is still precarious, especially when preservation reagents produce variable signatures within a same water sample. Future research should focus on the possibilities to identify and eliminate background noise from flow cytometric signatures of freshwater samples.

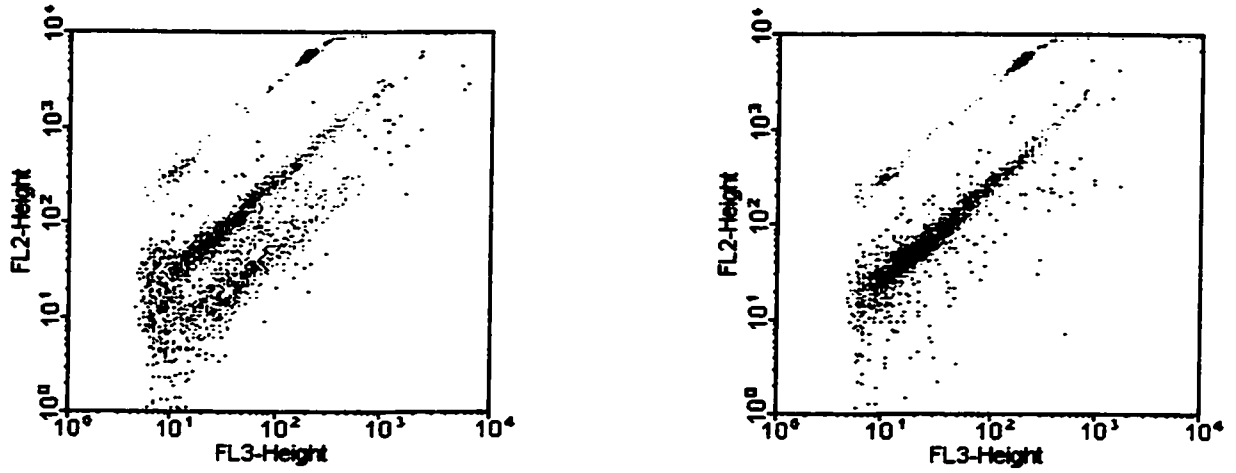
Table 2.1 Picocyanobacteria (PC) abundance determined by flow cytometry and epifluorescence microscopy. With flow cytometry, counts have been made separately for phycoerythrin-rich and phycocyanin-rich PC. Counting of PC with epifluorescence microscopy was done with green excitation (510-560 nm) which does not allow the distinction of the two types. One sample for each lake was analysed. **LAC**: light attenuation coefficient.

	Flow cytometry			Epifluorescence	
Lake	Phycoerythrin-rich ($10^4 \cdot ml^{-1}$)	Phycocyanin-rich ($10^4 \cdot ml^{-1}$)	PC total ($10^4 \cdot ml^{-1}$)	PC total ($10^4 \cdot ml^{-1}$)	LAC
Big Rideau	8.41	0.24	8.66	14.08	0.41
Upper Rideau	7.32	1.37	8.69	7.77	0.71
Indian	5.85	2.11	7.95	16.22	0.57
Opinicon	3.34	5.69	9.04	10.77	0.75

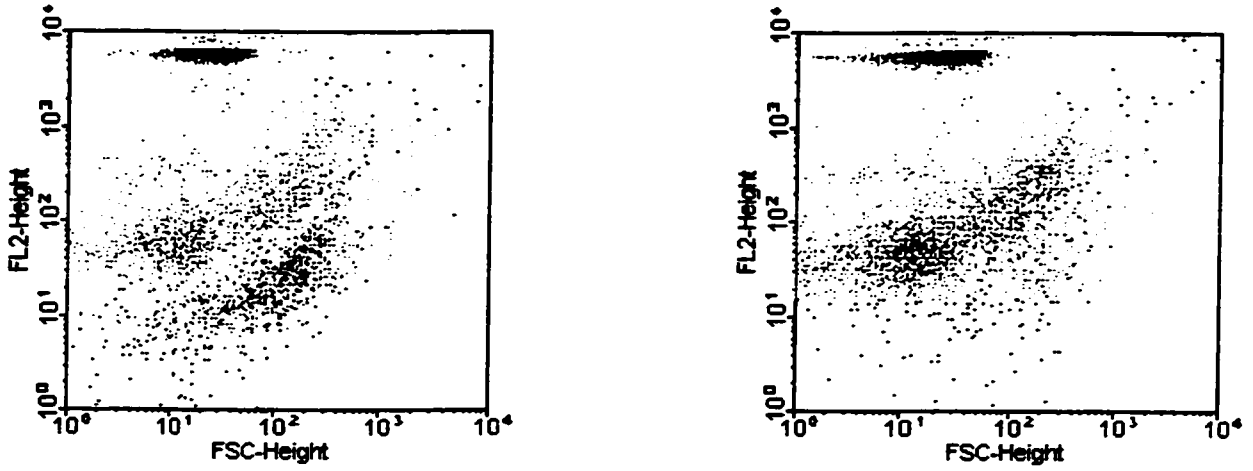
Figure 2.1 Flow cytometric signatures of West Bay (left column) and Green Bay (right column) from Bob's lake, Ont. (July 12th 2000). **A)** Green dots clustered from the FL2 vs FL3 dot plot are phycoerythrin-rich PC while the blue dots, only significant in West Bay, are phycocyanin-rich PC. Red dots are the FLB. **B)** Dot plots of FL2 (phycoerythrin fluorescence) vs FSC (cell relative size) and **C)** FL3 (chl *a* fluorescence) vs FSC show that both type of PC have a similar size range. A threshold value was set on the chl *a* fluorescence.

West Bay **Green Bay**

A



B



C

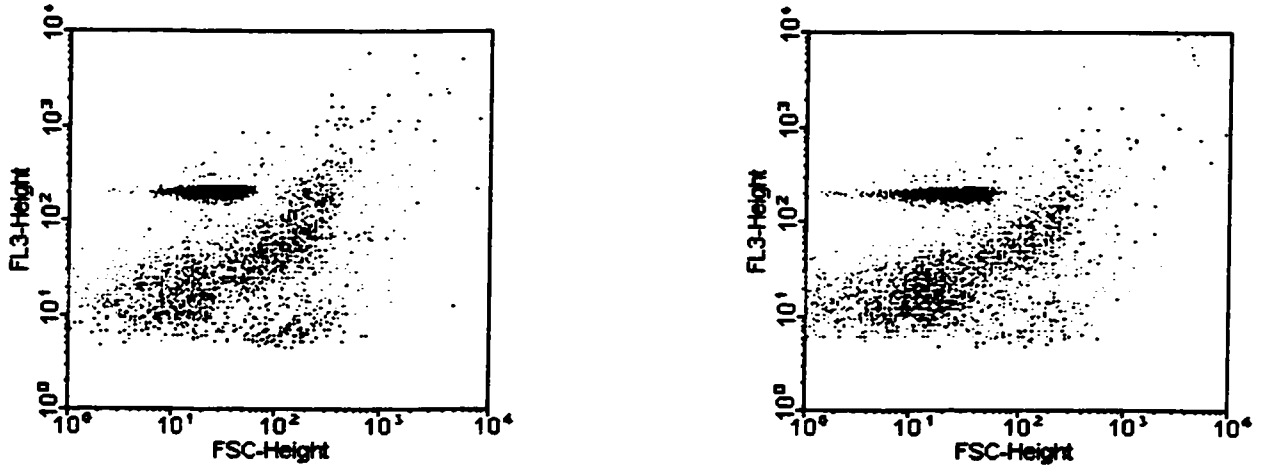
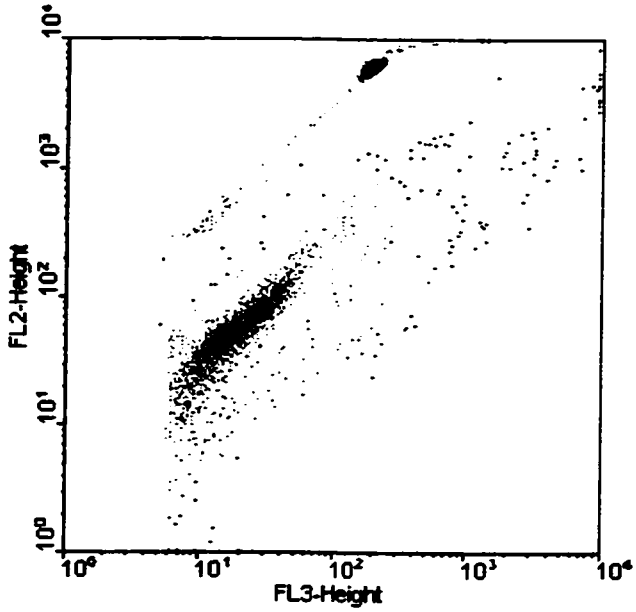
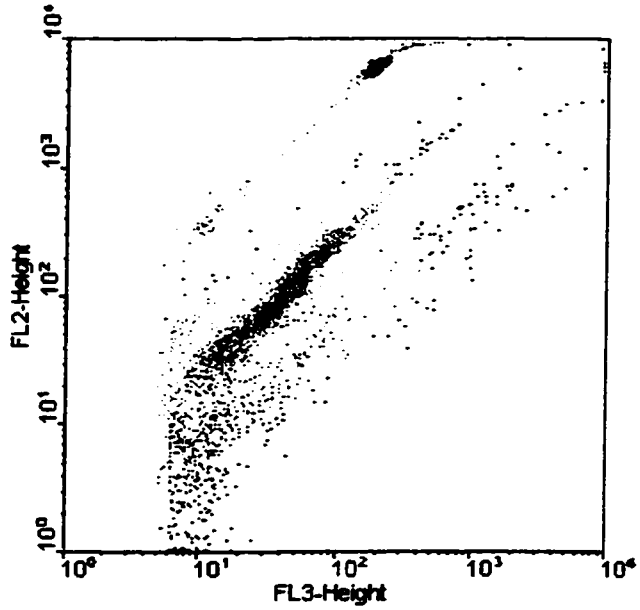


Figure 2.2 Flow cytometric signature of four lakes of the Rideau Canal waterway. Signatures are similar except for the formation of a cluster of dots from Upper Rideau lake through Opinicon lake which was identified as phycocyanin-rich picocyanobacteria (blue events) while the cluster above it would be phycoerythrin-rich PC (green events). Dots in red are the fluorescently labelled beads (FLB) added to the water samples. FL2 is phycoerythrin fluorescence and FL3 is chlorophyll a fluorescence.

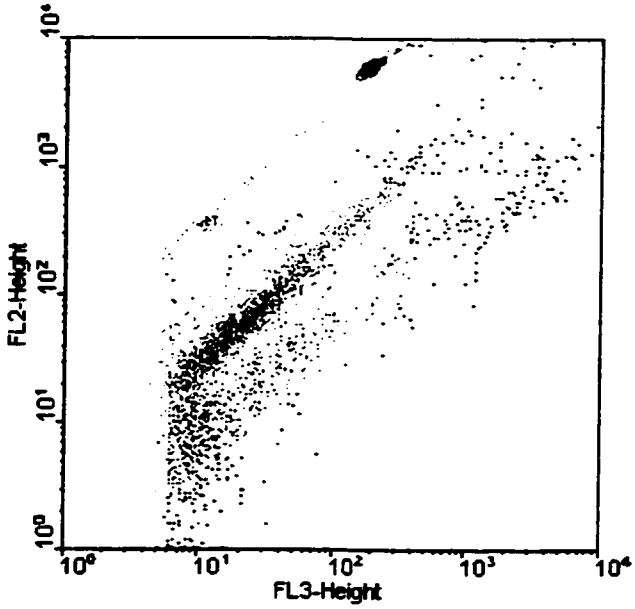
Big Rideau



Upper Rideau



Indian



Opinicon

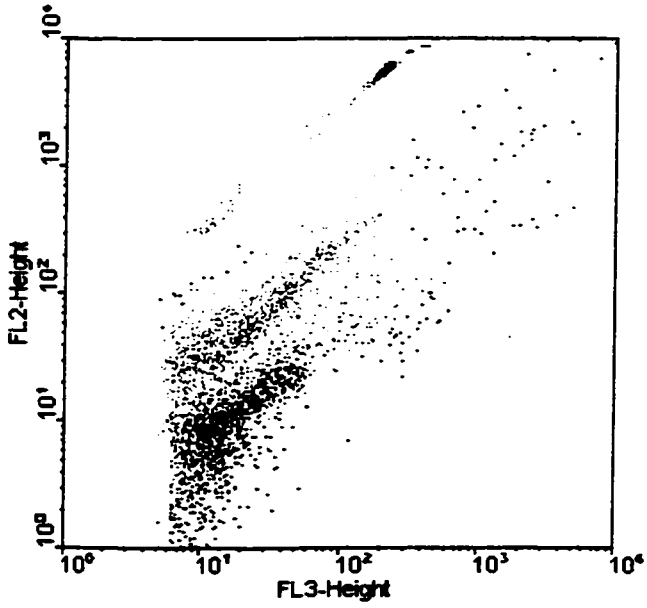
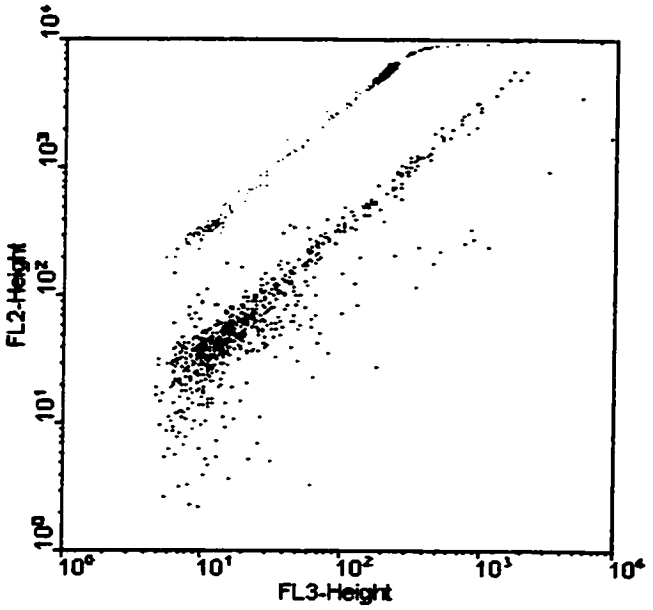


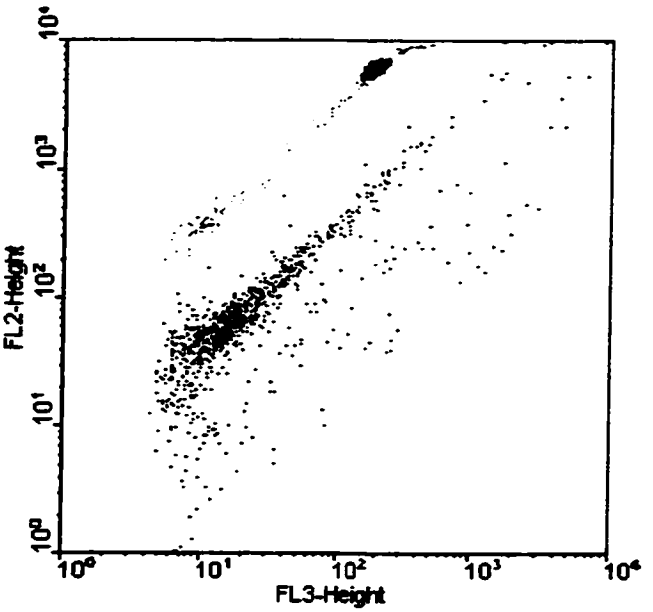
Figure 2.3 Flow cytometric signatures of water systems having similar physical and chemical variables: A) lakes Simon and Barrière, two lakes connected; B) two bays from lake Memphrémagog. Red dots are the FLB. FL2 is phycoerythrin fluorescence and FL3 is chl *a* fluorescence.

A)

Lake Simon

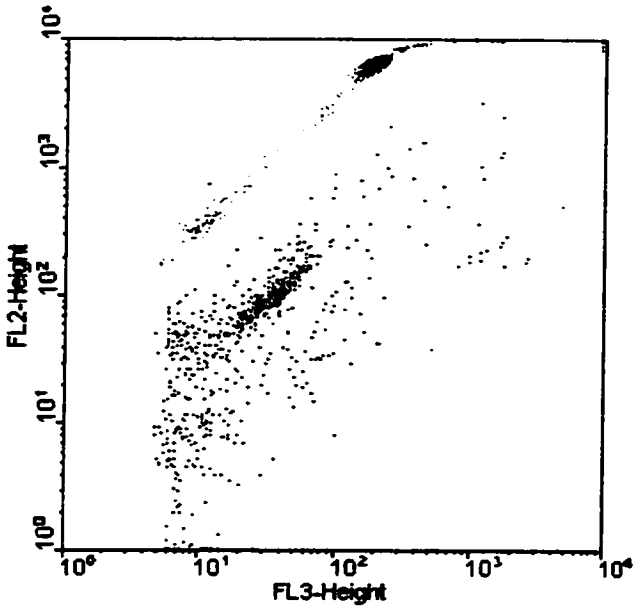


Lake Barrière



B) Lake Memphrémagog

Magog Bay



Sargent Bay

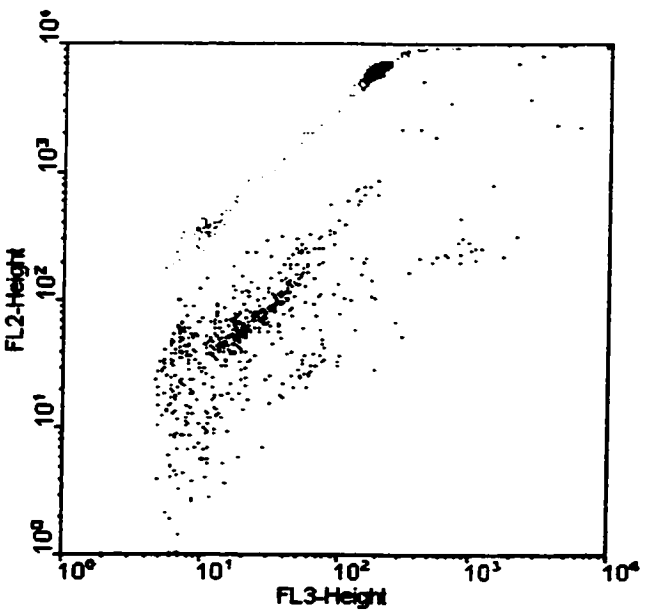
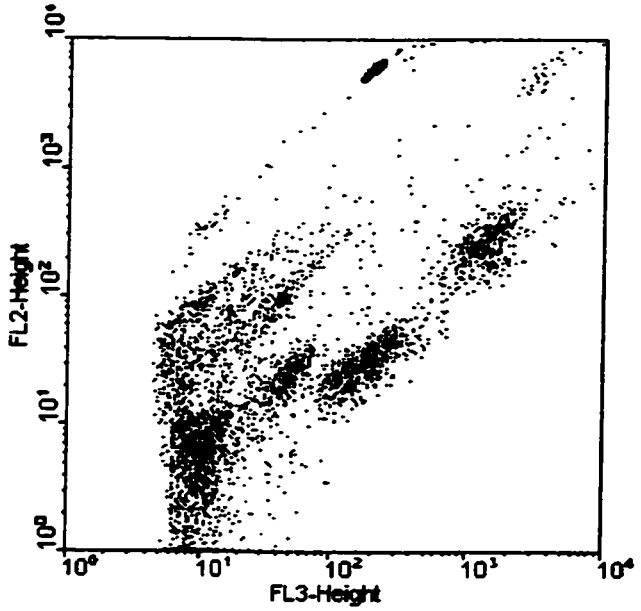
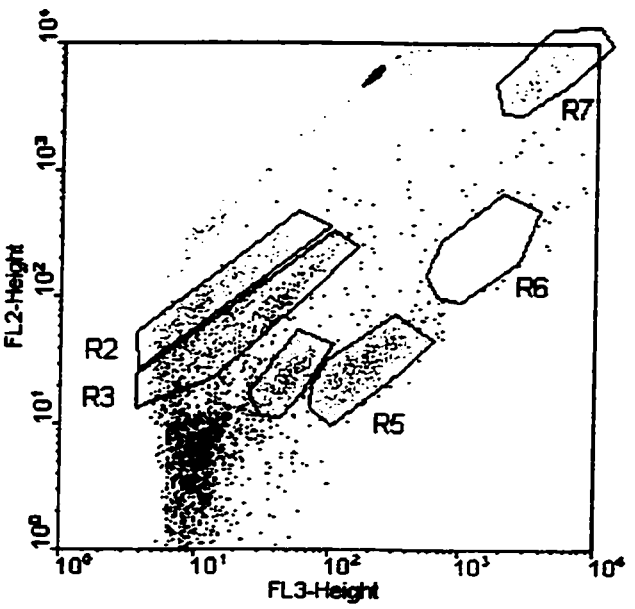


Figure 2.4 Flow cytometric signature of Lake Onondaga, New York State. **A)** initial signature from FL2 (phycoerythrin fluorescence) as a function of FL3 (chl *a* fluorescence); **B)** gates are created around clusters of dots (R2 to R7), FLB are in red (limits of the gate not showed); **C)** same as in *B)* although limits of the gates are invisible; **D)** FL2 vs FSC (cell relative size) dot plot showing size range of clustered cells.

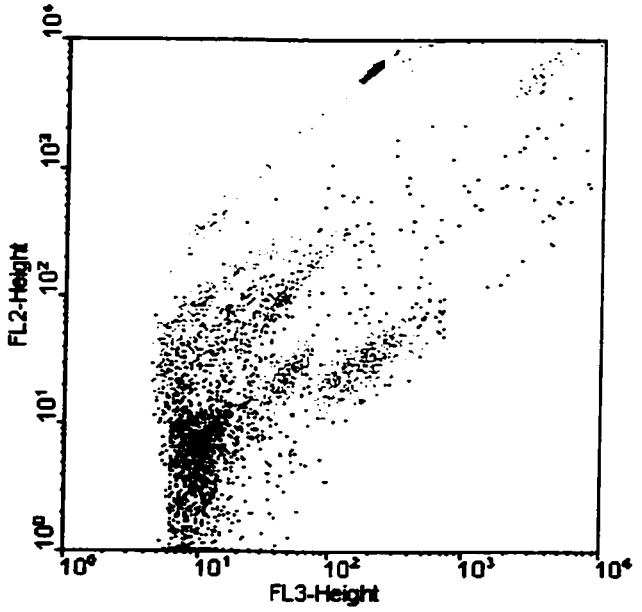
A


FL2-Height
FL3-Height
10^0
10^1
10^2
10^3
10^4

B


R7
R6
R2
R3
R5
FL2-Height
FL3-Height
10^0
10^1
10^2
10^3
10^4

C


FL2-Height
FL3-Height
10^0
10^1
10^2
10^3
10^4

D

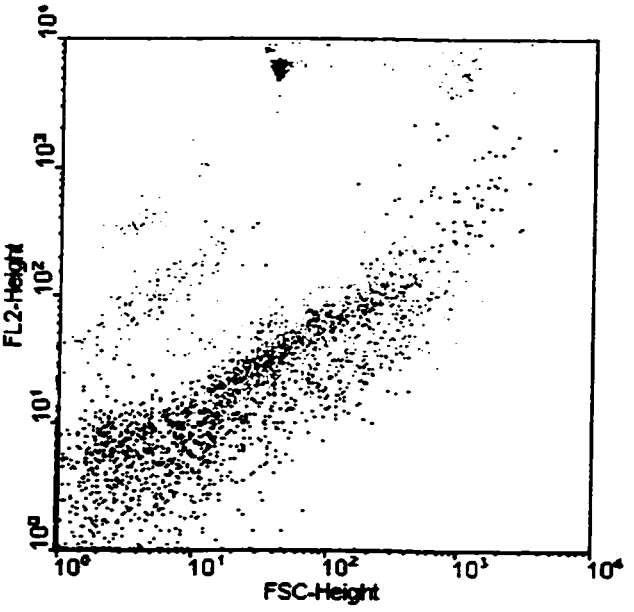

FL2-Height
FSC-Height
10^0
10^1
10^2
10^3
10^4

Figure 2.5 Comparison of PC counts from four lakes using flow cytometry and epifluorescence microscopy. For each lake, the four separated water samples represent treatments, with triplicates for each treatment. Error bars are the standard deviation of the mean. Dashed line is the 1:1 reference line. r_s value of the relationship for all points is 0.62.

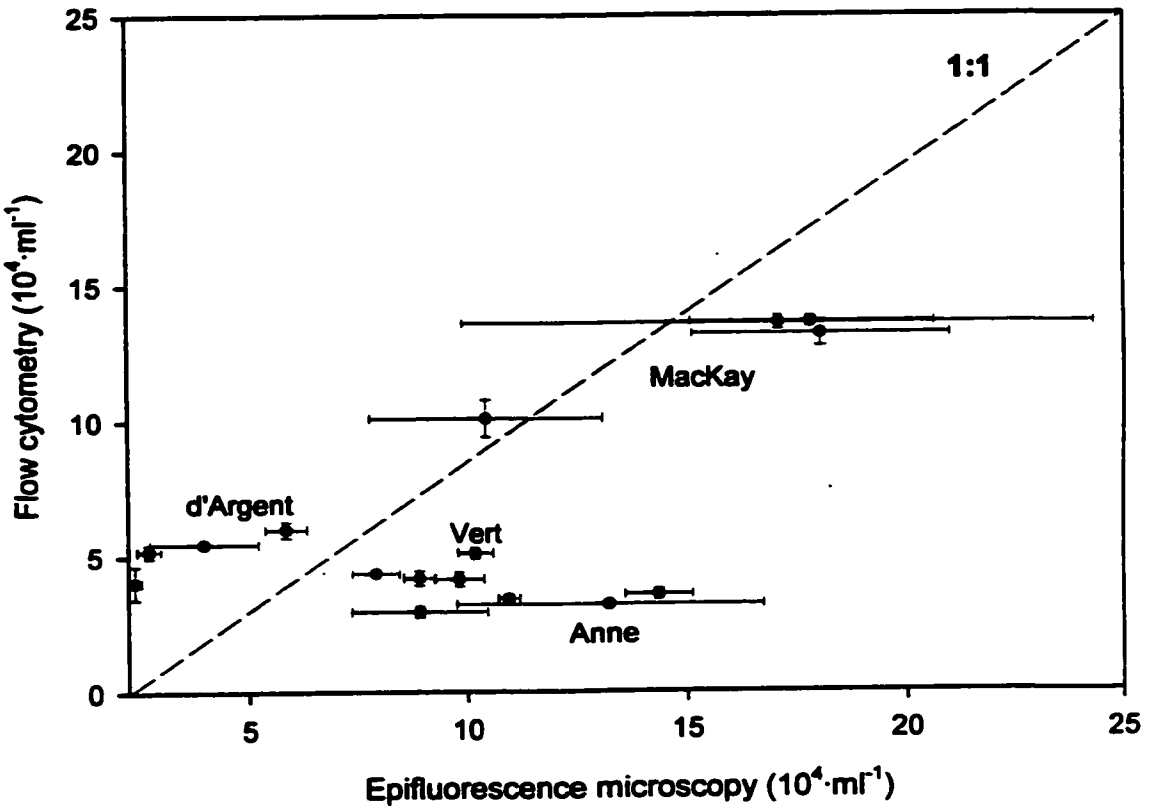
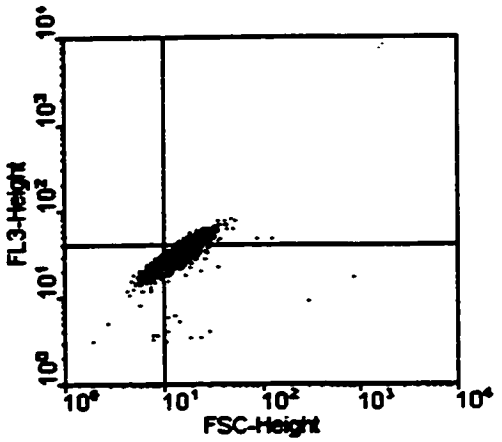
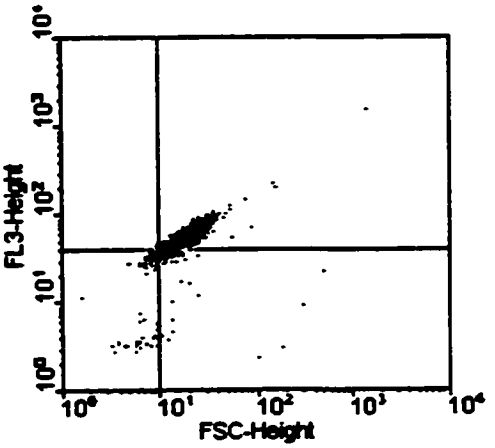

25
20
15
10
5
0
5
10
15
20
25
1:1
MacKay
d'Argent
Vert
Anne
Flow cytometry (10^4·ml^{-1})
Epifluorescence microscopy (10^4·ml^{-1})

Figure 2.6 Flow cytometric signature of *Synechococcus leopoliensis* cultures grown at different light intensities for 6 days with a light:dark cycle of 14:10. This species does not contain phycoerythrin. Lines were added to the plots to aid in the comparisons.

A) 100 $\mu E \cdot m^{-2} \cdot s^{-1}$



B) 28 $\mu E \cdot m^{-2} \cdot s^{-1}$



C) 28 $\mu E \cdot m^{-2} \cdot s^{-1}$ (green light)

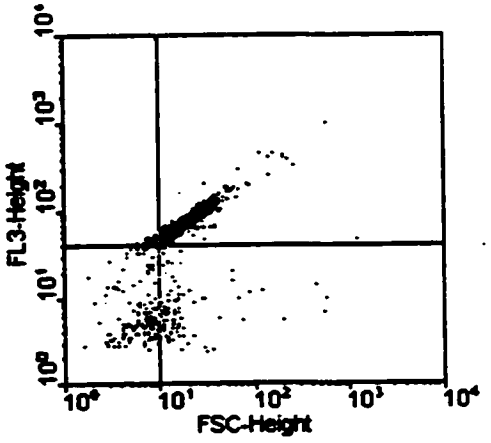
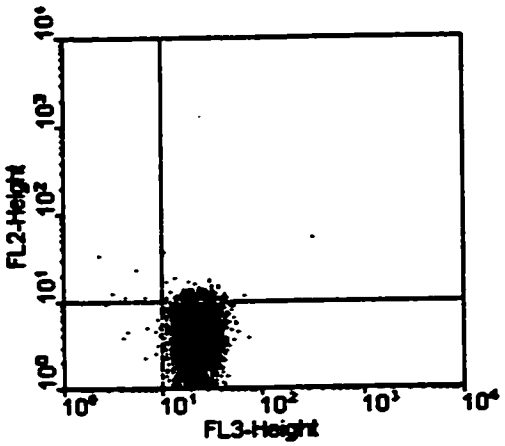
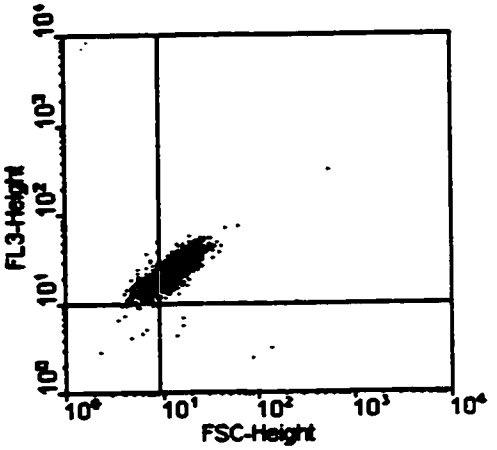


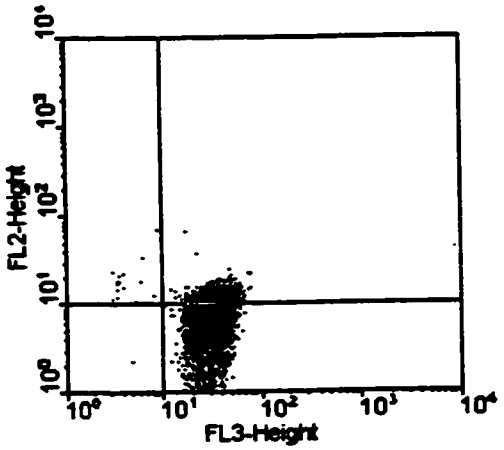
Figure 2.7 Effect of preservation on the flow cytometric signature of *Synechococcus leopoliensis* cultures grown for 6 days at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. Cultures were either fresh (non-preserved) or preserved with 1% glutaraldehyde or 1% paraformaldehyde. Lines were added to the plots to aid in the comparisons. FL2 is the phycoerythrin fluorescence, FL3 is chl *a* fluorescence and FSC is the relative cell size.

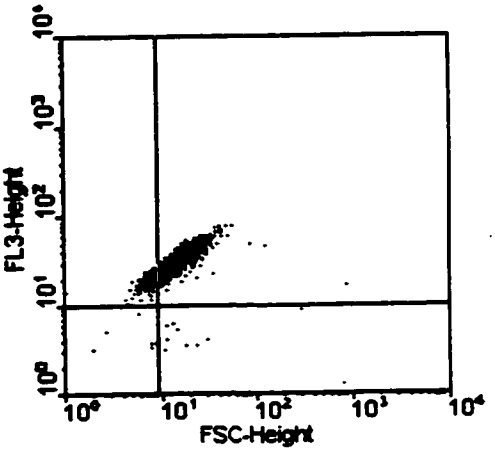
A) Non-preserved



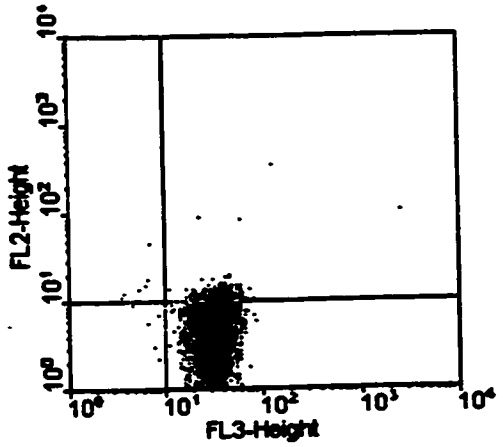


B) Glutaraldehyde 1%





C) Paraformaldehyde 1%



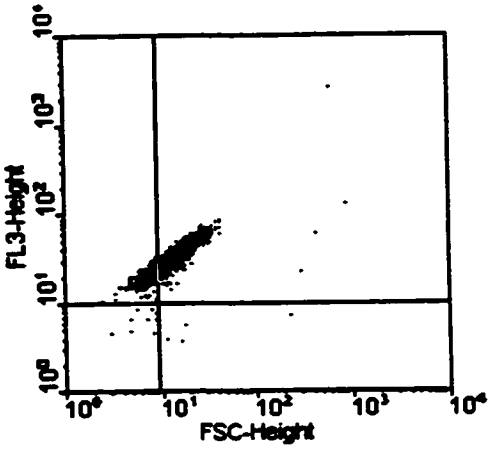
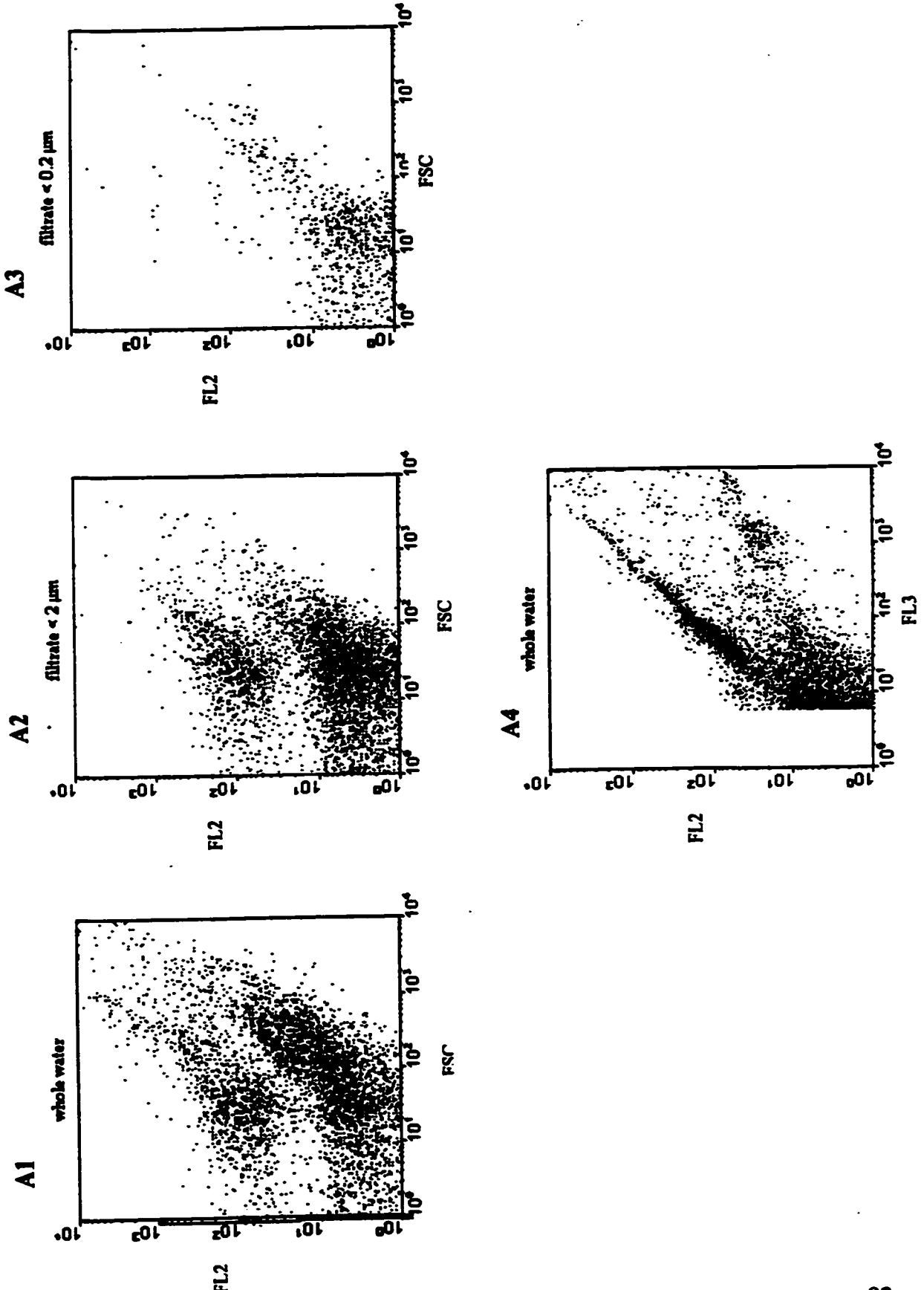
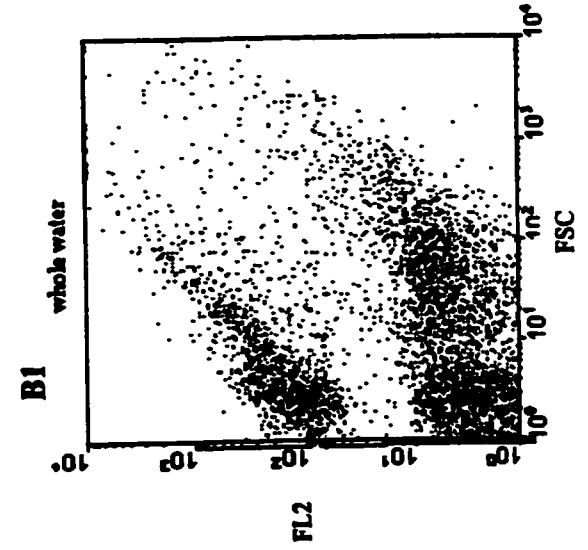


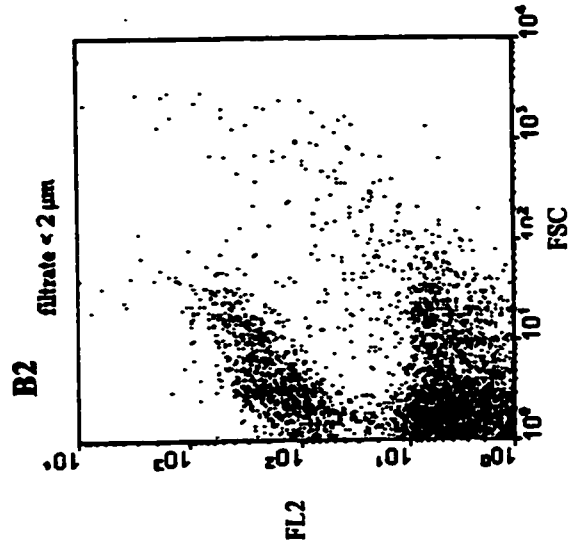
Figure 2.8 Effect of filtration and preservation on the flow cytometric signature of water samples from Bob's lake (West Bay), Ont. (September 3rd 2001). Water samples were either preserved with **A)** 1% glutaraldehyde; **B)** 1% paraformaldehyde or **C)** non-preserved (fresh). Water samples were either unfiltered or filtered through 2 µm or 0.2 µm Nuclepore filters. FL2 is the phycoerythrin fluorescence, FL3 is chl *a* fluorescence and FSC is the cells relative size. Dot plots of filtrates < 0.2 µm contain 1 000 events while whole water and filtrates < 2 µm contain 5 000 events.

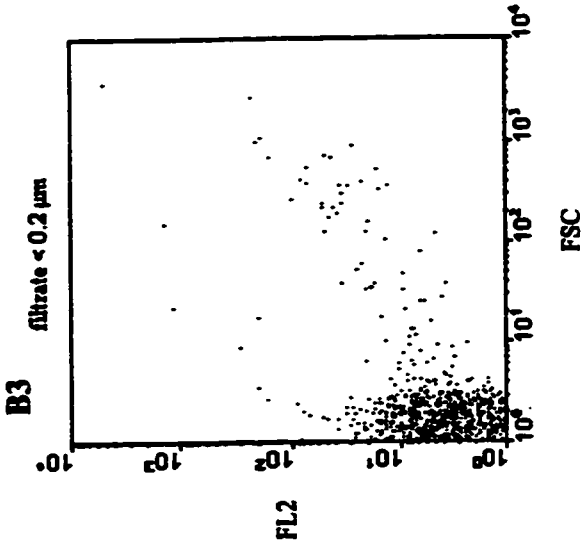
A) Glutaraldehyde

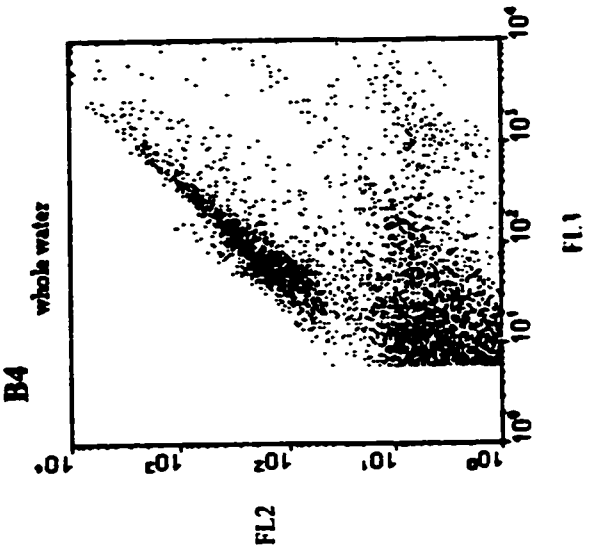


B) Paraformaldehyde

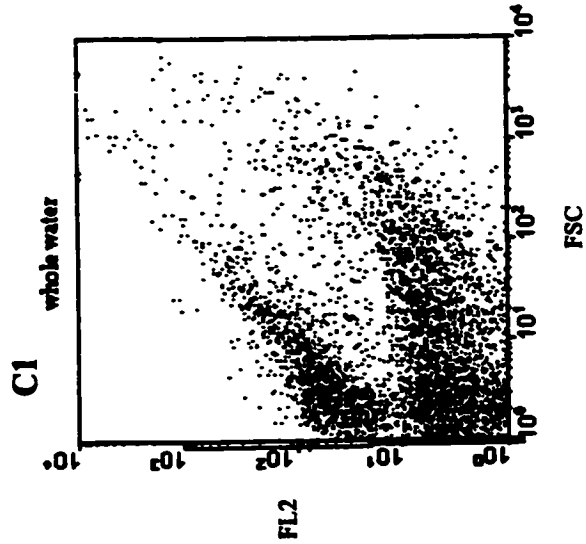


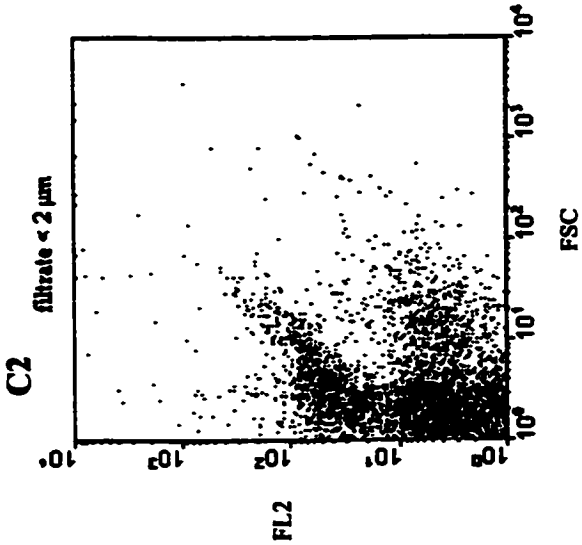


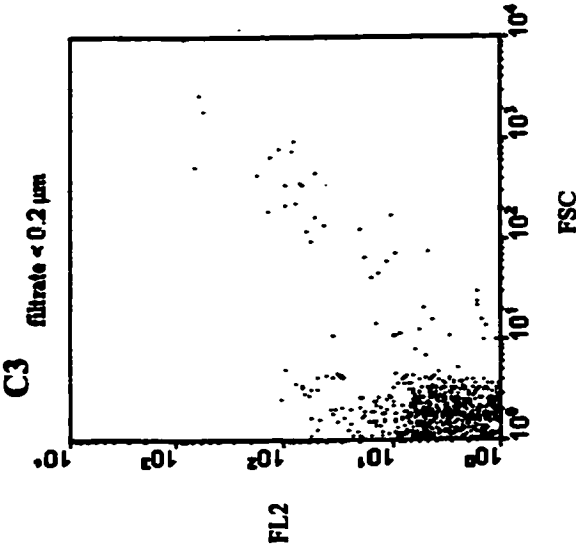




C) Non-preserved







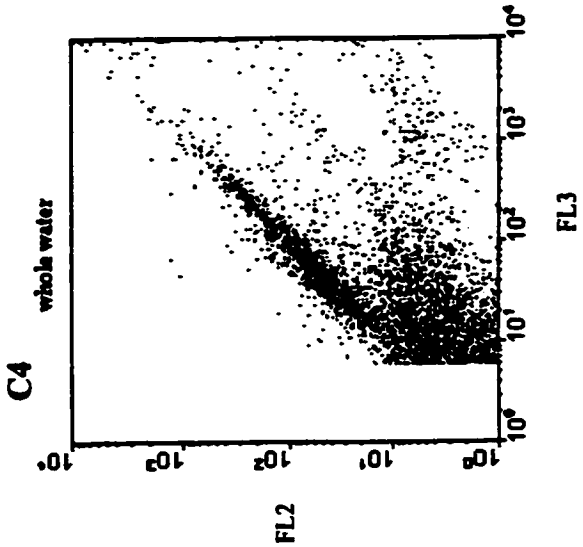
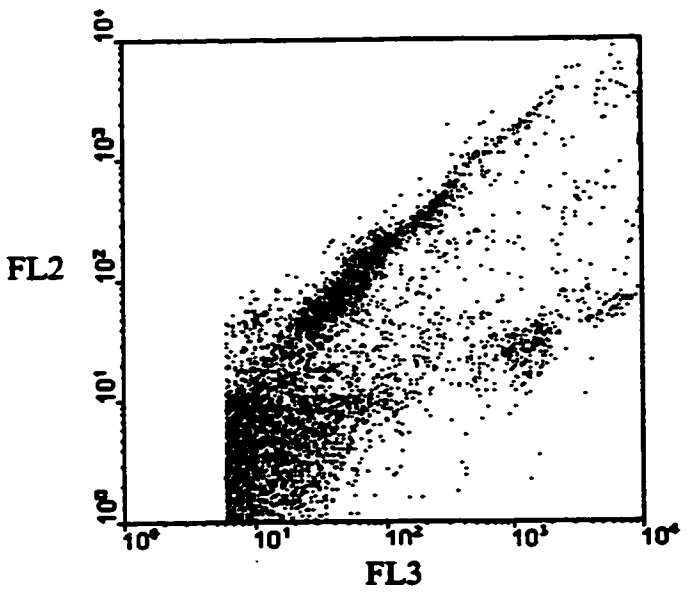
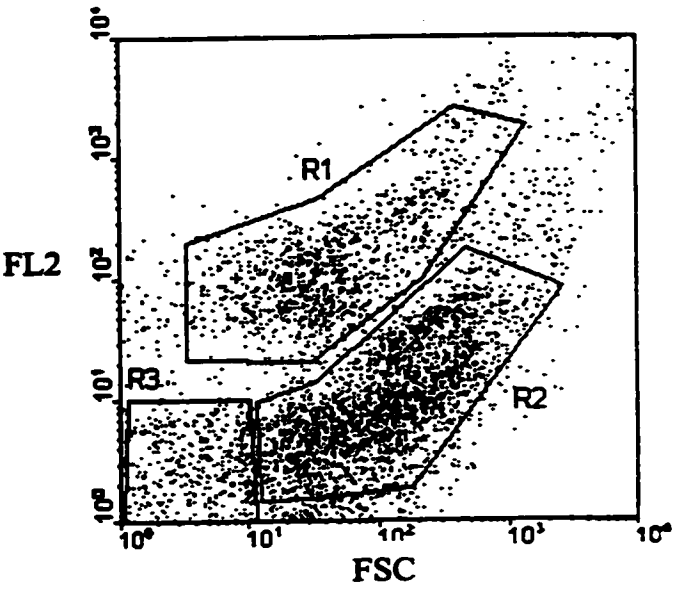


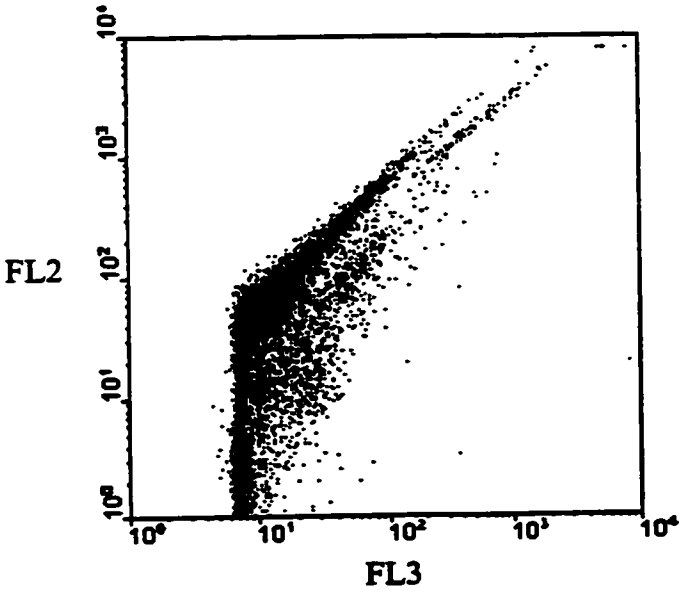
Figure 2.9 Comparison of flow cytometric signatures by **A)** FACScan and **B)** FACS Vantage for lake water sample of Bob's lake (West Bay), Ont. (September 3rd 2001). Dot plots of FL2 vs FL3 and FL2 vs FSC show differences in clusters distribution between the two cytometers.

A) FACScan





B) FACS Vantage



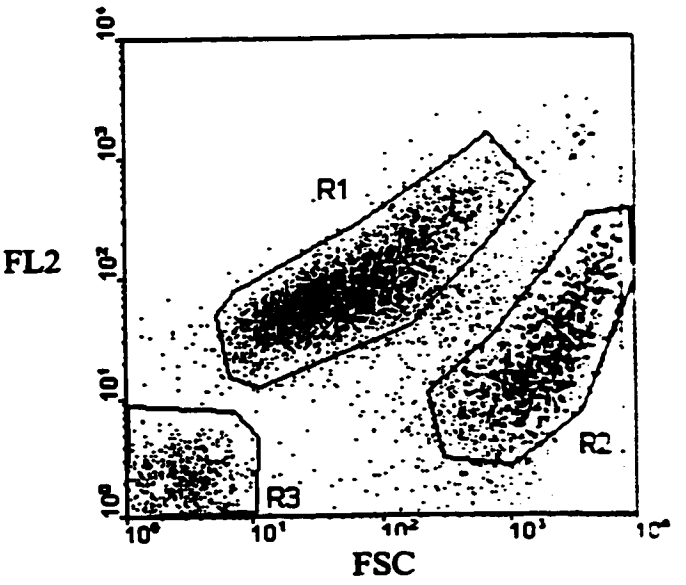
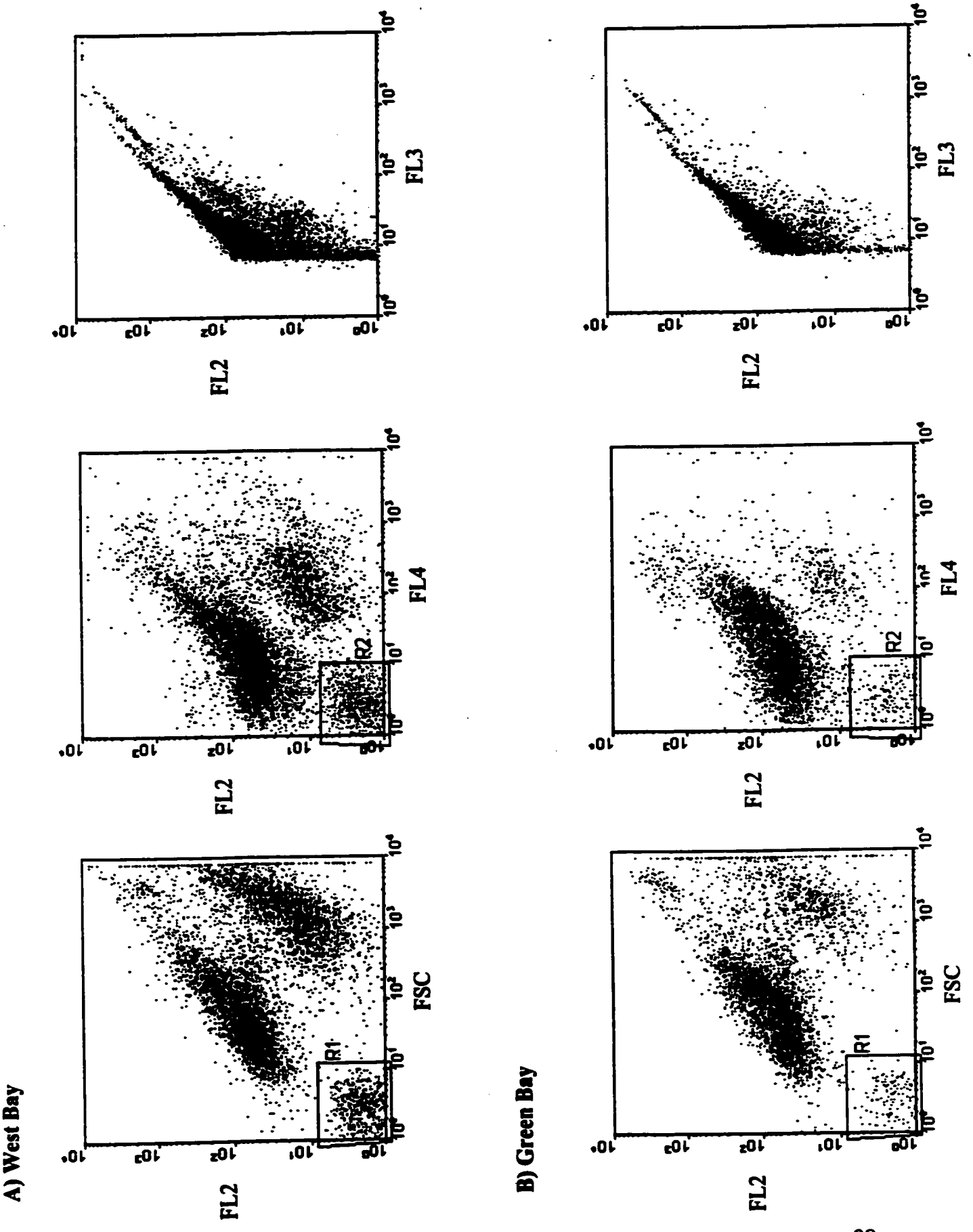


Figure 2.10 Comparison of flow cytometric signatures of West Bay and Green Bay from Bob's lake (September 3rd 2001) with a FACS Vantage able to detect phycocyanin fluorescence (FL4). Dot plots of FL2 vs FSC and FL2 vs FL4 show the two PC populations as well as background noise (low FL2, FL4 and FSC relative values). In the FL2 vs FL3 dot plots, the light-tinted dots are the events clustered from FL2 vs FSC and FL2 vs FL4 plots.


A) West Bay
B) Green Bay
FL2
FL3
FL4
FSC
R1
R2

SUMMARY AND CONCLUSION

From the comparison of a series of oligotrophic to mesotrophic lakes, it seems that the abundance of picocyanobacteria was more closely related to loss rates than physical and chemical variables. Growth rates for PC may be almost always maximal in the surface waters such that the observed abundance is more closely related to loss processes rather than any one physical or chemical variable. Loss rate of PC was the main variable that best explained the variation in intrinsic growth rates among lakes. The predation pressure from heterotrophic microorganisms can stimulate a microbial population to increase its productivity in order to compensate for the increasing losses. Studies of picoplanktonic size algae should focused on their growth and the loss processes occurring in their environment instead of dealing only with population size.

Epifluorescence microscopy, even though time consuming, remains the best method, in my point of view, to study PC when determination of cell abundance is critical. The use of flow cytometry was not as helpful as it could be for a new user of this technology. Species identification is hard to achieve even in marine environments. Perhaps with a lot of experience, some researchers are able to interpret correctly flow cytometric signals and quantify precisely the data. Still, the approach should be to use both methods: epifluorescence to observe phytoplankton characteristics (length, pigmentation) and identify if possible to genus and flow cytometry to quantify the abundance of the phytoplanktonic groups. However, chances are that the application of flow cytometric signatures of freshwater systems will increase with a better understanding of background noise and variation among lakes.

REFERENCES

Agawin, N.R.S., C.M. Duarte, and S. Agustí. 1998. Growth and abundance of *Synechococcus* sp. in a Mediterranean bay: seasonality and relationship with temperature. Mar. Ecol. Prog. Ser. **170**: 45-53.

Agawin, N.R.S., C.M. Duarte, and S. Agustí. 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol. Oceanogr. **45**: 591-600.

Allman, R., A.C. Hann, A.P. Phillips, K.L. Martin and D. Lloyd. 1990. Growth of *Azotobacter vinelandii* with correlation of coulter cell size, flow cytometric parameters, and ultrastructure. Cytometry **11**: 822-831.

Azam, F., F. Fenchel, J.G. Field, L.A. Meyer-Reid and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10**: 257-263.

Bautista, B. and F. Jiménez-Gómez. 1996. Ultraphytoplankton photoacclimation through flow cytometry and pigment analysis of Mediterranean coastal waters. Sci. Mar. **60**: 233-241.

Burnison, B.K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll a analysis of phytoplankton. Can. J. Fish. Aquat. Sci. **37**: 729-733.

Burns, C.W. and J.G. Stockner. 1991. Picoplankton in six New Zealand lakes: Abundance in relation to season and trophic state. Int. Revue ges. Hydrobiol. **76**: 523-536.

Callieri, C., E. Amicucci, R. Bertoni and L. Vörös. 1996. Fluorometric characterization of two picocyanobacteria strains from lakes of different underwater light quality. Int. Revue ges. Hydrobiol. **81**: 13-23.

Campbell, L. and E.J. Carpenter 1986. Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus* spp. using the sea water dilution and selective inhibitor techniques. Mar. Ecol. Prog. Ser. **33**: 121-129.

Chisholm, S.W., R.J. Olson, E.R. Zettler, R. Goericke, J.B. Waterbury and N.A. Welschmeyer. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **334**: 340-342.

Collier, J.L. 2000. Flow cytometry and the single cell in phycology. J. Phycol. **36**: 628-644.

Corzo, A., F. Jiménez-Gómez, F.J.L. Gordill, R. García-Ruíz and F.X. Niell. 1999. *Synechococcus* and *Prochlorococcus*-like populations detected by flow cytometry in a eutrophic reservoir in summer. J. Plankton Res. **21**: 1575-1581.

Crosbie, N.D. and M.J. Furnas. 2001. Abundance, distribution and flow-cytometric characterization of picophytoprokaryote populations in central (17°S) and southern (20°S) shelf waters of the Great Barrier Reef. J. Plankton Res. **23**: 809-828.

del Giorgio, P.A., D.F. Bird, Y.T. Prairie and D. Planas. 1996. Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. Limnol. Oceanogr. **41**: 783-789.

Fahnenstiel, G.L., L. Sicko-Goad, D. Scavia and E.F. Stoermer. 1986. Importance of picoplankton in Lake Superior. Can. J. Fish. Aquat. Sci. **43**: 235-240.

Fahnenstiel, G.L., H.J. Carrick, C.E. Rogers and L. Sicko-Goad. 1991a. Red fluorescing phototrophic picoplankton in the Laurentian great lakes: what are they and what are they doing? Int. Revue ges. Hydrobiol. **76**: 603-616.

Fahnenstiel, G.L., T.R. Patton, H.J. Carrick and M.J. McCormick. 1991b. Diel division cycle and growth rates of *Synechococcus* in lakes Huron and Michigan. Int. Revue ges. Hydrobiol. **76**: 657-664.

Fahnenstiel, G.L. and H.J. Carrick. 1992. Phototrophic picoplankton in lakes Huron and Michigan: abundance, distribution, composition, and contribution to biomass and production. Can. J. Fish. Aquat. Sci. **49**: 379-388.

Gaedke, U. and T. Weisse. 1998. Seasonal and interannual variability of picocyanobacteria in Lake Constance (1987-1997). Arch. Hydrobiol. Spec. Issues Advanc. Limnol. **53**: 143-158.

Glibert, P.M., T.M. Kana, R.J. Olson, D.L. Kirchman and R.S. Alberte. 1986. Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. J. Exp. Mar. Biol. Ecol. **101**: 199-208.

Hahn, M.W., E.R.B. Moore and M.G. Hölfe. 1999. Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. Appl. Environ. Microb. **65**: 25-35.

Hall, J.A., D.P. Barrett and M.R. James. 1993. The importance of phytoflagellate, heterotrophic flagellate and ciliate grazing on bacteria and picophytoplankton sized prey in a coastal marine environment. J. Plankton Res. **15**: 1075-1086.

Hauschild, C.A., H.J.G. McMurter and F.R. Pick. 1991. Effect of spectral quality on growth and pigmentation of picocyanobacteria. J. Phycol. **27** : 698-702.

Hofstraat, J.W., W.J.M. van Zeijl, M.E.J. de Vreeze, J.C.H. Peeters, L Peperzak, F. Colijn and T.W.M. Rademaker. 1994. Phytoplankton monitoring by flow cytometry. J. Plankton Res. **16**: 1197-1224.

Hudson, J.J., W.D. Taylor and D.W. Schindler. 2000. Phosphate concentrations in lakes. Nature **406**: 54-56.

Jeffrey, S.W. and G.F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz **167** : 191-194.

Johnson, P.W. and J.McN. Sieburth. 1979. Chroococoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. Limnol. Oceanogr. **24**: 928-935.

Kalff, J. and R. Knoechel. 1978. Phytoplankton and their dynamics in oligotrophic and eutrophic lakes. Annu. Rev. Ecol. Syst. **9**: 475-495.

Kudoh, S., J. Kanda and M. Takahashi. 1990. Specific growth rates and grazing mortality of chroococcoid cyanobacteria *Synechococcus* spp. in pelagic surface waters in the sea. J. Exp. Mar. Biol. Ecol. **142**: 201-212.

Landry, M.R. 1994. Methods and controls for measuring the grazing impact of planktonic protists. Mar. Microb. Food Webs **8**: 37-58.

Landry, M.R., J. Kirshtein and J. Constantinou. 1996. Abundances and distributions of picoplankton populations in the central equatorial Pacific from 12°N to 12°S, 140°W. Deep-sea Res. II **43**: 871-890.

Li, W.K.W. 1994. Phytoplankton biomass and chlorophyll concentration across the North Atlantic. Sci. Mar. **58**: 67-79.

Li, W.K.W. 1995. Composition of ultraphytoplankton in the central North Atlantic. Mar. Ecol. Prog. Ser. **122**: 1-8.

Liu, H., L. Campbell and M.R. Landry. 1995. Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. Mar. Ecol. Prog. Ser. **116**: 277-287.

Liu, H., L. Campbell, M.R. Landry, H.A. Nolla, S.L. Brown and J. Constantinou. 1998. *Prochlorococcus* and *Synechococcus* growth rates and contributions to production in the Arabian Sea during the 1995 Southwest and Northeast Monsoons. Deep Sea Res. II **45**: 2337-2352.

Liu, H., R.R. Bidigare, E. Laws, M.R. Landry and L. Campbell. 1999. Cell cycle and physiological characteristics of *Synechococcus* (WH7803) in chemostat culture. Mar. Ecol. Prog. Ser. **189**: 17-25.

Maeda, H., A. Kawai and M.M. Tilzer. 1992. The water bloom of cyanobacterial picoplankton in Lake Biwa, Japan. Hydrobiologia **248**: 93-103.

Marie, D., F. Partensky, S. Jacquet and D. Vaulot. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYGR Green I. Appl. Environ. Microb. **63**: 186-193.

Marie, D., C.P.D. Brussaard, R. Thyrhaug, G. Bratbak and D. Vaulot. 1999. Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl. Environ. Microb. **65**: 45-52.

Monger, B.C. and M.R. Landry. 1992. Size-selective grazing by heterotrophic nanoflagellates: an analysis using live-stained bacteria and dual-beam flow cytometry. Arch. Hydrobiol. Beih. Ergebn. Limnol. **37**: 173-185.

Müller, H. 1996. Selective feeding of a freshwater chrysomonad, *Paraphysomonas* sp., on chroococcoid cyanobacteria and nanoflagellates. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. **48**: 63-71.

Ning, X. and D. Vaulot. 1992. Estimating *Synechococcus* spp. growth rates and grazing pressure by heterotrophic nanoplankton in the English Channel and the Celtic Sea. Acta Oceanol. Sinica **11** : 255-273.

Ning, X., J.E. Cloern and B.E. Cole. 2000. Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay. Limnol. Oceanogr. **45**: 695-702.

Olson, R.J., S.W. Chisholm, E.R. Zettler and E.V. Armbrust.1990. Pigments, size and distribution of *Synechococcus* in the North Atlantic and Pacific oceans. Limnol. Oceanogr. **35**: 45-58.

Olson, R.J., E.R. Zettler and M.D. DuRand. 1993. Phytoplankton analysis using flow cytometry. In Handbook of methods in aquatic microbial ecology. Edited by Paul F. Kemp et al., pp. 175-186.

Pick, F.R. 1991. The abundance and composition of freshwater picocyanobacteria in relation to light penetration. Limnol. Oceanogr. **36**: 1457-1462.

Pick, F.R. 2000. Predicting the abundance and production of photosynthetic picoplankton in temperate lakes. Verh. Internat. Verein. Limnol. **27**: 1884-1889.

Pick, F.R. and D.A. Caron. 1987. Picoplankton and nanoplankton biomass in Lake Ontario: relative contribution of phototrophic and heterotrophic communities. Can. J. Fish. Aquat. Sci. **44**: 2164-2172.

Pick, F.R. and D.R.S. Lean. 1987. The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. N. Z. J. Mar. Freshwater Res. **21**: 425-434.

Pick, F.R. and M. Agbeti. 1991. The seasonal dynamics and composition of photosynthetic picoplankton communities in temperate lakes in Ontario, Canada. Int. Revue. ges. Hydrobiol. **76**: 565-580.

Pinel-Alloul, B., N. Bourbonnais and P. Dutilleul. 1996. Among-lake and within-lake variations of autotrophic pico- and nanoplankton biomass in six Quebec lakes. Can. J. Fish. Aquat. Sci. **53**: 2433-2445.

Porter, J., J. Diaper, C. Edwards and R. Pickup. 1995. Direct measurements of natural planktonic bacterial community viability by flow cytometry. Appl. Environ. Microb. **61**: 2783-2786.

Porter, J., D. Deere, M. Hardman, C. Edwards and R. Pickup. 1997. Go with the flow – use of flow cytometry in environmental microbiology. FEMS Microbiol. Ecol. **24**: 93-101.

Raven, J.A. 1998. The twelfth Tansley Lecture. Small is beautiful: the picophytoplankton. Funct. Ecol. **12**: 503-513.

Regional Municipality of Ottawa-Carleton. 1993. Surface water quality technical report 1993. Regional Municipality of Ottawa-Carleton, Surface Water Branch, R.O. Pickard Environmental Centre, Gloucester, ON K1J 8G8, Canada.

Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge.

Rhew, K. and C.A. Ochs. 2000. Spatial patterns in autotrophic picoplankton abundance in a reservoir examined using microcosm experiments. Int. Revue. ges. Hydrobiol. **85**: 395-412.

Rippka, R. 1988. Recognition and identification of cyanobacteria. Methods in Enzymology **167**: 28-67.

Sherr, B.F., E.B. Sherr, T.L. Andrew, R.D. Fallon and S.Y. Newell. 1986. Trophic interactions between heterotrophic protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. Mar. Ecol. Prog. Ser. **32**: 169-179.

Sherr, E.B. and B.F. Sherr. 1993. Protistan grazing rates via uptake of fluorescently labelled prey. In Handbook of methods in aquatic microbial ecology. Edited by Paul F. Kemp et al., pp. 695-701.

Søndergaard, M. 1991. Phototrophic picoplankton in temperate lakes: seasonal abundance and importance along a trophic gradient. Int. Revue ges. Hydrobiol. **76**: 505-522

Stockner, J.G. 1991. Autotrophic picoplankton : Community composition, abundance and distribution across a gradient of oligotrophic British Columbia and Yukon territory lakes. Int. Revue ges. Hydrobiol. **76**: 483-492.

Stockner, J.G. and N.J. Antia. 1986. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. **43**: 2472-2503.

Stockner, J.G. and K.S. Shortreed. 1991. Autotrophic picoplankton: community composition, abudance and distribution across a gradient of oligotrophic British Columbia and Yukon Territory lakes. Int. Revue ges. Hydrobiol. **76**: 581-601.

Suttle, C.A. and A.M. Chan. 1994. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl. Environ. Microbiol. **60**: 3167-3174.

Takamura, N. and Y. Nojiri. 1994. Picophytoplankton biomass in relation to trophic state and the TN:TP ratio of lake water in Japan. J. Phycol. **30**: 439-444.

Vanni, M.J. and J. Temte. 1990. Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. Limnol. Oceanogr. **35**: 697-709.

Vörös, L., C. Callieri, K. V-Balogh and R. Bertoni. 1998. Freshwater picocyanobacteria along a trophic gradient and light quality range. Hydrobiologia **369-370**: 117-125.

Waterbury, J.B., S.W. Watson, R.R.L. Guillard and L.E. Brand. 1979. Widespread occurrence of a unicellar, marine, planktonic cyanobacterium. Nature **277**: 293-294.

Watson, S. and J. Kalff. 1981. Relationship between nannoplankton and lake trophic status. Can. J. Fish. Aquat. Sci. **38**: 960-967.

Wehr, J.D. 1989. Experimental tests of nutrient limitation in freshwater picoplankton. Appl. Environ. Microbiol. **45**: 1196-1201.

Wehr, J.D. 1990. Predominance of picoplankton and nanoplankton in eutrophic Calder lake. Hydrobiologia **203**: 35-44.

Wehr, J.D. 1991. Nutrient and grazer-mediated effects on picoplankton and size structure in phytoplankton communities. Int. Revue ges. Hydrobiol. **76**: 643-656.

Wehr, J.D. 1993. Effects of experimental manipulations on light and phosphorus supply on competition among picoplankton and nanoplankton in an oligotrophic lake. Can. J. Fish. Aquat. Sci. **50**: 936-945.

Weisse, T. 1988. Dynamics of autotrophic picoplankton in Lake Constance. J. Plankton Res. **10**: 1179-1188.

Wilkinson, L. 2000. Systat 10 for windows. SPSS Inc. Chicago, Ill.

Appendix IA Physical and chemisty variables of 50 sites sampled once in July or August 2000. **SA**: surface area; **Z_{max}**: maximum depth; **Z_{mean}**: mean depth of the lake; **LAC**: light attenuation coefficient; **TP**: total phophorus; **RP** : reactive phosphate; **TKN**: total Kjeldahl . nitrogen; **Temp**: temperature of epilimnion; **Cond**: conductivity of epilimnion; **SD**: Secchi disk.

Region/ Lake	Lat.	Long.	SA (km^2)	Z_{max} (m)	Z_{mean} (m)	LAC	TP ($\mu g \cdot l^{-1}$)	TKN ($\mu g \cdot l^{-1}$)	NH_3 ($\mu g \cdot l^{-1}$)	NO_2+NO_3 ($\mu g \cdot l^{-1}$)	RP ($\mu g \cdot l^{-1}$)	Temp. (°C)	Cond. (µS/cm)	pH	SD (m)	chla *a* total ($\mu g \cdot l^{-1}$)	chla *a* < 2 µm ($\mu g \cdot l^{-1}$)
Outaouais, Que.																	
Argile	45° 52'	75° 34'	4,51	43	13,3	0,62	11,5	290	6	0	1,5	22	80	8,5	3,00	6,55	1,74
Barrière	45° 53'	75° 06'	3,11	73		0,43	2,5	189	1,5	115	1	20	38	8,0	4,25	2,01	0,39
Black	45° 29'	75° 51'	0,01	9,5	3,1	0,68	11,5	398	4	0	3	21	80	8,5	2,75	9,44	1,44
Forgeron	46° 03'	75° 37'	0,55	38,0	11,5	0,84	15,5	550	127	35	2	19,5	61	6,7	2,75	12,55	1,57
Mulvihill	45° 29'	75° 51'	0,01	3,7	1,1	0,62	10,5	342	4	0	2,5	21	125	8,2	2,75	11,55	2,34
Orignal	45° 52'	75° 35'	0,37	13,3		0,33	8,5	290	4	0	2	24	195	8,9	4,50	4,79	1,42
Philippe	45° 36'	76° 01'	1,76	17,4	8,7	0,59	6,5	248	5	0	2	24	68	8,4	4,25	4,06	0,37
Renaud	45° 36'	76° 02'	0,10	4,3	1,0	0,35	24,0	535	5	0	0	23	68	7,6	2,00	13,33	2,33
Simon	45° 55'	75° 04'	30,73	108	48,7	0,39	1,0	179	3	120	2	19	38	8,2	4,50	1,83	0,34
Taylor	45° 36'	76° 03'	0,60	12,2	4,7	0,42	4,5	318	6	0	2,5	24	70	8,2	4,00	4,30	0,95
Vert	45° 54'	75° 36'	0,24	25,3		0,82	5,0	273	4,5	0	2	21	186	8,6	4,50	4,76	n/a
Ottawa, Ont.																	
Constance	45° 24'	75° 58'	1,45	4,0		1,07	40,0	860	39	0	6,5	20	348	8,5	1,50	16,96	1,18
MacKay	45° 27'	75° 40'	0,79	13		0,64	19,0	585	11	0	3	23	420	9,2	4,75	9,76	1,95
Rideau lakes, Ont.																	
Big Rideau	44° 42'	76° 13'	5,8	95	12,5	0,41	14,0	428	3	0	7,5	24,5	190	8,8	5,75	3,51	0,64
Bob's			9,0	23	11,0												
(Green Bay)	44° 38'	76° 35'				0,35	7,0	315	1	0	1,5	25,0	185	8,0	6,25	2,19	0,31
(West Bay)	44° 39'	76° 37'				0,50	9,5	372	1	0	2,5	24,0	130	7,6	4,50	4,28	0,93
Indian	44° 35'	76° 19'	2,66	26	10,1	0,57	15,0	400	4,5	0	6	23,0	180	8,9	3,10	5,62	0,06
Jack's	44° 41'	78° 04'	5,10	44	17,0	0,44	7,0	434	10	0	7	22,0	109	8,2	5,00	3,93	0,50
Opinicon	44° 33'	76° 20'	7,85	9,2	4,9	0,75	14,0	415	4	0	6	23,5	175	8,6	4,40	5,98	0,33
Otter	44° 46'	76° 07'	5,71	37	10,0	0,58	10,5	445	7,5	0	9	23,5	240	8,8	3,00	3,55	0,79
Upper Rideau	44° 40'	76° 21'	13,62	22	8,1	0,71	20,5	455	5	0	7,5	22,5	200	8,9	3,20	8,69	1,34
Upper Rock	44° 29'	76° 24'	0,76	44,5	20,5	n/a	10,0	470	8	0	7	24,0	200	9,1	4,25	2,22	0,66
Westport Sand	44° 40'	76° 25'	2,08	12,8	6,7	0,49	19,0	416	10,5	0	7,5	23,0	195	8,8	6,00	4,71	0,36
Wolfe	44° 40'	76° 29'	7,5	31	10,5	0,42	9,5	340	3,5	0	7,5	23,5	200	8,9	4,20	2,95	0,47

Appendix IA (continued)

Region/ Lake	Lat.	Long.	SA (km^2)	Z_{max} (m)	Z_{mean} (m)	LAC	TP ($\mu g \cdot l^{-1}$)	TKN ($\mu g \cdot l^{-1}$)	NH_3 ($\mu g \cdot l^{-1}$)	NO_2+NO_3 ($\mu g \cdot l^{-1}$)	RP ($\mu g \cdot l^{-1}$)	Temp. (°C)	Cond. ($\mu S/cm$)	pH	SD (m)	chla *a* total ($\mu g \cdot l^{-1}$)	chla *a* < 2 µm ($\mu g \cdot l^{-1}$)
Laurentians, Que.																	
Achigan	45° 56'	73° 57'	5,32	26	12,5	0,65	4,5	250	10	20	1	23,0	230	7,5	3,50	4,65	0,84
Anne	45° 49'	74° 18'	1,18	22	10,0	0,58	6,0	265	3	0	1,5	23,0	180	7,3	4,50	3,69	0,42
Connelly	45° 53'	73° 57'	1,21	20	7,7	0,62	16,0	350	10	0	3	24,0	365	7,5	3,25	10,68	3,59
Croche	45° 59'	74° 00'	0,18	11	5,3	0,81	4,5	275	3,5	0	3,5	24,0	20	6,9	3,25	2,44	0,71
En coeur	45° 58'	74° 00'	0,47	8	3,2	0,62	5,0	285	3	0	1,5	23,0	250	n/a	3,25	11,70	3,17
Hugues	45° 48'	74° 15'				0,64	7,0	320	10,5	10	1,5	22,0	200	n/a	3,25	6,18	1,74
Morency	45° 55'	74° 02'	0,26	20	8,6	0,22	5,0	304	3	0	1	23,0	410	7,4	7,50	2,69	0,91
Montaubois	45° 55'	74° 04'	0,16	32	13,9	0,27	7,0	235	3,5	0	2	22,5	320	7,3	6,25	4,27	0,62
Ours	45° 57'	74° 03'	0,14	17	7,2	1,30	20,5	525	12	0	1,5	23,0	310	7,2	2,25	13,85	3,48
Pin rouge	45° 57'	74° 02'	0,15	13	4,7	2,45	14,0	460	13,5	0	3	23,0	45	6,4	1,75	16,92	2,08
Estrie, Que.																	
d'Argent	45° 18'	72° 18'	1,0	15,6	4,5	0,80	5,0	311	4	0	2	22,5	88	7,5	2,50	10,45	1,94
Bowker	45° 42'	72° 21'	2,5	57,9	23,9	0,31	1,0	149	8	15	0	21,0	50	7,5	5,25	2,95	0,66
Brome	45° 14'	72° 30'	14,39	12,2	5,9	1,13	31,0	655	4	0	4,5	22,0	110	8,2	1,50	36,65	5,73
Brompton	45° 42'	72° 14'	11,9	42,3	12,5	0,66	3,0	245	11	60	0	21,5	56	7,4	3,25	4,31	0,39
Choinière	45° 25'	72° 37'	4,75			0,90	28,0	503	40,5	45	1	22,0	126	7,4	2,25	14,88	1,31
Fraser	45° 38'	72° 17'	1,6	19,8	8,6	0,59	4,5	260	7,5	0	1	22,0	52	7,5	4,75	5,66	1,60
Lovering	45° 17'	72° 15'	4,94	22,9	8,8	0,69	7,5	358	16,5	40	1	22,5	86	7,7	3,25	6,56	1,43
Magog	45° 30'	72° 04'	10,95	19,2	8,0	0,57	15,0	345	8	10	0	23,0	151	8,3	3,75	8,97	1,02
Massawippi	45° 21'	72° 00'	17,9	84,9	40,2	0,51	7,5	308	8,5	290	1,5	23,0	218	8,5	3,50	8,46	1,99
Memphrémagog			97,9		16												
(Magog Bay)	45° 16'	72° 11'		33,5	12,8	0,48	4,0	233	13	10	1	22,5	132	8,1	6,00	4,24	0,65
(Sargent Bay)	45° 10'	72° 17'				0,41	8,0	238	7,5	0	0	23,0	132	8,1	6,00	3,01	0,63
Montjoie	45° 40'	72° 10'	3,3	21,3	8,0	0,63	12,0	275	9	0	1	22,0	54	7,3	3,25	4,44	0,89
Stukely	45° 18'	72° 16'	4,0	30,5	13,1	0,50	1,5	247	4,5	0	1	21,5	48	7,7	3,25	6,36	0,71
Orford	45° 22'	72° 15'	1,29	39,5	17,5	0,45	1,0	222	6	0	1	22,0	167	8,3	3,25	5,23	0,90
New York State, U.S.																	
Oneida	43° 06'	76° 13'	210	16,7	6,8	1,84	42,0	900	16	0	5,5	22,5	319	9,1	1,50	68,98	11,40
Onondaga	43° 14'	76° 48'	12	19,5	10,5	0,69	27,5	655	9	1115	4,5	23,5	1809	8,2	1,60	19,05	4,05

Appendix IB Mean growth and loss rates measured from the selective metabolic inhibitor technique where n represents the number of replicates and SD, the standard deviation of the mean.

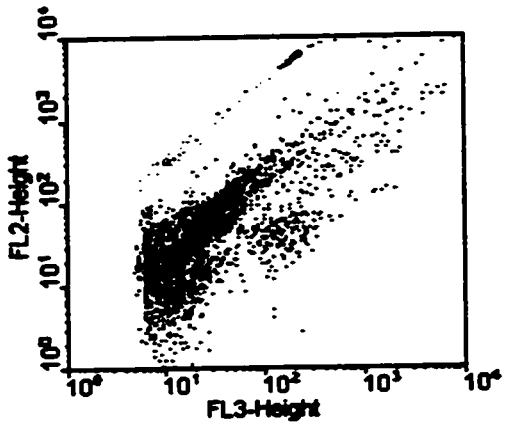
Region / Lake	Growth rate (d^{-1}) +/- SD	Loss rate (d^{-1}) +/- SD	n
Outaouais, Que.			
Argile	0,43 (0,22)	0,43 (0,35)	2
Barrière	-0,18 (0,28)	0,01 (0,45)	2
Black	0,09 (0,19)	-0,07 (0,44)	2
Forgeron	0,16 (0,49)	-0,25 (0,39)	3
Mulvihill	0,49 (0,07)	0,27 (0,17)	2
Orignal	0,06 (0,10)	0,05 (0,15)	2
Philippe	0,54 (0,37)	0,59 (0,06)	2
Renaud	0,33 (0,31)	-0,33 (0,34)	2
Simon	0,07 (0,49)	-0,05 (0,11)	2
Taylor	0,14 (0,32)	-0,14 (0,07)	2
Vert	0,35 (0,04)	0,22 (0,06)	3
Ottawa, Ont.			
Constance	1,52 (0,19)	1,25 (0,40)	3
MacKay	0,65 (0,54)	0,56 (0,12)	3
Rideau lakes, Ont.			
Big Rideau	0,11 (0,22)	0,33 (0,13)	3
Bob's			
(Green Bay)	0,94 (0,07)	0,42 (0,07)	2
(West Bay)	0,53 (0,13)	0,33 (0,09)	3
Indian	0,72 (0,35)	0,45 (0,46)	3
Jack's	0,37 (0,09)	0,12 (0,14)	3
Opinicon	0,43 (0,03)	0,49 (0,12)	2
Otter	-0,31 (0,11)	-0,20 (0,14)	3
Upper Rideau	0,63 (0,08)	0,60 (0,27)	3
Upper Rock	0,63 (0,15)	0,54 (0,42)	3
Westport Sand	-0,02 (0,29)	0,58 (0,51)	3
Wolfe	0,85 (0,20)	1,02 (0,16)	3
Laurentians, Que.			
Achigan	-0,01 (0,34)	0,05 (0,15)	3
Anne	0,32 (0,24)	0,22 (0,18)	3
Connelly	0,51 (0,58)	0,56 (0,17)	3
Croche	0,50 (0,07)	0,11 (0,26)	3
En coeur	0,73 (0,16)	0,32 (0,17)	3
Hugues	0,22 (0,15)	0,13 (0,26)	3
Morency	0,28 (0,08)	0,22 (0,10)	3
Montaubois (Ogilvy)	0,16 (n/a)	-0,24 (0,26)	3
Ours	0,40 (0,18)	0,07 (0,05)	3
Pin rouge	0,23 (0,64)	-0,05 (0,21)	3

Appendix IB (continued)

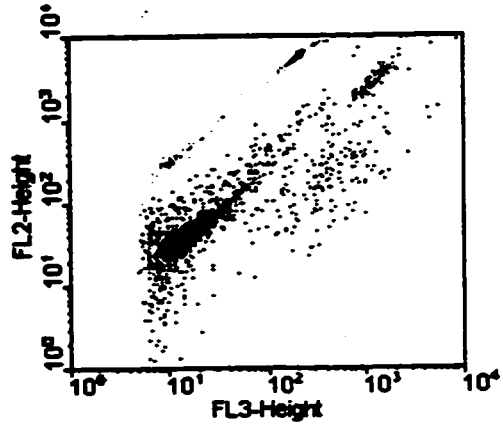
Region / Lake	Growth rate (d^{-1}) +/- SD	Loss rate (d^{-1}) +/- SD	n
Estrie, Que.			
d'Argent	0,44 (0,38)	0,70 (0,12)	3
Bowker	0,38 (0,30)	0,49 (0,09)	3
Brome	1,01 (0,06)	1,22 (0,03)	3
Brompton	-0,15 (0,29)	-0,30 (0,23)	3
Choinière	1,01 (0,28)	0,29 (0,12)	3
Fraser	1,93 (0,17)	0,87 (1,76)	3
Lovering	-0,11 (0.06)	-0,27 (0.18)	3
Magog	0,55 (0,27)	0,12 (0,10)	3
Massawippi	0,92 (0,25)	0,68 (0,38)	3
Memphrémagog			
(Magog Bay)	0,39 (0,18)	0,07 (0,17)	3
(Sargent Bay)	0,23 (0,17)	0,31 (0,29)	3
Montjoie	0,56 (0,07)	0,10 (0,31)	3
Orford	-0,19 (0,19)	0,05 (0,13)	3
Stukely	0,27 (0,07)	0,06 (0,09)	3
New York State, U.S.			
Oneida	n/a	n/a	3
Onondaga	0,09 (0,20)	-0,09 (0,10)	3

Appendix IIA Flow cytometric signature of 48 temperate freshwater lakes sampled in summer 2000. Dot plots represent phycoerythrin fluorescence (FL2) as a function of chl *a* fluorescence (FL3) for each cell. For each plot, the series of dots in the upper part represent the fluorescently labelled beads (FLB). Flow cytometry analyses were done with a FACScan (Becton Dickinson).

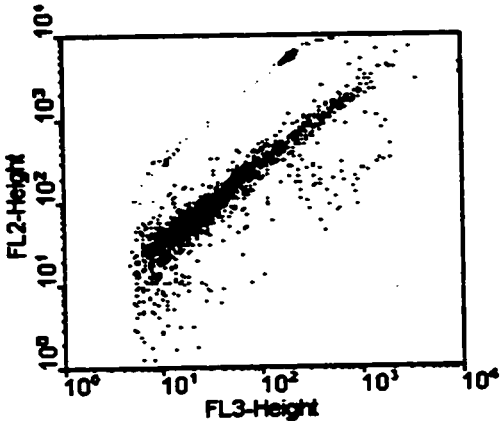
1- Mulvihill



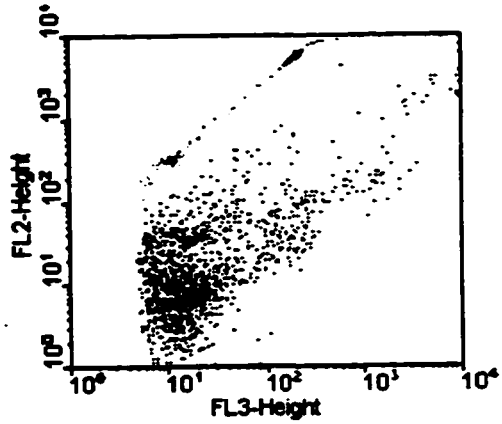
2- Orignal



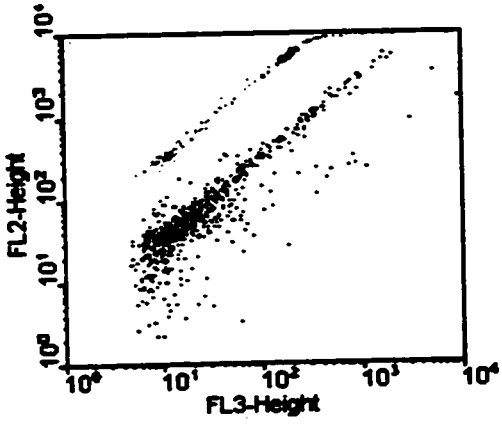
3- Otter



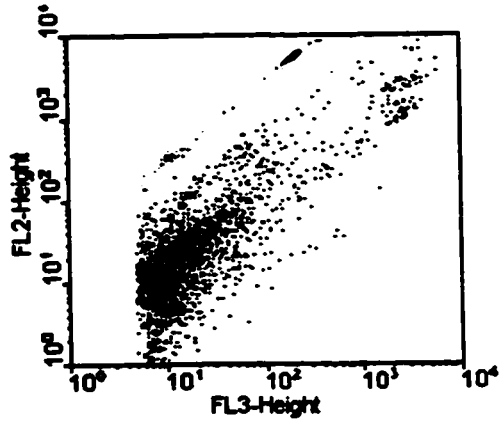
4- Ours



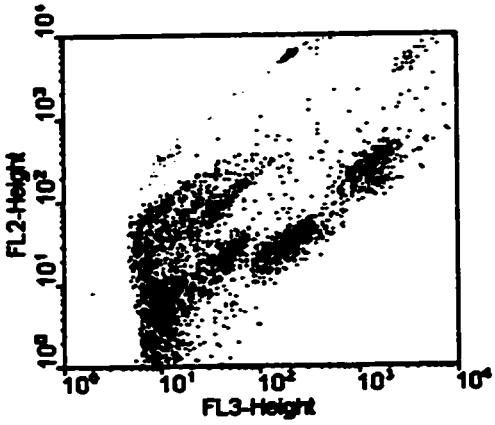
5- Simon



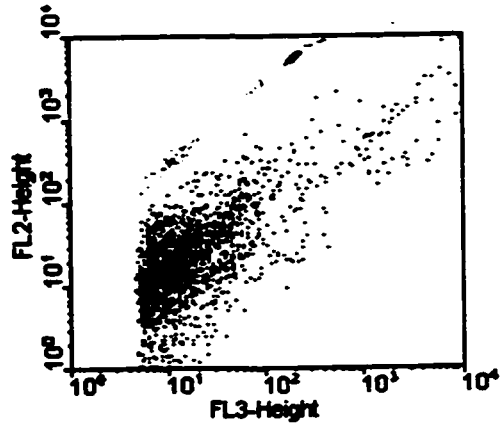
6- Oneida



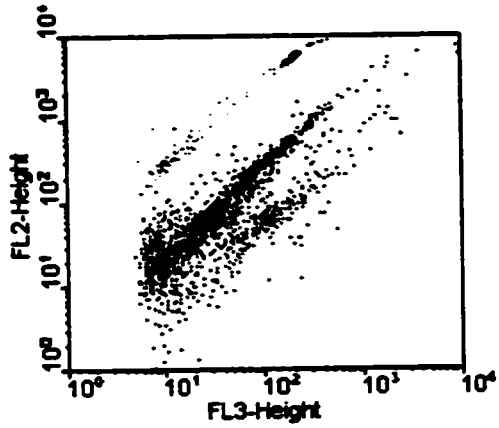
7- Onondaga



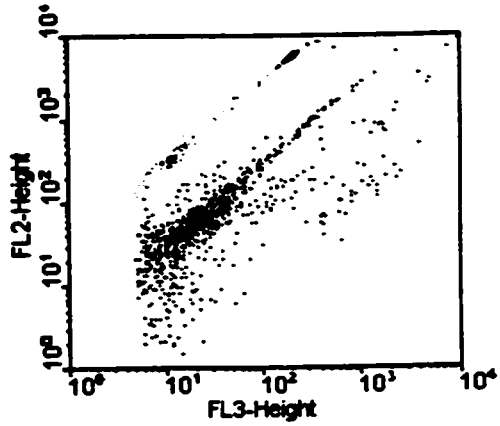
8- Constance



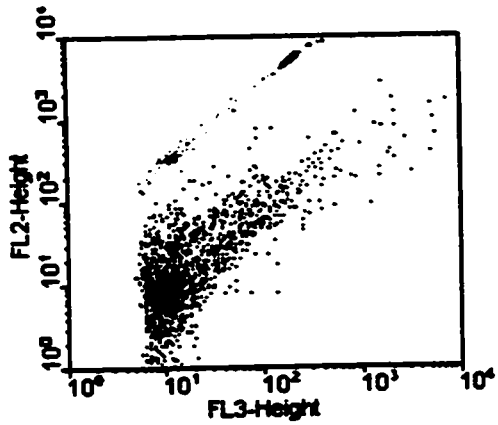
9- Anne



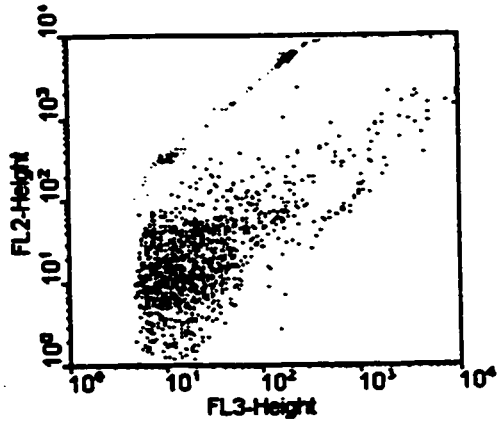
10- Morency



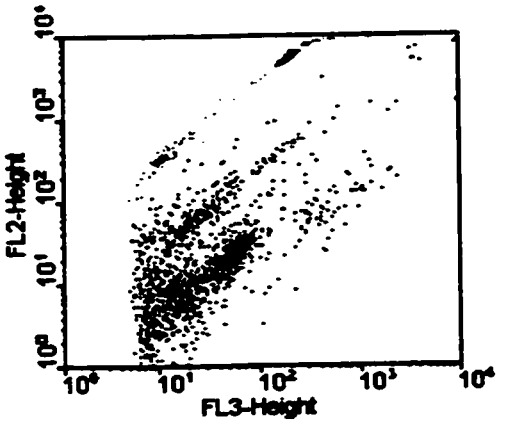
11- Croche



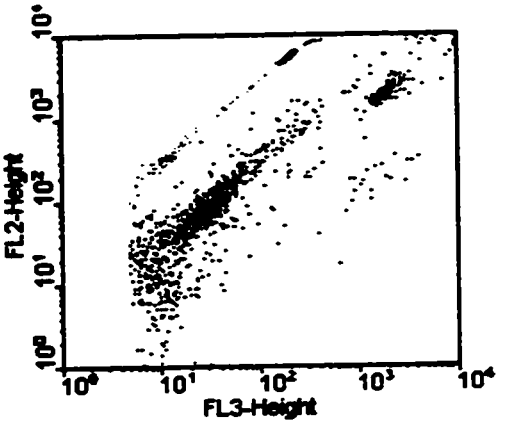
12- Pin Rouge



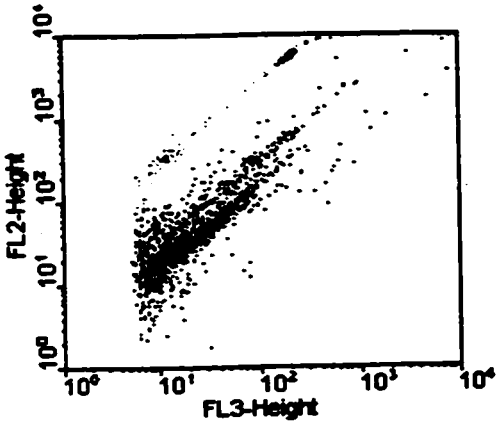
13- Magog



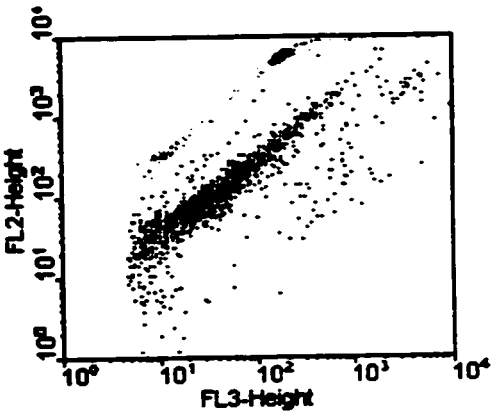
14- Massawippi



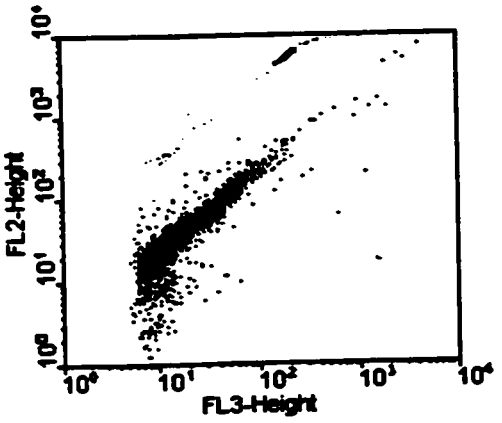
15- Bowker



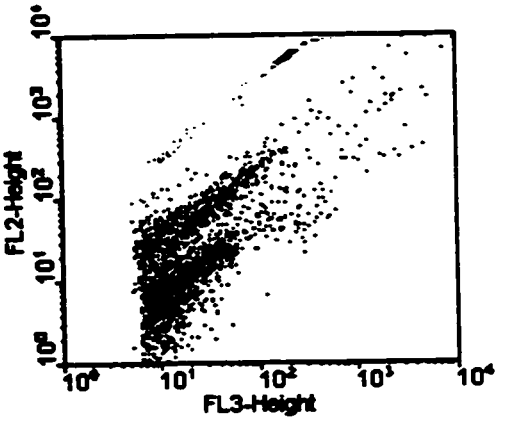
16- Vert



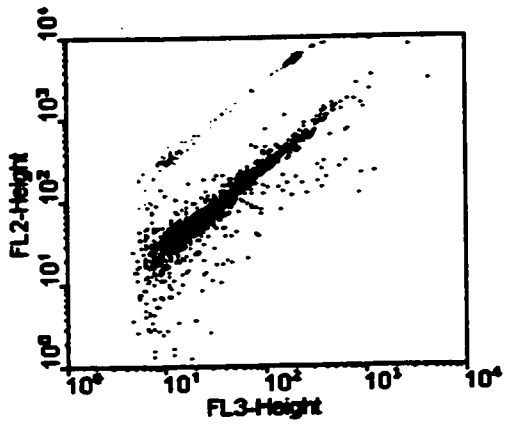
17- Westport Sand



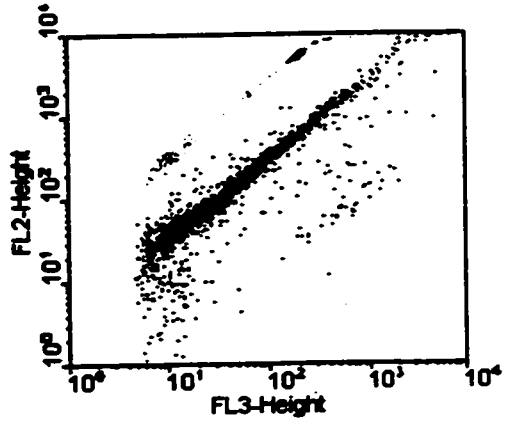
18- Opinicon



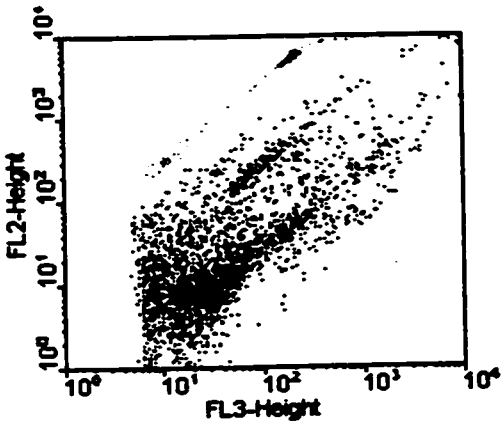
19- Bob's (Green bay)



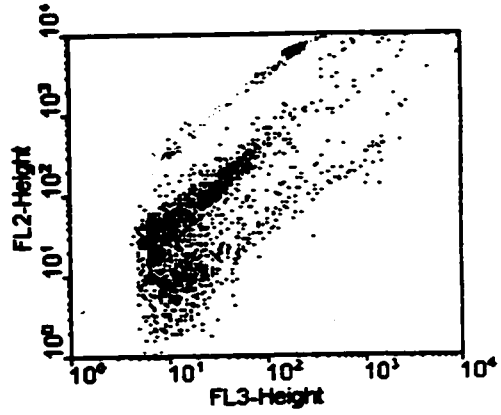
20- Brome



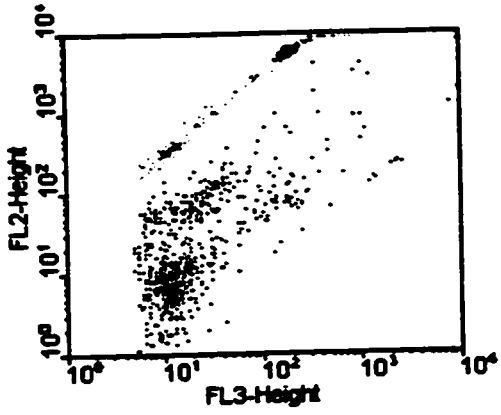
21- Orford



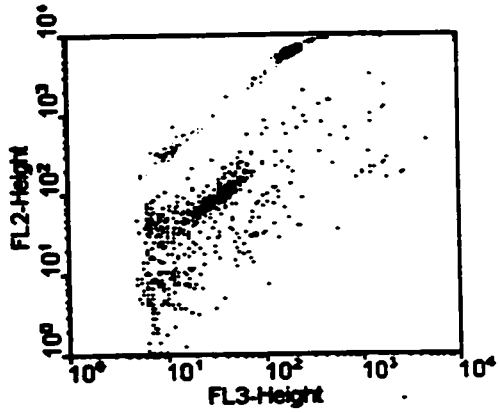
22- Lovering



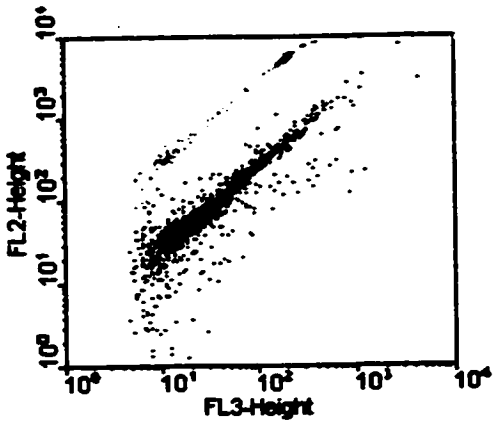
23- Montjoie



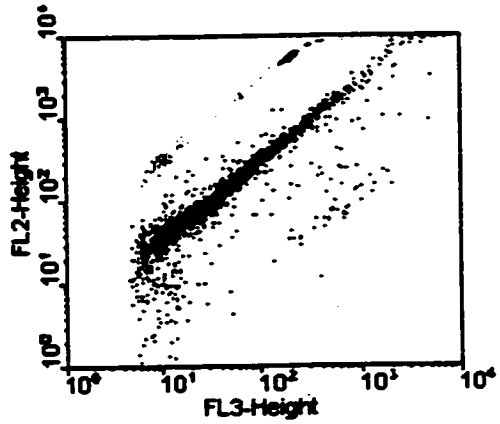
24- Memphrémagog (Magog bay)



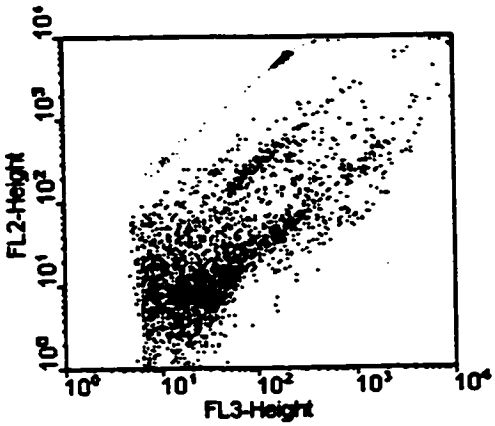
19- Bob's (Green bay)



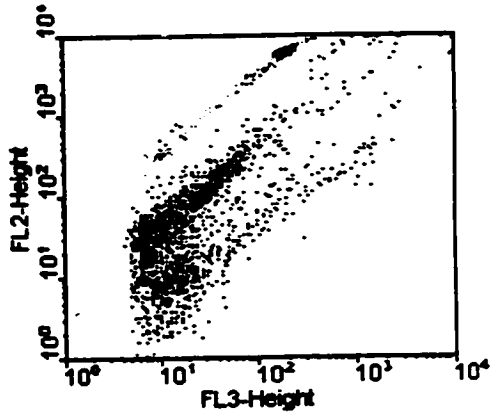
20- Brome



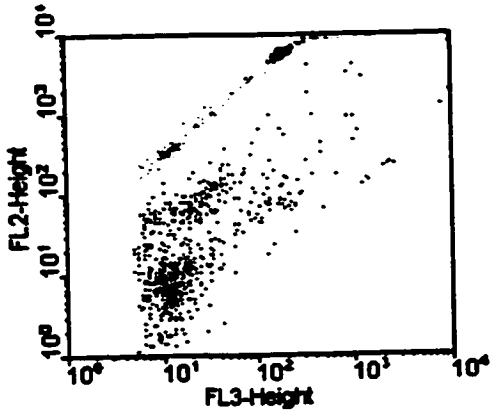
21- Orford



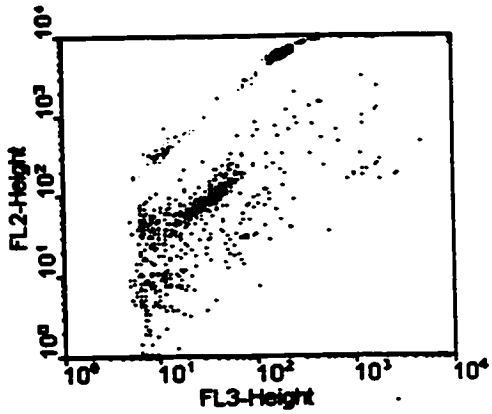
22- Lovering



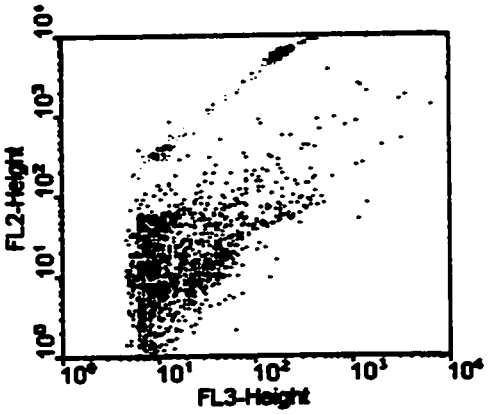
23- Montjoie



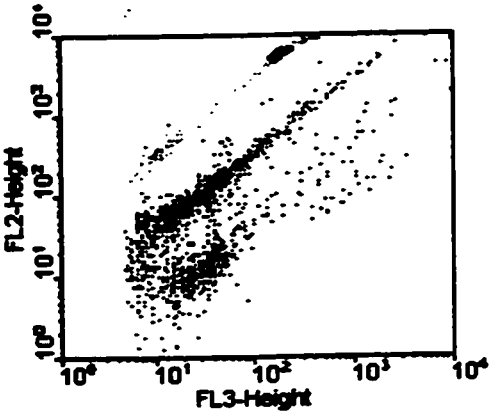
24- Memphrémagog (Magog bay)



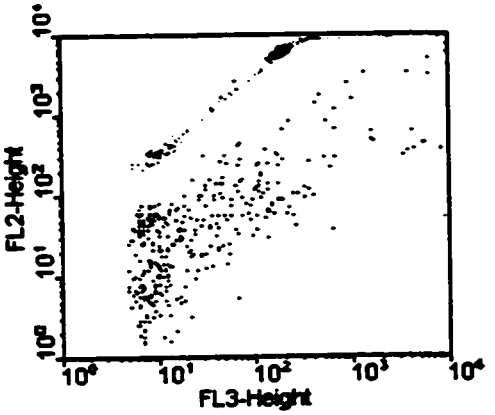
25- Choinière



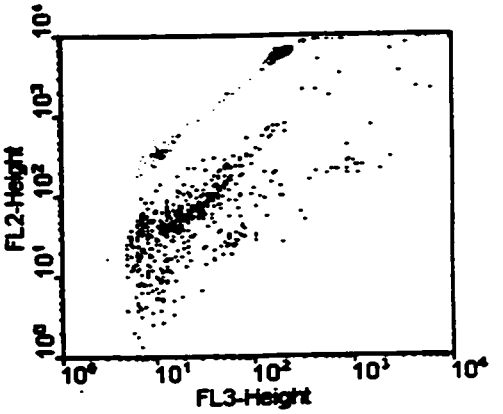
26- Stukely



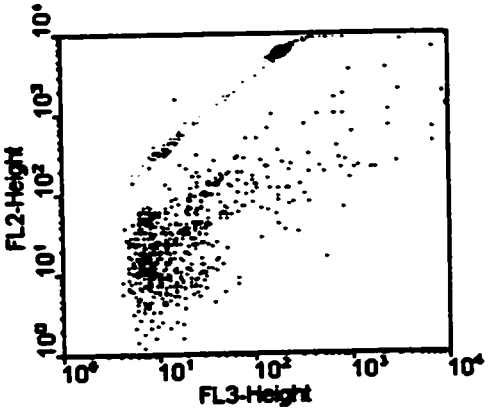
27- Fraser



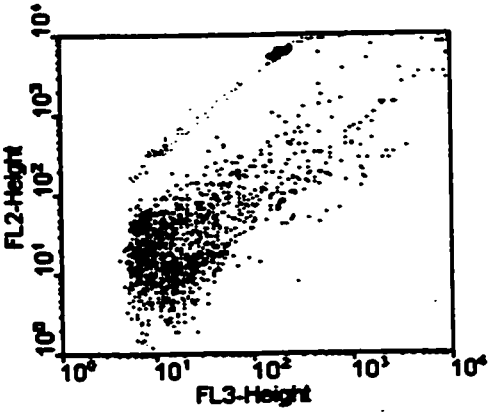
28- Memphrémagog (Sargent bay)



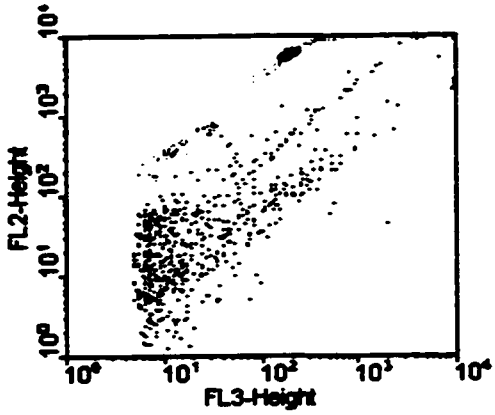
29- Brompton



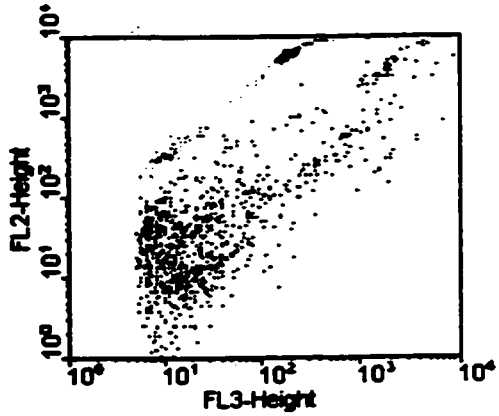
30- Hugues



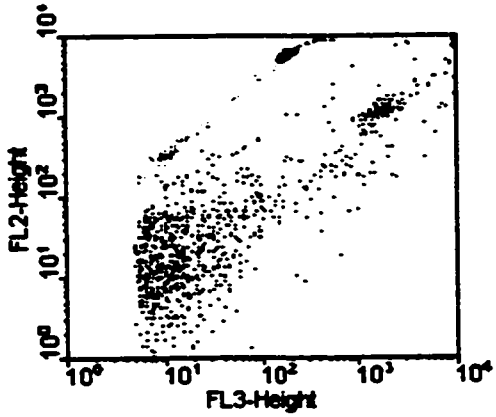
31- Achigan



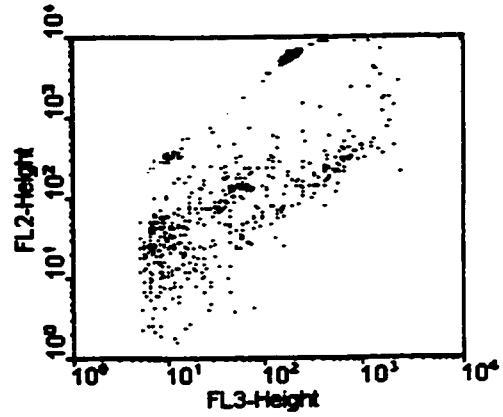
32- En Coeur



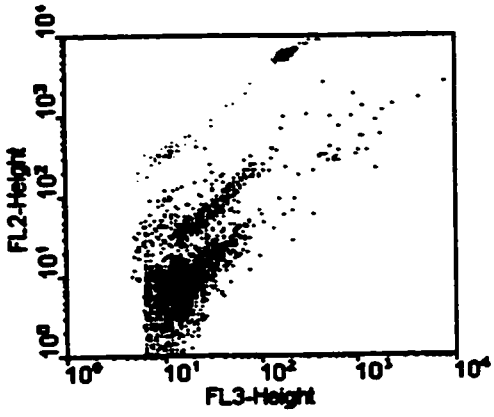
33- Connely



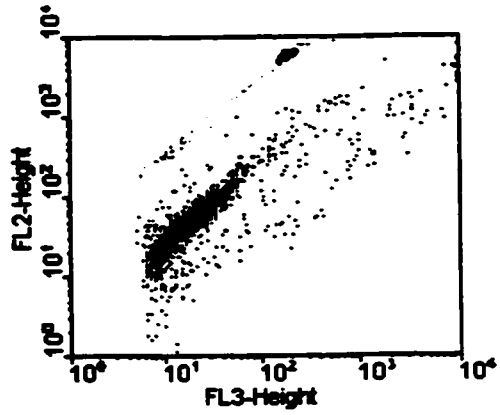
34- Montaubois



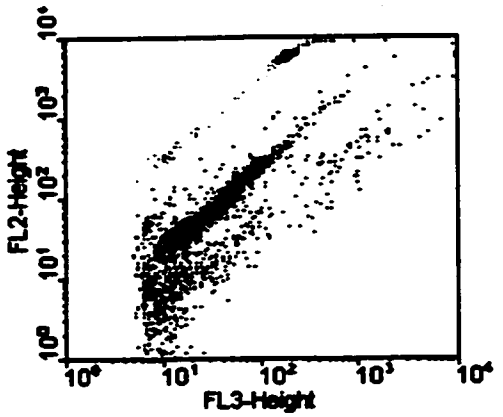
35- Upper Rock



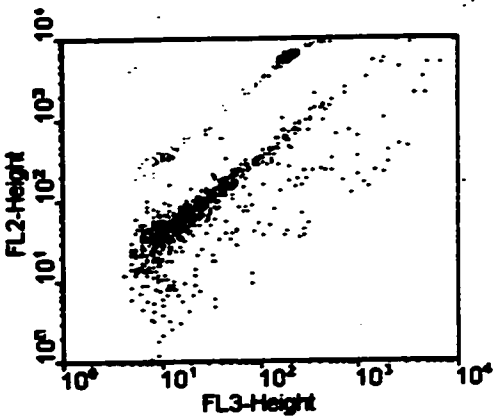
36- Big Rideau



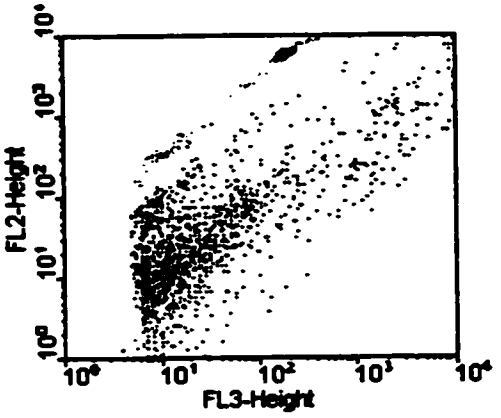
37- Upper Rideau



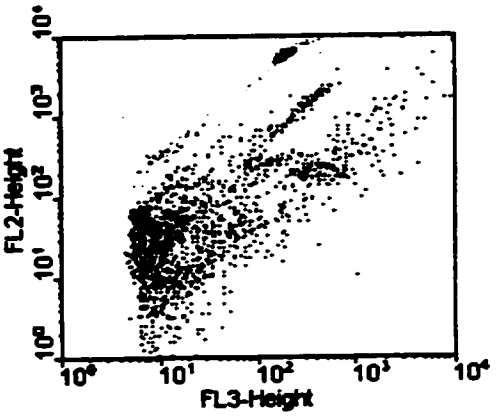
38- Barrière



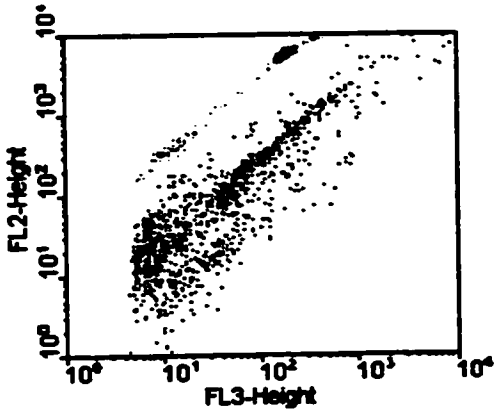
39- Forgeron



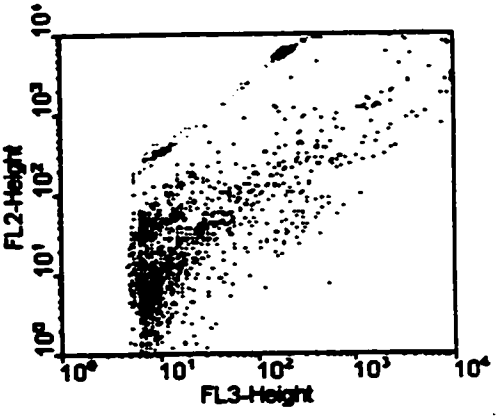
40- Black



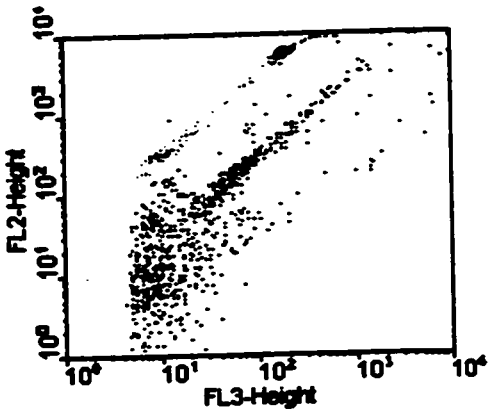
41- Taylor



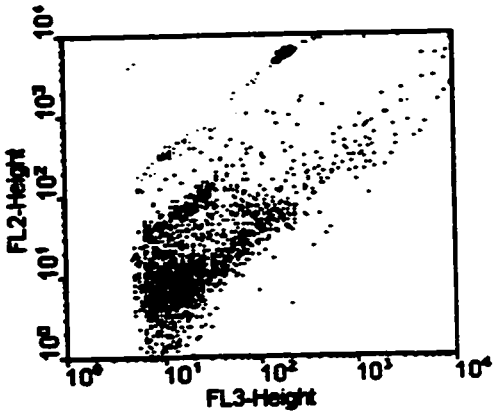
42- Renaud



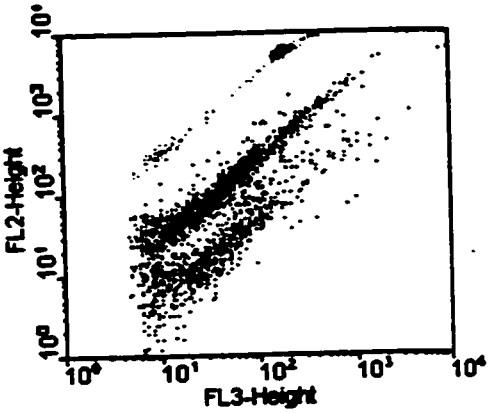
43- Argile



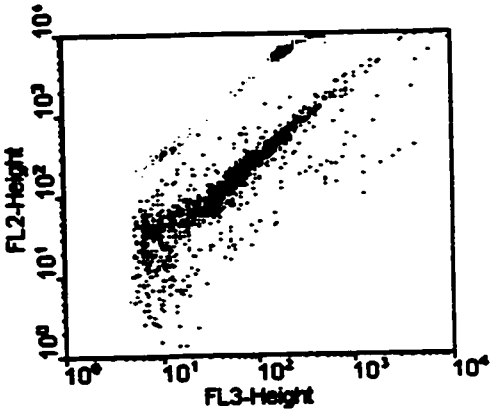
44- d'Argent



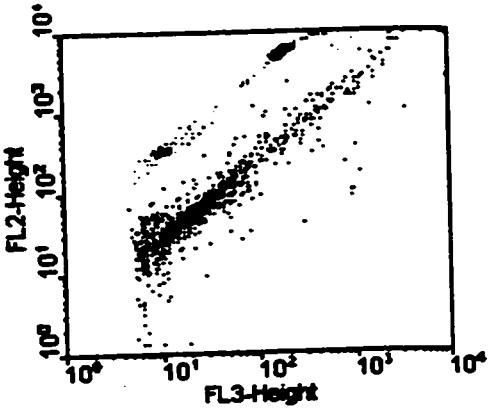
45- Bob's (West bay)



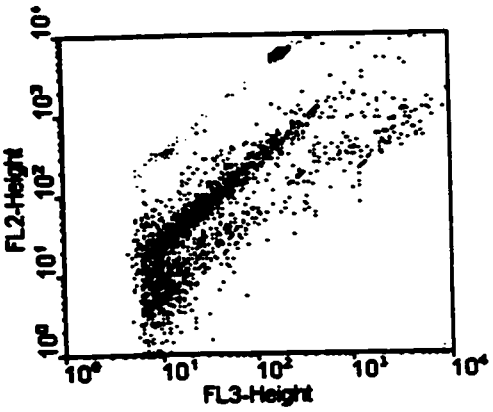
46- Jack's



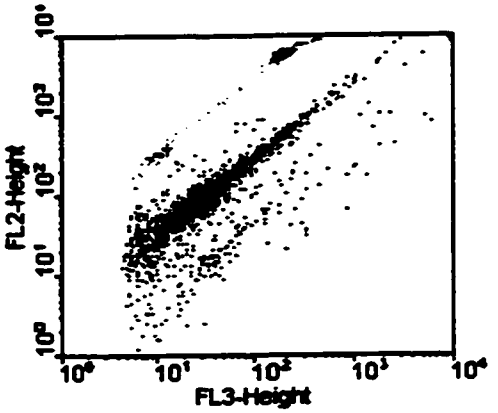
47- Wolfe



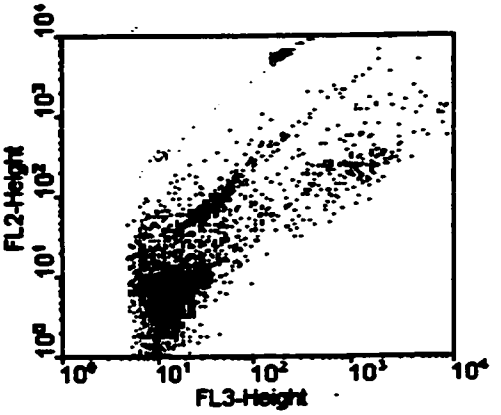
48- Indian



49- Philippe



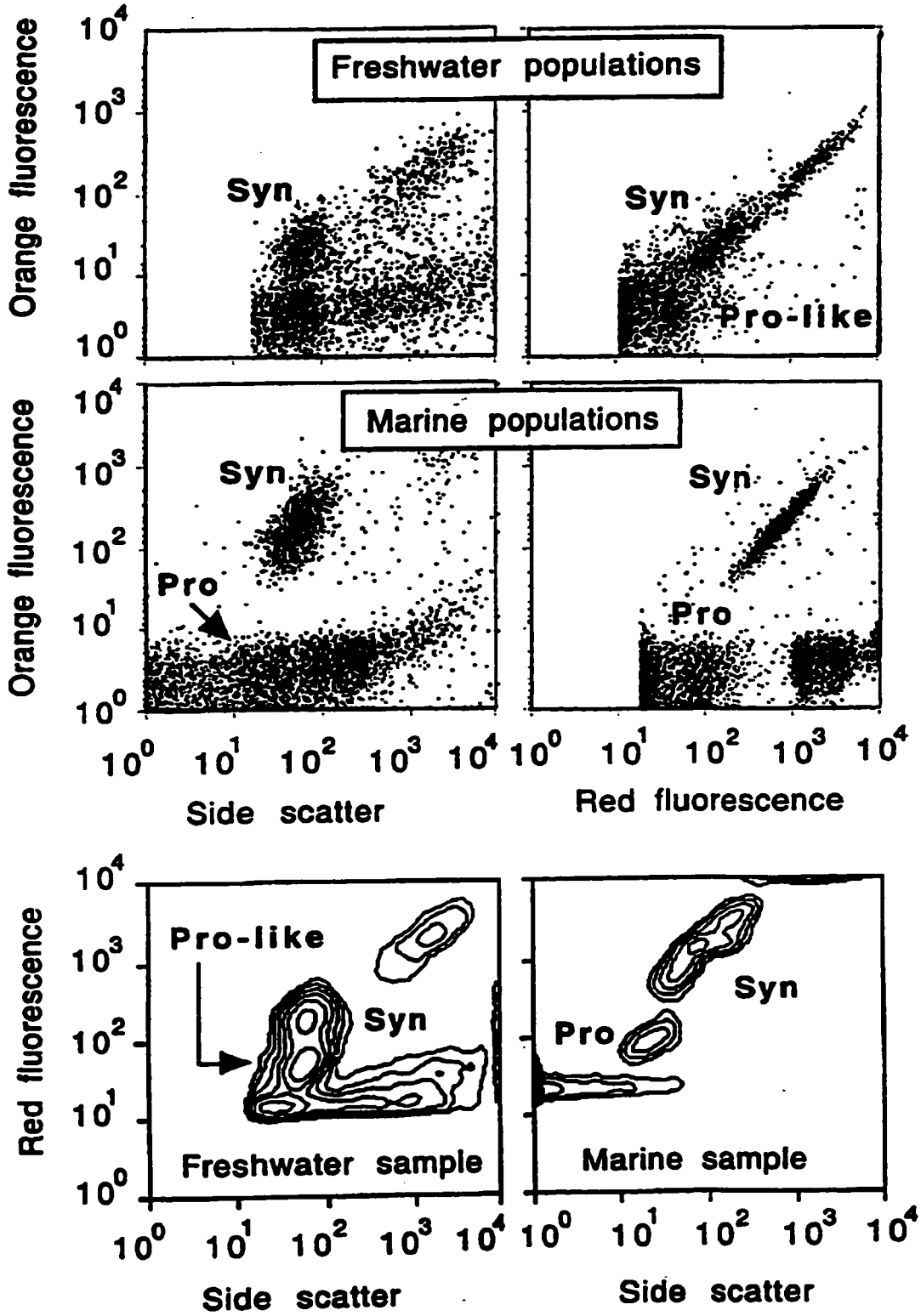
50- MacKay



Appendix IIB Picocyanobacteria (PC) abundance determined by flow cytometry and epifluorescence microscopy for 4 lakes sampled during summer 2000, from inhibitory incubations with ampicillin (A: ampicillin; C: control) as described in chapter 1. Counting of PC with epifluorescence microscopy was done with green filter which does not allow to distinguish the two types while with flow cytometry, distinction of phycoerythrin-rich and phycocyanin-rich PC was made. For each treatment, total PC abundance is the mean of three replicates with its standard deviation (SD).

		Flow cytometry				Epifluorescence	
Lake	Treatment	Phycoerythrin-rich ($10^4 \cdot ml^{-1}$)	Phycocyanin-rich ($10^4 \cdot ml^{-1}$)	PC total ($10^4 \cdot ml^{-1}$)	+/- SD ($10^4 \cdot ml^{-1}$)	PC total ($10^4 \cdot ml^{-1}$)	+/- SD ($10^4 \cdot ml^{-1}$)
Anne	C 0 h	2.50	0.71	3.21	0.02	13.26	3.50
	A 0 h	2.61	0.81	3.42	0.14	10.98	0.25
	C 24 h	2.69	0.89	3.58	0.19	14.37	0.77
	A 24 h	2.15	0.79	2.94	0.20	8.91	1.57
d'Argent	C 0 h	0.96	4.48	5.44	0.12	3.98	1.24
	A 0 h	0.98	4.20	5.18	0.24	2.73	0.27
	C 24 h	1.32	4.56	5.98	0.29	5.86	0.47
	A 24 h	1.01	3.02	4.03	0.61	2.41	0.15
MacKay	C 0 h	2.47	11.14	13.61	0.25	17.11	7.20
	A 0 h	2.31	10.90	13.21	0.46	18.06	2.92
	C 24 h	2.78	10.87	13.65	0.18	17.85	2.76
	A 24 h	2.31	7.79	10.10	0.68	10.45	2.68
Vert	C 0 h	4.10	0.08	4.18	0.26	8.90	0.36
	A 0 h	7.07	0.07	4.14	0.26	9.82	0.58
	C 24 h	4.97	0.14	5.11	0.20	10.20	0.41
	A 24 h	4.23	0.13	4.36	0.06	7.90	0.54

Appendix IIC Reprint of Figure 1 from Corzo et al. (1999). Orange fluorescence is for phycoerythrin (FL2) and red fluorescence is for chlorophyll *a* (FL3).



Appendix IID Reprint of Figure 2 from Collier (2000). PC/APC abbreviation is the for the fluorescence of phycocyanin and allophycocyanin, a pigment similar to phycocyanin but with slightly different fluorescence emission wavelengths. Events were identified as phycoerythrin-rich PC (red), phycocyanin-rich PC (blue), eukaryotes (black) and background noise (yellow). Water samples were analysed with a FACS Calibur (Becton Dickinson).