Titre: Title:	Establishment of an early warning system for cyanobacteria using an online multi-probe system measuring physicochemical parameters, chlorophyll and phycocyanin
Auteur: Author:	Natasha McQuaid
Date:	2009
Type:	Mémoire ou thèse / Dissertation or Thesis
Référence: Citation:	McQuaid, N. (2009). Establishment of an early warning system for cyanobacteria using an online multi-probe system measuring physicochemical parameters, chlorophyll and phycocyanin [Mémoire de maîtrise, École Polytechnique de Montréal]. PolyPublie. https://publications.polymtl.ca/8492/

URL de PolyPublie: PolyPublie URL:	https://publications.polymtl.ca/8492/
Directeurs de recherche: Advisors:	Sarah Dorner, & Michèle Prévost
Programme: Program:	Non spécifié

UNIVERSITÉ DE MONTRÉAL

ESTABLISHMENT OF AN EARLY WARNING SYSTEM FOR CYANOBACTERIA USING AN ONLINE MULTI-PROBE SYSTEM MEASURING PHYSICOCHEMICAL PARAMETERS, CHLOROPHYLL AND PHYCOCYANIN

NATASHA MCQUAID
DÉPARTEMENT DES GÉNIES CIVIL, GÉOLOGIQUE ET DES MINES
ÉCOLE POLYTECHNIQUE DE MONTRÉAL

MÉMOIRE PRÉSENTÉ EN VUE DE L'OBTENTION
DU DIPLÔME DE MAÎTRISE ÈS SCIENCES APPLIQUÉES
(GÉNIE CIVIL)
JUILLET 2009

Your file Votre référence
ISBN: 978-0-494-57260-3
Our file Notre référence
ISBN: 978-0-494-57260-3

UNIVERSITÉ DE MONTRÉAL

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

Ce mémoire intitulé:

ESTABLISHMENT OF AN EARLY WARNING SYSTEM FOR CYANOBACTERIA USING AN ONLINE MULTI-PROBE SYSTEM MEASURING PHYSICOCHEMICAL PARAMETERS, CHLOROPHYLL AND PHYCOCYANIN

présenté par: MCQUAID Natasha

en vue de l'obtention du diplôme de: Maîtrise ès sciences appliquées

a été dûment accepté par le jury d'examen constitué de :

M. BARBEAU Benoit, Ph.D., président

Mme DORNER Sarah, Ph.D., membre et directrice de recherche

Mme PRÉVOST Michèle, Ph.D., membre et codirectrice de recherche

Mme HUDON Christiane, Ph.D, membre

Dédicace

For Gera,

Acknowledgments

I would like to thank my director Sarah Dorner for her valuable guidance, her accessibility, and for entrusting me with this project. I am also grateful for the direction I received from my co-director Michèle Prévost. I must thank Yves Fontaine, Mireille Blais, Jacinthe Mailly and Julie Philibert for always making time to help me with my field and laboratory work. I would also like to recognize the work of the Centre d'Expertise en Analyses Environmentales du Québec (CEAEQ) in Quebec's Ministry of the Environment and Serge Paquet from Université du Québec à Montréal's (UQAM) Biological Sciences department whose work was invaluable to the project. I would like to thank the managers of the drinking water treatment plants for permitting us to sample their source waters. It is essential that I thank Arash Zamyadi, without whom I would never have accomplished what I have with this project. I would like to thank my family for their infallible support of almost everything I do. Finally, I am grateful for all of my friends at the Drinking Water Industrial Chair who are generous friends and good pep-talkers.

Abstract

An online multi-probe system (MPS) measuring *in vivo* phycocyanin (PC) fluorescence (a pigment specific to fresh water cyanobacteria), chlorophyll fluorescence, pH, dissolved oxygen, temperature, specific conductivity and turbidity was used to monitor cyanobacteria in the raw water and source water of two drinking water treatment plants (DWTP) in Quebec. The goal of this study was to validate the use of *in vivo* PC fluorescence in laboratory and environmental conditions and to use these results to propose an online alert system to monitor potentially toxic cyanobacteria in the raw water of DWTPs in Quebec.

Sampling was conducted in 2007 and 2008 in the raw water (before treatment) and source water (water above and surrounding the intake) of DWTPs on Missisquoi Bay and the Yamaska Reservoir. Potentially toxic cyanobacteria were monitored using both conventional laboratory methods (taxonomic analysis, pigment extraction and toxin analysis) and a novel online *in vivo* PC fluorescence probe.

The estimations of cyanobacterial density generated by the internal calibration of the *in vivo* PC fluorescence probe were inaccurate in both laboratory and environmental conditions. The use of the PC probe's generic, and therefore standardized, ratio fluorescent units (RFU) are instead suggested for the establishment of an alert system for cyanobacterial monitoring in Quebec.

High and significant correlations were derived between online *in vivo* PC fluorescence (measured by the PC probe on the MPS) and all measured parameters measured from grab samples. However, cyanobacterial biovolume was retained as the best option for a locally adapted alert level framework.

The results of the monitoring campaigns yielded observations of large spatio-temporal variations of cyanobacterial abundance, cyanobacterial species and cyanotoxin

concentrations in both the raw and source water of the DWTPs. In 2008, despite the measurement of highly toxic cyanobacterial blooms in the source water of both DWTPs, high cyanobacterial biovolumes and cyanotoxin concentrations were only present in the raw water of the DWTP on Missisquoi Bay. At the Yamaska Reservoir, high cyanobacterial biovolumes and cyanotoxin concentrations were observed at the surface and on the shore of the DWTP's source water, but low cyanobacterial biovolumes and cyanotoxin concentrations were measured in the plant's raw water.

In 2008, a multiple linear regression analysis of the parameters measured by the MPS determined that turbidity and ΔpH (the 24h change in pH) at Missisquoi Bay best predicted *in vivo* PC fluorescence. A multiple linear regression analysis at the Yamaska Reservoir deemed specific conductivity and dissolved oxygen as the best predictors of *in vivo* PC fluorescence. All predictive parameters can be easily monitored by DWTPs vulnerable to cyanobacterial blooms in their source water. This could allow DWTP operators to be better prepared to treat influxes of cyanobacteria cells and cyanotoxins.

High and significant correlations were derived between the maximum potential microcystin concentration (MPMC) and cyanobacterial biovolume (both measured in grab samples). The results of *in vivo* PC fluorescence validation were used to determine Alert Level Thresholds (ALT) for cyanobacterial monitoring at both monitored DWTPs.

The use of online *in vivo* PC fluorescence to monitor ALT could allow DWTP operators to quickly adjust their treatment to increases of potentially toxic cyanobacteria in the raw water of their DWTP.

The presented Alert Level Threshold values, as well as the presented predictive values of *in vivo* PC fluorescence were determined specifically for the two monitored DWTP but the results upon validation of the methodology can be applied to other regions.

Résumé

La multisonde (MS) de la compagnie YSI mesure la fluorescence *in vivo* de la phycocyanine (PC) (le pigment particulier aux cyanobactéries), la fluorescence *in vivo* de la chlorophylle, la turbidité, le pH, l'oxygène dissous, la température, et la conductivité spécifique. La MS a été utilisée pour faire un suivi des cyanobactéries potentiellement toxiques dans l'eau brute, et l'eau à la source de deux usines d'eau potable au Québec. Le but de l'étude était de valider la sonde fluorimétrique PC *in vivo* en laboratoire et en conditions environnementales, et à partir de ces résultats, de créer un système d'alarme en ligne, pour la surveillance des cyanobactéries dans l'eau brute des usines d'eau potable québécoises.

Les estimations de la densité cyanobactérienne générées par la calibration interne de la sonde fluorimétrique PC étaient imprécises tant pour les travaux effectués en laboratoire que pour les conditions environnementales. Par contre, il y avait une corrélation forte et significative entre la mesure *in vivo* PC (mesurée avec la sonde fluorimétrique en ligne sur la MS) et le biovolume cyanobactérien (mesuré par des échantillons d'eau ponctuels). De plus, il y existait une corrélation forte et significative entre la concentration maximum potentielle de *microcystine-LR* et le biovolume cyanobactérien des échantillons pris de l'eau brute des deux usines.

En 2007 et 2008, une campagne d'échantillonnage a été réalisée dans l'eau brute et l'eau à la source de 2 usines de traitement d'eau potable situées à la baie Missisquoi et au réservoir Yamaska. Les cyanobactéries potentiellement toxiques ont été suivies par des méthodes courantes (analyses taxonomiques, extractions de pigment, analyses de toxicité) et avec une sonde fluorimétrique de PC.

Les résultats des campagnes d'échantillonnage ont montré une grande variation spatio-temporelle de l'abondance des cyanobactéries, de l'espèce dominante des

cyanobactéries, et de concentration des cyanotoxines dans l'eau brute et dans l'eau à la source des usines d'eau potable. En 2008, des biovolumes élevés de cyanobactéries, et des concentrations élevées de cyanotoxines ont été détectés dans l'eau brute de l'usine d'eau potable à la baie Missisquoi seulement, alors que des efflorescences denses et toxiques étaient présentes dans l'eau à la source des deux usines.

En 2008, une analyse de régression multiple linéaire a été réalisée sur les paramètres mesurés en continu sur la MS. Cette analyse a confirmé que la turbidité et les changements journaliers de pH sont les meilleurs paramètres de prédiction des valeurs générées par la sonde fluorimétrique de PC. Au réservoir Yamaska, la même analyse a déterminé que la conductivité spécifique et l'oxygène dissous pouvaient bien prédire les valeurs de la sonde fluorimétrique de PC. Tous ces paramètres peuvent être suivis par les opérateurs d'usines d'eau potable vulnérables à des efflorescences de cyanobactéries dans l'eau de leurs ressources. Ce suivi permettrait aux opérateurs d'être mieux préparés pour gérer l'occurrence de concentrations élevées de cellules et de toxines dans l'eau brute de l'usine.

Les résultats de la validation de la sonde PC en laboratoire et conditions environnementales ont été utilisés pour proposer des seuils d'alarme pour la surveillance des cyanobactéries aux deux usines d'eau potable. L'utilisation de la fluorescence *in vivo* PC pour surveiller les seuils d'alarme aux usines d'eau potable pourrait aider les opérateurs à mieux gérer leur traitement d'eau en fonction de la quantité de cyanobactéries dans l'eau brute. Les seuils d'alarmes d'UFR et les paramètres prédictifs présentés ont été conçus pour les deux usines d'eau potable suivies, mais ils peuvent aussi être appliqués dans d'autres usines d'eau potable au Québec.

Condensé en français

Des sources d'eau potable sont plus vulnérables à la prolifération de cyanobactéries s'ils sont touchés par l'eutrophisation anthropogénique (Sivonen et al. 1999), des températures d'eau élevées et la stagnation de l'eau (Mur et al. 1999; Davis et al. 2006; Lehman et al. 2008). Les cyanobactéries sont plus compétitives que d'autres types de phytoplancton pour stocker le phosphore (Fogg et al. 1973) et ont un accès plus facile aux nutriments dans l'eau (Oliver et al. 2000), à la lumière du soleil (Lavoie et al. 2007) et au charbon inorganique (Pick et al. 1987). Les conditions dans lesquelles les cyanobactéries produisent des cyanotoxines ne sont pas bien comprises(Giani et al. 2005). Les façons les plus fréquentes que les humains sont exposées aux cyanotoxines sont la baignade et la consommation d'eau contaminée par des cyanotoxines. Ce contact peut avoir des conséquences variées en fonction de la dose, et le type de toxine (Yu 1995; Carmichael 2001; Carmichael et al. 2001).

Pour suivre les recommandations de l'Organism Mondial de la Santé (OMS) sur le suivi des cyanobactéries dans les usines d'eau potable, des analyses fastidieuses et couteuses faites par du personnel hautement qualifié sont nécessaires. Pour cette raison, les opérateurs d'usine d'eau potable ne peuvent pas prendre des décisions rapides et informées pour traiter des cyanobactéries potentiellement toxiques dans l'eau brute de leurs usines. Deux études ont surveillé les seuils d'alertes de l'OMS pour les cyanobactéries avec la fluorescence in vivo de phycocyanine (PC) (au lieu des méthodes courantes) (Ahn et al. 2007; Izydorczyk et al. 2009). La fluorescence *in vivo* du PC est une méthode précise et rapide pour détecter des cyanobactéries dans leurs environnements naturels (Yentsch et al. 1979). Par contre, la précision des résultats générés par cette méthode dépend de la qualité de l'appareil utilisé (Millie et al. 2002).

Objectif

L'objectif de cette étude est d'utiliser une multisonde (MS), incluant une sonde fluorimétrique de PC, pour établir un système d'alarme pour les cyanobactéries potentiellement toxiques dans l'eau brute des usines d'eau potable au Québec.

Matériel et Méthodes

Deux usines d'eau potable dans le sud du Québec, Canada, ont été suivies durant l'été et l'automne de 2007 et 2008. Une multisonde (MS) 6600V2-4 de YSI (YSI, Yellow Springs, Ohio) a été installé au réservoir Yamaska en 2007 entre la rivière et le réservoir Yamaska (le réservoir alimente une usine d'eau potable). En 2008, la MS a été installée à l'intérieur de l'usine sur le réservoir pour mesurer l'eau brute entre juillet et octobre 2008. L'eau de la ressource a été suivie en continu par une MS attachée à une bouée et suspendue au-dessus de la prise d'eau de l'usine située au fond du réservoir. L'eau brute de l'usine sur la baie Missisquoi a été mesurée avec la MS en été et automne de 2007 et 2008. En 2008 seulement, des profils de profondeur ont été documentés deux fois par semaine au-dessus de la prise deux de l'usine avec la MS.

La MS a huit sondes : la turbidité, le pH, l'oxygène dissous, la température, la conductivité spécifique, la profondeur, la chlorophylle (fluorescence), et la phycocyanine (fluorescence). Le MS a pris et enregistré une lecture toutes les 30 minutes et les données ont été téléchargées deux fois par semaine. Des échantillons d'eau ont été récupérés en même temps que le téléchargement de donnée ou les profils de profondeur. Les échantillons étaient pris deux fois par semaine pour valider les lectures de phycocyanine (PC) et chlorophylle sur la MS. Des analyses taxonomiques ont été faites sur des échantillons conservés au lugol, et des analyses pour mesurer la concentration de microcystine-LR avec des kits ELISA (Abraxis, Warminster, Pennsylvania, USA) ont été faites sur des échantillons gelés. La chlorophylle a été

extraite avec de l'éthanol chaud et la phycocyanine avec une solution de phosphate de sodium. Les deux extractions ont été lues au spectrophotomètre.

Cinq suspensions de différentes densités de *M. aeruginosa* ont été préparées dans cinq seaux. La sonde PC a été calibrée avec un point. Toutes les sondes sur la MS ont pris des mesures en parallèle à un intervalle de 0.5 seconde durant l'essai. Des échantillons en triplicata ont été pris et fixés avec le lugol pour des analyses taxonomiques. Des extractions de chl-*a* et de PC ont été faits sur les cinq suspensions. Des régressions multiples linéaires ont été faites sur les données de la saison de chaque MS.

Résultats et Discussion

Dynamiques de phytoplancton – 2008

Des échantillons d'eau pris à la baie Missisquoi et au réservoir Yamaska ont démontré une grande variation spatio-temporelle de la composition du phytoplancton en 2008. Dans l'eau brute de l'usine sur le réservoir Yamaska, les cyanobactéries potentiellement toxiques n'ont jamais dominé le phytoplancton, par contre, ils ont dominé le phytoplancton dans cinq échantillons recueillis dans l'eau brute de l'usine sur la baie Missisquoi.

La variation spatiale aux deux sites aurait pu être due aux variations phytoplanctoniques diurnes dans la colonne d'eau, l'état physique de la colonne d'eau (mélangées par vagues ou calme), et le moment dans le cycle de vie d'une efflorescence de cyanobactéries. En 2008, il y avait un risque plus élevé de contamination de l'eau brute par des cyanotoxines à la baie Missisquoi qu'au réservoir Yamaska.

Dynamiques cyanobactériennes 2007-2008

En 2008, sur la même journée, mais à des endroits différents sur la baie Missisquoi, il y avait de grandes variations d'espèces cyanobactériennes, de concentration de cyanotoxines, et d'abondance de biovolumes cyanobactériens dans les échantillons d'eau recueillis. De plus, une variation importante interannuelle d'abondance cyanobactérienne et d'espèces dominantes de cyanobactéries a été mesurée à la baie Missisquoi. Les résultats de la sonde PC *in vivo* ont bien interprété les variations de biovolume total de cyanobactéries mesurées dans les échantillons d'eau prises avant, durant et après les efflorescences de cyanobactéries dans la baie Missisquoi.

Les lectures de la sonde PC et les résultats des analyses sur les échantillons d'eau prises en 2007 et 2008 ont indiqué que les efflorescences de cyanobactéries n'ont pas influencé la qualité de l'eau à l'entrée de l'usine sur le réservoir Yamaska. La qualité d'eau de l'usine était bonne même quand il y avait des accumulations denses et toxiques de cyanobactéries dans le réservoir.

En 2008, la distribution de la concentration de la cyanotoxines MC-LC dans l'eau brute de l'usine sur le réservoir Yamaska était beaucoup plus faible que dans l'eau brute de l'usine sur la baie Missisquoi.

La validation en laboratoire et en condition environnementale de la sonde PC sur la MS.

Les résultats des essais de calibration ont démontré une corrélation forte et significative entre les vrais comptes taxonomiques et les estimations de la sonde PC. La relation entre les deux variables était linéaire, mais les estimations de la densité cyanobactérienne générées par la sonde PC étaient 20 % plus faibles que les vrais comptes microscopiques.

En 2007 et 2008, aux deux sites d'échantillonnage, les vrais comptes microscopiques étaient plus élevés que les estimations générées par la sonde PC. Les estimations de cyanobactéries (cellules/mL) générées par la sonde PC avaient mal interprété la densité réelle de cyanobactéries puisqu'il y avait une abondance de très petites cyanobactéries dans l'eau des deux sites.

Corrélation entre la fluorescence *in vivo* de PC et les 4 paramètres de seuils d'alarme (SA) pour surveiller les cyanobactéries en laboratoire et en condition environnementale.

Toutes les corrélations calculées entre la sonde PC et les paramètres de SA étaient plus fortes en condition de laboratoire qu'en condition environnementale. Parmi les analyses faites en condition environnementale, il y avait une meilleure corrélation entre les mesures de la sonde PC et le biovolume cyanobactérien (mm^3/L) qu'entre la sonde PC et la densité cyanobactérienne (cellules/mL). La densité cyanobactérienne avait la pire corrélation avec les mesures de la sonde PC en conditions environnementales. Dans les essais en laboratoires et en conditions environnementales, les corrélations entre la sonde PC et les extractions de PC (µg/L) étaient fortes. Les corrélations entre la sonde PC et les extractions de chl-*a* (µg/L) en laboratoire et en condition environnementaux étaient les plus forts de tous les paramètres SA mesurée.

Dans l'eau brute des deux usines suivies, le biovolume cyanobactérien est les extractions de chl-*a* étaient les deux paramètres les plus fortement corrélés avec les donnés de la sonde PC. Ces deux paramètres ont été fortement considérés pour être la base d'un système d'alarme pour les cyanobactéries aux usines d'eau potable au Québec.

Sélection d'un paramètre pour indiquer le risque de contamination de MC-LR avec la sonde PC aux usines d'eau potable au Québec.

En 2008, au réservoir Yamaska, il y avait peu de variabilité des niveaux d'alerte de l'OMS dans lesquels les échantillons d'eau se situaient. Tous les échantillons étaient considérés « Niveau 1 » pour tous les paramètres pour évaluer.

En 2008, dans l'eau brute de l'usine sur la baie Missisquoi, le risque d'occurrence de cyanotoxines sur les journées avec des densités importantes de petites cyanobactéries était surestimé. La densité cyanobactérienne avait une corrélation modérée avec les concentrations de MC-LR dans les échantillons d'eau pris à l'eau brute de l'usine. La densité cyanobactérienne est un paramètre peu fiable parce qu'il y a un changement important interannuel des espèces cyanobactériennes à la baie Missisquoi et une grande variation de volume cellulaire des cyanobactéries identifié dans ces échantillons.

Dans les échantillons d'eau pris dans l'eau brute de l'usine sur la baie Missisquoi, les extractions de PC (µg/L) ont corrélé avec les concentrations mesurées de MC-LR faiblement. Les extractions PC étaient aussi faiblement corrélées avec les lectures de la sonde PC dans l'eau brute de l'usine, donc les extractions de PC n'étaient pas assez fiables pour être utilisées pour un système d'alarme pour la détection de cyanobactéries à la baie Missisquoi.

Les extractions de chl-*a* (µg/L) étaient interprétées par la sonde PC plus facilement que tous les autres paramètres mesurés, mais ils avaient une corrélation médiocre avec les concentrations de MC-LR présentes. Puisque la corrélation avec les cyanotoxines est l'élément le plus important pour les usines l'eau potable donc, la chl-*a* n'est pas le paramètre SA idéal.

Le biovolume cyanobactérien avait la meilleure corrélation avec la concentration de MC-LR dans les échantillons pris dans l'eau brute de l'usine sur la baie Missisquoi. Le biovolume cyanobactérien avait aussi une bonne corrélation avec les lectures de la sonde PC dans l'eau brute de l'usine. De ce fait, le biovolume cyanobactérien est le meilleur paramètre pour utiliser comme base pour un système d'alerte contre les cyanobactéries dans l'eau brute des usines d'eau potable.

Proposition d'un système d'alerte pour les deux usines d'eau potable suivies.

Basé sur les échantillons pris dans l'eau brute de l'usine sur la baie Missisquoi en 2008, les valeurs de la sonde PC qui représentaient les seuils pour les niveaux d'alerte de biovolume cyanobactérien de l'OMS étaient : 0.1 RFU et 4.1 RFU. Les seuils d'alerte pourraient être utilisés pour suivre les cyanobactéries par les opérateurs de l'usine sur la baie Missisquoi en 2009.

En 2007, une analyse régression multiple linéaire sur les données générées par la MS à la baie Missisquoi a montré que l'oxygène dissous, la turbidité et le ΔpH étaient les paramètres qui prédisaient mieux les lectures de la sonde PC. En 2008, la même analyse au même site a déterminé que seulement la turbidité et le ΔpH était les facteurs qui corrélaient mieux avec la sonde PC.

La conductivité spécifique et l'oxygène dissous étaient les paramètres qui corrélaient mieux avec les données de la sonde PC à l'usine sur le réservoir Yamaska.

Conclusions

Des échantillons d'eau pris à la baie Missisquoi et au réservoir Yamaska ont démontré une grande variation spatio-temporelle de la composition du phytoplancton en 2008. En 2008, il y avait un risque plus élevé de contamination de l'eau brute par des

cyanotoxines à la baie Missisquoi qu'au réservoir Yamaska. En 2007 et 2008, aux deux sites d'échantillonnage, les vrais comptes microscopiques étaient plus élevés que les estimations générées par la sonde PC.

Le biovolume cyanobactérien est le meilleur paramètre pour utiliser comme base pour un système d'alerte contre les cyanobactéries dans l'eau brute des usines d'eau potable. Les valeurs de la sonde PC qui représentaient les seuils pour les niveaux d'alerte de biovolume cyanobactérien l'OMS étaient : 0.1 RFU et 4.1 RFU. À la baie Missisquoi en 2008, la turbidité et le ΔpH étaient les paramètres qui prédisaient mieux les lectures de la sonde PC dans une analyse de régression multiple linéaire.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1 Introduction

Expanding human populations are having adverse and accelerated impacts on limited sources of fresh water all over the world. Several countries and regions have reported that severe anthropogenic eutrophication of their fresh water sources has made them vulnerable to toxic cyanobacterial blooms (Galvez et al. 2003; Davis et al. 2006; Levi et al. 2006). The World Health Organization (WHO) considers that toxic cyanobacterial blooms are ubiquitous (Sivonen et al. 1999). Exposure to cyanotoxins exerts consequences on human health ranging from mild skin irritations to fatality, depending on the type of toxin, the method of exposure, and the concentration of the toxin (Carmichael et al. 2001; Chorus et al. 2001).

1.1. Guidelines and recommendations for cyanobacteria and cyanotoxins in drinking water

Action is being taken to avoid the potential health problems which could arise from cyanotoxin concentrations in treated drinking water. In 1999, the World Health Organization (WHO) established guidelines to help monitor cyanobacteria in drinking water treatment plants (DWTP) (Bartram et al. 1999). In addition to the WHO's recommended values, countries such as Brazil, New Zealand, Australia, and Canada have all adopted recommendations for the maximum acceptable concentrations of cyanotoxins in treated drinking water. The United States has also put cyanotoxins on their "Contaminant Candidate List" (AWWARF 2006). In, Canada, the Quebec Ministry of the Environment, Sustainable Development and Parcs adopted a ten year plan (2007-2017) to better understand potentially toxic cyanobacteria, to take actions to prevent their proliferation, and to manage affected water bodies (MDDEP 2007).

All guidelines and recommendations are based on conventional cyanobacterial monitoring methods: taxonomic counts and species identification, pigment extraction and measurement, and cyanotoxin identification and quantification. These methods

are precise, but also costly, slow, and require highly trained personnel as well as expensive equipment. Consequently, these monitoring methods are maladapted to the immediate decision making needs of drinking water treatment plants (DWTP) susceptible to toxic cyanobacterial development in their source waters. This delay leaves the communities served by the DWTP vulnerable to cyanotoxin contamination in the event of inadequate water treatment.

1.2 Cyberinfrastructure for environmental monitoring

Cyberinfrastructure is an organized comprehensive collection of information technologies (computers, storage, data, networks, scientific instruments) which can be coordinated to continuously monitor environmental parameters (Kido et al. 2008). Novel online cyanobacterial monitoring tools which could be integrated into new or existing cyberinfrastructure for environmental monitoring could complement conventional monitoring methods. This type of infrastructure is used for interdisciplinary, long-term scientific enquiries (Harward et al. 2008) and reduces problems often encountered during conventional environmental monitoring such as time limitations, geographical restrictions, and individual capabilities (Berman 2008).

This study assesses the validity of a monitoring tool which could be integrated into an eventual province-wide cyberinfrastructure network for cyanobacterial monitoring. The monitoring tool used in this research is a submersible *in vivo* phycocyanin fluorescence probe which estimates cyanobacterial abundance *in situ*. This technology has been used in recent studies to measure cyanobacterial abundance at the entrance of DWTPs (Ahn et al. 2007; Izydorczyk et al. 2009).

1.3 Project description

This study aims to determine le validity of tools for the eventual establishment of an alert system for monitoring cyanobacteria using a novel online submersible multi-

probe system (MPS) which measures physico-chemical water parameters in parallel with chlorophyll and phycocyanin fluorescence. The continuous monitoring of many bloom-related factors provides an opportunity to better understand cyanobacterial blooms, and environmental conditions leading to a bloom. Ultimately, such a tool could help ensure the maintenance of a safe drinking water supply in the event of cyanobacterial proliferation in a DWTP's raw water.

This research is part of a study funded by the Fonds québécois de recherche sur la nature et les technologies and aims to 1) validate the use of an *in vivo* phycocyanin fluorescence probe to monitor cyanobacteria, and 2) test the *in vivo* phycocyanin probe at two DWTP's in Quebec and 3) establish an alert system for potentially toxic cyanobacteria at DWTPs in Quebec using a novel multi-probe system.

Chapter 2 Critical Literature Review

2.1 Eutrophication and its consequences

Eutrophication is caused by the presence of excessive nutrients such as nitrogen (N) and phosphorous (P) in a water body, leading to an increase in plant production. Bacterial degradation of decaying plant biomass results in depleted dissolved oxygen concentration, which can provoke fish mortality, and the release of toxic substances and phosphates from sediments. A release of phosphate from sediments closes a positive feedback loop, increasing eutrophication and biomass growth and decreasing dissolved oxygen concentration (Janus et al. 1981b). Eutrophication was recognized as a source of pollution in the middle of the twentieth century and since then the phenomenon has become more prevalent, compromising the quality of drinking water supply, as well as recreational activities (Bartram et al. 1999) .

Since phosphorous is the limiting nutrient for plant, algae and cyanobacteria growth in freshwater aquatic ecosystems (Lavoie et al. 2007) it is used for categorizing the trophic levels of water bodies. Even if it is not possible to set firm boundary values between trophic groups, a water body's level of eutrophication is categorized according to its total phosphorous and/or chlorophyll *a* concentrations (see Table 2-1).

Table 2-1. Trophic Categories. Adapted from (Janus et al. 1981a) and (Lavoie et al. 2007)

Trophic Category	P Conc	Chl *a* Conc.
Oligotrophic	< 10 µg P L^{-1}	5 µg Chl*a* L^{-1}
Mesotrophic	10-30 µg P L^{-1}	5-16 µg Chl*a* L^{-1}
Eutrophic	30-100 µg P L^{-1}	16-25 µg Chl*a* L^{-1}
Hypertrophic	> 100 µg P L^{-1}	>25 µg Chl*a* L^{-1}

Eutrophication is a natural process; however, it can be accelerated by human activities such as sewage discharges and poor agricultural practices which can lead to excessive runoff of nitrogen and phosphorous rich fertilizer into adjacent waters. Natural events such as flooding, intense rain storms or rapid snow melt which transports eroded nutrient rich soils into receiving waters (Janus et al. 1981b). Yet, it is important to realize that even though anthropogenic eutrophication creates favorable conditions for cyanobacterial proliferation, dense toxic blooms have been documented in pristine, or near-pristine, watersheds in Australia and Switzerland (Sivonen et al. 1999).

2.2 Occurrence of cyanobacteria blooms worldwide

A cyanobacterial bloom is an excessive proliferation of cyanobacteria which can be seen with the naked eye, and cyanobacterial scum is a very dense accumulation at the surface of a lake, river or reservoir (Blais 2007). It is important to note the distinction between eutrophication, which is a long term state, and a bloom event which generally lasts only a few days or weeks.

Toxic cyanobacterial blooms are not isolated to one region or country; freshwater toxic blooms have been reported in 45 countries (Codd et al. 2005) and surveys carried out by the World Health Organization indicate that toxic blooms, particularly microcystin producing cyanobacteria, are ubiquitous (Sivonen et al. 1999).

The situation in Southern Quebec

In Quebec, the documented number of lakes which have had at least one cyanobacterial bloom greater than or equal to 20 000 cells/ml has increased from 34 in 2004 to 108 in 2008 (MDDEP 2008a). This increase in bloom events prompted the Ministry of Sustainable Development, the Environment and Parks of Quebec to initiate a program to monitor toxic cyanobacteria in treated and raw water of six drinking water plants (Robert 2008). The Ministry has also published an action plan for 2007-

2017 to deal with current issues and to research practical solutions to prevent an aggravation of the problem.

2.3 Cyanobacteria and cyanotoxins

Cyanobacteria, unlike green algae, are prokaryotic organisms meaning that their cellular structure is more similar to bacteria than to other types of eukaryotic algae. However, like other types of algae, they are photoautotrophs with two photosystems (PSI and PSII) located in their thykaloid membrane. This thykaloid membrane houses the reaction centers, where the PSI, PSII and the Terminal Emitter Pigments receive light energy and use it to fix CO_2 to provide energy for the cell (Fogg et al. 1973). Even though eukaryotic algae photosystems are similar to those in cyanobacteria, the light harvesting complexes (LHC) or antenna pigments, which absorb sunlight, are very different. The LHC in green algae are chl *b* and *c* and are integrated into the algae's thykaloid membrane, however cyanobacterial LHC are phycobilisomes which are attached to the periphery of the thykaloid membrane (Figure 2.1). The three existing types of phycobilisome pigments are phycocyanin (PC) and allophycocyanin (APC) in blue-green cyanobacteria, and phycoerythrin (PE) in red cyanobacteria (Oliver et al. 2000).

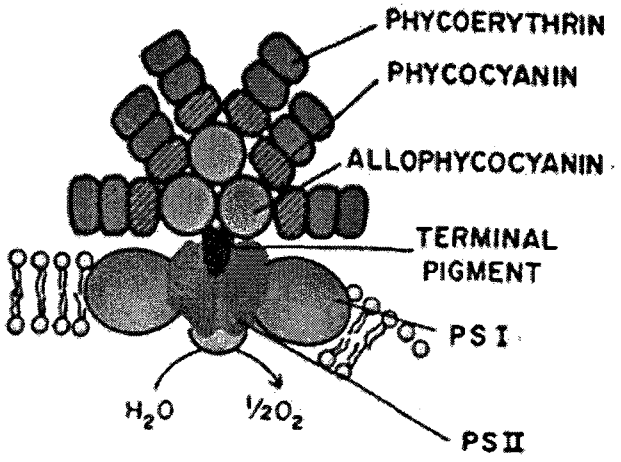


Figure 2.1 Schematic LHC in cyanobacteria: (A) hemidiscoidal phycobilisome. Adapted from (Beutler 2003) Note that generally, cyanobacteria found in freshwater have a similar structure but without phycoerythrin.

Cyanobacteria are primitive organisms which have strong competitive advantages over other phytoplankton not the least of which is their ability to change their environment to their advantage (Lavoie et al. 2007). In bloom conditions, cyanobacteria reduce the concentration of CO_2 in water because of its use for photosynthesis, therefore increasing the water's pH (Shapiro 1997). This creates a limiting condition for the growth of most algae, yet cyanobacteria may be able to use bicarbonate ions as well as CO_2 as sources of inorganic carbon making cyanobacteria more competitive at high pH values (Pick et al. 1987). Increasing pH values are also associated with the decomposition of cyanobacterial blooms and their associated production of ammonia (Blais 2002).

Cyanobacteria are also better adapted for accessing nutrients and sunlight than other phytoplankton. Some cyanobacterial species have gas vacuoles within their cell wall allowing them to migrate vertically throughout the water column to better access light at the surface and nutrients released by sediments (Lavoie et al. 2007). Cyanobacteria can also accumulate and store more phosphorous (luxury consumption) in the form of polyphosphate than other microalgae within their cell wall for subsequent growth in phosphorous depleted water conditions (Fogg et al. 1973). Some species can fix N_2

from the atmosphere, and most can store N for later use. They are the only known micro-algae able to access N in these ways, and are therefore at a significant competitive advantage when sources of inorganic nitrogen are low (Oliver et al. 2000). Yet, despite all of their competitive advantages, cyanobacteria do not usually dominate the water bodies all year long because they do not thrive in nutrient deficient conditions nor in cold, or rapidly flowing, water (Lavoie et al. 2007).

Cyanotoxins are generally sorted into three categories corresponding to what they damage: hepatoxins (liver), neurotoxins (nervous system) and dermatoxins (skin).
As many as 46 species of cyanobacteria have been known to produce toxins (Ernst et al. 2005), but processes and conditions which activate their production are not fully understood. One hypothesis is that toxin production increases cyanobacteria's competitive advantage (Codd 1995). Researchers have documented that population dynamics (Giani et al. 2005), their stage of growth (Sivonen et al. 1999), high nutrient concentrations (Rantala et al. 2006), Photosynthetically Active Radiation (PAR), water temperature (Albay et al. 2005), or combinations of these factors, can induce microcystin toxin production. However due to the large number of species and variants within species (80 known strains of the hepatoxin microcystin alone (Funari et al. 2008)) scientists have not yet been able to pinpoint the precise reasons for toxin production with certainty.

2.4 Triggers of cyanobacteria blooms

The development of a potentially toxic cyanobacteria bloom is a complex process which can be triggered by a combination of environmental conditions. As previously mentioned, one key trigger of cyanobacterial development is anthropogenic eutrophication and research has been done regarding the connection between land use and bloom events. For example, a study by (Burford et al. 2007) compares lakes in watersheds with varying degrees of forested land. In watersheds with mostly

agricultural and urban land use there was a higher frequency and magnitude of toxic cyanobacteria blooms than in watersheds with more forest cover. Another study used a water quality model to show the correlation between external nutrient inputs from agricultural land and recurrent cyanobacterial blooms (Burger et al. 2007).

During eutrophication, nutrient increases are often important triggers of bloom development. When P is available to cyanobacteria in as little as 1-2 μg/L their growth is no longer limited by the nutrient (Mur et al. 1999). Studies have shown external P loading can come from runoff from agricultural or livestock land uses (Zhang et al. 2004; Iitala et al. 2005; Iqbal et al. 2006), or from inadequate sewage treatment (Sivonen et al. 1999). Phosphorous in sediments accumulated at the bottom of lakes and reservoirs can be released into the water column for years after their initial deposit depending on the water temperature, the degree of sediment disturbance, the mixing conditions, and the sediment chemistry of a particular lake or reservoir (Mur et al. 1999; Chowdhury et al. 2006). In the event of very high P concentrations, total nitrogen will often become the limiting factor for CB abundance (Davis et al. 2006; Wongsai et al. 2007). Nitrogen has been shown to be the most limiting nutrient in coastal or marine ecosystems (Quibliera et al. 2008). Rainfall, and its associated nutrient inputs, has also been suggested as a condition which favors cyanobacteria abundance (Ahn et al. 2002).

However, nutrients can play a secondary role to low stream flow, high water temperature, thermal stratification, and light penetration in triggering cyanobacterial blooms when the level of eutrophication is very serious (Davis et al. 2006; Lehman et al. 2008). High pH and low dissolved oxygen have also been correlated with high densities of cyanobacteria (Wongsai et al. 2007).

Climate change, with the dual effect of a global temperature increase as well as an increase in the frequency and intensity of precipitation events, could also become a major driver for cyanobacteria blooms in the future. In Quebec, cyanobacteria

contribute to the deterioration of the quality of drinking and recreation sources of water by more frequent or intense blooms provoked by climate change (Bourque et al. 2008; Ministère du Développement Durable 2008).

2.5 Cyanotoxin provoked health problems

The occurrence of cyanobacteria and their associated toxins can range from a mild annoyance to a potential danger to humans and animals in contact with affected waters. These organisms can cause inconveniences such as the development of a murky water color and surface scum, as well as, unappealing tastes and odors compounds such as geosmin or methyl isoborneol (MIB) which can make the production of potable water challenging (Izaguirre et al. 2004).

The three most common ways humans are exposed to cyanotoxins are: 1) oral and dermatological exposure through the recreational use of water, 2) the consumption of contaminated drinking water or health food products, or 3) inhalation while in the shower (Carmichael 2001). Toxins release from dying cells into the water column near a treatment plant's water intake could also increase the risk of drinking water contamination (Jones et al. 1994). The presence of cyanobacterial cells and toxins increases the stress on the drinking water treatment plant's (DWTP) normal treatment.

Table 2-2 Health risks associated with cyanotoxin microcystin (Falconer et al. 1999)

Cyanobacteria/mL	Potential microcystin conc.	Health Risks
20, 000	**2-4 µg/L(10 µg/L possible)**	**Gastrointestinal and skin irritations**
100, 000	**10-20 µg/L (50 µg/L possible)**	**Lung and liver disease**
Scum	**1 mg/L**	**Possible poisoning**

There have been many documented incidents of illness as a result of exposure to cyanotoxins (Appendix B), but arguably the most tragic event of human contact with cyanotoxins occurred in a dialysis center in Brazil when 76 patients died because the water used for their treatment was contaminated with high concentrations (19.5μg/l) of the hepatoxin microcystin (Carmichael et al. 2001). Many cases of domestic animals and livestock poisoned from contaminated water sources have been reported along with incidents of toxins affecting plankton, fish and crustaceans (Chorus et al. 2001). Unfortunately, substantial challenges lie ahead since current research is best familiar with the affects of acute toxicity (Table 2-2). One hypothesis on the effects of chronic exposure to cyanotoxins is made by Chinese researchers who linked a high prevalence of liver cancer to hepatitis B, aflatoxins in the diet, and the consumption of microcystin in surface water (Yu 1995). However, the impact of chronic exposure to low doses of toxins remains largely unknown (Erdner et al. 2008).

2.6 Standards and recommendations for cyanobacteria monitoring.

In 1999, the WHO published a monitoring framework based on Australia's Alert Level Framework for drinking water plants (Burch et al. 2009). In order to ensure safe drinking water to consumers, the WHO recommends actions to be taken by DWTP operators once Alert Level Thresholds (ALT) are surpassed in the plant's raw water. The ALT can be measured by either: cyanobacterial densities, cyanobacterial biovolumes, or phytoplanktonic chl-*a* concentrations (Table 2-3). When grab samples confirm that any of the three criteria exceed an ALT operators are recommended to increase monitoring frequency and public awareness. In 2008, Quebec's Ministry of the Environment proposed an operational threshold level of 20 000 cyanobacteria cells/mL to compliment the WHO's monitoring recommendations (MDDEP 2008b).

Table 2-3 Alert level monitoring framework for DWTPs.

Alert level	Criteria	Actions for Drinking Water Treatment Plants
Vigilance	< 2000 cyanobacteria/mL, or < 1 µg/L Chl*a*, or < 0.2 mm3/L	No Bloom
1	2 000 – 100 000 cyanobacteria/mL, or 1 µg/L – 50 µg/L Chl*a*, or 0.2-10 mm3/L	Weekly counts cells Weekly monitoring of cyanotoxins Public warning
2	> 100 000 cyanobacteria/mL, or > 50 µg/L Chl*a*, or > 10 mm3/L	Weekly counts cells Weekly monitoring of cyanotoxins Increase information to public warning Alternative water source to be considered

Adapted from (Bartram et al. 1999)

In 1997, Korea's Ministry of the Environment established an "alert system for algal bloom" which uses the categories "Caution", "Warning" and "Outbreak". It is based on cyanobacteria densities (500, 5 000 and 1 000 000 cells/mL) and chlorophyll *a* concentrations (15, 25, and 100 µg/L) (Ahn et al. 2007). In 2007, an alternative alert level framework based on phycocyanin concentrations (µg/L) was proposed for Korean DWTPs (Table 2-4).

Table 2-4. Proposed Alert Levels for cyanobacterial monitoring by phycocyanin concentration in Korean DWTPs (Ahn et al. 2007).

Alert level	Criteria
No bloom	< 0.1 µg/L-PC
Caution	Between 0.1 µg/L and 30 µg/L - PC
Warning	Between 30 µg/L and 700 µg/L - PC
Outbreak	> 700 µg/L - PC

Maximum concentrations of common cyanotoxins in drinking water have also been proposed. The WHO recommends a maximum concentration of 1 µg/L of the

hepatoxin microcystin in drinking water at all times. This guideline value is based on the following equation (Bartram et al. 1999):

$$Guideline\ value = TDI*bw*P/L$$

Where:

TDI = Total daily intake µg MC/kg of consumer size (0.04 was used)
bw = An average adult body weight (60 kg used)
P = Proportion of total daily intake of the contaminant which is ingested from the drinking water needs (assumed to be 0.8)
L = Typical daily water intake in litres (2 liters used)

Several other similar guidelines and recommendations have been made in other regions (Table 2-5). Although no binding legislation exists in Canada concerning the allowable concentration of cyanobacterial cells or toxins in recreation and drinking water, many regions globally have adopted the recommendations developed in Australia and recommended by the World Health Organization.

Table 2-5 Worldwide guidelines and standards for cyanotoxins in treated drinking water. Adapted from AWWARF (2006)

Country/ Region/ Continent	Guidelines/Standards	Comments/explanations
World Health Organization	Microcystin: 1.0 µg/L	Published in 'WHO Guidelines for Drinking Water", 1996
Canada	1.5 µg/L toxins as microcystin LR MAC	Maximum acceptable concentrations (MAC) are derived from the tolerable daily intake (TDI), which is in tern derived from the No-observed adverse affect level (NOAEL)
Quebec	1.5 µg/L Anatoxin	
Australia	1.3 µg/L toxins as microcystin LR	
Africa	None found	
Asia	None found	
European Union and United Kingdom	Assumed to be the same as WHO recommendations. No specific values found	Guidelines indicated that "water should not contain algae" and that were measured in terms of MACs
New Zealand	≤ 1 potentially toxic cyanobacterium in 10ml sample. MAC for toxins: Anatoxin (as STX-eq) 3.0 µg/L ; Anatoxin-a (S) : 1.0 µg/L ; Cylindrospermopsin: 1.0 µg/L ; Microcystin: 1.0 µg/L ; Saxitoxins: 1.0 µg/L ; Nodularin: 1.0 µg/L ; LPS endotoxin: 3.0 µg/L	MACs are based on WHO guidelines. Standards provide compliance criteria and compliance is monitored.
Brazil	Microcystin: 1.0 µg/L Saxitoxin: 3.0 µg/L Cylindrospermopsin: 15 µg/L	Guidelines for microcystin are mandatory, and guidelines for eq-saxitoxin and eq- cylindrospermopsin are recommended.
United States of America	None currently known	Cyanotoxins are on the Contaminant Candidate List (CCL) and the Environmental Protection Agency is pushing for their inclusion in official legislation.

2.7 Advantages and disadvantages of monitoring methods

Traditional monitoring methods include taxonomic enumerations and pigment extractions which are fastidious and costly because they necessitate analysis by highly trained personnel and expensive equipment. These methods are also inconvenient for DWTP operators, who often receive analysis results days after sampling, therefore preventing them from responding quickly to a contamination event of toxic cyanobacteria.

A faster and less expensive method of monitoring cyanobacterial concentrations at the entrance of DWTP is the use of *in vivo* (intra-cellular) phycocyanin fluorescence technology (Leboulanger et al. 2002; Izydorczyk et al. 2005; Gregor et al. 2007; Seppala et al. 2007; Brient et al. 2008). Two recent studies have proposed the use of *in vivo* fluorescence to replace traditional methods of monitoring the cyanobacteria alert levels recommended by the WHO in lakes and reservoirs used as sources of drinking water by treatment plants (Ahn et al. 2007; Izydorczyk et al. 2009).

2.8 Fluorescence mechanism in cyanobacteria

In vivo fluorescence monitoring of algal biomass is based on the ability to measure the light emitted by photosynthetic phytoplankton in their natural habitat after they have absorbed light energy. There are 3 possible uses of light absorption by algal LHC pigments: 1) photosynthesis, 2) energy lost to heat, 3) fluorescence (absorption of light at one wavelength and its emission at a longer wavelength). All actions are in competition, and when one is optimized, the yield of the others decreases (Oliver et al. 2000). In cyanobacteria, the phycobiliproteins in their LHC absorb light at different wavelengths depending on which pigments are present (Figure 2.2).

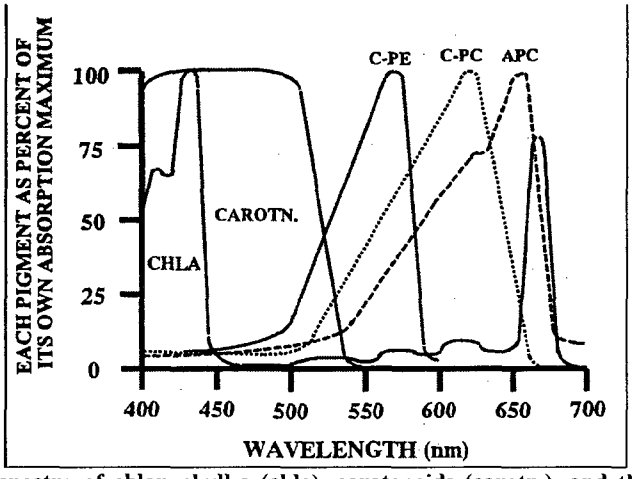


Figure 2.2 Absorption spectra of chlorophyll-a (chla), carotenoids (carotn.), and the phycobiliproteins C-phycoerythrin (C-PE) in red cyanobacteria, cphycocyanin (C-PC) in blue cyanobacteria and allophycocyanin (APC) in both red and blue. Chla and Carotenoids are in LHC of other algal species. The absorption spectrum for each pigment is shown relative to its absorption peak. Reproduced from : Oliver, R. L., & Ganf, G. G. (2000). Freshwater blooms. In B. A. Whitton & M. Potts (éds.), The Ecology of Cyanobacteria. (pp. 149-194). the Netherlands: Kluwer Academic Publishers. With kind permission of Springer Science and Business Media (Appendix H)

In cyanobacteria, the light absorption processes takes place in PBSs, PS II and PS I (van Thor et al. 1998). About 95% of light energy absorbed by the antenna pigments is transferred to the TE and then to the PSI or PSII, a small fraction (1-2%) of the light energy is fluoresced, and the rest is lost to heat (Figure 2.3) (Maxwell et al. 2000). A similar process occurs in other algal species.

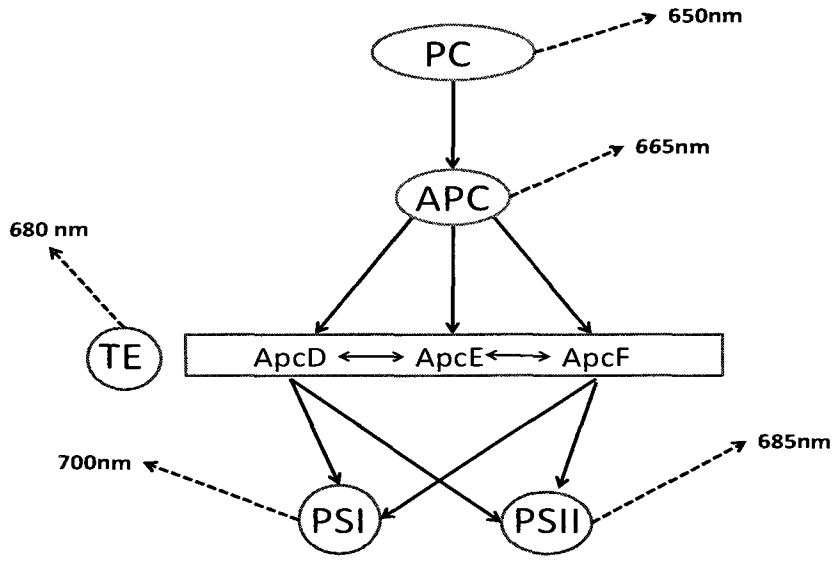


Figure 2.3 Fluorescence and energy transfer characteristics. Data presented here are representative of cyanobacteria containing the phycobilin phycocyanin (PC) and allophycocyanin (APC). The terminal emitters (TE) ApcD, ApcE, and ApcF and Photosystem I and II (PSI and PSII) all contain chlorophyll. Solid lines represent pathways of absorbed light energy. Dotted lines represent light energy fluoresced. Fluoresced wavelengths were measured at room temperature. Adapted from (van Thor et al. 1998).

The phycobilins are highly fluorescent (Beutler et al. 2002) and in freshwater, phycocyanin is responsible for the majority of the fluorescent energy emitted by cyanobacteria. Even though the TE, PSI and PSII can all act as light energy absorbers and emitters, their combined fluorescent effect is minimal compared to the emissions from PC. This is because the majority of Chl*a* (80-90%) in cyanobacteria is located in PSI (Bryant 1986), which is very efficient at trapping energy and therefore weakly fluorescent (van Thor et al. 1998). The photoemission of light energy by pigments is the process upon which *in vivo* fluorescence monitoring is based.

2.9 Studies using online *in vivo* fluorescence to estimate chl-*a* content of phytoplankton, including cyanobacteria.

The monitoring of different phytoplankton groups using online, *in vivo* fluorescence allows the rapid estimation of the abundance of different algal groups in their natural habitat. While the first studies focused exclusively on the identification of green algae (Lorenzen 1966), subsequent studies demonstrated that various algal spectral groups were distinguishable by measuring their particular absorption wavelengths (Yentsch et al. 1979; Millie et al. 2002).

Table 2-6 Spectral groups of microalgae. Adapted from Beutler et al. 2002.

Migroalgae group	Spectral groups	Accessory pigment(s)
Chlorophyta	Green	Chlorophyll a/b carotenoids
Cyanobacteria (fresh water) ; Glaucophyta	Blue	Phycobilisomes (Phycocyanin)
Heterokontophyta	Brown	Chlorophyll a/c
Rhodophyta (some salt water cyanobacteria)	Red	Phycobilisomes (phycoerythrin)
Cryptophyta	Mixed	Phycobiliprotein Chlorophyll *a/c*

The use of submersible online probes is becoming a common monitoring tool for the continuous monitoring of transient phytoplankton proliferations. There are two commonly used methods of monitoring cyanobacteria by *in vivo* fluorescence (Table 2-7). One method measures exclusively fluorescence emission by phycocyanin from the LHC of cyanobacteria and the other estimates the biomass of 5 algal groups (Table 2-6) characterized by the fluorescence excitation "fingerprint" due to the composition

of their LHC. Up until now, no research has been published using the YSI phycocyanin probe.

Table 2-7. Non exhaustive summary of research on in vivo fluorescence to measure cyanobacteria or microalgae abundance.

Spectro-fluorometers used	Authors	excitation wavelengths used	Emission wavelength measured	Probe type
Absorption by LCH of many algal groups is measured	Beutler et.al. 2002	Five LEDs at: 450 nm 525 nm 570 nm 590 nm and 610nm	690nm	FluroProbe (bbe Moldaenke, Kiel, Germany)
	Leboulanger et al., 2002	Same as Beutler	690nm	FluroProbe (bbe Moldaenke, Kiel, Germany)
	Gregor et al. 2007	Same as Beutler	690nm	FluroProbe (bbe Moldaenke, Kiel, Germany)
	Izydorczyk et al. 2008	Same as Beutler	690nm	Algae Online Analyser (AOA) fluorometer (bbe Moldaenke, Kiel, Germany)
	Parésys et al.2005	625 +/-20nm 430 +/- 30nm 540 +/- 15nm	690nm	Phytosensor prototype
Phycocyanin probe only Only the LHC of cyanobacteria are measured	Izydorczyk et al. 2005	630nm	660nm	Turner Fluorometer 10AU-005
	Seppala et al. 2007	620 nm	650 nm	Turner Fluorometer 10-AU
	Ahn, 2007	620 nm	645nm	FluroProbe (bbe Moldaenke, Kiel, Germany) (only phycocyanin probe used)
	Brient et al., 2008	620nm	655nm	TriOS miroFlu-blue

2.10 Advantages and limitations of in vivo fluorescence

In vivo fluorescence with submersible probes provides advantages such as sampling in the benthic zone of water bodies (Gregor et al. 2007), monitoring multiple points quickly near recreational areas (Brient et al. 2008), and the rapid assessment of water quality at the entrance of DWTPs (Cagnard et al. 2006).

It must be mentioned that even though the estimation of cyanobacteria by *in vivo* fluorescence has been demonstrated to be possible and accurate, the quality of the instrument's estimations will depend on the specificity of the instrument and/or the algorithm used for conversion (Millie et al. 2002). Rigorous testing of instruments in the field is nevertheless highly recommended. Additionally, pigment fluorescence can be affected by exposure to sunlight intensity, nutrient availability, turbidity in the water matrix, cell age and history (Gregor et al. 2007).

Another factor which could interfere with the use of *in vivo* phycocyanin fluorescence is the fact that phycocyanin is not specific to cyanobacteria. Some *Cryptophyceae* or *Rhodophyceae* contain PC (Brient et al. 2008) and cyanobacteria can produce different amounts of phycocyanin depending on existing nutrient and light conditions (van Thor et al. 1998).

2.11 Summary and Research Needs

Anthropogenic eutrophication of drinking water sources have made them vulnerable to cyanobacterial proliferation (Sivonen et al. 1999), especially in sunny and warm conditions in stagnant waters (Mur et al. 1999; Davis et al. 2006; Lehman et al. 2008). Cyanobacteria are more competitive than other types of phytoplankton at storing P (Fogg et al. 1973) and better at accessing nutrients (Oliver et al. 2000), sunlight (Lavoie et al. 2007) and inorganic carbon (Pick et al. 1987). The precise conditions in

which cyanobacteria produce cyanotoxins are not fully understood (Giani et al. 2005). Human exposure to cyanotoxins generally occurs while bathing in or drinking water containing toxic cyanobacteria. Exposure to cyanotoxins can have mild to fatal consequences depending on the dose, method of exposure and type of toxin (Yu 1995; Carmichael 2001; Carmichael et al. 2001).

Following the WHO's alert level framework, cyanobacterial monitoring requires costly and fastidious work to be done by highly trained personnel, therefore DWTP managers are unable to make rapid and informed decisions regarding potentially toxic cyanobacterial contamination inside their DWTPs. The WHO suggests their alert level framework can be adapted to local needs and protocols (Bartram et al. 1999). To address the problems of costly and fastidious analyses, two studies have used *in vivo* fluorescence monitor DWTPs with locally adapted threshold values for cyanobacterial chlorophyll (Izydorczyk et al., 2008) and phycocyanin concentrations (Ahn et al. 2007) . *In vivo* phycocyanin fluorescence is a rapid and precise method to identify cyanobacteria in their natural environment (Yentsch et al. 1979) but the results of the method depend on the quality of the fluoroprobe used (Millie et al. 2002).

The aim of this research is to use a multi-probe system, which includes an *in vivo* PC fluorescence probe, to establish an accurate, rapid and user-friendly warning system to monitor potentially toxic cyanobacteria at two DWTPs in southern Quebec.

Chapter 3 Hypotheses and Research Objectives

The general goal of this study is to establish an alert system using a multi-probe system for potentially toxic cyanobacteria at DWTPs in Quebec. The establishment of an alert system which can rapidly detect cyanobacteria in Quebec is important given the potential health problems which can arise from the consumption of drinking water containing cyanotoxins (Carmichael 2001). This study is expected to succeed in this goal because two similar studies have used *in vivo* fluorescence to monitor potentially toxic cyanobacteria at DWTPs (Ahn et al. 2008; Izydorczyk et al., 2008) and others have recommended this technology as a cyanobacterial monitoring tool (Leboulanger et al. 2002; Beutler et al. 2004; Izydorczyk et al. 2005; Brient et al. 2008). This will be accomplished through the use of a novel online submersible multi-probe system which measures multiple physico-chemical parameters in parallel with chlorophyll and phycocyanin fluorescence. The continuous monitoring of multiple bloom-related factors provides an opportunity to describe cyanobacterial bloom conditions in real time, which could help DWTPs to react more quickly to the presence of potentially toxic cyanobacterial blooms in their raw water.

The hypotheses and specific objectives of the research are the following:

1) The *in vivo* PC fluorescence probe provides an accurate method for the detection and quantification of cyanobacterial abundance in laboratory and environmental conditions.

    Specific Objective (1a): To compare the *in vivo* PC fluorescence probe's estimations of cyanobacterial density with the true cyanobacterial density as determined by microscopic counts.

Specific Objective (1b): To evaluate the correlations between *in vivo* PC fluorescence and 4 conventional methods of monitoring cyanobacterial abundance (cyanobacterial density, cyanobacterial biovolume, phytoplanktonic chl-*a* concentration and phytoplanktonic PC concentration) in laboratory and environmental conditions.

2) The multi-probe system is a useful and sensitive method for monitoring cyanobacteria in southern Quebec, Canada.

Specific Objective (2a): To monitor the spatio-temporal, and interannual variations of cyanobacteria and cyanotoxins by both conventional laboratory methods and novel online *in vivo* PC fluorescence at two DWTPs in Quebec.

Specific Objective (2b): To conduct statistical analyses on the correlations between the *in vivo* PC fluorescence probe and other parameters (turbidity, pH, dissolved oxygen, specific conductivity, chlorophyll fluorescence, and temperature) measured continuously by the multi-probe system.

3) The PC probe can be used to monitor cyanobacteria within an existing Alert Level Framework for DWTPs.

Specific Objective (3a): To evaluate the correlations between the maximum potential microcystin concentrations (µg/L) in grab samples and 4 parameters (cyanobacterial density, cyanobacterial biovolume, phytoplanktonic chl-*a* concentration and phytoplanktonic PC concentration) in the raw water of two DWTPs in Quebec.

Specific Objective (3): To calculate the PC probe's ratio fluorescent units (RFUs) which correspond to existing alert level thresholds in one cyanobacterial monitoring framework (WHO or Korean).

Chapter 4 Materials and Methods

4.1 Site descriptions and Sampling Locations

Sampling sites were chosen on the basis of historical taste & odour and cyanobacterial bloom records at drinking water treatment plants (DWTP). Two DWTP in southern Quebec, Canada, prone to cyanobacterial activity were monitored during the summer and autumn of 2007 and 2008. The managers of both DWTPs where sampling was carried out requested that the exact locations and names of the communities disserved by the DWTPs remain confidential.

The first field site was located on the Missisquoi Bay in Lake Champlain. This Bay is eutrophic and has an average depth of 2.8 m and a surface area of 77.5 km^2. The Bay's inputs are groundwater, tributary rivers and runoff from predominantly agricultural lands (Galvez et al. 2003). Water from the Bay' tributary rivers have been classified as "poor" or "very poor" by Quebec's bacterial and physic-chemical indicators due to high turbidity and excessive concentrations of nitrogen and phosphorous (Simoneau 2007). It is a source of drinking water for 4 100 residents (StatisticsCanada 2006a) and also serves as a recreational site for boating, fishing, and swimming activities.

On-line 6600V2-4 multi-probe systems (MPS) from YSI (YSI, Yellow Springs, Ohio) were used to monitor raw water inside the Missisquoi Bay DWTP from August to early November in 2007, and from May to October in 2008. Vertical depth profiles were taken above the DWTP's intake point and in the pelagic zone with the MPS once or twice a week. The water intake was approximately 4 meters below the surface at the bottom of the Bay.

The second field site was located in the Yamaska River Basin. Over half of the land in the Yamaska River Basin is used for intensive agricultural practices. Upstream from the sampling site, the Yamaska River's water quality is euthrophic and considered very poor by the Quebec Minister of the Environment because of chronically high turbidity and elevated concentrations of phosphorous, fecal coliforms and chlorophyll *a* (Hébert 2005; Berryman 2008). Between 1999-2005 the measured median phosphorous concentration in the Yamaska River was 99 µg/L (Berryman 2008). The Yamaska Reservoir is fed by the Yamaska River and holds enough water to supply a population of 47 000 residents (StatisticsCanada 2006b) with thirty days of water. The water level in the reservoir is controlled by the water treatment plant's operators. The reservoir is aerated, and lime is added to control the pH as part of the DWTP's pre-treatment.

In 2007, one MPS was installed at the junction between the Yamaska River and the Yamaska Reservoir which feeds a DWTP. However the MPS was relocated to measure raw water of the DWTP from July to October, 2008. Additionally, continuous source water monitoring was carried out in the Yamaska Reservoir by attaching a MPS to a buoy and suspending it 1 meter below the water surface, above the DWTP's water intake, located at the bottom of the Reservoir. The Yamaska's Reservoir's depth varied between 4-6 meters according to the DWTP's need.

4.2 MPS specifications calibration, and validation

MPS specifications

YSI probes were chosen because they are a relatively low cost tool making them more likely to be used by small municipalities than other more expensive probes using similar technology. The MPS comprises eight probes: turbidity, pH, dissolved oxygen (DO), temperature, specific conductivity, depth, chlorophyll fluorescence (CHL fluo), and phycocyanin fluorescence (PC fluo). The phycocyanin probe aims to excite

cyanobacteria's phycocyanin at 590nm and to measure the pigment's emission at 660nm with the probe's photodiode. The chlorophyll probe follows the same procedure but aims to excite chlorophyll pigments at 470nm and then to read the light emitted from chlorophyll at 680nm. The photodiodes of both probes use an algorithm to convert the light information into ratio fluorescent units (RFUs) and then use a conversion factor to estimate the concentration of either cyanobacteria (cells/ml) or chlorophyll (µg/L). This conversion from RFU to eq-cyanobacteria/mL, which is referred to as the probes "internal calibration" in this study, is calculated based the results of calibration work using the PC probe to measure a laboratory monoculture of *M. aeruginosa* (YSI 2006).

MPS calibration

The MPSs were calibrated once a month in the field. The three MPS installed field sites recorded measurements every 30 minutes and the data was downloaded twice a week. The turbidity, pH and conductivity probes were calibrated with products purchased from the manufacturer (YSI, Yellow Springs, Ohio) for this purpose and a one point calibration with de-ionized water was done for the chlorophyll, dissolved oxygen and PC probes.

Laboratory validation

The morning of validation experiments, a sample of a purified culture of *Microcystis aeruginosa* from the Missisquoi Bay with a biovolume of 75.3µm^{3}was fixed with lugol and counted. Based on the cyanobacterial density in the stock culture, suspensions of estimated cyanobacterial densities of 2 000, 10 000, 50 000, 100 000 and 250 000cells/ml of *M. aeruginosa* were prepared in 5 buckets each containing 5 liters of tap water (water was left to de-gas for 24 hours to remove any residual chlorine content). Buckets with cyanobacterial suspensions were mixed constantly with a magnetic bar during the experiment.

The PC probe was calibrated with one point: 0 cyanobacteria/mL (bucket with only tap water). All probes on the MPS took measurements in parallel, and at an interval of 0.5 seconds during sampling. Once the probe was calibrated, it was used to measure each of the 5 prepared cyanobacterial suspensions. All measurements were saved to the MPS's memory immediately after sampling. Triplicate grab samples were collected and fixed with lugol for taxonomic analysis (cyanobacterial density and biovolume estimations) in parallel with MPS measurements of each of the 5 suspensions. Triplicate grab samples were taken in each of the 4 suspensions at the same moment as measuring with the PC probe. The five suspensions were then filtered over Watman GF-C fiber glass filters under a green light and the filters were wrapped in aluminum foil and frozen at -80°C until chl-*a* and PC extractions and measurements were performed (see environmental validation for extraction methods).

Validation of the MPS in environmental conditions

Unconcentrated raw water samples to validate the readings of the phycocyanin and chlorophyll probes were collected bi-weekly in clean opaque plastic 4 liter bottles at the same time that depth profiles were conducted and data were downloaded. All samples were kept in coolers on ice until they were processed later the same day. All bottles were washed, rinsed three times with tap water and three times with de-ionized water and left to drip dry.

Taxonomic counts with species identification, biomass and biovolume calculations were performed by the Centre d'Expertise en Analyses Environmentales du Québec (CEAEQ) in Quebec's Ministry of the Environment and Université du Québec à Montréal's (UQAM) Biological Sciences department on field samples conserved in lugol according to previously established methods (Lund et al. 1958 ; Wetzel 2000).

Because cyanobacteria cell volumes can vary greatly in size (from 0.5μm^3 to 1022μm^3 in our samples), taxonomic results derived from grab samples are reported in terms of

cyanobacterial biovolume (mm^3/L) rather than cyanobacterial density (cell/mL). By using biovolume, results are more standardized, therefore giving a clearer picture of cyanobacteria abundance regardless of cell size.

Samples for cyanotoxin analysis were collected in duplicate and frozen at -15C until processed by ELISA kits (Abraxis, Warminster, Pennsylvania, USA) for microcystin LR (MC-LR) content. Total (intra and extra cellular) toxins in samples were measured after three freeze thaw cycles to lyse the cells. Water samples for phytoplanktonic chlorophyll-*a* and phycocyanin extractions were collected in opaque plastic bottles and filtered over Watman GF-C fiber glass filters under a green light. The filters were wrapped in aluminum foil and frozen at -80°C until analyzed. Chlorophyll-*a* was extracted with 90% hot ethanol and read with a spectrophotometer at an absorbance of 665nm and 750nm (Nush 1980). For phycocyanin extractions, filters were placed in 0.01M sodium phosphate (pH 7.0 , 0.15M NaCl) (Otsuki et al. 1994) and put through 3 freeze/thaw cycles to lyse cells. The suspension was then centrifuged at 3500g for 10min, the supernatant was centrifuged at 13000g for 90min at 4°C and read with by spectrophotometer at an absorbance of 652nm with a correction for turbidity at 615nm (Bennett et al. 1973).

4.3 Statistical Analyses

All analyses were done on Statistica 8 (Statsoft, Tulsa, Oklaholma, USA). Daily averages were calculated for all data generated by the MPSs to reduce the interdependency of the temporal data. Negative values which represent readings outside the probes limit of detection were removed from the MPS data. Log transformations to ensure normally distributed parameters were performed on PC fluorescence for the Missisquoi Bay model. All parameters except water temperature, dissolved oxygen and PC fluorescence were log-transformed for the model derived from data collected inside the DWTP of the Yamaska Reservoir.

Separate multiple linear regression analyses were performed on data collected by each MPS. Forward and Backward stepwise selections were used to determine the best predictors of the dependent variable, phycocyanin (PC) fluorescence. Independent variables were the other parameters measured by the MPS: chlorophyll (CHL) fluorescence, turbidity, pH, specific conductivity, temperature (Temp), and dissolved oxygen. The daily range of pH (ΔpH) and dissolved oxygen (ΔDO) were also included in the regression analyses as independent variables.

The MPS's manufacturer states that the PC probe is unable to properly estimate the PC concentration of cyanobacterial scum accumulations; therefore any samples obtained in these conditions were not included in statistical analyses and only analyzed for toxin content and phytoplankton dynamics.

Chapter 5 Results and Discussion

5.1 Laboratory and environmental validation of the *in vivo* PC fluorescence probe on the MPS.

Chapter 5.1 aims to determine the accuracy of the PC probe's cyanobacterial density estimations and which of the currently used cyanobacterial monitoring parameters is/are best monitored by the online PC probe on the MPS.

5.1.1 Validation of the PC probe's estimations of cyanobacterial density in laboratory and environmental conditions (2007-2008)

Laboratory conditions

During the laboratory experiment, *in vivo* PC fluorescence readings had a standard deviation of 285 eq/cyanobacteria/mL in water with no cyanobacteria which is consistent with specifications from the manufacturer (YSI 2006). The standard deviation of the PC probe readings varied between 85 to 534eq-cyanobacteria/ml during the experiment and increased in amplitude with higher densities of *Microcystis aeruginosa* as was also observed by Brient et al. 2008. The correlation between *in vivo* PC fluorescence as measured by the PC probe and true microscopic points are presented below (Figure 5.1)

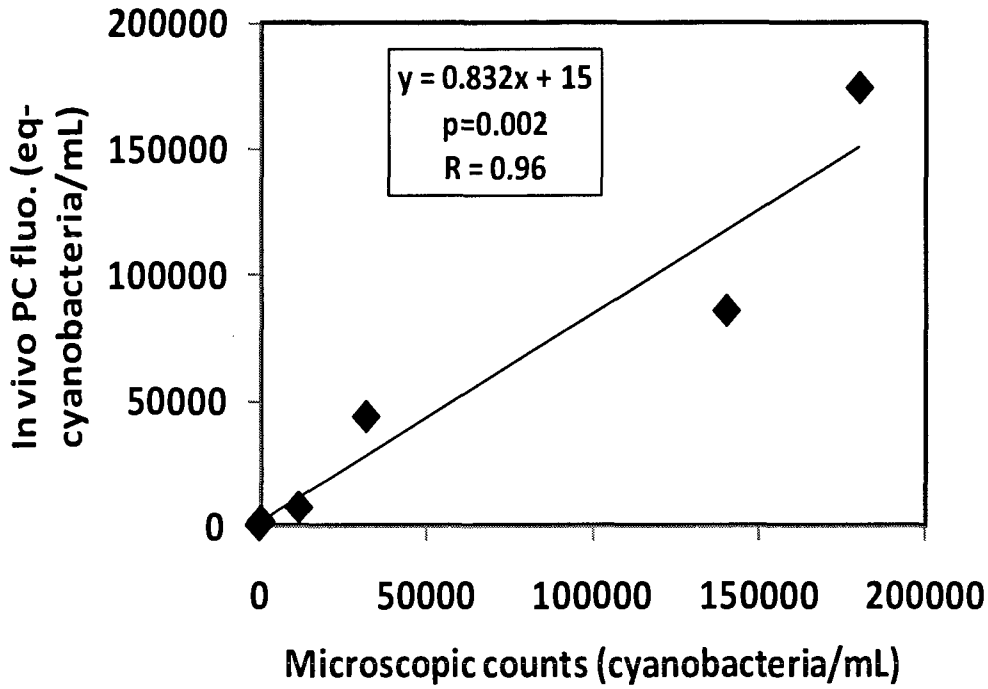


Figure 5.1 Correlation between *in vivo* PC fluorescence measured by the PC probe on the MPS and true microscopic counts of *M. aeruginosa*

There was a strong and significant correlation between the two variables (r = 0.96, p=0.002) and the relationship between the variables is linear, however, the slope of the line indicates that the PC probe underestimated the true microscopic counts by approximately 20%. This is contrary to another study which also tested the calibration for cellular density estimations of the YSI PC probe. Zamyadi et. al calibrated the PC probe using a two point calibration curve (0 and 50 000 cyanobacteria/mL of *M. aeruginosa*) and then measured different densities of the same species with the PC probe. The results were linear but showed an over-estimation of the true amount of cyanobacteria in suspensions by the PC probe by about 20%. This same study found that the PC probe measurements overestimated the true cyanobacterial microscopic counts by approximately 80% when the PC probe was calibrated with *M. aeruginosa* and then used to measure a suspension of cyanobacteria with a cell volume three times as large (*A. circinalis*).

Nevertheless, cyanobacterial density estimations by the PC probe have proven to be accurate in environmental conditions when a rapid, onsite, two point calibration was performed. This two point calibration used de-ionized water (0 cyanobacteria/mL) and a known cyanobacterial density (cells/mL) measured onsite by microscopy (Mosisch 2008). This could mean that in order to accurately calibrate the PC probe's measurement of eq-cyanobacteria/mL, the quality and speed of the microscopic counts are more important factors than the cellular volume of the cyanobacteria being measured.

Environmental conditions

In 2007, at both the Missisquoi Bay and the Yamaska Reservoir, the true cyanobacterial densities were much higher than the *in vivo* PC probe estimations (Table 5-1) due to an abundance of small cyanobacteria which were misinterpreted by the internal calibration of the PC probe.

Table 5-1. Comparison of true cyanobacterial densities and cyanobacterial densities estimated by the *in vivo* PC probe in 2007. Data collected by Arash Zamyadi.

Sampling location	Sampling date	Total cyanobacteria (cells/mL) microscopy	*Aphanothece clathrata brevis* (cells/mL) microscopy	*Aphanocapsa delicatissima* (cells/mL) microscopy	Estimated *in vivo* PC fluorescence by probe (eq-cyanobacteria /mL)
Missisquoi Bay	08/20/07	18 675	11 492	6 076	693
Missisquoi Bay	09/05/07	41 111	20 782	17 084	1 060
Missisquoi Bay	09/10/07	65 232	34 872	27 519	1 828
Entrance Yamaska Reservoir	10/01/07	29 946	25 978	0	923
Entrance Yamaska Reservoir	10/09/07	111 495	69 479	38 042	1 273

Both *Aphanothece clathrata brevis* and *Aphanocapsa delicatissima* have cell volumes of 0.5μm^3 whereas the average cyanobacterial cell volume was 91.5μm^3 at the Missisquoi Bay and 74.4μm^3 at the Yamaska River in 2007 samples. *Aphanothece*

clathrata brevis and *Aphanocapsa delicatissima* represented between 87%-96% of total cyanobacterial densities on monitored sampling days. Due to their small biovolume, and therefore their low cellular concentrations of PC, the probe's internal calibration underestimated the total cyanobacterial density present.

In 2008, the cyanobacterial densities (cells/ml) estimated by the PC probes on all three online MPS were between 2 and 520 times lower than the true microscopic counts calculated from grab samples taken at regular intervals during the season (Figure 5.2). Again, this underestimation of true cyanobacterial densities is hypothesized to be due to fact that the PC probe's internal calibration is ill adjusted to the cyanobacterial species present at our field sites.

In 2007 and 2008, between 2 and 12 cyanobacterial species, all with different cell volumes, were present in every grab sample taken at both the Yamaska Reservoir and the Missisquoi Bay. Given the variability of cyanobacterial species at the our monitored field sites, future monitoring using the PC probe should only be interpreted using Ratio Fluorescent Units (RFUs) (the true measurements of the probe before they are converted to eq-cyanobacteria/mL) rather than the probe's estimated cyanobacterial density.

Since RFUs are the generic, and therefore standard, values of the YSI PC probes, only a one point calibration is necessary. This reduces the possible calibrations errors presented above when calibrating with two points for cyanobacterial density. It is hypothesized that RFUs can be used to interpret cyanobacterial biovolume since the amount of PC in cyanobacteria, and therefore the strength of the florescence signal, is proportional to cyanobacterial cell volume. However, given that 4 parameters are regularly used to measure cyanobacterial abundance, this study investigated which of the 4 could best be interpreted by *in vivo* PC fluorescence at DWTPs in Quebec, Canada.

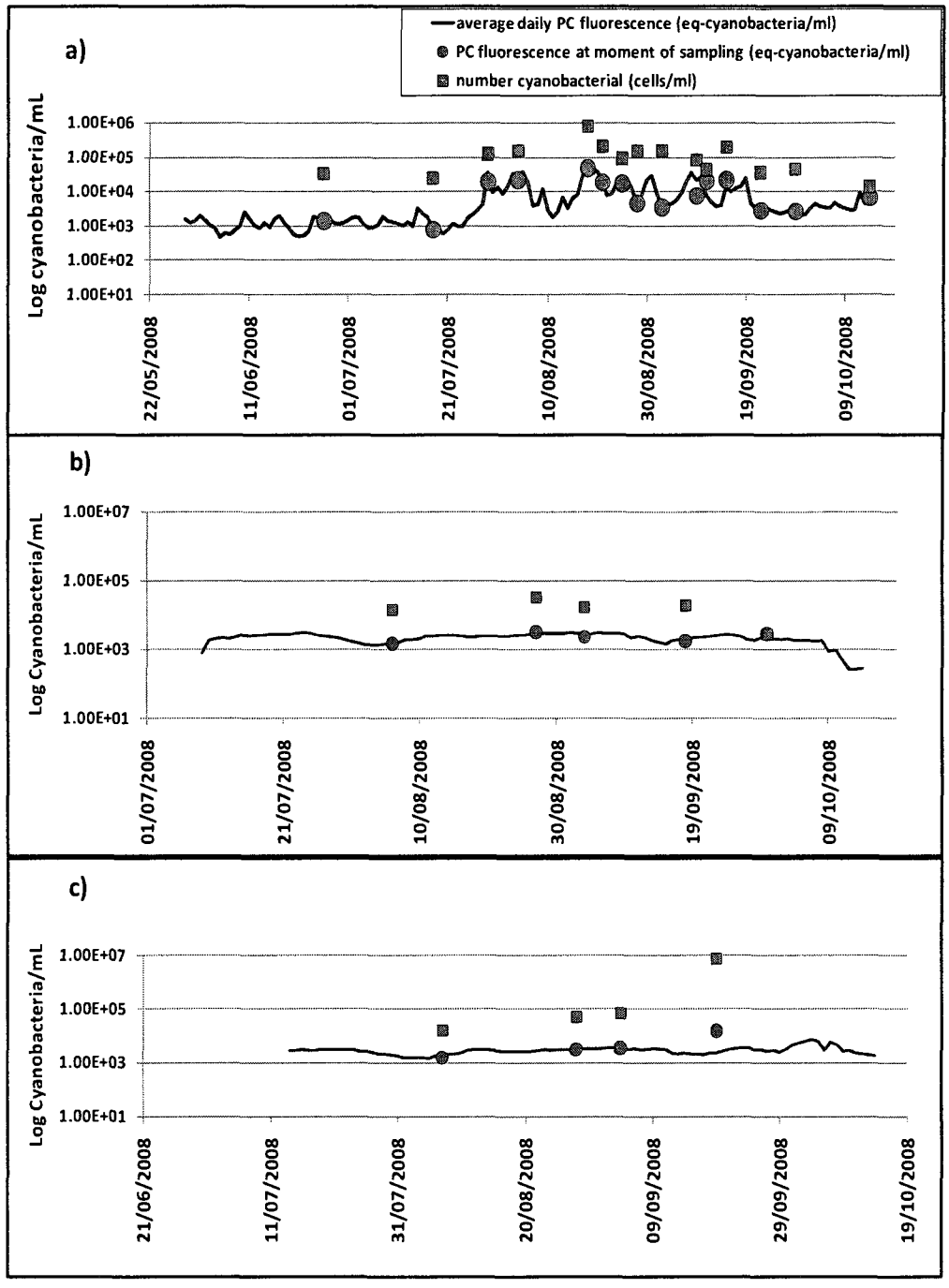


Figure 5.2. Comparison between estimations of cyanobacterial density by online *in vivo* PC fluorescence readings by the PC probe and the true cyanobacterial densities determined by microscopic counts. Graph "a" presents results from monitoring in raw water inside DWTP on Missisquoi Bay, "b" from raw water inside DWTP on the Yamaska Reservoir, and "c" from above the DWTP's water intake in the Yamaska Reservoir.

5.1.2 Correlations between *in vivo* PC fluorescence and 4 ALT parameters for cyanobacterial monitoring in laboratory and environmental conditions.

The PC probe readings were correlated with the 4 ALT parameters measured from grab samples taken during the calibration experiments (cell/mL, mm^3/L, µg/L chl-*a*, µg/L PC) (Table 5-2). For evaluations of the PC probe's performance in environmental conditions, online PC probe results were compared to ALT parameters measured from grab samples collected at both the Missisquoi Bay and Yamaska Reservoir collected in 2007 and 2008 (Table 5-2).

Table 5-2 Correlations between *in vivo* phycocyanin fluorescence and other measured parameters (2007-2008) in laboratory and environmental conditions. Data from 2007 only included in regressions analysis between PC fluo and cells/mL and mm^3/L in environmental conditions.

Parameters	*In vivo* PC fluorescence R			
	Laboratory	n value	Environmental	n value
Cyanobacterial concentration (cells/ml)	0.97 **	5	0.60*	37
Biovolume (mm^3/L)	0.97 **	5	0.77*	37
PC extractions (µg/L)	0.93 **	5	0.77*	32
Chlorophyll *a* extractions (µg/L)	0.998 **	5	0.85*	32

* $p < 0.01$, ** $p < 0.001$

All correlations in laboratory conditions are much stronger than those in environmental conditions. This was to be expected since there are many factors which can interfere with the PC probes readings in environmental conditions. For example, in both laboratory and environmental conditions, the quantity or PC in cyanobacteria and its ability to fluoresce depends on its stage of growth (Oliver et al. 2000). In environmental conditions, depending on the type of turbidity present during sampling, the effect on the PC probe readings can be different. Previous work observed the overestimation of cyanobacterial abundance by a PC probe in the presence of mineral turbidity (Zamyadi et al. 2008). However, another study observed a decrease in

fluorescence yield, and therefore an underestimation of cyanobacterial abundance, due to the absorption of the probe's signal by suspended particles (Brient et al. 2008). False positive readings of cyanobacteria RFUs can also be generated in the presence of eukaryotic algae if the excitation and detection wavelengths used to detect cyanobacteria are not precise (Beutler et al. 2002; Gregor et al. 2007; Zamyadi et al. 2008). Finally, fluorescence-quenching errors can cause an underestimation of cyanobacterial abundance by *in vivo* PC fluorescence when used in the photic zone (where there is sufficient light for photosynthesis to occur) (Beutler et al. 2002). This quenching of the phycocyanin pigment decreases its fluorescence yield due to the transfer of energy to the photosynthetic process when the cells are exposed to sunlight (Leboulanger et al. 2002).

Cellular density (cells/mL) and biovolume (mm^3/L) correlations with *in vivo* PC fluorescence

In the laboratory, analyses between *in vivo* PC fluorescence and both cyanobacterial density and cyanobacterial biovolume were almost identical in terms of correlation ($r = 0.97$), the significance of the regressions ($p < 0.01$), and the slopes of the lines of the two regressions ($y \approx 1.2x$) (Figure 5.3). One possible explanation for the similarity of the results is the experiments were done with a monoculture, therefore eliminating the impact of different cyanobacterial biovolumes (Brient et al. 2008).

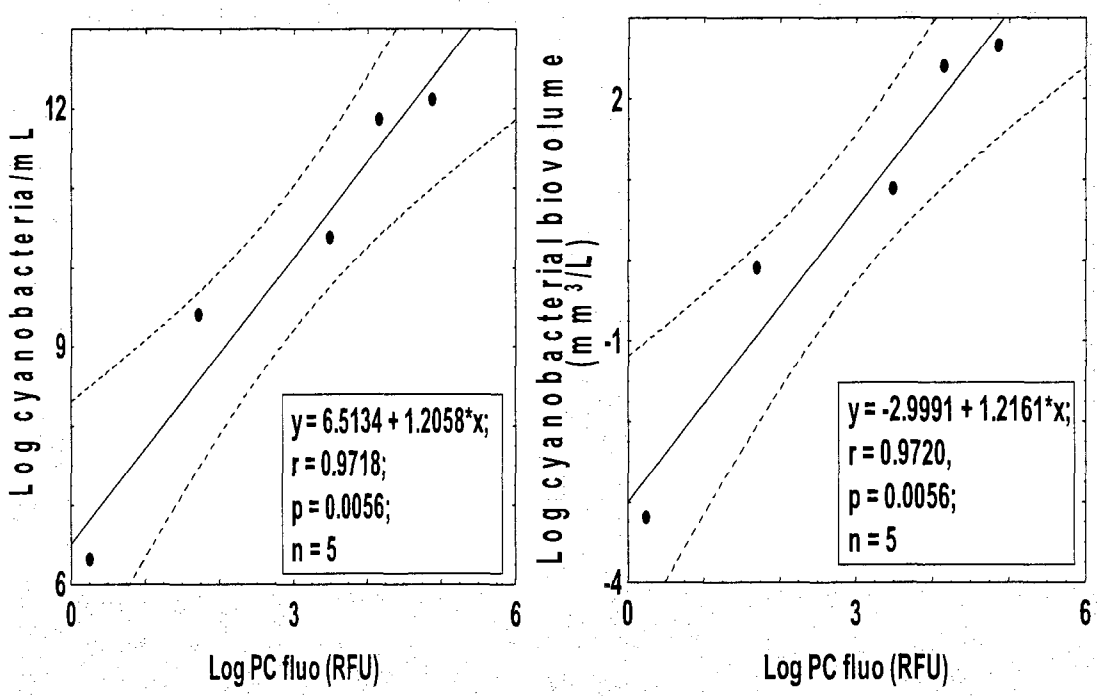


Figure 5.3 Simple linear regressions between: log transformed *in vivo* PC fluorescence and log transformed cyanobacterial density (on left) and log transformed *in vivo* PC fluorescence and cyanobacterial biovolume (on right).

Analysis done on field work in 2007 and 2008 showed a significant relationship between in vivo PC fluorescence and cyanobacterial density ($p < 0.00008$) which followed the same trends as previously reported (Izydorczyk et al. 2005; Gregor et al. 2007; Brient et al. 2008). However, the correlation coefficient measured between the two variables was the lowest of all measured parameters at $r = 0.60$ (Figure 5.4). The comparatively low correlation between the variables in environmental conditions is due to the non-standardized size of cyanobacterial cell volumes (0.5 to 1022 μm^3) in grab samples.

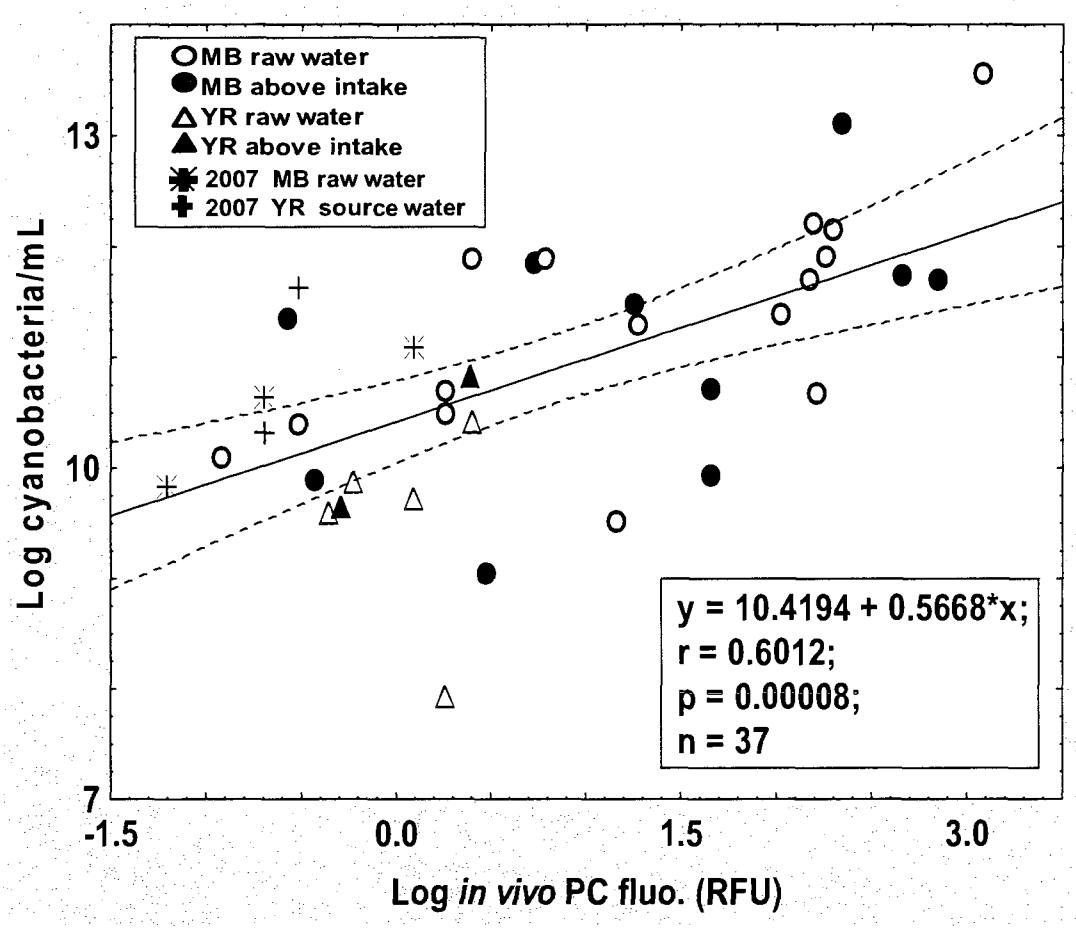


Figure 5.4 Relationship between log transformed in vivo PC fluorescence data and log transformed cyanobacterial density derived from samples taken in source and raw water from both field sites.

Environmental samples collected in 2007 and 2008 show a good correlation between *in vivo* PC fluorescence and cyanobacterial biovolume ($r = 0.77$ $p < 0.00001$).

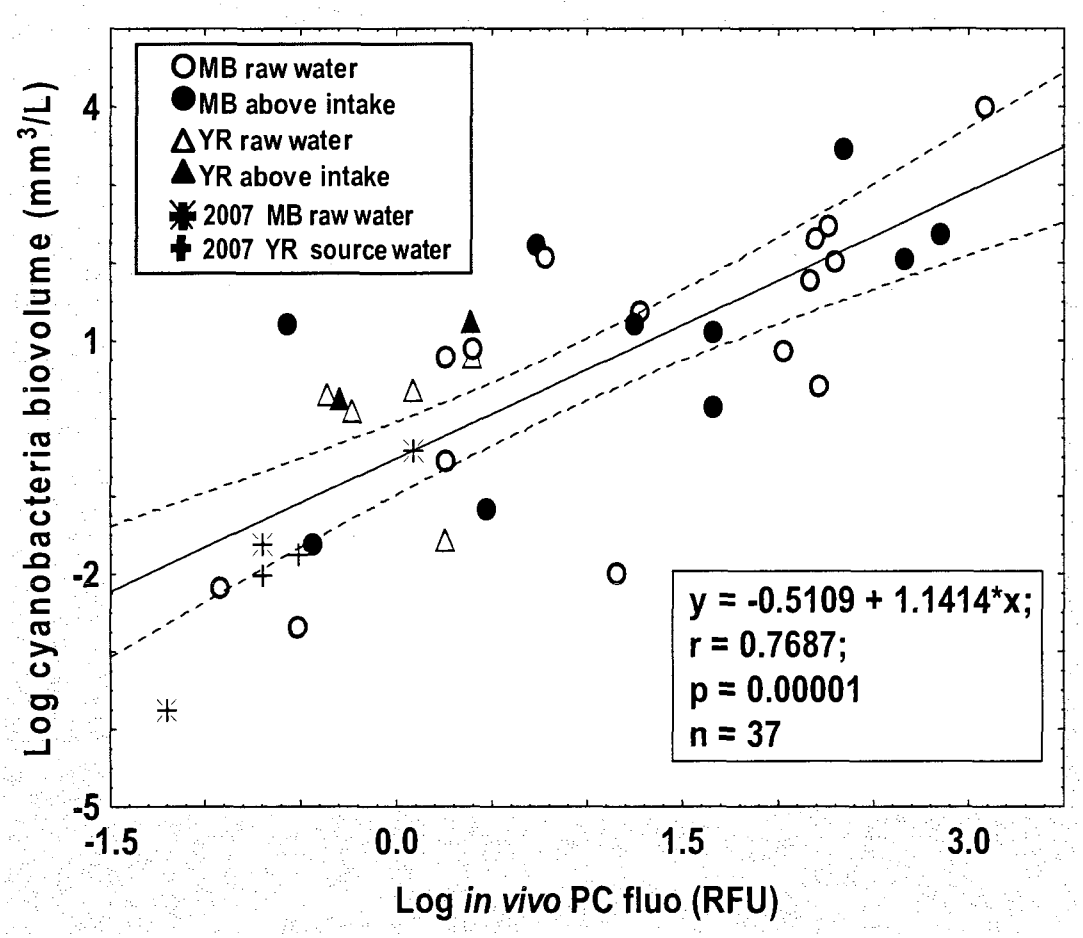


Figure 5.5 Relationship between log transformed *in vivo* PC fluorescence data and log transformed cyanobacterial biovolume (mm^3/L) derived from samples taken in source and raw water from both field sites in 2007 and 2008.

This strong and significant correlation between biovolume and PC fluorescence was reflected in similar monitoring campaigns (Izydorczyk et al. 2005; Seppala et al. 2007; Izydorczyk et al. 2009). Cyanobacterial biovolume had a higher correlation with *in vivo* PC fluorescence than cyanobacterial density because biovolume is a standard measurement which is easier for the PC probe to interpret when measuring many different species of cyanobacteria (Brient et al. 2008). This unvarying nature gives

biovolume a clear advantage over cyanobacterial density when of assessing risk of potentially toxic cyanobacteria in the raw water DWTPs.

PC extractions and *in vivo* PC fluorescence

In the laboratory, the correlation coefficient between *in vivo* PC fluorescence and extracted PC was also high and the regression was significant and linear (R = 0.93; p = 0.02) (Figure 5.6).

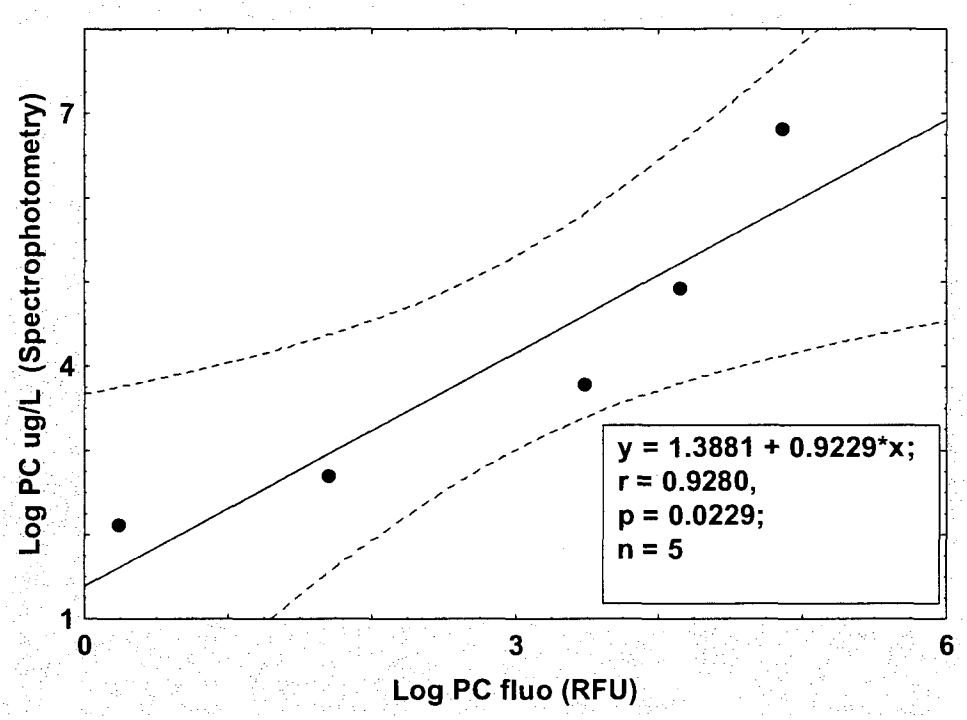


Figure 5.6 Relationship between PC in vivo fluorescence and extracted PC measured by spectrophotometry

In environmental samples, the highly correlated relationship between *in vivo* PC fluorescence and extracted phytoplanktonic PC measured by spectrophotometry (R = 0.77. p = 0.00001) are comparable to field work done in Korea (Ahn et al. 2007). The correlations between *in vivo* PC fluorescence and extracted PC (μg/L) in both laboratory and environmental samples were stronger than correlations with cyanobacterial biovolume and density, as would be expected as they are measuring the same parameter (PC) via two different methods.

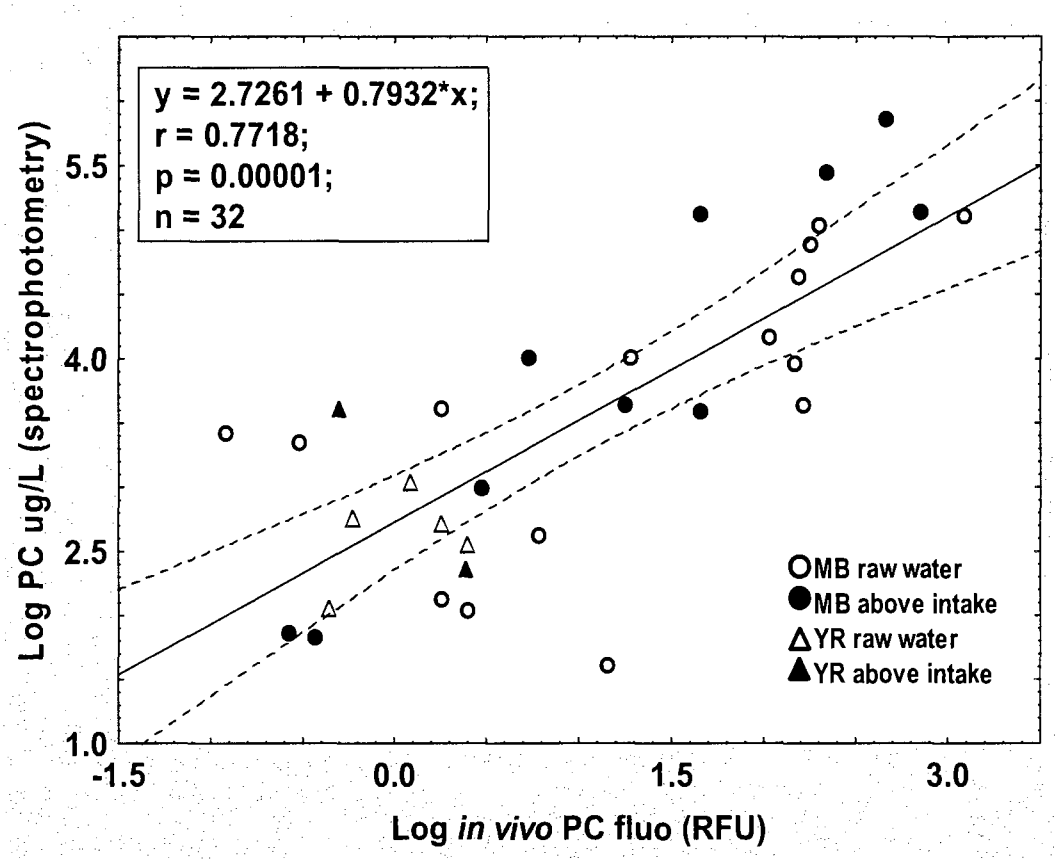


Figure 5.7 Relationship between log transformed *in vivo* PC fluorescence data and log transformed cyanobacterial PC concentration (µg/L) derived from samples taken in source and raw water from both field sites in 2008.

In addition to being better correlated, another advantage that PC extractions has over both cyanobacterial densities and cyanobacterial biovolumes is that highly trained personnel are not required to perform the extractions like they are with the other two parameters. However, phytoplanktonic PC is present in other types of phytoplankton such as cryptophyta (Brient et al. 2008) which were present in grab samples collected at both field sites in 2008 (Appendix C). This non exclusive source of PC could generate false readings of cyanobacterial abundance by the PC probe if crytophyta is present in high concentrations. Hence, if using phytoplanktonic PC concentrations for cyanobacterial monitoring, periodic complementary taxonomic analysis would be necessary to validate the probe's readings.

Phytoplanktonic chl-*a* extractions and *in vivo* PC fluorescence

In both laboratory (r = 0.998; Figure 5.8) and environmental conditions (r = 0.85; Figure 5.9) *in vivo* PC fluorescence was best correlated with extracted cyanobacterial chl-*a* (μg/L).

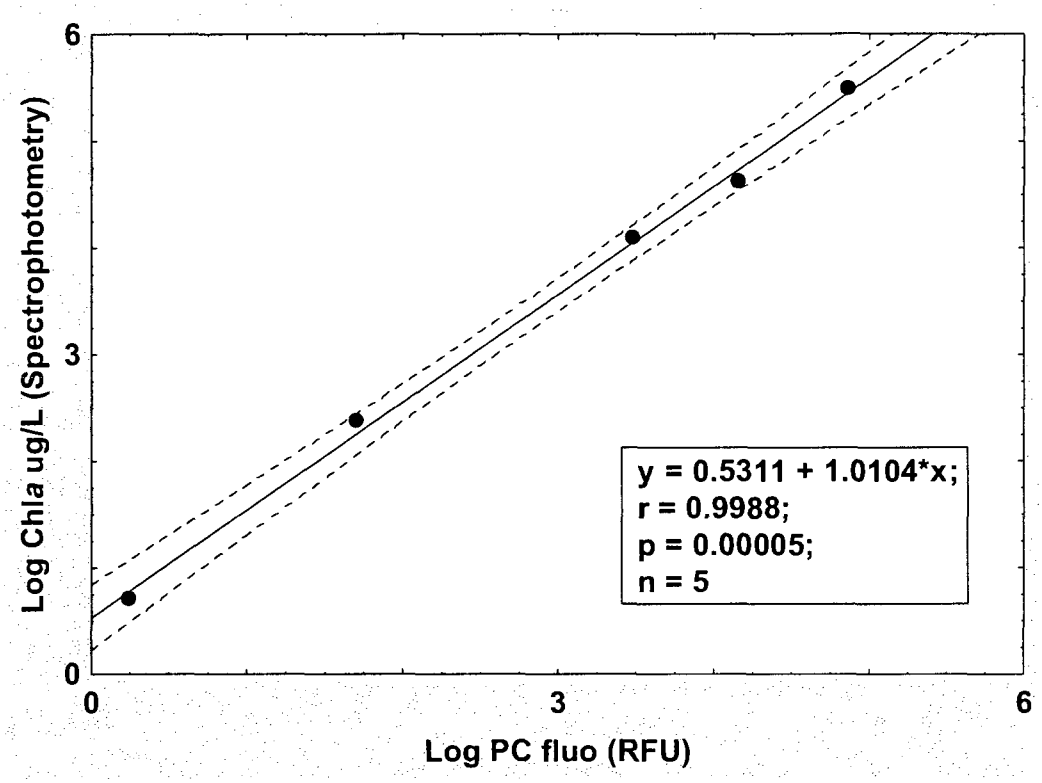


Figure 5.8 Relationship between PC in vivo fluorescence and extracted chl-*a* measured by spectrophotometry.

This is unsurprising because many laboratory (Beutler et al. 2002; Leboulanger et al. 2002; Parésys et al. 2005; Gregor et al. 2007), and environmental studies (Seppala et al. 2007; Izydorczyk et al. 2009) have also reported strong correlations between these two variables. This relationship is based on the assumption that cyanobacterial abundance and the amount of chl-*a* in cyanobacteria increase and decrease at the same

rate (Bartram et al. 1999; WHO 2003). However, Ahn et. al (2007) has shown that this relationship is not always linear.

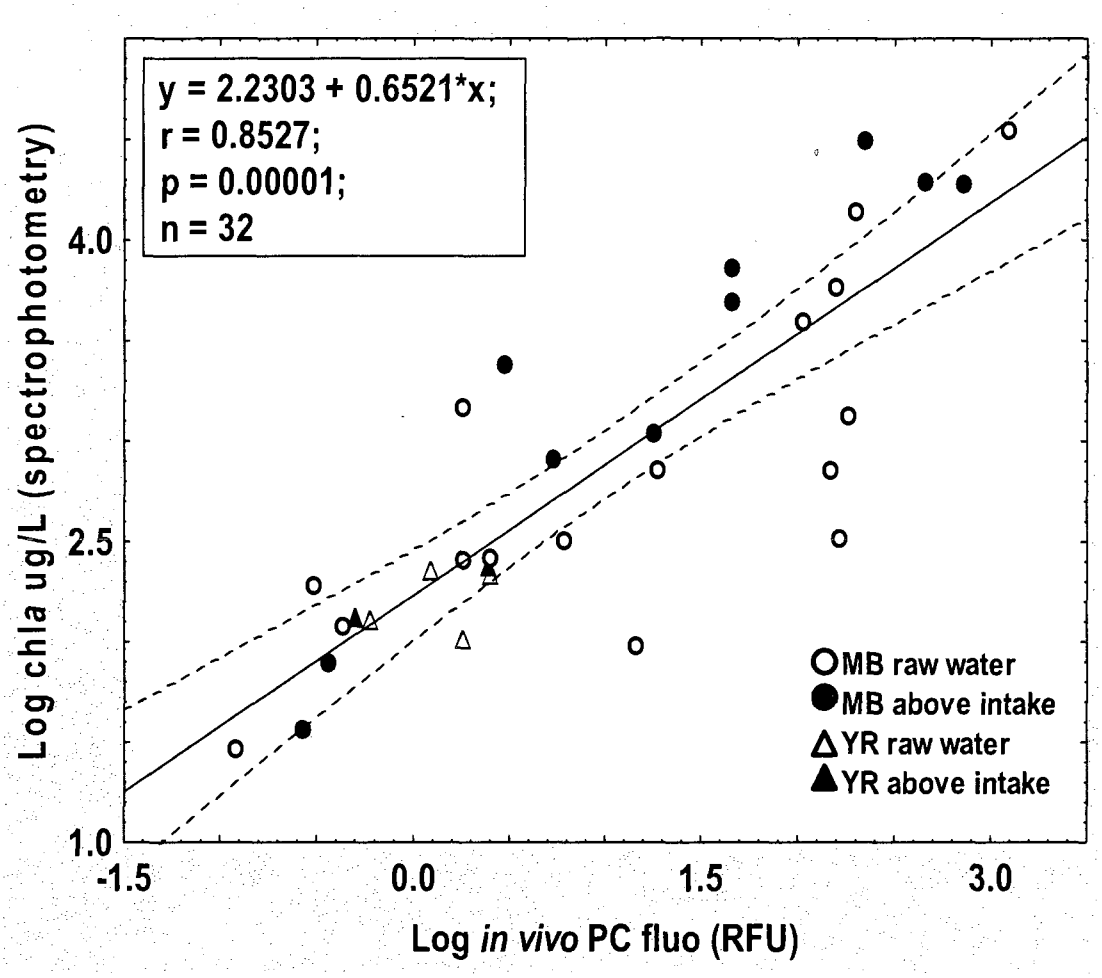


Figure 5.9 Relationship between log transformed *in vivo* PC fluorescence data and log transformed chl *a* concentration (µg/L) derived from samples taken in source and raw water from both field sites.

This relationship between *in vivo* PC fluorescence and extracted chl-*a* exists because large amounts of chl-*a* are present in cyanobacteria. Yet, chl-*a* is poorly detected by the CHL fluorescence probe due to its location in the organism. It is housed in the poorly fluorescent Photosystem I (van Thor et al. 1998) which makes it difficult to detect by *in vivo* chlorophyll fluorescence (Gregor et al. 2005; Seppala et al. 2007).

This very strong correlation between extracted µg/L-chl-*a* and *in vivo* PC fluorescence as measured by the PC probe is counterintuitive because chlorophyll is primarily recognized as a good indicator of total phytoplankton biovolume (van Thor et al. 1998; Oliver et al. 2000) which is no exception in collected grab samples from Missiquoi Bay and Yamaska Reservoir (r = 0.85; n = 25; p = 0.000001; Figure 5.10b). Yet, cyanobacteria contribute significantly to the total chl-*a* content of environmental samples taken at our field sites since the relationship between extracted chl-*a* and cyanobacterial biovolume is (R = 0.66; n = 25; p = 0.0003) (Figure 5.10a).

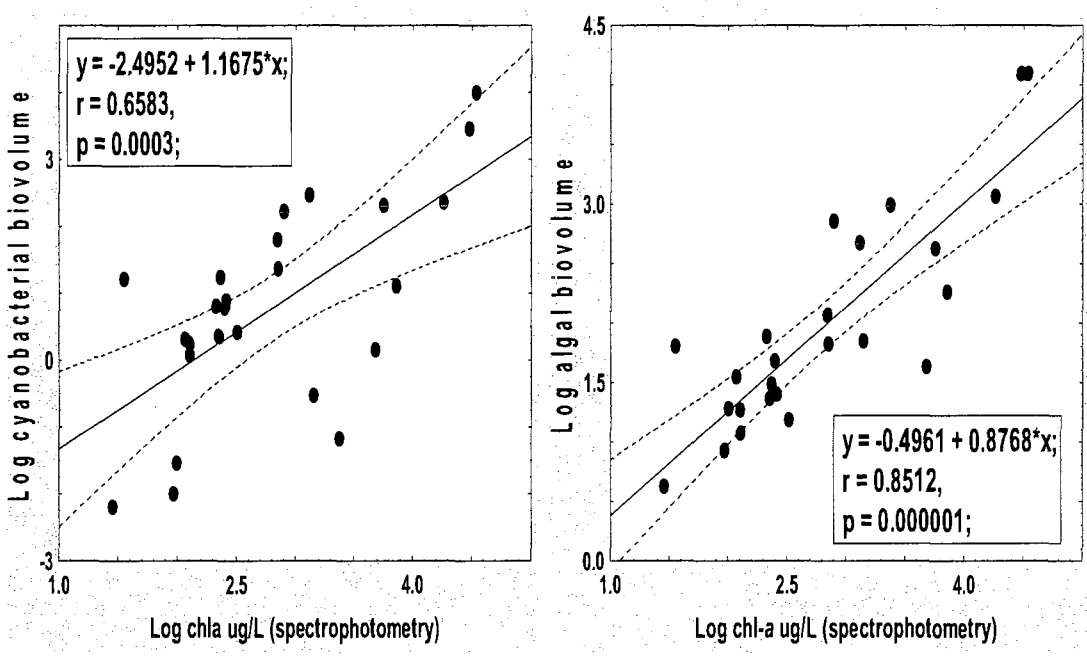


Figure 5.10. Samples analyzed are from raw water samples taken inside both DWTPs and above both water intakes: a) Chl-*a* measured by spectrophotometry versus cyanobacterial biovolume; b) Chl-*a* measured by spectrophotometry against the total biovolume from other phytoplankton groups.

Phytoplanktonic chl-*a* is easily detected by the PC probe, yet chl-*a* not being exclusive to cyanobacteria, nor an indicator of cyanotoxins, is disadvantageous for monitoring purposes. In a study which adapted the WHO's framework to Lake Champlain, USA, chl-*a* concentration thresholds were poor indicators of cyanotoxin concentrations and were therefore not included in the monitoring framework (Watzin et al. 2006).

Cyanobacterial biovolume, with its significant correlation with *in vivo* PC fluorescence, and its ability to identify potentially toxic species of cyanobacteria, should be included in a locally adapted alert level framework for cyanobacteria monitoring by *in vivo* PC fluorescence at DWTPs in Quebec, Canada.

5.2 Cyanobacterial monitoring at two drinking water treatment plants in Southern Quebec

Section 5.2 describes the results from the monitoring campaigns in 2007 and 2008 at both the Missisquoi Bay and the Yamaska Reservoir. See appendix C for complete phytoplanktonic analysis.

5.2.1 Cyanobacterial and cyanotoxin monitoring with conventional laboratory methods and the *in vivo* PC fluorescence.

This section describes interannual and spatio-temporal variations of cyanobacteria and their associated toxins at the Missisquoi Bay and the Yamaska reservoir as monitored by conventional grab samples and novel continuous *in vivo* PC fluorescence measured by a PC probe over two field seasons (2007-2008). All 2007 data was collected by A. Zamyad (unpublished data)

Missisquoi Bay Raw water inside DWTP (2007)

In 2007, only three raw water samples were analyzed inside the DWTP due the lack of bloom development in the Bay. These grab samples had cyanobacterial biovolumes between 0.023mm^3/L and 0.655mm^3/L, toxicities less than 0.1µg/L of MC-LR, and were dominated by *Anabaena spiroides crassa.*

Continuous *in vivo* PC fluorescence monitoring inside the DWTP by the PC probe on the MPS detected a seasonal average of 1 200 eq-cyanobacteria/mL with no significant peaks in cyanobacterial abundance between August and November 2007 (Zamyadi 2007).

Continuous *in vivo* PC fluorescence monitoring showed that there were low concentrations of cyanobacteria and cyanotoxins in the Missisquoi Bay between August and November 2007. This was contrary to the reports of high cyanobacterial abundances detected in both the Bay and the DWTP in previous years (Blais 2002; MDDEP 2008a; Robert 2008).

Missisquoi Bay 2008 – Raw water inside DWTP

Results collected in 2008 from the Missisquoi Bay's DWTP had similar results past monitoring campaigns by Quebec's Ministry of the Environment. The Ministry reported that 88-100% of raw water samples analyzed between 2004-2006 had cellular densities over 2 000 cyanobacteria/mL with a peak density of 1.9 million cells/mL on one sampling day in 2006. Cyanotoxin monitoring in 2005 detected a maximum concentration of 4.5µg/L MC-LR in raw water at the entrance of the DWTP on Missisquoi Bay (Robert 2008).

Laboratory method

One hundred percent of samples analyzed at the entrance of the DWTP which specifically targeted periods with expected cyanobacterial blooms had cellular densities over 2 000 cells/mL (see Table 5-5 for all cyanobacterial densities measured from grab samples taken at both field sites in 2008). Between the end of July and mid-September 2008, cyanobacterial blooms were detected by both conventional and novel *in vivo* PC monitoring technology in source and raw water of the Missisquoi Bay's DWTP.

Inside the DWTP, total biovolume measurements determined *Microcystis* spp. and *Anabaena* spp. to be the most dominant cyanobacterial genera during the season. *Microcystis aeruginosa* was not always dominant but was present in all analyzed samples (Figure 5.11a). The highest cyanobacterial biovolume (54.1mm^3/L) measured inside the DWTP occurred on August 18th and the lowest (0.576mm^3/L) on June 26th (Figure 5.11b).

In 2008, all samples collected inside the DWTP had higher cyanobacterial biovolumes than those taken in 2007 with the exception of one collected on June 26th 2008. This demonstrates that interannual cyanobacterial abundance can fluctuate greatly. Additionally, historical data show that the most frequently identified species in the raw water of the DWTP at Missisquoi Bay between 2004-2006 were *Anabaena flos-aquae, Aphanizomenon flos-aquae* and *Microcystis flos-aquae* (Robert 2008). However, in 2007 the prevailing species was *Anabaena spiroides crassa*, and in 2008 *Microcystis aeruginosa* and *Anabaena flos-aquae* dominated the cyanobacterial fraction of the phytoplankton. Hence, the interannual variation of the dominant species of cyanobacteria is highly changeable and presents no trends.

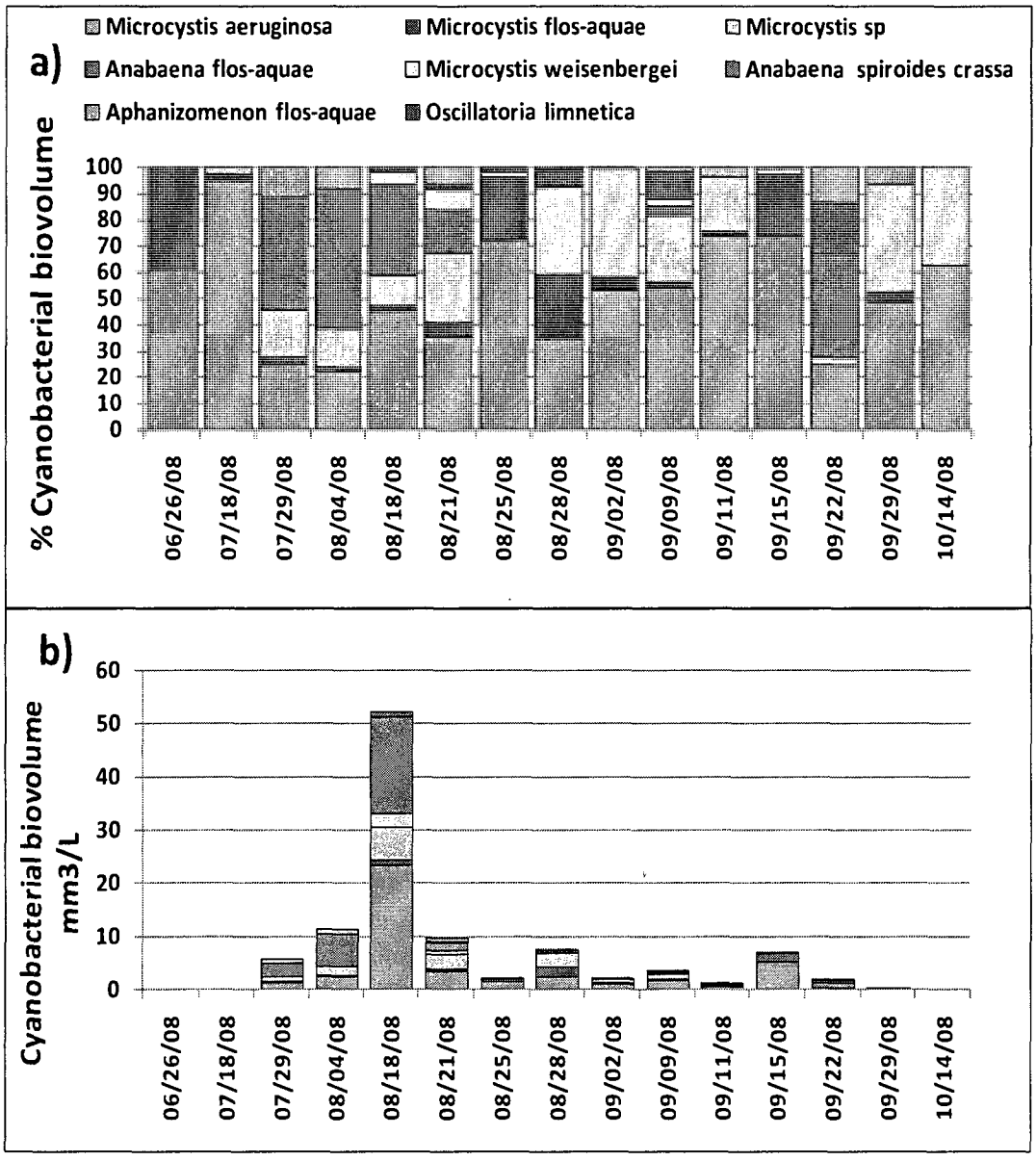


Figure 5.11 Distribution of cyanobacterial biovolume of the most abundant species inside the DWTP on Missisquoi Bay: a) Cyanobacterial species shown as percent of total cyanobacterial biovolume sample each sampling day, b) total biovolume of the most abundant cyanobacterial species present each sampling day. Some samples presented in figure "a" are not visible on figure "b" due to low biovolumes.

***In vivo* PC fluorescence monitoring**

Like in 2007, the DWTP's raw water was measured continuously by a PC probe (measuring *in vivo* PC fluorescence) on the MPS installed at the entrance of the plant's raw water in 2008.

During pre-bloom conditions from May 29th and July 29th the PC probe estimated a daily average of 1 300 eq-cyanobacteria/mL in raw water. Between July 29th and September 15th cyanobacteria were detected in raw water inside the DWTP; the PC probe detected peaks in cyanobacteria abundance which averaged out to the probe's estimated densities of 15 000 eq-cyanobacteria/mL. The PC probe's average estimated cyanobacterial density dropped to 3 600 eq-cyanobacteria/mL during the post-bloom period from September 16th to October 14th.

The PC probe readings corresponded well to the relative increases and decreases of total cyanobacterial biovolumes measured by grab samples during the pre-bloom, bloom and post-bloom periods (Figure 5.11). As previously mentioned, PC probe's estimated cyanobacterial densities were not accurate but there are a strong linear relationships between *in vivo* PC fluorescence and cyanobacterial densities in our laboratory and environmental samples.

Cyanotoxin monitoring

In 2008, measured MC-LR concentrations in raw water inside the Missiquoi Bay's DWTP varied from 0.15 to 14µg/L (Figure.5.12).

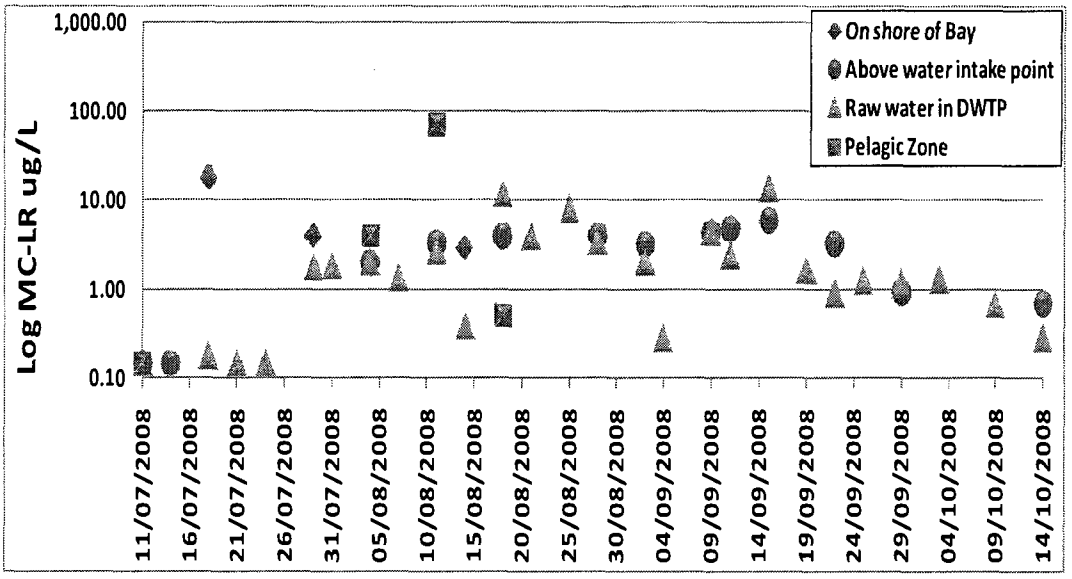


Figure.5.12. Spatio-temporal distribution of cyanotoxin (μg/L of MC-LR) at Missisquoi Bay.

When raw water samples inside DWTPs contained microcystin concentrations higher than 1μg/L (the WHO's recommendation for treated drinking water) they could pose a risk to human health if the physical removal of cells and/or the treatment of dissolved toxins are inadequate or unavailable. Of samples taken inside Missisquoi Bay's DWTP, 45% had concentrations >1μg/L MC-LR where as only 15% were above the same concentration in the DWTP on the Yamaska Reservoir (Appendix F).

Missisquoi Bay 2008 – Source water of DWTP

Laboratory method

In grab samples taken above the Missisquoi Bay's DWTP water intake, the cyanobacterial genera with the highest daily biovolume (mm^3/L) alternated between *Microcystis* spp. and *Anabaena* spp. throughout the season (Figure.5.13a). All analyzed samples had cyanobacterial biovolumes under $10mm^3/L$ except one taken on August 28th which had a biovolume of $616mm^3/L$ (Figure.5.13b).

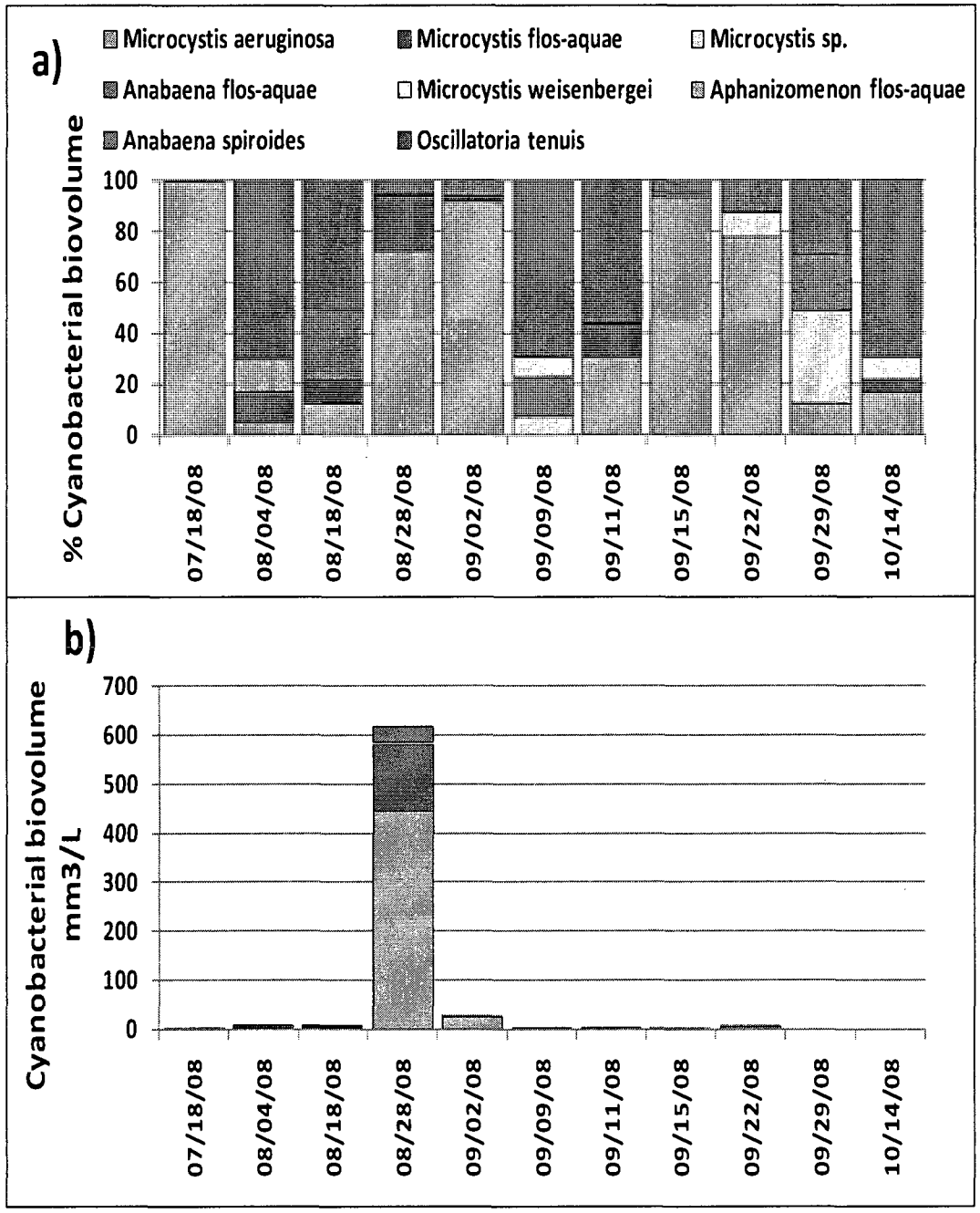


Figure.5.13 Distribution of cyanobacterial biovolume of most abundant species above the DWTP's intake point on Missisquoi Bay: a) Cyanobacterial species shown as percent of total cyanobacterial biovolume sample each sampling day, b) total biovolume of most abundant cyanobacterial species present each sampling day. Some samples presented in figure a) are not visible on figure b) due to low biovolumes.

Taxonomic analysis was also performed on samples taken in dense scum accumulations on July 29th and August 14th (Appendix E). In both samples there was an almost uniform distribution of the cyanobacterial biovolume of *Microcystis aeruginosa*, *Anabana spiroides*, and *Microcystis flos-aquae,* however the total biovolume on August 14th was 2816mm^3/L and only 38mm^3/L on July 29th.

In 2001, results generated when monitoring Missisquoi Bay's photic zone (zone where photosynthesis can be performed because of sufficient light penetration) within a 200m radius of the DWTP showed large spatio-temporal variations of cyanobacterial abundance. Results produced in 2002 are much like 2008's season, but very different from 2007 results in terms of cyanobacterial abundance (Blais 2002). It is not yet understood why Missisquoi Bay has had dense cyanobacterial blooms events in some years (e.g. 2002 and 2008) and none in other years (e.g. 2007). Potential interannual differences in precipitation and temperature are hypothesized to explain some of the variability but such hypotheses have not yet been tested.

Spatial variation of cyanobacteria and cyanotoxins

During the 2008 season, the toxicity of grab samples taken above the water intake in the Bay fluctuated between 0.15 and 6µg/L. The toxicity detected in grab samples taken at other locations in the Bay, including dense cyanobacterial scum varied between 0.15 and 72µg/L (Figure.5.12).

On August 18th and September 15th cyanobacterial biovolumes of 10.6mm^3/L and 3mm^3/L with MC-LR concentrations of 4µg/L and 6µg/L (Figure.5.12) were measured above the intake point outside the DWTP. The most abundant species identified in the samples were *Anabaena spiroides* and *Anabaena flos-aquae* on August 18th, and *Microcystis aeruginosa* on September 15th (Figure.5.13). All of the most abundant species are able to produce MC-LR (Appendix A) therefore it is reasonable to have detected concentrations of the cyanotoxin in samples. On the same days as mentioned

above, cyanobacterial biovolumes of 54.1mm^3/L and 7.5mm^3/L (Figure 5.11) with MC-LR concentrations of 12µg/L and 14µg/L (Figure.5.12) were measured inside the DWTP. The same cyanotoxin producing species were detected inside the plant with the exception of *Anabaena spiroides* which was not present.

Depth profiles using PC *in vivo* fluorescence was used to understand the spatial distribution of cyanobacteria throughout the water column as per and above the DWTP's water intake. The use of *in vivo* PC fluorescence was also used by (Beutler et al. 2002; Leboulanger et al. 2002; Brient et al. 2008) to detect variations in cyanobacterial concentrations throughout the water column. The depth profiles detected homogeneous cyanobacterial concentrations throughout the water column and above the DWTP's intake on both August 18th and September 9th. The Missisquoi Bay is relatively shallow (mean depth of 4m), thus mixing by waves and wind on both sampling days (Figure 5.14a). This mixing could have permitted higher concentrations of toxic cyanobacteria to enter the DWTP, thereby creating a more difficult situation for operators of the DWTP to ensure that <1µg/L MC was present in drinking water. Treated drinking water was not measured for the presence of cyanotoxins.

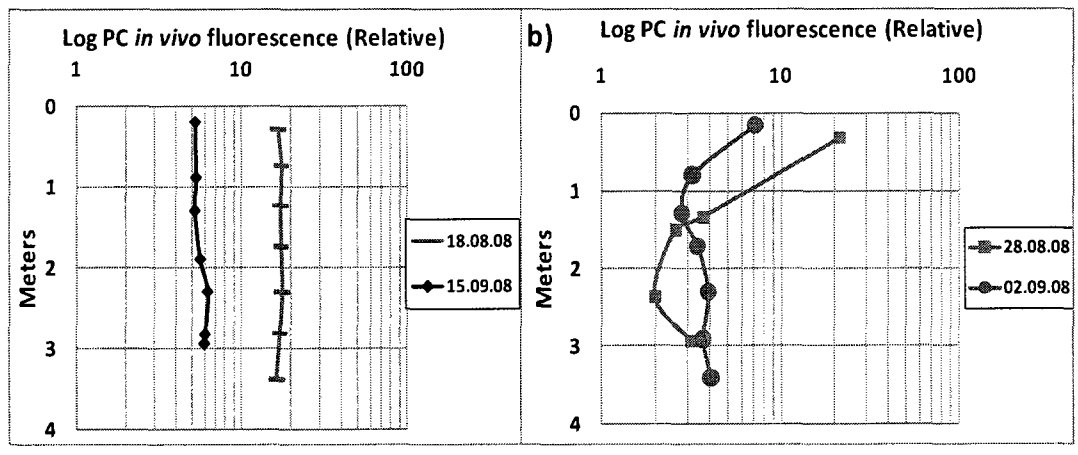


Figure 5.14. Phycocyanin depth profiles with *in vivo* PC fluorescence probe above the water intake on the Missisquoi Bay; a) on windy days with energetic mixing of water column; b) on calm days with very little movement of the water column.

On the other hand, the opposite situation was also true on August 28th and September 2nd when biovolumes of 619mm^3/L and 32mm^3/L, which were dominated by MC-LR producing *Microcystis aeruginosa* (Figure.5.13) were detected above the DWTP's intake point. Samples from raw water inside the plant on the same days measured cyanobacterial biovolumes of only 7mm^3/L and 4mm^3/L (Figure 5.11) made up of *Microcystin* producing *Microcystis aeruginosa, Microcystis flos-aquae and, Microcystis weisenbergei.* Toxicity analyses on both sampling days indicated that concentrations of MC-LR measured inside the DWTP were slightly lower (3.5µg/L and 2µg/L) than concentrations measured above the water intake outside the DWTP (4µg/L and 3.3µg/L) (Figure.5.12).

Depth profiles by *in vivo* PC fluorescence read dense accumulations of cyanobacteria near the water's surface over the intake point and much less in the water matrix below the surface (Figure 5.14b). Depth profiling results follow the same pattern of cyanobacterial spatial distribution as those presented in Gregor et al. (2007) with the exception that our profile was done in a much more shallow Bay (average depth of 4m). Dense accumulations of cyanobacteria near the water's surface without water column mixing could provide lower risk conditions for cyanotoxin contamination of the DWTP's water. However, the diurnal variation of water column stratification with regards to cyanobacterial densities should be studied further.

There were often large variations of cyanobacterial species, cyanotoxin concentrations and cyanobacterial abundance in grab samples taken on the same day but at different locations at the Missisquoi Bay.

Yamaska Reservoir 2007

As no other research has been conducted on the Yamaska Reservoir other than this study, interannual comparisons of cyanobacteria their associated toxins are limited to data collected in 2007-2008.

At entrance of reservoir

In 2007, two samples taken at the entrance of the Yamaska Reservoir had cyanobacterial biovolumes of $0.133mm^3/L$ and $0.173mm^3/L$ and were mostly composed of *Woronichiana naegeliana* and *Anabaena spiroides crassa.* Continuous monitoring *in vivo* PC monitoring at the entrance of the reservoir yielded a seasonal average of 1 300 eq-cyanobacteria/mL as estimated by the PC probe on the MPS. Like data collected by the PC probe inside the Missisquoi Bay's DWTP in 2007, there were no significant peaks of *in vivo* PC fluorescence throughout the season. Also like the results from Missisquoi Bay in 2007, the PC probe greatly underestimated the true cyanobacterial densities in grab samples (Table 5-1).

On shore of reservoir

In October of 2007 three spatially clustered highly toxic cyanobacterial blooms were sampled and measured with the probe (Table 5.3). *Anabaena flos-aquae, Anabaena circinalis, Anabaena planktonica, Aphanizomenon flos-aquae, Microcystis aeruginosa* and *Woronichinia* spp. were present in bloom events at the Yamaska Reservoir, with a dominance of *Woronichinia* spp.

Table 5-3.Toxicity and spatio-temporal PC *in vivo* fluorescence values taken on three field days in the Yamaska Reservoir in 2007.

Date	Sample toxicity	PC probe reading (eq-cyanobacteria/mL) at time of sampling		
	MC-LR µg/L (intra and extra-cellular)	In scum	At entrance of Reservoir	Inside DWTP
09.10.07	490	n.a.*	n.a.*	n.a.*
11.10.07	>500	183,000	15,000	n.a.*
16.10.07	125	99,600	1,250	1,650

*n.a.: data not available

PC fluorescence probe readings in other locations on the reservoir indicated that the bloom did not seem to affect water quality at the entrance of the Reservoir or inside the DWTP. These preliminary results led to a more intensive study in 2008.

Yamaska Reservoir 2008 – Raw water inside DWTP

Laboratory method

In 2008, raw water samples taken in inside the DWTP in August identified *Aphanizomenon* spp., *Woronichinia* spp. and *Anabaena* spp. as the most abundant genera.

Unlike 2007, when *Woronichinia* was the dominant genus at the end of the season, *Microcystis aeruginosa* dominated the phytoplankton at the end of September (Figure.5.15a). Different from the high biovolumes detected at the entrance of Mississquoi Bay's DWTP, all analyzed samples taken inside Yamaska Reservoir's DWTP had cyanobacterial biovolumes lower than $2mm^3/L$ (Figure.5.15b).

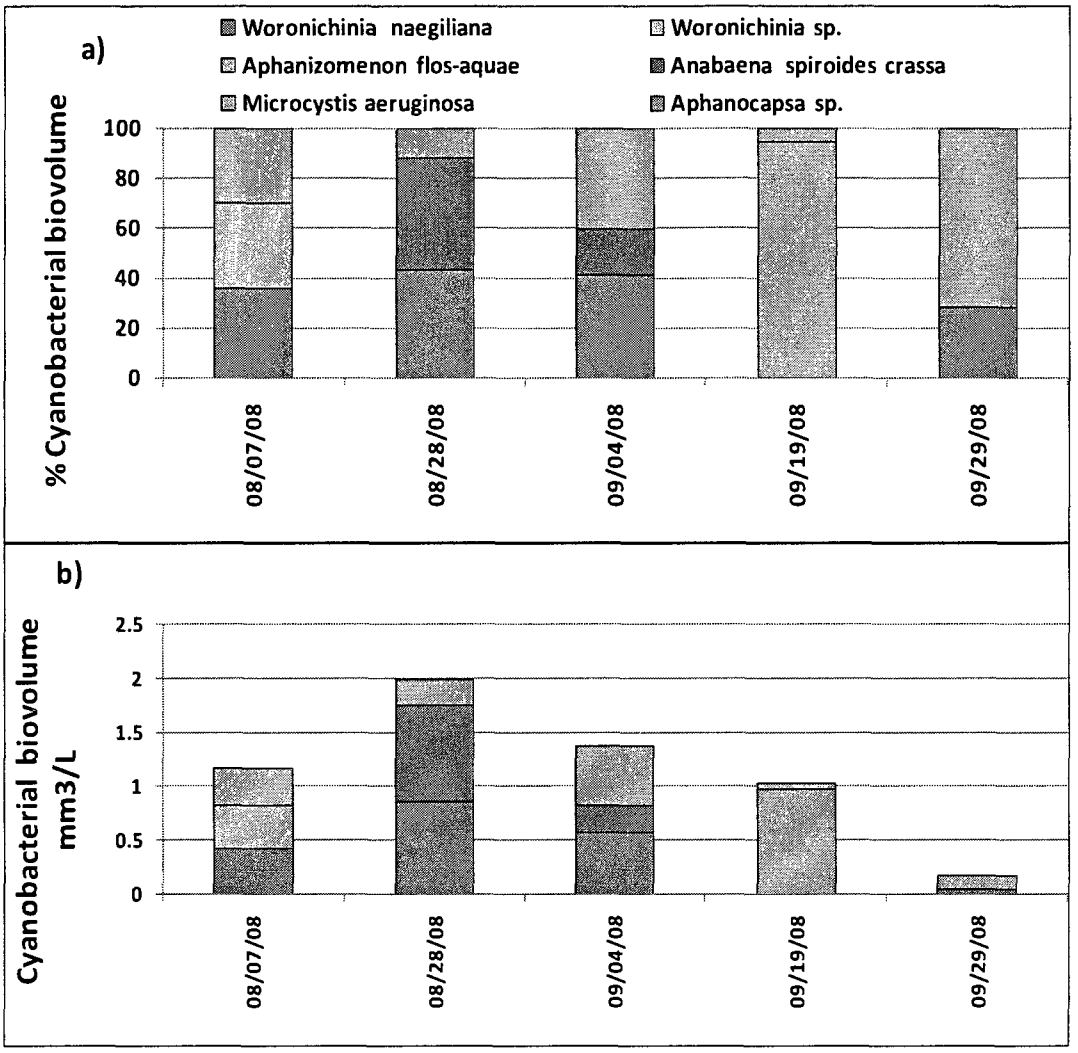


Figure.5.15 Distribution of cyanobacterial biovolume of most abundant species of in samples taken in raw water inside the DWTP on reservoir: a) Biovolume of cyanobacterial species shown as percent of total grab sample each sampling day, b) Biovolume of most abundant cyanobacterial species present each sampling day.

In vivo PC fluorescence monitoring of cyanobacteria inside the DWTP

In 2008, continuous cyanobacterial monitoring yielded a seasonal average of 2 700 eq-cyanobacteria/mL in the DWTP's raw water as estimated by the PC probe. No significant peaks in *in vivo* PC fluorescence were observed in raw water inside the DWTP for the entire monitoring season. However, as in 2007, highly clustered and toxic cyanobacterial blooms developed in the reservoir during the 2008 season.

Cyanotoxin monitoring in raw water of DWTP

In 2008, the measured cyanotoxin concentrations from samples taken inside the Yamaska Reservoir's DWTP never yielded MC-LR concentrations higher than 2µg/L (Figure 5.16).

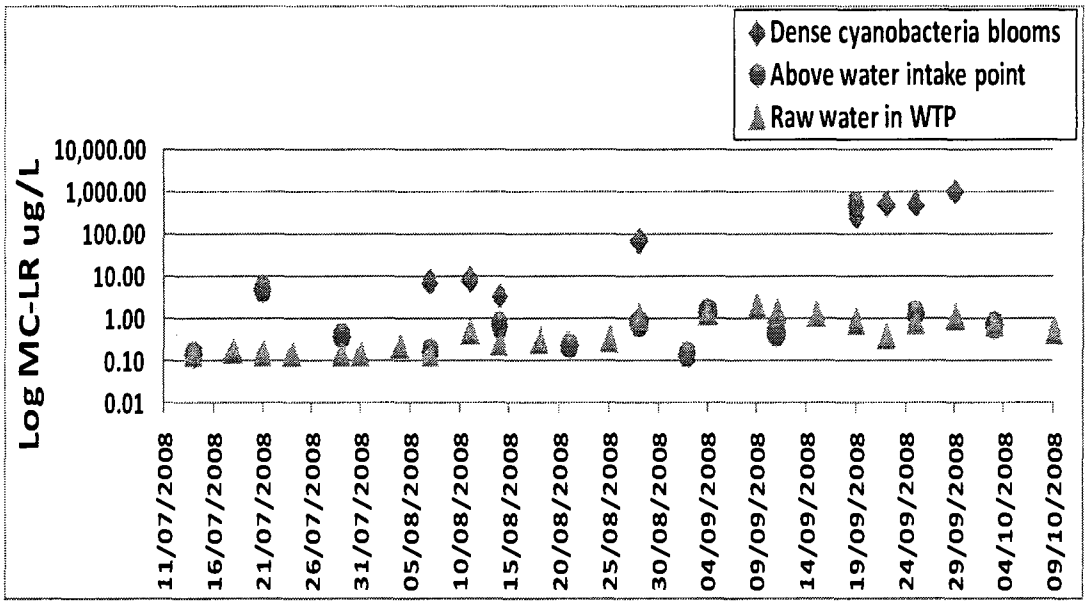


Figure 5.16. Spatio-temporal distribution of cyanotoxin (µg/L of MC-LR) inside and outside of DWTP near Yamaska River.

In 2008, the distribution of MC-LR concentrations detected in the raw water of Yamaska Reservoir's DWTP was much more stable and the concentrations were lower than raw water samples taken inside the Missisquoi Bay's DWTP.

Yamaska Reservoir 2008 – Source water of DWTP

Laboratory method

In the Reservoir outside the DWTP the dominant species changed from *Woronichinia naegiliana* to *Anaebana flos-aquae* to *Microcystis aeruginosa* as the season progressed (Figure.5.17a). Contrary to the Missisquoi Bay where bloom events occurred were first detected on July 29th, the largest detected cyanobacterial bloom above the water intake occurred at the end of September 2008 (Figure.5.17b).

Dense accumulations of cyanobacterial scum were detected by the edge of the Yamaska Reservoir on three sampling days in September and October (Appendix C). Here again there is evidence of a shift in the dominant species from *Aphanezomenon flos-aquae* to *Woronichinia naegiliana* to *Microcystis aeruginosa* with by far the highest biovolume present latest in the season on September 29th. Dense cyanobacterial accumulations on the shore of the reservoir could be due to the wind which tended to push cyanobacteria away from the intake point.

Spatial variation of cyanobacteria and cyanotoxins at the Yamaska Reservoir

On the 19th, 22nd, 25th and 29th of September the MC-LR concentrations near the South-West shore of the Yamaska Reservoir were two orders of magnitude higher than the concentrations detected inside the DWTP on the same days (Figure 5.16). The dense cyanobacterial accumulations were as toxic and located on the same side of the Yamaska Reservoir as those measured in 2007.

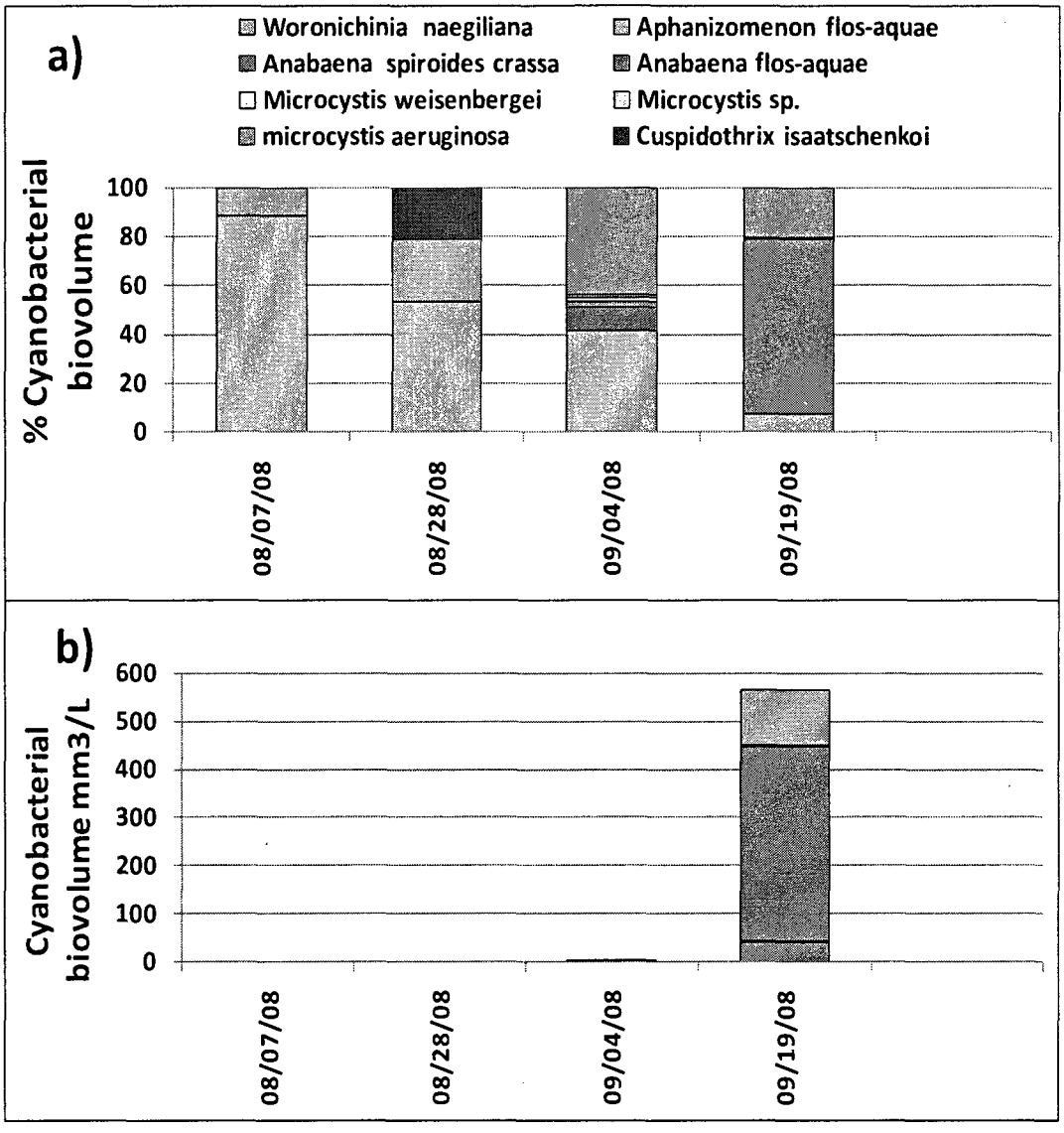


Figure.5.17. Distribution of the most abundant cyanobacterial species. Samples taken above water intake in the Yamaska Reservoir outside the DWTP: a) Biovolume of cyanobacterial species shown as percent of total grab sample each sampling day, b) Biovolume of most abundant cyanobacterial species present each sampling day. Some samples presented in figure a) are not visible on figure b) due to low biovolumes.

Depth profiles above the Yamaska Reservoir DWTP's intake point using *in vivo* PC fluorescence showed low concentrations of homogenously distributed cyanobacteria throughout the water column on August 7th, 28th and September 4th (Figure 5.18). The low *in vivo* PC fluorescence values corresponded to low cyanobacterial biovolumes both inside (between 1.3mm^3/L and 2mm^3/L) and outside (between 1.2mm^3/L and 3.4mm^3/L) the DWTP on the same days (Figure.5.15 and Figure.5.17). MC-LR concentrations were under 0.15µg/L inside the DWTP and under 0.17µg/L above the water intake on all days (Figure 5.16). Online PC probe readings on the above mentioned sampling days were: 1600, 3 000 and 2 800eq-cyanobacteria/mL respectively, confirming that only low concentrations of cyanobacteria entered the DWTP's intake. Contrary to results generated at the Missisquoi Bay, the uniform concentrations of cyanobacteria throughout the water column did not increase cyanotoxin concentration inside DWTP because the cyanobacterial and cyanotoxin density above the water intake was very low.

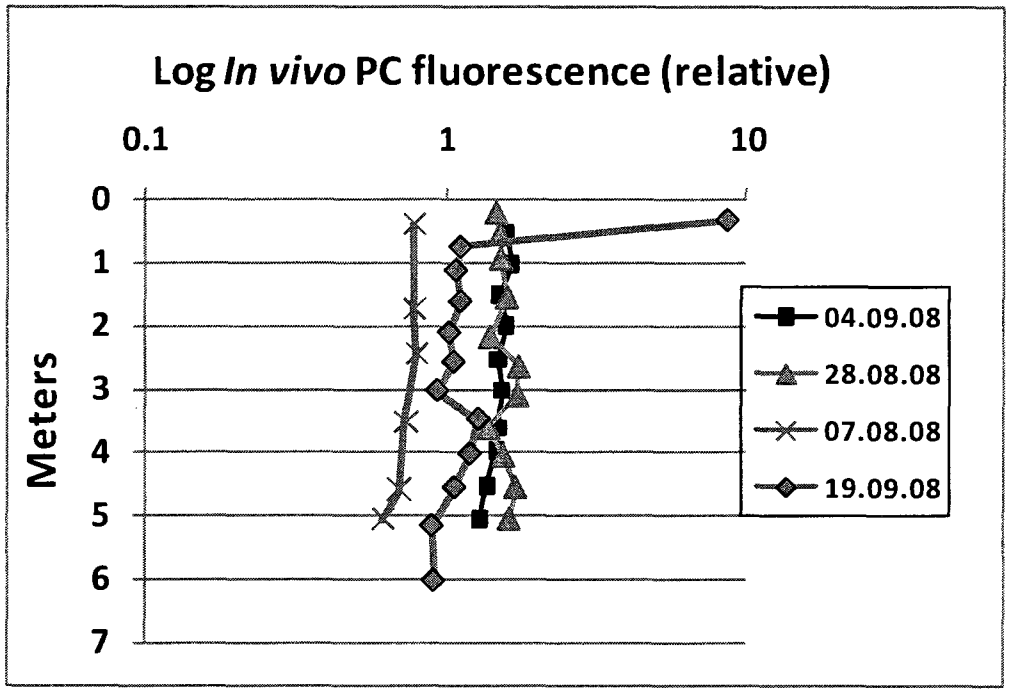


Figure 5.18. Depth profiles with PC *in vivo* fluorescence in the Yamaska Reservoir above the water intake .

On September 19th an accumulation of cyanobacteria developed at the surface of the reservoir with a biovolume of 569mm^3/L (Figure.5.17) and a MC-LR concentration of 489µg/L (Figure 5.16). A depth profile taken above the water intake on the same day indicated cyanobacterial concentrations were dense near the surface but dropped an order of magnitude as lower depths were monitored (Figure 5.18). Despite the highly toxic cyanobacteria at the surface, only a cyanobacterial biovolume of only 1mm^3/L (Figure.5.15) was measured inside the DWTP with a toxicity of 0.9µg/L MC-LR (Figure 5.16). These results are similar to those collected on days without water column mixing in the Missisquoi Bay. Over all, despite highly toxic accumulations near the water intake, there was little cyanotoxin contamination of the water entering the DWTP on the Yamaska Reservoir. Although diurnal variations of depth profiles are not available, the online probe measuring the DWTP's raw water confirmed that only low densities of cyanobacteria (1 400 eq-cyanobacteria/mL) migrated to the intake.

5.2.3 Correlations between *in vivo* PC fluorescence and other parameters of the MPS (2007-8).

Multiple regression analysis was performed to try to determine which of the monitored parameters on the MPS (turbidity, CHL fluorescence, pH, dissolved oxygen, ΔpH, Δ dissolved oxygen, specific conductivity, and temperature) co-occur when high values of *in vivo* PC fluorescence are measured. This knowledge would give DWTP operators a better idea of which parameters should be carefully monitored during periods when the source water of plants are vulnerable to cyanobacterial blooms.

Results of the Multiple Linear Regression models from the 2007-8 seasons are presented in Table 5-4. A CHL probe malfunction in the middle of the sampling season forced the creation of two multiple linear regression models at Missisquoi

Bay's DWTP: the first used the entire temporal data series but excluded CHL fluorescence data, and the second included CHL fluorescence but excluded several weeks of temporal data from the other probes on the MPS.

Studies have used multiple linear regressions to link chl-*a* concentrations (μg/L) to *in vivo* fluorescence data (Seppala et al. 2007) and to link *in vivo* fluorescence to empirical bloom related factors (Ahn et al. 2007). However, this study's multiple linear regression using continuous online data parameters has not been looked at until now.

Table 5-4. Results from multiple linear regression analysis on data collected by the MPS at both Missisquoi Bay and the reservoir on Yamaska River in 2007-8. Y value = PC fluorescence and X values = sp. cond., CHL fluo, Turb, pH, DO , Delta pH, Delta DO and water temperature.

Temporal information	Site/MPS location	Significant parameters	Multiple R	P value	Correlation between x values
2007 Whole season	Missisquoi Bay	Dissolved O_2 Turbidity Delta pH	0.70	0.000001	Delta pH and Dissolved O_2 (-0.33)
2008 Whole season	Missisquoi Bay (no chl)/ inside DWTP,	**Turbidity Delta pH**	0.71	0.000001	none
2008 Whole season except data from 07/24/08 to 08/18/08 *	Missisquoi Bay / inside DWTP,	Turbidity, Dissolved O_2 Delta pH, CHL fluo.	0.78	0.000001	Turbidity and CHL fluorescence (0.33)
2008 Whole season	Yamaska / inside DWTP,	Sp. Cond, Dissolved O_2	0.78	0.000001	none
2008 Whole season	Yamaska / Under buoy: Above water intake	Temp, pH	0.47	0.00013	Temperature and pH (0.67)

* Data from not included form because of chlorophyll probe malfunction

Missisquoi Bay multiple linear regression models

In 2007 the data collected by the MPS at Missisquoi Bay's DWTP deemed dissolved oxygen, turbidity and delta pH to be the most significant factors for predicting PC fluorescence with a correlation coefficient of R = 0.70.

In 2008, the same site produced regression models with the same predictive parameters, a stronger correlation coefficient (R = 0.71 for the first model without CHL fluorescence), and determined turbidity to be the most important factor correlated to *in vivo* PC fluorescence. Cyanobacterial blooms can cause a significant increase in turbidity values when present in large numbers. This change from the 2007 to 2008 regression model could be due to the increased concentration of cyanobacteria detected inside the DWTP in 2008 and their associated turbidity values (Zamyadi, 2008 unpublished work). This is also coherent with work which found a correlation coefficient of (R^2=0.531) between turbidity and *in vivo* PC fluorescence (Ahn et al. 2007).

Delta pH was deemed the second most important value associated with *in vivo* PC fluorescence, cyanobacteria increase the water's pH by reducing the concentration of CO_2 in the water while performing photosynthesis in bloom conditions (Shapiro 1997). The decomposition of cyanobacterial blooms and their associated production of ammonia (Blais 2002) also increases pH values in water. Hence, increases in pH, could be a warning for DWTP operators that raw water should be carefully treated to avoid cyanotoxins in drinking water sent to consumers.

Since turbidity and pH are parameters which are already monitored at all DWTPs in Quebec, this model presents a low cost monitoring strategy for operators to be prepared for potential cyanobacterial blooms in raw water of the plant.

Yamaska Reservoir multiple linear regression models

Specific conductivity and dissolved oxygen were the most important independent variables at the Yamaska Reservoir's DWTP (Table 5-4). The correlation coefficient was relatively strong at (R = 0.78) but the range of PC fluorescence used to make this model is very narrow, and so the models predictive power is limited to conditions of low PC fluorescence values.

Regression analysis on data collected by the MPS over the water intake in the Yamaska Reservoir yielded a very poor correlation coefficient (R = 0.47) and strongly correlated x-parameters. This could be because of the unusual nature of the Yamaska Reservoir which was aerated, the pH controlled, and had low turbidity, therefore reducing the impact of the factors deemed important at Missisquoi Bay. Therefore, the 2008 models from Missisquoi Bay could be more accurate than all other models presented because they interpret a larger range of PC fluorescence than data collected either in 2007 at Missisquoi Bay or from Yamaska Reservoir. Between the two models generated from 2008 data at Missisquoi Bay's DWTP, the first was the most reliable because it includes the complete temporal data set from May to October (pre, during, and post bloom events) and there were no significant correlations between the predictive independent variables generated.

5.3. The integration of *in vivo* PC fluorescence into an existing cyanobacterial monitoring framework for the two monitored DWTPs.

The main reason for cyanobacterial monitoring in sources of drinking water is to protect consumers of potable water against cyanotoxin provoked health problems. However, monitoring cyanobacterial abundance alone cannot predict the occurrence, the concentration, or even the type of cyanotoxin produced by cyanobacteria. This is because each cyanotoxin can be produced by more than one cyanobacterial species and

the same species is able to generate multiple toxins (Appendix B). Furthermore, within a single species, different genotypes occur, allowing some to possess the gene for a given toxin and others not (Funari et al. 2008). Finally, specific environmental conditions (Albay et al. 2005; Rantala et al. 2006) and population dynamics (Sivonen et al. 1999; Giani et al. 2005) are hypothesized to be necessary to trigger cyanotoxin production. Consequently, in this study rather than trying to correlate the measured cyanotoxin occurrence in the raw water of DWTPs, the "worst case scenario" or "maximum potential cyanotoxin concentration" was correlated with cyanobacterial abundance indicators and *in vivo* PC fluorescence.

5.3.1 Evaluation of the correlations between the maximum potential microcystin concentrations (µg/L) in grab samples and both conventional and *in vivo* parameters for cyanobacterial monitoring.

The maximum potential microcystin concentrations (MPMC) were calculated for samples collected at the monitored DWTPs. Microcystin producers *Microcystis* spp. and *Anabaena* spp. were the two dominant genera identified in raw water samples taken over the 2008 season. The WHO calculates that a *Microcystis* spp cell may contain up to 0.2 pg of microcystin per cell (Bartram et al. 1999; Codd et al. 2005). The maximum potential microcystin concentration for *Anabaena* spp. was calculated at 0.1pg/cell of microcystin based on the concentration of MC-LR measured in the raw water sample taken at the Missisquoi Bay DWTP on August 18th, 2008. This sample was chosen because it contained the highest *Anabaena* spp. biovolume of all samples taken throughout the season. Therefore, for the purpose of this study, the maximum potential microcystin concentration (MPMC) is 0.2pg/*Microcystis* spp. and it is 0.1pg/*Anabaena* spp.

The MPMC for all samples taken in the raw water of DWTPs at both sites were correlated with the 4 parameters which measured cyanobacterial abundance. All

correlations between log transformed maximum potential microcystin concentrations (μg/L) and cyanobacterial monitoring parameters produced correlations ranging from r = 0.67 to r = 0.92 with significant results ($p < 0.01$) (Figure 5.19). Correlations between the true measured microcystin concentrations and cyanobacterial abundance methods are presented in Appendix G.

The correlation between concentrations of extracted phytoplanktoninc PC and the MPMC was the weakest of all measured parameters in this study (r = 0.67), but the correlation was still highly significant ($p = 0.01$). Yet, in another study, phycocyanin was shown to be highly correlated with cyanobacterial biomass (and therefore cyanobacterial biovolume) (Otsuki et al. 1994) and cyanobacterial biomass capable of producing the cyanotoxin MC-LR has been correlated with total MC-LR concentration (Giani et al. 2005). However, given the low correlation between parameters measured in our samples, PC is not the best method to assess the risk of microcystin occurrence in the DWTPs' raw water.

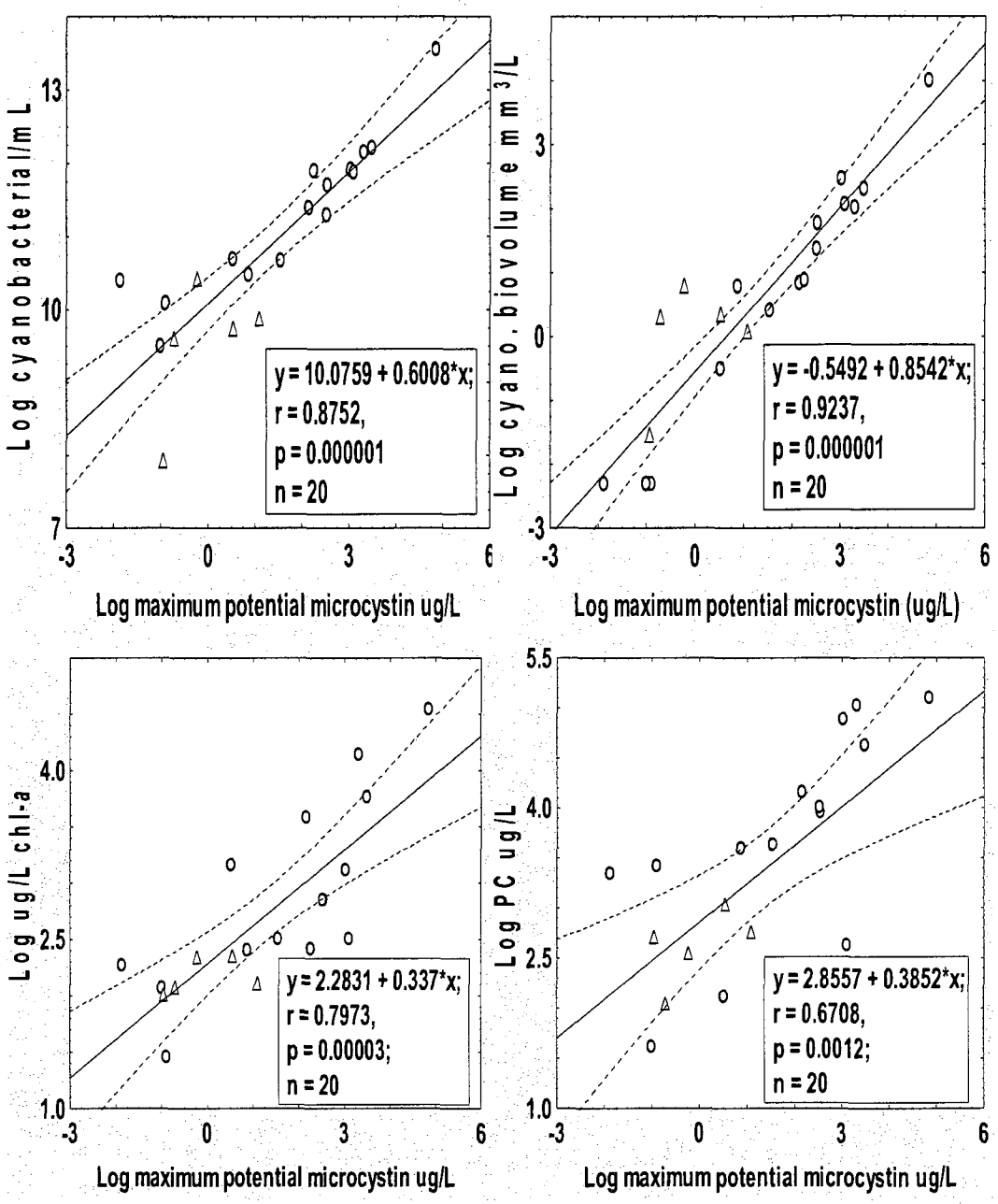


Figure 5.19 The correlations between log transformed maximum potential microcystin concentrations presented clockwise : log cyanobacterial density (top left); log cyanobacterial biovolume (top right); log extracted PC concentration (bottom right); and log of extracted chl-*a* concentration (bottom left). Samples were taken from raw water of both DWTPs in 2008. Circles represent samples from the raw water at the Missisquoi Bay's DWTP and, triangles the raw water form Yamaska Reservoir's DWTP.

Extracted phytoplanktonic chl-*a* concentration in grab samples had a strong correlation MPMC ($r = 0.80$; $p = 0.00003$, Figure 5.19). This is consistent with work done by (Izydorczyk et al. 2009) who found a correlation of ($r = 0.7$) between extracted chl-*a* and intracellular microcystin. In addition, chl-*a* is the easiest parameter for the *in vivo* PC fluorescence probe to interpret in the laboratory ($r = 0.998$) and field samples ($r = 0.85$) (Table 5-2) making it a promising monitoring option. Nevertheless, even if phytoplanktonic chl-*a* correlates well with a sample's MPMC and with *in vivo* PC fluorescence, it is not specific to cyanobacteria. Given this, Watzin et. al (2006) excluded chl-*a* concentration from their monitoring program since the WHO alert level threshold values were not good indicators of microcystin concentration in grab samples.

The correlations between the maximum potential microcystin concentration (MPMC) and both cyanobacterial density ($r = 0.88$ $p = 0.000001$) and biovolume ($r = 0.92$, $p = 0.000001$) were the strongest of all measured parameters. This is unsurprising since these are the two parameters particular to cyanobacteria, whereas the other methods measure phytoplanktonic pigment concentration. The correlation between biovolume and MPMC had the highest correlation of all other parameters measured in this study (Figure 5.19) and the results are similar to previous work performed in Quebec and in Poland (Giani et al. 2005; Izydorczyk et al. 2005).

At the Missisquoi Bay in 2008, biovolume was a far more accurate reflection of true cyanobacterial abundance than cyanobacterial density. All grab samples taken inside the DWTP contained *Aphanothece clathrata brevis* which has a small cell volume of $0.5\mu m^3$ (the average cyanobacterial cell volume of samples from the Missisquoi Bay was $126\mu m^3$). This cyanobacterium represented over 50% of the cyanobacterial density (cells/ml) on 5 days, yet, it never corresponded to more than 20% of total cyanobacterial biovolume (mm^3/L). In fact, *Aphanothece clathrata brevis* corresponded to more than 2% of total cyanobacterial biovolume only three times

during the whole season (Figure 5.20). Consequently, even though *Aphanothece clathrata brevis* had noticeable impacts on the total cyanobacterial density (cells/mL) of samples taken throughout the season, it had little influence on total cyanobacterial biovolumes sampled. Hence, using cyanobacterial biovolume as a monitoring parameter, rather than cyanobacterial density, gives the decision maker a better idea of true cyanobacterial abundance.

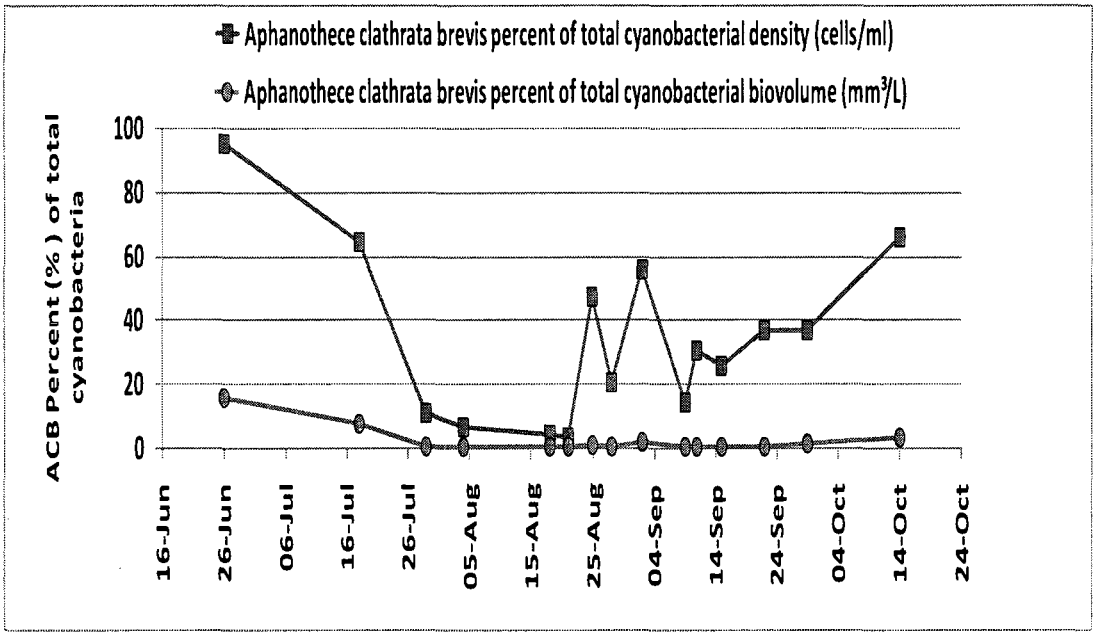


Figure 5.20. The percent of total cyanobacterial density (squares) and cyanobacterial biovolume (circles) that *Aphanothece clathra brevis* represented in samples taken in raw water inside the DWTP on Missisquoi Bay.

However, cyanobacterial density analysis is used to monitor recreational and drinking water sources effectively (WHO framework) in Vermont, USA and Australia (Watzin et al. 2006; Burch et al. 2009). Both programs only count cyanobacteria which are potentially toxic and have a history of bloom development in the area. This pre-screening for only relevant species could be used in Quebec if taxonomic experts were available to perform analyses. However, since cyanobacterial biovolume correlates better with *in vivo* PC fluorescence than cyanobacterial density, it is the best monitoring option for our two field sites.

5.3.2 PC probe's ratio fluorescent units (RFUs) which correspond to existing alert level thresholds in a cyanobacterial or cyanotoxin monitoring framework (WHO or Korean).

Table 5-5 presents all results obtained from grab samples collected in the raw water of DWTPs during the 2008 season at both Missisquoi Bay and Yamaska Reservoir. Results from analyzed water samples are presented in terms of the WHO and the proposed Korean frameworks for cyanobacterial and cyanotoxin monitoring (see section 2.7 for details). The maximum potential microcystin concentration (MPMC) for all samples is also presented in table 5-5. Results from grab samples are visually coded according to each framework's criteria:

WHO alert level framework for cyanobacteria:	The proposed Korean alert system for algal blooms:	WHO's recommendations for *Microcystin* concentrations in treated water:
Alert Level 2*	***Warning****	***Greater than or equal to 1µg/L-MC***
Alert level 1	**Caution**	Less than 1µg/L-MC
Vigilance level	No bloom	N/A

In the Korean system there is a fourth category named "Outbreak" but none of our grab samples fell into this category. The recommended actions for DWTPs are different for each cyanobacterial monitoring system (WHO and Korean system) (Bartram et al. 1999; Ahn et al. 2007); therefore the actions to be taken when thresholds are breached will not be discussed. The focus will instead be on the idea that depending on the criteria used to judge if a threshold is breached, the resulting Alert Level categorization can be different.

Table 5-5. Analyses on samples taken inside the DWTP at Missisquoi Bay and the Yamaska Reservoir (untreated water).

2008		WHO monitoring parameters			Proposed Korean monitoring parameter	WHO recommendation	MPMC
DWTP Location	Date	Cells/ml	Chl *a* µg/l	Bio.vol. mm^3/L	PC µg/L	Inter and extra cellular MC-LR (µg/L)	Microcystin µg/L
Missisquoi Bay	26-Jun	32 490	9.7	0.1	28.4	0.01	0.15
	18-Jul	23 873	4.3	0.1	*30.4**	0.2	0.41
	29-Jul	*119 411**	17.2	5.9	*52.6**	*1.76***	*12.63***
	04-Aug	*148 650**	22.7	*11.7**	*132.1**	*2.09***	*20.81***
	18-Aug	*765 625**	*94.1**	*54.1**	*163.7**	*12***	*127.45***
	21-Aug	*201 309**	42.9	*10.1**	*102.4**	*4.32***	*32.68***
	25-Aug	88 213	36	2.3	*64.2**	*8***	*8.55***
	28-Aug	*143 937**	12.2	7.8	13.8	*3.53***	*22.33***
	02-Sep	*146 078**	11.2	2.4	7.6	*2.07***	*9.54***
	09-Sep	80 193	17.3	3.9	*54.9**	*4.42***	*12.41***
	11-Sep	42 795	12.3	1.5	*37.6**	*2.43***	*4.67***
	15-Sep	*189 599**	*62.7**	7.5	*152.2**	*14***	*27.38***
	22-Sep	35 410	11.1	2.2	*36.6**	0.91	*2.37***
	29-Sep	43 739	23.5	0.6	8.3	*1.19***	*1.68***
	14-Oct	13 372	7.9	0.1	5	0.28	0.37
Yamaska Reservoir	07-Aug	14548	7.9	1.35	7.7	0.13	0.50
	28-Aug	33045	10.3	2.19	12.7	*1.06***	0.80
	04-Sep	16704	10.5	1.41	20.6	*1.44***	*1.72***
	19-Sep	19217	8.2	1.07	15.7	0.88	*2.98***
	29-Sep	2743	7.4	0.21	15	*1.08***	0.39

A concentration higher than 1µg/L of MC-LR in raw water of the DWTP represents a threshold value at which the maintenance of an adequate treatment barrier becomes critical. The WHO considers that long-term exposure to 1µg/L of MC-LR can cause adverse affects on human health (Bartram et al. 1999). However, this threshold is considered a maximum concentration for lifetime exposure to MC-LR and therefore exposure to higher concentrations for a short amount of time can be acceptable.

When comparing the maximum potential microcystin concentrations (MPMC) to the measured values in grab samples in Table 5-5 the MPMC were as high as an order of magnitude higher than the measured MC-LR concentrations on two days (August 4th and 18th). These two data sets are significantly different (t = -4.78. p = 0.0001, Figure 5.21). Given this, and that laboratory analysis is needed to identify the cyanotoxin producing gene in cyanobacteria, it is crucial to use the MPMC values (rather than measured microcystin values) to assess the true risk of cyanotoxin contamination in DWTPs.

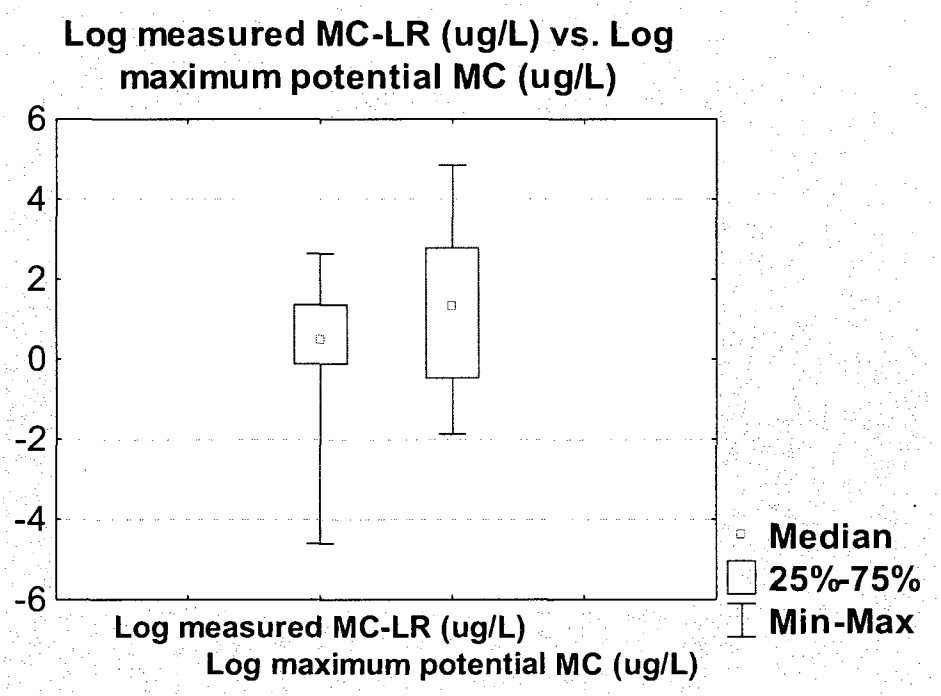


Figure 5.21. T-test of dependent values comparing the measured concentrations of MC-LR to the maximum potential microcystin concentrations.

At the Yamaska Reservoir, there was little variability in the alert level categorization at the Yamaska Reservoir. The alert level remained at "Level 1" (WHO) and "Caution" (Korean) for all parameters in all samples (Table 5-5). The cyanotoxin threshold of 1μg-MC was crossed three times, but each time cyanotoxin concentrations were close to threshold value. On both August 28th and September 29th the concentrations of

measured MC-LR were higher than the MPMC for the given samples. This underestimation of the maximum potential toxin concentrations is most likely due to the presence of dissolved cyanotoxins from cyanobacterial outside the plant. Regardless, all 4 cyanobacterial monitoring parameters accurately reflected the low concentrations of cyanotoxins measured in grab samples from the DWTP's raw water.

However, at the Missisquoi Bay, the four parameters did not predict the measured and potential microcystin concentrations with the same accuracy. Chl-*a* has been shown to increase with cyanobacterial biomass (and therefore cyanobacterial biovolume) in conditions of cyanobacterial dominance of the phytoplankton (Seppala et al. 2007). This was the case on August 18th (Appendix C) and is hypothesized to be the case on September 15th when chl-*a* accurately reflected the high potential risk of cyanotoxin occurrence in the Missisquoi Bay's DWTP's raw water (Table 5-5). Given this, chl-*a* could be a good indicator of cyanobacterial abundance in bloom conditions, however, phytoplanktonic chl-*a* didn't categorized any sampling day as "vigilance" even when there was low cyanobacterial abundance. This was due to the presence of algae other than cyanobacteria which increased the phytoplanktonic chl-*a* concentration of samples (Appendix C). It is for this reason that chl-*a* could be at risk of over estimating the potential microcystin occurrence in DWTP's raw water as was discussed in Watzin et al. (2006). For this reason, chl-*a* should not be included in future monitoring campaigns at both field sites.

Cyanobacterial density was a conservative monitoring parameter at the Missisquoi Bay in 2008. In every case that cyanobacterial density breached the WHO's second ALT, MC-LR concentration was higher than 1µg/L. On sampling days with the highest cyanobacterial densities (i.e. August 18th, 24th and September 15th) larger, potentially toxic, cyanobacteria dominated making density a more effective parameter in bloom conditions. However, there were relatively high cyanobacterial densities (13 000cells/mL - 35 000 cells/mL), on the three sampling days with the lowest MPMC

values (June 26th, July 18th, and October 14th) (Figure 5.20). This is due to the aforementioned abundance of small cyanobacteria therefore over-estimating the risk of toxin occurrence on days with low total cyanobacterial biovolume. Cyanobacterial density should only be used as a monitoring parameter if pre-screening for larger, potentially toxic, species is possible.

PC is also good indicator potential cyanotoxin occurrence on high risk days such as August 18th and September 15th when both high concentrations of PC and of the MPMC values were measured. This is because on August 18th, when the highest extracted concentration of PC was detected (163.7µg/L) (Table 5-5), 93% of total phytoplankton was comprised of cyanobacteria (Appendix C). The "Warning" threshold of the proposed Korean framework corresponded relatively well with the 1µg/L threshold in both true and MPMC values of grab samples (Table 5-5).

Cyanobacterial biovolume was the only monitoring parameter which consistently indicated low risk of cyanobacterial contamination by categorizing samples as "vigilance level" on three days with the lowest measured and potential cyanotoxin concentrations (June 26th, July 18th and October 14th, Table 5-5). Alert level 1 as defined by biovolume corresponded better than any other parameter to the 1µg/L of MPMC. Biovolume also categorized alert level 2 on three of the highest days at risk of microcystin contamination of the raw water. Due to the accurate reflection of the MPCP at high and low cyanobacterial abundance, to its excellent correlation with MPMC values and its good correlation with *in vivo* PC fluorescence, biovolume is considered the best cyanobacterial monitoring parameter for the two monitored DWTPs.

Corresponding PC probe readings (RFUs) for biovolume ALTs

New proposed *in vivo* PC fluorescence Alert level threshold values are presented in Table 5-6. The *in vivo* PC fluorescence ALT were calculated based on the

cyanobacterial biovolume measured in grab samples collected from the raw water of the Missisquoi Bay's DWTP in 2008. The proposed *in vivo* PC fluorescence ALT was calculated based on the regression line from Figure 5.22.

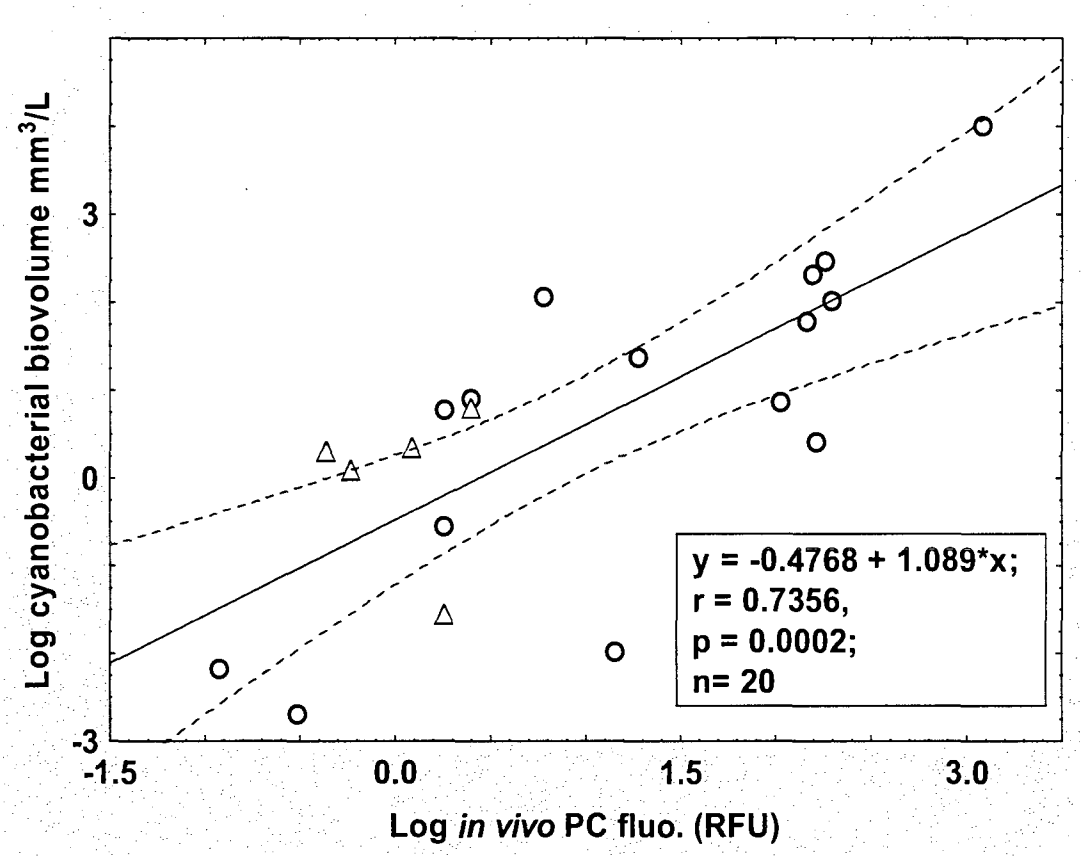


Figure 5.22 Correlation between log transformed *in vivo* PC fluorescence and Log transformed cyanobacterial biovolume from samples taken in the raw water of the Missisquoi Bay and the Yamaska Reservoir in 2008.

$$y = -0.4768 + 1.089x$$

Where

y = log transformed RFU value of the *in vivo* PC fluorescence probe on the MPS

x = log transformed WHO alert level threshold value for cyanobacteria biovolume (Bartram et al. 1999)

Results reflect two series of data and are not to be used to predict cyanotoxins in raw water of DWTPs. The formulas were derived from linear regressions between *in vivo* PC fluorescence probe values and grab sample values of biovolume taken in raw water of the DWTP at the Missisquoi Bay.

Table 5-6 WHO (Bartram et al. 1999) Alert level thresholds and the corresponding ratio fluorescent units as determined by the in vivo PC probe.

Alert Levels	Cyanobacterial Biovolume	
	Actual WHO ALT (mm^3/L)	Proposed ALT *in vivo* PC fluorescence (RFU)
Level 1 (WHO)	0.2	0.1
Level 2 (WHO)	10	4.1

Similar estimation of *in vivo* PC probe values based on results from a local monitoring campaign have been performed by (Ahn et al. 2007; Brient et al. 2008; Izydorczyk et al. 2009), but these studies all used different monitoring tools making it impossible to compare the particular RFU values obtained.

Therefore, based on the results of the 2008 season, the presented RFU ALTs could be used to monitor cyanobacteria in the raw water of the monitored DWTP in the 2009 season. DWTP operators could follow the recommended actions outlined in the WHO's guidelines (see section 2.7) when the PC probe RFU values breach the above ATL. This online monitoring system could reduce the risk of cyanotoxins in treated drinking water because it would allow a faster response to the raw water dangerous concentrations of potentially toxic cyanobacteria.

Chapter 6 Conclusions

1a) The estimations of cyanobacterial density generated by the internal calibration of the *in vivo* PC fluorescence probe were inaccurate in both laboratory and environmental conditions. The use of the PC probe's generic, and therefore standardized, ratio fluorescent units (RFU) are instead suggested for the establishment of an alert system for cyanobacterial monitoring in Quebec.

1b) The high and significant correlations between *in vivo* PC probe RFU values and all cyanobacterial monitoring parameters from grab samples taken in source and raw water of both DWTPs. However, cyanobacterial biovolume was retained as the best option for a locally adapted alert level framework.

2a) Large spatio-temporal variations of cyanobacterial abundance were observed at both field sites throughout the 2008 season. The annual variation of cyanobacterial dominant species was both unpredictable and highly changeable in source and raw water of both DWTPs. However, the impacts that this variability had on the DWTPs was different at both field sites:

> At the Missisquoi Bay in 2008, on online PC fluorescence detected a large increased the abundance of cyanobacteria and their associated toxins in the raw water of the DWTP as compared to 2007 results. This made the treatment of cyanobacterial cells and cyanotoxins much more challenging in 2008 than in 2007 for operators of the DWTP at the Missisquoi Bay. In bloom conditions, depth profiles above the DWTP's intake using *in vivo* PC fluorescence demonstrated that the mixing of the water column by waves and wind can cause high concentrations of cyanobacteria and their associated toxins to enter the plant.

At the Yamaska Reservoir, although high concentrations of cyanobacteria and cyanotoxins were detected in the source water of the DWTP on several occasions, very low concentrations of cyanobacteria and cyanotoxins were measured in the raw water of the DWTP in 2008. This was due to the accumulation of cyanobacteria and cyanotoxins at the surface of the reservoir and little water column mixing.

2b) In 2008, the parameters on the MPS which best predicted cyanobacteria as detected by *in vivo* PC fluorescence were turbidity and Δ pH at Missisquoi Bay and specific conductivity and dissolved oxygen at the Yamaska Reservoir. All parameters can be easily monitored by DWTPs which are vulnerable to cyanobacterial blooms in their source water in order to be better prepared to treat cyanobacteria and cyanotoxins in their raw water.

3a) Cyanobacterial biovolume had the highest correlation with the maximum potential microcystin concentration of all other measured parameters taken from grab samples collected in the raw water of both DWTPs.

3b) RFU values which corresponded to cyanobacterial biovolumes measured in grab samples collected in the raw water of both DWTPs were: 0.1 RFU for the WHO's Alert Level 1 and 4.1 RFUs for WHO's Alert Level 2.

Recommendations for future work

The use of RFU rather than the estimations generated by the PC probe are recommended to standardize the results generated by the probe. An increase in the frequency of depth profiles could help better understand the diurnal variations of cyanobacteria in the water column above DWTPs water intakes. More online data would increase the predictive power of multiple linear regression models generated at both sites. Suggestions for future monitored parameters are: nutrients such as phosphorous and nitrogen, wind speed and direction, hours of daylight, and rain accumulation.

References

Ahn, C.-Y., Chung, A.-S., & Oh, H.-M. (2002). Rainfall, phycocyanin, and N:P ratios related to cyanobacterial blooms in a Korean large reservoir. *Hydrobiologia, 474*, 117-124.

Ahn, C. Y., Joung, S. H., Yoon, S. K., & Oh, H. M. (2007). Alternative alert system for cyanobacterial bloom, using phycocyanin as a level determinant. *Journal of Microbiology, 45*(2), 98-104.

Albay, M., Matthiensen, A., & Codd, G. A. (2005). Occurrence of Toxic Blue-Green Algae in the Kucukcekmece Lagoon (Istanbul, Turkey). *Environmental Toxicology 20*(3), 277 - 284.

AWWARF. (2006). *Management Strategies for Algal Toxins. An International Review.*

Bartram, J., & Chorus, I. (Eds.). (1999). *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management.* London: WHO.

Bennett, A., & Bogorad, L. (1973). COMPLEMENTARY CHROMATIC ADAPTATION IN A FILAMENTOUS BLUE-GREEN ALGA. *THE JOURNAL OF CELL BIOLOGY, 58*, 419 -435.

Berman, F. (2008). Making Research and Education Cyberinfrastructure Real. *Educause Review, 43*(4), 18-24.

Berryman, D. (2008). *Faits Saillants 2004-2006 :État de l'écosystème aquatique – Bassin versant de la rivière Yamaska*

Beutler, M. (2003). *Spectral fluorescence of chlorophyll and phycobilins as an in-situ tool of phytoplankton analysis – models, algorithms and instruments.* Christian-Albrechts-Universität, Kiel.

Beutler, M., Wiltshire, K. H., Meyer, B., Moldaenke, C., Luring, C., Meyerhofer, M., Hansen, U. P., & Dau, H. (2002). A fluorometric method for the determination of algal populations in vivo and in situ. *Photosynthesis Research, 72*, 39-53.

Beutler, M., Wiltshire, K. H., Reineke, C., & Hansen, U. P. (2004). Algorithums and practical fluorescence models of the photosynthetic apparatus of red cyanobacteria and Cryptophyta designed for the fluorescence detection of red cyanobacteria and cryptophytes. *Aquatic Microbial Ecology, 35*, 115-129.

Blais, S. (2002). La problématique des cyanobactéries (algues bleu-vert) à la baie Missisquoi en 2001. *Agrosol, 13*(2), 103-110.

Blais, S. (2007). *Guide d'identification des fleurs d'eau de cyanobactéries. Comment les distinguer des végétaux observés dans nos lacs et nos rivières.*

Bourque, A., & Simonet, G. (2008). Quebec. In D. S. Lemmen, J. Lacroix, E. Bush & F. J. Warren (éds.), *From Impacts to Adaptation: Canada in a Changing Climate* (pp. 171-226). Ottawa: Government of Canada.

Brient, L., Lengronne, M., Bertrand, E., Rolland, D., Sipel, A., Steinmann, D., Baudin, I., Legeas, M., Le Rouzic, B., & Bormans, M. (2008). A phycocyanin probe as a tool for monitoring cyanobacteria in freshwater bodies. *Journal of Environmental Monitoring, 10*(2), 248-255.

Bryant, D. A. (1986). The Cyanobacteria Photosynthetic Apparatus: Comparisons to Those of Higher Plants and Photosynthetic Bacteria. *Photosynthetic picoplankton, 214*, 423-500.

Burch, M., Brookes, J., Regel, R., Daly, R., Lewis, D. C., Antenucci, J., Lambert, M., House, J., Velzeboer, R., Hobson, P., Linden, L., Shaw, G., Smith, M., Saint, C., Rasmussen, P., Monis, P., Giglio, S., Fergusson, K., Schembri, M., Barbez, P., Campbell, R., Sourzat, A., Baker, S., Baker, P., Newcombe, G., Ho, L., Wang, H., Meyne, T., Cook, D., Humpage, A., Froscio, S., Falconer, I., Papageorgio, J., Nicholson, B., Hoefel, D., Fontaine, F., Lewanowitsch, T., Stewart, I., McDowall, B., & Slyman, N. (2009). The Alert Levels Framework for Drinking Water. In *Cyanobacteria Management and Implications for Water Quality: Outcomes from the Research Programs of the Cooperative Research Centre for Water Quality and Treatment* (pp. 36). Salisbury: The Cooperative Research Centre for Water Quality and Treatment.

Burford, M. A., Johnson, S. A., Cook, A. J., Packer, T. V., Taylor, B. M., & Townsley, E. R. (2007). Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs. *Water Research, 41*(18), 4105-4114.

Burger, D. F., Hamilton, D. P., & Pilditch, C. A. (2007). Modelling the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in a shallow polymictic lake. *Ecological Modelling, 2 1 1* 411–423.

Cagnard, O., Baudin, I., Lemoigne, I., & Keith, C. (2006). *Assesment of emerging optic sensors*. Le Pecq Suez Environnement Cirsee.

Carmichael, W. (2001). Health Effects of Toxin-Producing Cyanobacteria: "The CyanoHABs". *Human and Ecological Risk Assessment, 7*, 1393-1407.

Carmichael, W., Azevedo, S., An, J. S., Molica, R., Jochimsen, E., Lau, S., Rinehart, K. L., Shaw, G. R., & Eaglesham, G. K. (2001). Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins. *Environmental Health Perspectives, 109*(7), 663-668.

Chorus, I., Falconer, I. R., Salas, H. J., & Bartram, J. (2001). Health Risks Caused by Freshwater Cyanobacteria in Recreational Waters. *Journal of Toxicology and Environmental Health, Part B,, 3* 323-347.

Chowdhury, M., & Al Bakri, D. (2006). Diffusive nutrient flux at the sediment-water interface in Suma Park Reservoir, Australia. *Hydrological Sciences Journal, 51*(1), 144-156.

Codd, G. (1995). Cyanobacterial toxins: Occurrence, properties and biological significance *Water science and technology 32* 149 -156

Codd, G. A., Morrison, L. F., & Metcalf, J. S. (2005). Cyanobacterial toxins: risk management for health protection. *Toxicology and Applied Pharmacology, 203* 264– 272.

Davis, J. R., & Koop, K. (2006). Eutrophication in Australian rivers, reservoirs and estuaries – a southern hemisphere perspective on the science and its implications. *Hydrobiologia, 559*, 23–76.

Erdner, D. L., Dyble, J., Parsons, M. L., Stevens, R. C., Hubbard, K. A., Wrabel, M. L., Moore, S. K., Lefebvre, K. A., Anderson, D. M., Bienfang, P., Bidigare, R. R., Parker, M. S., Moeller, P., Brand, L. E., & Trainer, V. L. (2008). Centers for Oceans and Human Health: a unified approach to the challenge of harmful algal blooms. *Environmental Health, 7*.

Ernst, B., Dietz, L., Hoeger, S. J., & Dietrich, D. R. (2005). Recovery of MC-LR in Fish Liver Tissue. *Environmental Toxicology, 20*(4), 449-458.

Falconer, I., Bartram, J., Chorus, I., Kuiper- Goodman, T., Utkilen, H., Burch, M., & A. Codd, G. (1999). Safe Levels and Safe Practices In J. Bartram & I. Chorus (éds.), *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management* (pp. 161-182). London and New York: E & FN Spon.

Fogg, G. E., Stewart, W. D. P., Fay, P., & Waslsby, A. E. (1973). *The blue-green algae.* San, Diego, California, USA,: Academic Press, Inc.

Funari, E., & Testai, E. (2008). Human health risk assessment related to cyanotoxins exposure. *Critical Reviews in Toxicology, 38*(2), 97-125.

Galvez, R., & Levine, S. (2003). *Canada-USA Agreement on the Eutrophication Control and Protection of Lake Champlain* Quebec: Laval University, Quebec, Canada. Laval University, Quebec, Canada and Prof. Rubenstein School, U. of Vermont, Burlington, VT, USA.

Giani, A., Bird, D. F., Prairie, Y. T., & Lawrence, J. F. (2005). Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. *Can. J. Fish. Aquat. Sci., 62*, 2100–2109

Gregor, J., & Marsalek, B. (2005). A simple in vivo fluorescence method for the selective detection and quantification of freshwater cyanobacteria and eukaryotic algae. *Acta hydrochim. hydrobiol., 33*(2), 142-148.

Gregor, J., Marsalek, B., & Sipkova, H. (2007). Detection and estimation of potentially toxic cyanobacteria in raw water at the drinking water treatment plant by in vivo fluorescence method. *Water Research, 41*(1), 228-234.

Harward, V. J., Feng, C.-C., Beng Lim, H., Iqbal, M., Mueller-Wittig, W., Vöss, G., Wenqiang, W., & Bailey, P. H. (2008). A Cyberinfrastructure for Environmental Data Integration and Management. Singapore-MIT Alliance for Research and Technology (SMART) Center for Environmental Sensing and Monitoring (CENSAM).

Hébert, S. (2005). *Compariason entre l'Indice de la qualité générale de l'eau du Québec (IQBP) et l'Indice du qualité de l'eau du CCME pour la protection de la vie aquatique.*

Iitala, A., Stalnackeb, P., Deelstrac, J., Loigua, E., & Pihlakd, M. (2005). Effects of large-scale changes in emissions on nutrient concentrations in Estonian rivers in the Lake Peipsi drainage basin. *Journal of Hydrology, 304*, 261–273.

Iqbal, M. Z., Brown, E. J., & Clayton, M. E. (2006). Distribution of phosphorus in a biologically restricted lake in Iowa, USA. *Journal of Hydrology 326* 349–366.

Izaguirre, G., & Taylor, W. D. (2004). A guide to geosmin- and MIB-producing cyanobacteria in the United States *Water science and technology 49* 19 -24

Izydorczyk, K., Carpentier, C., Mrowczynski, J., Wagenvoort, A., Jurczak, T., & Tarczynska, M. (2009). Establishment of an Alert Level Framework for

cyanobacteria in drinking water resources by using the Algae Online Analyser for monitoring cyanobacterial chlorophyll a. *Water Research, 43*, 989 – 996.

Izydorczyk, K., Tarczynska, M., Jurczak, T., Mrowzynski, J., & Zalewski, M. (2005). Measurement of Phycocyanin Fluorescence as an Online Early warning system for cyanobacteria in reservoir intake water *Environmental Toxicology, 20*, 425-430.

Janus, L., & Vollenweider. (1981a). *The OECD Cooperative Programme on Eutrophication: Canadian Contribution*. Burlington, Ontario, Canada: National Water Research Institute.

Janus, L., & Vollenweider, R. (1981b). *The OECD Cooperative Programme on Eutrophication : Canadian Contribution*. Burlington, Ontario, Canada: National Water Research Institute.

Jones, G. J., & Orr, P. T. (1994). Release and Degradation of Microcystin Following Algicide Treatment of a Microcystis Aeruginosa Bloom in a Recreational Lake, As Determined by HPLC and Protein Phosphatase Inhibition Assay. *Water Research 28*, 871-876.

Kido, M. H., Mundt, C. W., Montgomery, K. N., Asquith, A., Goodale, D. W., & Kaneshiro, K. Y. (2008). Integration of Wireless Sensor Networks into Cyberinfrastructure for Monitoring Hawaiian "Mountain-to-Sea" Environments. *Environmental Management 42*, 658-666.

Lavoie, I., Laurion, I., Waren, A., & Vincent, W. (2007). *Les fleurs d'eau de cyanobactéries* (revue de littérature): INRS.

Leboulanger, C., Dorigo, U., Jacquet, S., Le Berre, B., Paolini, G., & Humbert, J.-F. (2002). Application of a submersible spectrofluorometer for rapid monitoring of freshwater cyanobacterial blooms: a case study. *Aquatic Microbial Ecology, 30*, 83-89.

Lehman, P. W., Boyer, G., Satchwell, M., & Waller, S. (2008). The influence of environmental conditions on the seasonal variation of Microcystis cell density and microcystins concentration in San Francisco Estuary. *Hydrobiologia, 600*(1), 187-204.

Levi, Y., Harvey, M., & Cervantès, P. (2006). *Rapport sur l'évaluation des risques liés à la présence de cyanobactéries et de leurs toxines dans les eaux destinées à l'alimentation, à la baignade et autres activités récréatives*. Paris: Agence française de sécurité sanitaire de l'environnement et du travail.

Lorenzen, C. (1966). A Cethod for the Continuous Measurement of *in vivo* Chlorophyll Concentration. *Deep-Sea Research, 13*, 223-227.

Lund, J. W. G., Kipling, C., & Le Cren, E. D. (1958). The Inverted Microscope Method of Estimating Algal Numbers and the Statistical Basis of Estimations by Counting. *Hydrobiologia 11*(2), 143-170

Maxwell, K., & Johnson, G. (2000). Chlorophyll fluorescence a practical guide. *Journal of experimental botany, 51*, 659-668.

MDDEP. (2007). *Plan d'intervention détaillé sur les algues bleu-vert 2007-2017.*

MDDEP. (2008a). Bilan des lacs et cours d'eau touchés par les fleurs d'eau de cyanobactéries au Québec. Consulté le http://www. mddep.gouv.qc.ca/ eau/algues-bv/bilan/liste_comparative.asp.

MDDEP. (2008b). *Procedure to follow and information to the public if blue-green algae blooms are detected.*

Millie, D. F., Schofield, O. M. E., Kirkpatrick, G. J., Johnsen, G., & Evens, T. J. (2002). Using absorbance and fluorescence spectra to discriminate microalgae. *European Journal of Phycology, 37*, 313 — 322.

Ministère du Développement Durable et des Parc du Québec (2008). *Le Québec et les Changement Climatiques - Un défi pour l'avenir, Plan d'Action 2006 – 2012*

Mosisch, T. (2008). Results from SA Water River Murray Drought Response Field Team; RDI South Australia Water Workshop 2008 Adelaide.

Mur, L. R., Skulberg, O. M., & Utkilen, H. (1999). Cyanobacteria in the Environment. In I. Chorus & J. Bartram (éds.), *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management.* London: St Edmundsbury Press.

Nush, E. A. (1980). Comparison of different methods for chlorophyll and phaeopigment determination. *Arch Hydrobiol 4*, 14-36.

Oliver, R. L., & Ganf, G. G. (2000). Freshwater blooms. In B. A. Whitton & M. Potts (éds.), *The Ecology of Cyanobacteria.* (pp. 149-194). the Netherlands: Kluwer Academic Publishers.

Otsuki, A., Omi, T., & Hashimoto, S. (1994). HPLC Fluorometric Determination of Natural Phytoplankton Phycocyanin and its Usefulness as Cyanobacterial

Biomass in Highly Eutrophic Shallow Lake *Water, Air and Soil Pollution, 76*, 383-396.

Parésys, G., Rigart, C., Rousseau, B., Wong, A. W. M., Fan, F., Barbier, J.-P., & Lavaud, J. (2005). Quantitative and qualitative evaluation of phytoplankton communities by trichromatic chlorophylll fluorescence excitation with special focus on cyanobacteria. *Water Research, 39*, 911-921.

Pick, F. R., & Lean, D. R. S. (1987). The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. *New Zealand Journal of Marine and Freshwater Research, 21*, 425-434.

Quibliera, C., Leboulanger, C., Sane, S., & Dufour, P. (2008). Phytoplankton growth control and risk of cyanobacterial blooms in the lower Senegal River delta region. *Water Research, 42* 1023 – 1034.

Rantala, A., Rajaniemi-Wacklin, P., Lyra, C., Lepisto, L., Rintala, J., Mankiewicz-Boczek, J., & Sivonen, K. (2006). Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (mcyE) PCR and associations with environmental factors. *Applied and Environmental Microbiology, 72*(9), 6101-6110.

Robert, C. (2008). *Résultats de cyanobactéries et cyanotoxines à sept stations de production d'eau potable (2004-2006).*

Seppala, J., Ylostalo, P., Kaitala, S., Hallfors, S., Raateoja, M., & Maunula, P. (2007). Ship-of-opportunity based phycocyanin fluorescence monitoring of the filamentous cyanobacteria bloom dynamics in the Baltic Sea. *Estuarine, Coastal and Shelf Science, 73*, 489-500.

Shapiro, J. (1997). The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. *Freshwater Biology, 37*, 307-323.

Simoneau, M. (2007). *État de l'écosystème aquatique du bassin versant de la baie Missisquoi: Fait Saillant 2001-2004*

Sivonen, K., & Jones, G. (1999). Cyanobacterial Toxins. In I. Chorus & J. Bartram (éds.), *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. WHO.* London: St Edmundsbury Press,.

StatisticsCanada. (2006a). 2006 Community Profiles - Missisquoi Bay Area. Consulté le June 3, 2009, tiré de http://www12.statcan.ca/census-recensement/2006/dp-pd/prof/92591 details/Page.cfm?Lang=E&Geo1 = CSD&Code1=2 446017

&Geo2 =
PR&Code2=24&Data=Count&SearchText=philipsburg&SearchType=
Begins&SearchPR=01&B1=All&Custom=.

StatisticsCanada. (2006b). Community Profiles 2006 - Yamaska River Area. Consulté le http://www12.statcan.ca/census-recensement/2006/dp-pd/prof/92-591/details/page.cfm?Lang=E&Geo1=CSD&Code1=2447015&Geo2=PR&Code2=24&Data=Count&SearchText=granby&SearchType=Begins&SearchPR=01&B1=All&Custom=.

van Thor, J. J., Mullineaux, C. W., Matthijs, H. C. P., & Hellingwerf, K. J. (1998). Light Harvesting and State Transitions in Cyanobacteria. *Botanica Acta, 111*, 430-443.

Watzin, M. C., Miller, E. B., Shambaugh, A. D., & Kreider, M. A. (2006). Application of the WHO Alert Level Framework to Cyanobacterial Monitoring of Lake Champlain, Vermont. *Environmental Toxicology 21*, 278–288.

Wetzel, R. G. (2000). *Limnological analysis* (3rd edition[e] éd.): Springer.

WHO. (2003). Guidelines for safe recreational water environments In (Volume 1 : Coastal and fresh waters, pp. 23). Geneva.

Wongsai, S., & Luo, K. (2007). Understanding environmental factors associated with cyanobacterial bloom. *3rd IASTED International Conference on Environmental Modelling and Simulation, EMS 2007, Honolulu, HI, United States* (pp. 25-30)Acta Press Calgary AB T3E 7J9 Canada.

Yentsch, C., & Yentsch, C. (1979). Fluorescence spectral signatures: The characterization of phytoplankton populations by the use of excitation and emission spectra *Journal of marine research, 37*(3), 471-483.

YSI. (2006). *6-Series Multiparameter Water Quality Sondes User Manual.* Yellow Springs, Ohio: YSI.

Yu, S. (1995). Primary prevention of hepatocellular carcinoma. *Journal of Gastroenterologic Hepatology, 10*, 674-682.

Zamyadi, A. (2007). *Occurrence and removal of cyanobacteria and their associated toxins in water resources of water treatment facilities in southern Québec.* Montreal: École Polytechnique de Montréal.

Zamyadi, A. *Rapid detection of harmful cyanobacteria in source waters with an online fluoroprobe: Method validation.* Quebec City, Canada. (2008).

Zhang, X. H., Wang, T. H., Yuan, W. Q., Duan, H. L., Zhang, W. H., & Li, C. X. (2004). Nonpoint pollution control for the Xili reservoir in Shenzhen, China. *2004 World Water and Environmental Resources Congress: Critical Transitions in Water and Environmental Resources Management, Salt Lake City, UT, United States* (pp. 495-503)American Society of Mechanical Engineers New York NY 10016-5990 United States.

Appendices

Appendix A - Potentially toxic species of cyanobacteria and their detected toxins.

Table A. Non-exhaustive list of potentially toxic species of cyanobacteria and their associated toxins (adapted from: (Zamyadi 2007) (Agence Française de Sécurité Sanitaire des Aliments (AFSSA) et l'Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET) 2006))

Species	Cyanotoxin	Species	Cyanotoxin
Anabaena circinalis	Anatoxin-a, Saxitoxins, Microcystins	Microcystis wesenbergii	Microcystins
Anabaena flos-aquae	Anatoxins (-a, -a(s), -b, -b(s), -c, -d), Microcystins	Microcystis sp.	Anatoxin-a
Anabaena lemmerman	Microcystins, Anatoxin-a(s)	Nodularia spumigena	Nodularins
Anabaena planktonica	Anatoxin-a	Nostoc sp.	Microcystins
Anabaena spiroides	Anatoxin-a, Microcystins	Oscillatoria formosa	Homoanatoxin-a
Anabaena sp.	Anatoxin-a	Oscillatoria limosa	Microcystins
Anabaenopsis milleri	Microcystins	Oscillatoria tenuis	Microcystins
Aphanizomenon flos-aquae	Anatoxin-a, Saxitoxins	Oscillatoria nigroviridis	Oscillatoxin-a
Aphanizomenon ovalisporum	Cylindrospermopsin	Oscillatoria sp.	Anatoxin-a
Aphanizomenon sp.	Anatoxin-a	Phormidium favosum	Anatoxin-a
Coelosphaerium naegelianum	Hépatotoxin	Planktothrix agardhii	Microcystins
Cylindrospermopsis raciborskii	Cylindrospermopsin, Saxitoxins	Planktothrix mougeotii	Microcystins
Cylindrospermum sp.	Anatoxin-a	Planktothrix rubescens	Microcystins
Hapalosiphon hibernicus	Microcystins	Planktothrix sp.	Anatoxin-a
Lyngbya gracilis	Debromoaplysiatoxin	Pseudanabaena sp.	Neurotoxin
Lyngbya majuscula	Lyngbyatoxin-a	Raphidiopsis sp.	Cylindrospermopsin
Lyngbya wollei	Saxitoxins	Schizothrix calcicola	Aplysiatoxins
Microcystis aeruginosa	Microcystins	Scytonema hofmanni	Scytophycins a et b
Microcystis botrys	Microcystins	Scytonema pseudohofmanni	Scytophycins a et b
Microcystis farlowian	Ichtyotoxin	Symploca muscorum	Aplysiatoxin
Microcystis flos-aquae	Microcystins	Trichodesmium erythraeum	Neurotoxin
Microcystis panniformis	Microcystins	Umezakia natans	Cylindrospermopsin
Microcystis viridis	Microcystins, Microviridin	Woronichinia naegeliana anciennement Gomphosphaeria naegelianum	Anatoxin-a

Appendix B - Examples of human health problems with cyanobacteria and their associated toxins

(Adapted from (Zamyadi 2007)Höger 2003; Svrcek and Smith 2004)

Location	Year	Cyanobacteria	Cyanotoxin	Human health outcome
Charleston, West Virginia, Ohio and Potomac River, US	1930-1931	Microcystis	Unknown	Gastrointestinal disease
Harare, Zimbabwe	1960-1965	*Microcystis aeruginosa*	Unknown	Gastrointestinal disease
Allegheny Country, Pennsylvania, Sewickley reservoir	1975	Schizothrix, Lyngbya, Plectinema, Phormidium		Gastrointestinal disease
Solomon Dam Palm Island, Australia	1979	Cylindrospermopsis		Gastrointestinal disease, liver damage, kidney damage, intestinal damage
Malpas Dam, Armidale, Australia	1981	Microcystis		liver damage
Armidale, Australia	1983	*Microcystis aeruginosa*	Unknown	liver damage, constipation and bloody diarrhea, kidney damange
Palm Island, Australia	1983	*Cylindrospermopsin raciborskii*	CYN	hepatoenteritis
Itaparica Dam, Brazil	1988	Anabaena, Microcystis		Gastrointestinal disease, 88 deaths
Rudyard reservoir, Staffordshire, UK	1989	*Microcystis aeruginosa*	MC-LR	Vomiting, dermatitis, pneumonia, diarrhea Gastrointestinal disease, nervous system affected
Towns at river Murray, Australia	1992	Anabaena circinalis		Multiple symptoms
Itaparica Dam, Brazil	1993	*Anabaena* sp., *Microcystis* sp.	Unknown	88 deaths, gastroenteritis
Scania, Sweden	1994	Planktothrix agardhii		Gastrointestinal disease
Nandong District, Jiangsu Province, Nanhui/Shanghai, Fusui, China	1994-1995	*M.aeruginosa*, *Plankothrix agardhii*, *Anabaena* sp., *Lyngbya* sp.	MC	primary liver cancer correlation
Caruaru, Brazil	1996	*Microcystis Anabaena, Cylindrosopermopsis*, *Aphanizomenon*	MC, CYN	liver damage, nervous system affected, Gastrointestinal disease, after hemodialysis by 98% of patients: 76 deaths

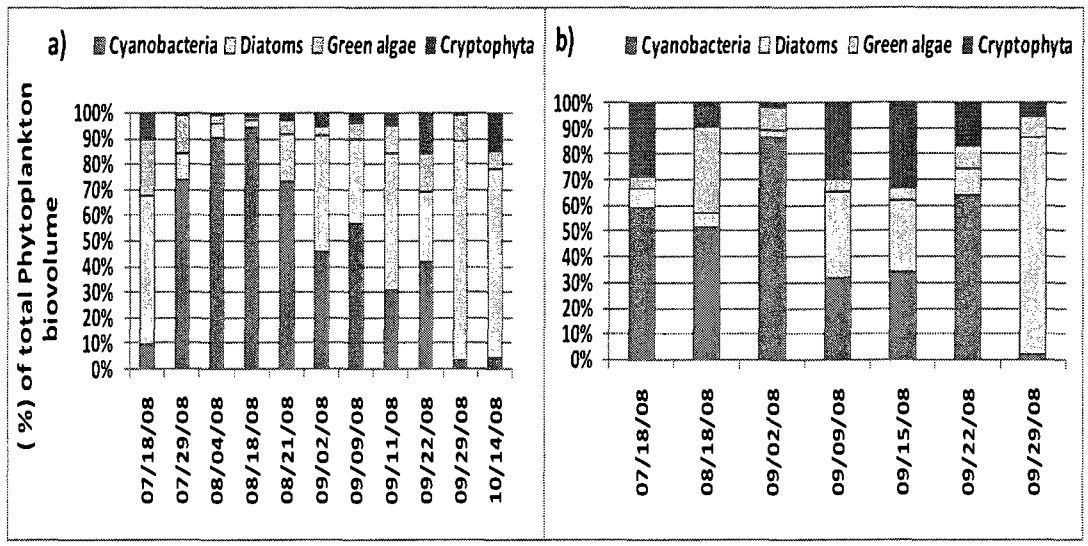
Appendix C - Monitoring of phytoplankton dynamics

In 2007, taxonomic analyses were only performed on cyanobacteria. As a result, no information is available on the overall phytoplankton dynamics at the Missisquoi Bay and Yamaska Reservoir for the 2007 season.

Missisquoi Bay 2008

In 2008, grab samples taken inside the Missisquoi Bay's DWTP showed that the cyanobacterial fraction of total phytoplankton varied considerably throughout the season. Cyanobacteria were present in 100% of raw water samples taken inside the DWTP between June 26th and October 14th 2008. In July, cyanobacteria comprised 10% of total phytoplankton biovolume, this rose to 93% by August 18th and then dropped to 3% at the end of the season when they were gradually overtaken by diatoms. The monitored phytoplankton dynamics at Missisquoi Bay were similar to those observed by (Gregor et al. 2007) who noted a dominance of cyanobacteria in August and September followed by their gradual decline as the season progressed.

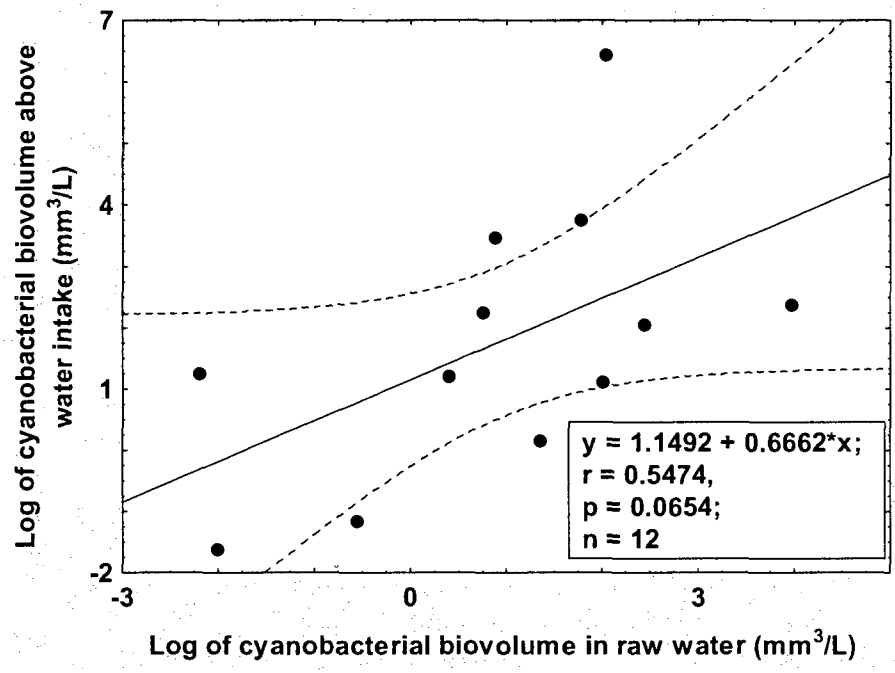
Water grab samples taken above the DWTP's water intake point in Missisquoi Bay also show variations in phytoplankton composition over the 2008 season. The cyanobacterial composition of grab samples varied between 30-60% of total phytoplankton composition for most of the season, however it made up 90% of total phytoplankton on September 2nd and only 2% on September 29th. As with samples taken inside the DWTP, diatoms dominated the phytoplankton at the end of the season.



Phytoplankton distribution of raw water grab samples taken a) inside Missisquoi Bay's DWTP and b) one meter below the water's surface, above the water intake on Missisquoi Bay in 2008.

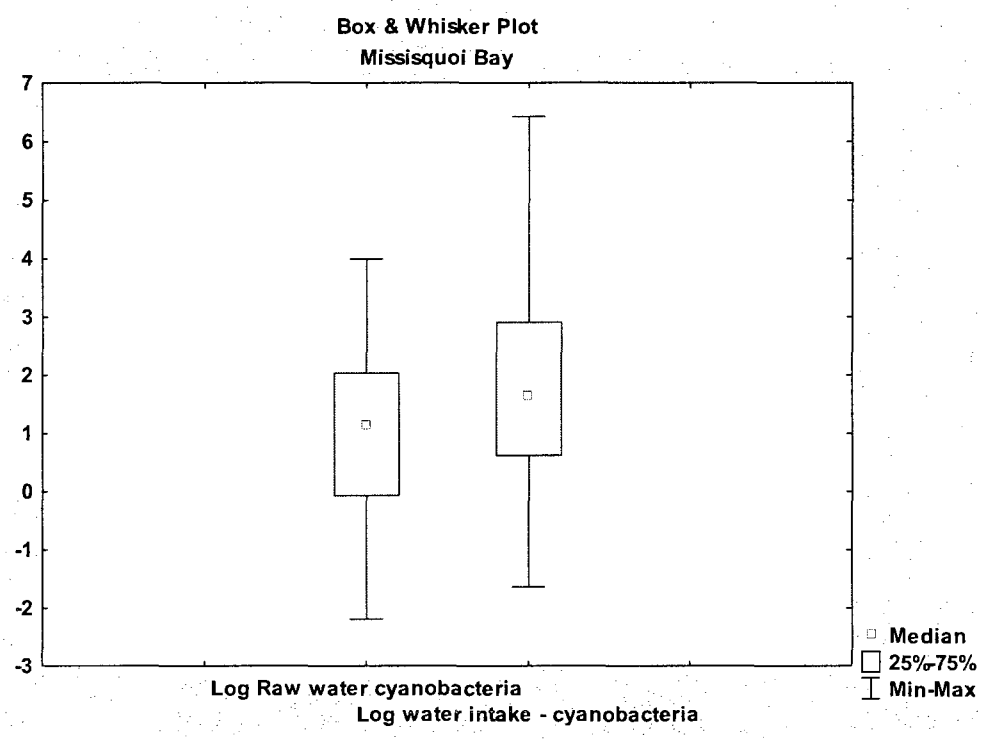
Interestingly, the phytoplankton distribution was not the same in samples taken on the same day but at different locations. For example, on July 18th, cyanobacterial content of total phytoplankton was only 10% in raw water inside the DWTP but made up as much as 60% of samples taken in source water above the water intake. The reverse situation was also true on September 2nd when there was a higher concentration of cyanobacteria above the water intake than in the plant's raw water.

This could be due to diurnal variations in phytoplankton composition of the water column as some cyanobacteria are able to move vertically through the water column to access nutrients or sunlight (Lavoie et al. 2007). Another factor could be the physical mixing of phytoplankton in the water column by waves and wind which can homogenize the water column sometimes permitting high concentration of toxic cyanobacterial to enter the DWTP. Finally, another factor for spatial discrepancies in phytoplankton distribution could be the life cycle stage of a cyanobacterial bloom; cells tend to float at the surface and form dense scum accumulations when the bloom is dying (Mur et al. 1999).



Relationship between the log of cyanobacterial biovolume in raw water inside Missisquoi Bay's DWTP and source water sampled above the intake.

Linear correlation analysis between samples taken inside and outside the DWTP showed no significant relationship between the two sampling locations ($p = 0.06$). Since samples inside and outside were taken at the same moment, this indicates that the spatial variation of cyanobacterial abundance did not follow the same trends inside and outside the DWTP.



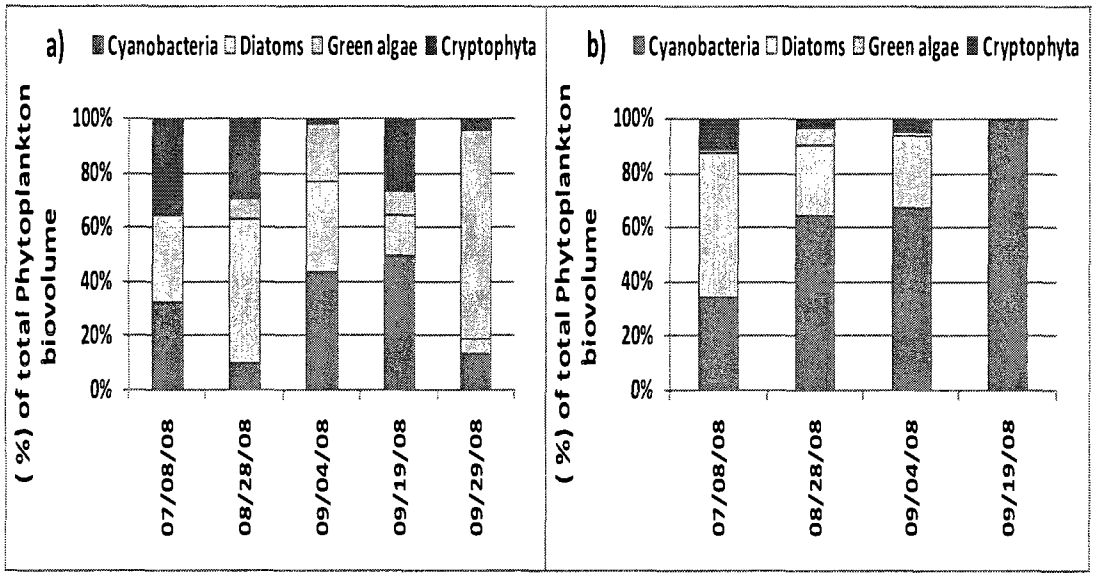
Matched paired t-test on log transformed data from raw water and source water of DWTP on Missisquoi Bay.

Matched t-test results showed that the cyanobacterial abundances in raw water and water sampled above the intake were not significantly different ($p = 0.16$, $n=12$). Therefore, despite the differing spatial variation trends between the two sampling sites on the Missisquoi Bay, in bloom conditions, the DWTP could be at risk of cyanotoxin concentrations in its raw waters.

Yamaska Reservoir 2008

At the Yamaska Reservoir's DWTP in 2008, all five grab samples collected between July 8th and September 29th contained cyanobacteria but cyanobacteria never dominated the phytoplankton. At the beginning of the season, diatoms and cryptophyta dominated the phytoplankton, cyanobacteria increased to represented between 40-50% of phytoplankton in September, and were then overtaken by a predominance of green algae at the end of the season.

Although cyanobacteria never dominated the phytoplankton in samples taken inside Yamaska Reservoir's DWTP, phytoplankton in water samples taken above the intake (in source water) were made up of between 60-98% cyanobacteria in August and September. Neither a simple linear regression nor a matched pair t-test analyses were possible on samples collected at the Yamaska Reservoir since the sample size was too small and not normally distributed.



Phytoplankton distribution of raw water grab samples taken a) inside Yamaska Reservoir's DWTP and b) one meter below the water's surface, above the water intake, in Yamaska Reservoir in 2008

When comparing the two sites, cyanobacteria dominated the phytoplankton inside Missisquoi Bay's DWTP in 5 grab samples whereas it was never dominant in samples taken inside Yamaska Reservoir's DWTP. In raw water at Missisquoi Bay's DWTP, potentially toxic cyanobacteria dominated the phytoplankton on the above mentioned 5 days. Consequently, in 2008, there was a higher risk of cyanotoxin contamination at the Missisquoi Bay plant than at the Yamaska River plant in the event of inadequate or failed treatment.

In 2008, like at the Missisquoi Bay, grab samples taken in raw water inside the Yamaska Reservoir's DWTP had different phytoplankton distributions from samples

collected above the water intake. However, contrary to results presented at the Missisquoi Bay, the cyanobacterial fraction of grab samples from source water was always higher than from raw water inside the plant. One hypothesis to explain this is that cyanobacteria phytoplankton in the Yamaska Reservoir were not often subject to mixing by wind and waves because the reservoir is well sheltered by surrounding trees. Hence, cyanobacteria may have more easily accumulated near the surface due to calm conditions.

Cryptophyta were present in all grab samples at both sites taken inside and outside the DWTPs. Since some cryptophyta contain phycocyanin, (PC) the pigment used to detect cyanobacterial using *in vivo* fluorescence, this could cause an over estimations of cyanobacterial abundance when cryptophyta is present.

Though, more research should be conducted on the possible interferences that cryptophyta could pose for *in vivo* PC measurements because a pigment's presence in an organism does not necessarily mean it will be detected by *in vivo* fluorescence. For example, chl-*a* in cyanobacteria is located in the weakly fluorescent PS I (Bryant 1986; van Thor et al. 1998) which makes it difficult to detect by *in vivo* Chlorophyll fluorescence (Seppala et al. 2007). It is possible that phycocyanin could be housed in a poorly fluorescent part of cryptophyta making it difficult to detect for by *in vivo* PC fluorescence.

Cyanobacteria were never dominant in samples taken inside Yamaska Reservoir's DWTP. In raw water at Missisquoi Bay's DWTP, potentially toxic cyanobacteria dominated the phytoplankton on 5 days. Consequently, in 2008, there was a higher the risk of cyanotoxin contamination at Missisquoi Bay than at the Yamaska River in the event of inadequate or failed treatment.

Appendix D - Total phytoplankton enumerations at both sites

Total phytoplantic biovolume found in raw water at entrance of drinking water treatment plant on Missisquoi Bay				
Sampling date	Cyanobacteria (mm^3/L)	Diatoms (mm^3/L)	Green algae (mm^3/L)	Cryptophyta (mm^3/L)
06/26/08	0.07	n.a	n.a	n.a
07/18/08	0.11	0.69	0.26	0.12
07/29/08	5.92	0.86	1.21	0.03
08/04/08	11.70	0.68	0.51	0.04
08/18/08	54.14	1.70	0.92	0.69
08/21/08	10.08	2.50	0.80	0.34
08/25/08	2.35	n.a	n.a	n.a
08/28/08	7.80	n.a	n.a	n.a
09/02/08	2.42	2.38	0.21	0.28
09/09/08	3.88	2.22	0.41	0.28
09/11/08	1.51	2.61	0.51	0.25
09/15/08	7.46	n.a	n.a	n.a
09/22/08	2.15	1.43	0.79	0.81
09/29/08	0.58	13.90	1.61	0.14
10/14/08	0.14	2.24	0.20	0.47

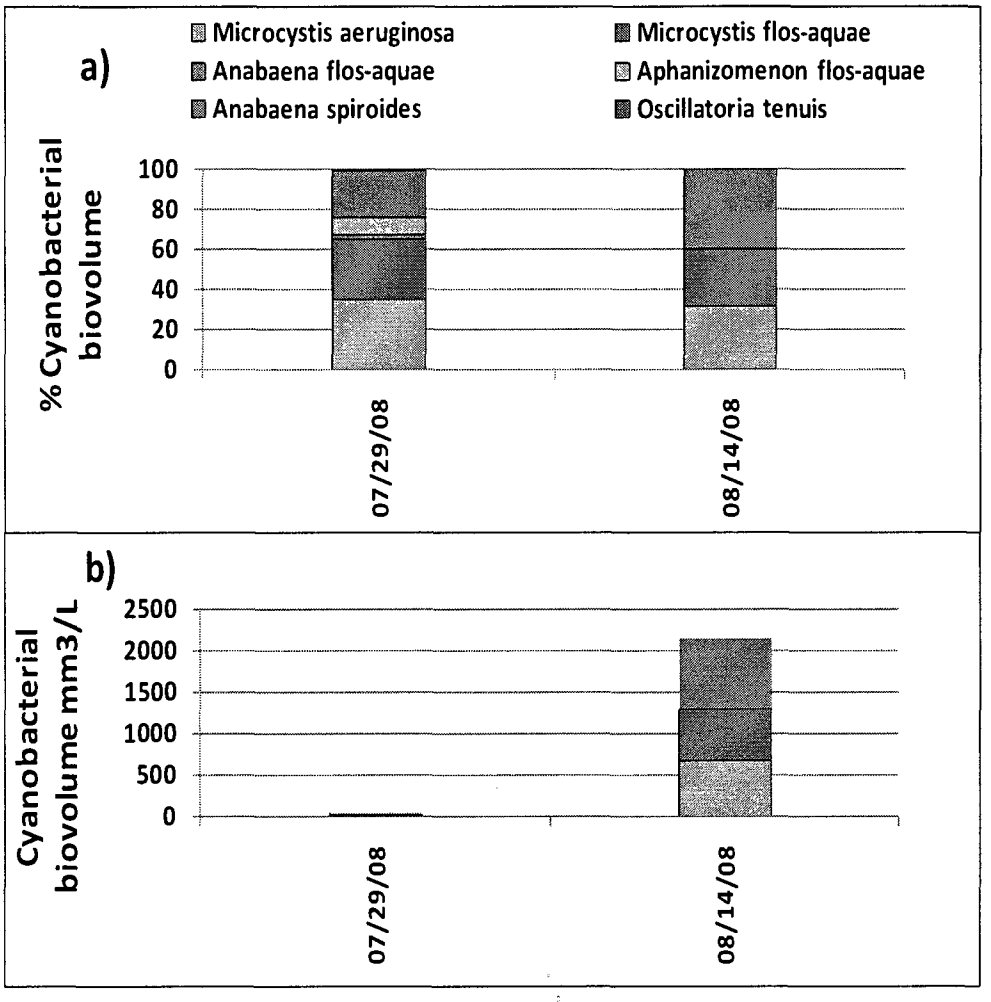
Total phytoplantic biovolume found in source water above the intake on Missisquoi Bay				
Sampling date	Cyanobacteria (mm^3/L)	Diatoms (mm^3/L)	Green algae (mm^3/L)	Cryptophyta (mm^3/L)
07/18/08	3.43	0.43	0.30	1.65
07/29/08	42.13	n.a	n.a	n.a
08/04/08	7.65	n.a	n.a	n.a
08/14/08	2914.07	n.a	n.a	n.a
08/18/08	10.57	1.07	6.76	1.97
08/28/08	618.87	n.a	n.a	n.a
09/02/08	31.72	0.91	3.20	0.75
09/09/08	1.16	1.23	0.17	0.17
09/11/08	3.32	n.a	n.a	n.a
09/15/08	3.01	2.43	0.17	2.93
09/22/08	9.27	1.51	1.32	1.32
09/29/08	0.31	12.29	1.26	0.77
10/14/08	0.19	n.a	n.a	n.a

Total phytoplantic biovolume found in raw water at the entrance of the drinking water treatment plant on Yamaska Reservoir				
Sampling date	Cyanobacteria (mm^3/L)	Diatoms (mm^3/L)	Green algae (mm^3/L)	Cryptophyta (mm^3/L)
07/08/08	1.35	1.36	0.04	1.46
08/28/08	0.31	1.63	0.24	0.92
09/04/08	1.41	1.11	0.68	0.08
09/19/08	1.07	0.31	0.20	0.57
09/29/08	0.21	0.10	1.23	0.07

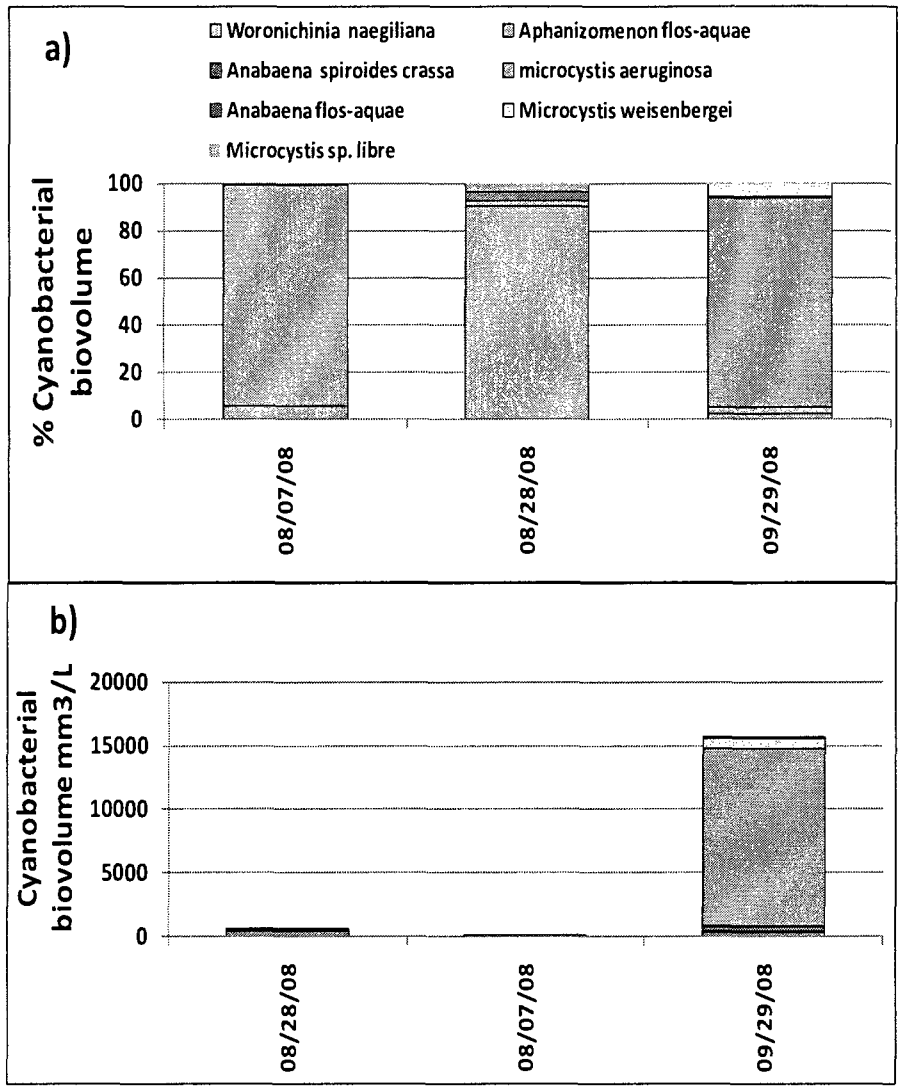
Total phytoplantic biovolume found in source water above the water intake on the Yamaska Reservoir				
Sampling date	Cyanobacteria (mm^3/L)	Diatoms (mm^3/L)	Green algae (mm^3/L)	Cryptophyta (mm^3/L)
07/08/08	1.28	1.96	0.07	0.41
08/28/08	3.41	1.38	0.31	0.00
09/04/08	2.95	1.16	0.08	0.20
09/19/08	568.74	0.00	0.19	4.74

Appendix E – Dense accumulations of cyanobacteria

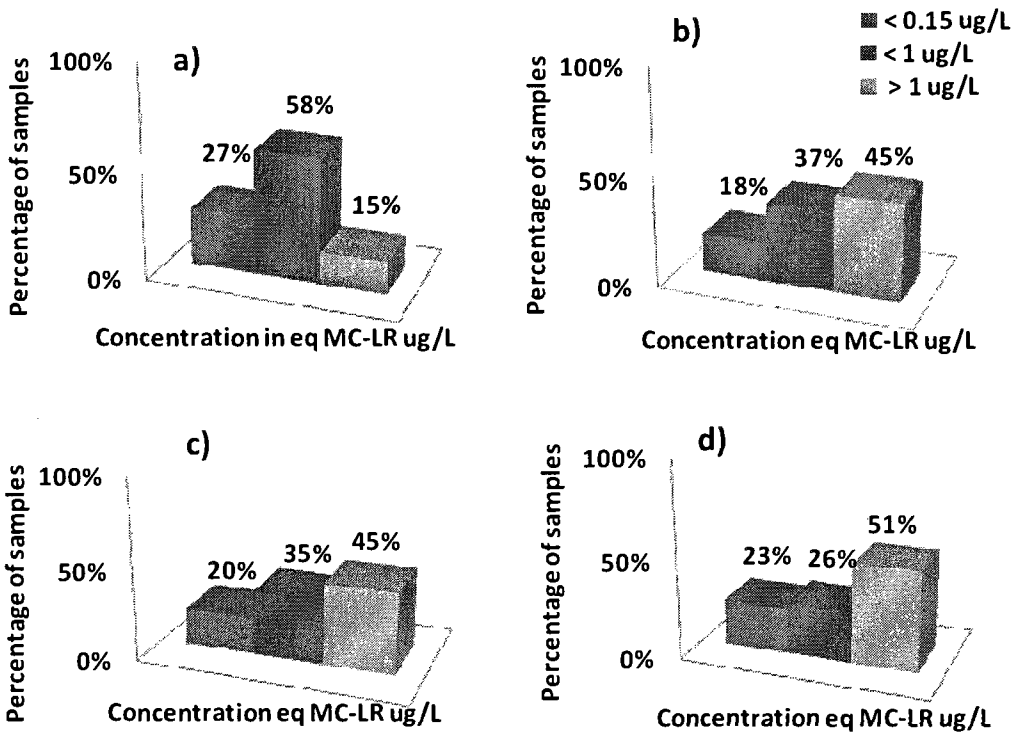
Dense accumulations of cyanobacteria pushed by the wind towards the shore at Missisquoi Bay.



Dense accumulations of cyanobacterial on the shore of Yamaska Reservoir on three sampling days.

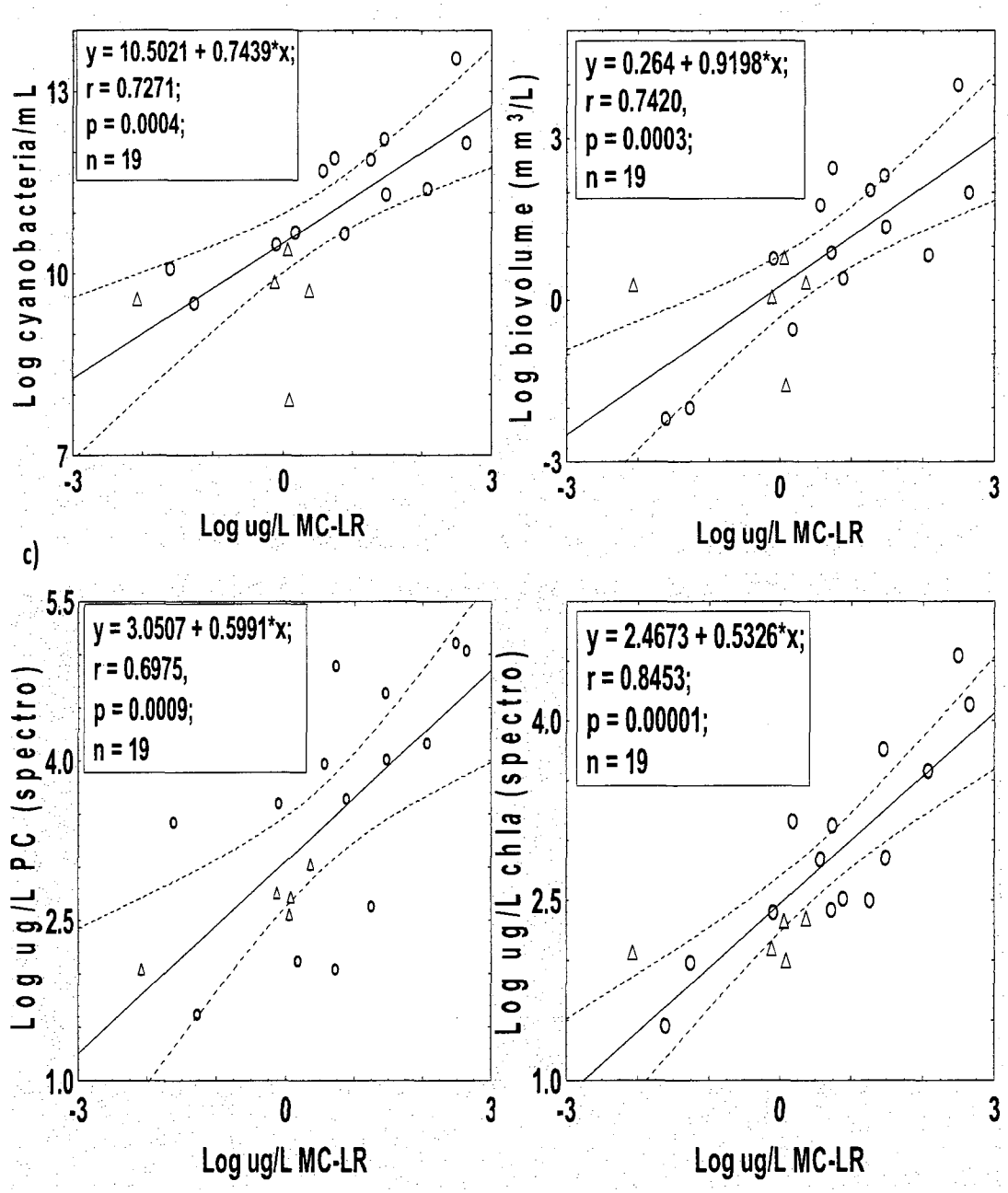


Appendix F - Distribution of detected cyanotoxins at both studied sites

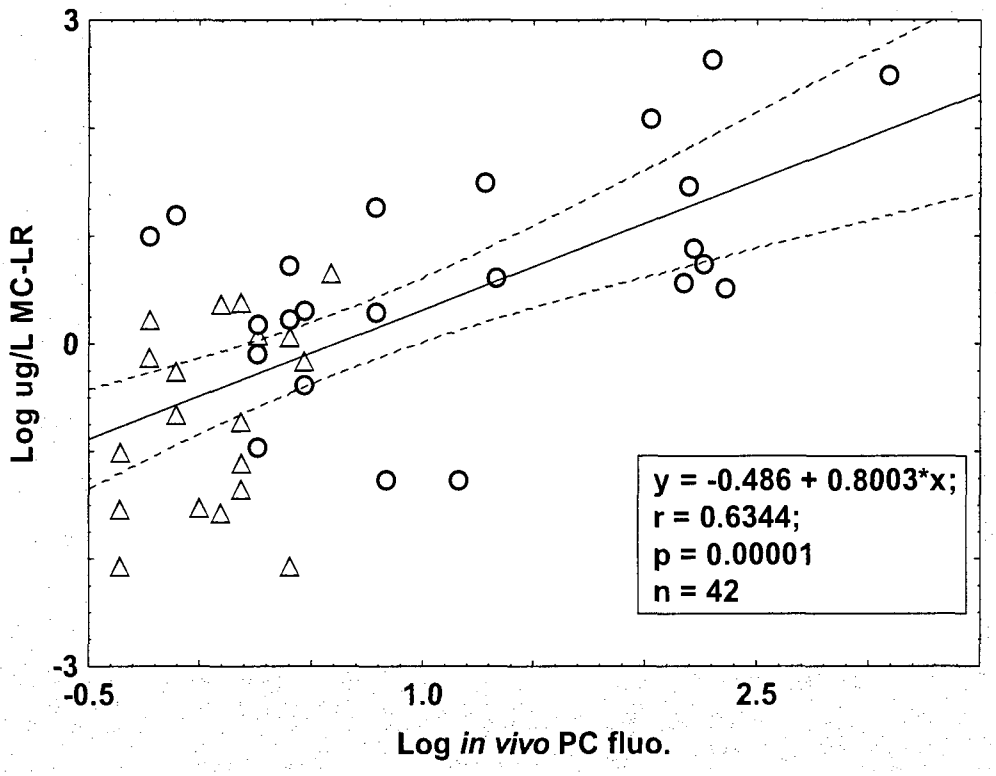


Distribution of toxicity results according to WHO recommendations (≤ 1µg/l MC-LR in treated water) and detection limit of ELISA kit (≤ 0.15µg/l MC-LR)at : a) WTP on Lemieux Reservoir; b) water outside WTP in Lemieux Reservoir; c) WTP on Missisquoi Bay; d) water outside WTP in Missisquoi Bay.

Appendix G – Correlations between measured MC-LR concentrations and both cyanobacterial abundance and *in vivo* PC fluorescence.



Correlations between. log transformed MC-LR concentrations and : a) log cyanobacterial density b) log cyanobacterial biovolume c) log extracted PC concentration and d) log of extracted chl-*a* concentration . Samples taken from raw water of both DWTPs in 2008.



Correlation between log transformed MC-LR concentrations (µg/L) and log transformed *in vivo* PC fluorescence in the raw water of Missisquoi Bay and Yamaska Reservoir's DWTPs.

Appendix H - Reproduction permission

Dear Mrs. McQuaid,

With reference to your request (copy herewith) to reprint material on which Springer Science and Business Media controls the copyright, our permission is granted, free of charge, for the use indicated in your enquiry.

This permission

- allows you non-exclusive reproduction rights throughout the World.
- permission includes use in an electronic form, provided that content is
  * password protected;
  * at intranet;
- excludes use in any other electronic form. Should you have a specific project in mind, please reapply for permission.
- requires a full credit (Springer/Kluwer Academic Publishers book/journal title, volume, year of publication, page, chapter/article title, name(s) of author(s), figure number(s), original copyright notice) to the publication in which the material was originally published, by adding: with kind permission of Springer Science and Business Media.

The material can only be used for the purpose of defending your dissertation, and with a maximum of 40 extra copies in paper.

Permission free of charge on this occasion does not prejudice any rights we might have to charge for reproduction of our copyrighted material in the future.

Kind regards,

—

Maaike Duine

Springer

Rights and Permissions

—

Van Godewijckstraat 30 | 3311 GX
P.O. Box 17 | 3300 AA
Dordrecht | The Netherlands
tel
+31 (0) 78 657 6537
fax
+31 (0) 78 657 6377
maaike.duine@springer.com

—

Van: SpringerAlerts@springeronline.com
[mailto:SpringerAlerts@springeronline.com]
Verzonden: Tuesday, August 04, 2009 8:39 PM
Aan: Permissions Europe/NL
Onderwerp: permission to reproduce fiqure

Hello,
I am writing my master's thesis at École Polytechnique in Montreal, and to complete my literature review, I would like to reproduce fiqure 7 on page 170 of this book :

Oliver, R. L., & Ganf, G. G. (2000). Freshwater blooms. In B. A. Whitton & M. Potts (éds.), The Ecology of Cyanobacteria. (pp. 149-194). the Netherlands: Kluwer Academic Publishers.

Would you grant me permission to do this? I would of course state that I received permission to do so.
Thank you
Natasha McQuaid