Empirical study of cyanobacterial toxicity along a trophic gradient of lakes

A. Giani, D.F. Bird, Y.T. Prairie, and J.F. Lawrence

Abstract: A series of 22 lakes in southern Quebec spanning a wide trophic range were sampled to develop models of changes in cyanobacterial abundance and toxicity. All lakes contained toxic cyanobacteria, and epilimnetic toxin content, expressed as microcystin equivalents, was best predicted by total nitrogen concentration and total phosphorus concentration (TP). Although phytoplankton biomass increased linearly with increases in TP among lakes, toxigenic biomass increased as greater than the squared power of TP. The only potentially toxigenic genera whose biomass was correlated with microcystin concentration were *Microcystis* and *Anabaena*. Surprisingly, the best model for toxic-species biomass was based on epilimnetic nitrogen. The level of the hepatotoxin microcystin per unit biomass in these organisms did not vary markedly among lakes, supporting the idea that environmental factors control the occurrence, but have only a limited effect on the toxicity, of potentially toxic species.

Résumé : Nous avons échantillonné une série de 22 lacs du sud du Québec couvrant une large gamme trophique afin de mettre au point des modèles du changement d'abondance et de toxicité des cyanobactéries. Tous les lacs contiennent des cyanobactéries toxiques et le contenu de toxines dans l'épilimnion, exprimé en équivalents de microcystine, est prédit le mieux par les concentrations d'azote total (TN) et de phosphore total (TP). Bien que la masse du phytoplancton s'accroisse de façon linéaire en fonction de l'accroissement de TP parmi les lacs, la biomasse des toxigènes augmente plus rapidement que la puissance au carré de TP. Les seuls genres potentiellement toxigènes dont la biomasse est en corrélation avec la concentration de microcystine sont *Microcystis* et *Anabaena*. De façon inattendue, le meilleur modèle de la biomasse des espèces toxiques est basé sur l'azote de l'épilimnion. La concentration chez ces organismes de l'hépatotoxine microcystine par unité de biomasse ne varie pas considérablement d'un lac à l'autre, ce qui appuie la proposition que les facteurs du milieu contrôlent la présence des espèces potentiellement toxiques, mais qu'ils ont un effet limité sur leur toxicité.

[Traduit par la Rédaction]

Introduction

Cyanobacteria are highly successful photoautotrophic prokaryotes of ancient lineage. They are a common feature of many aquatic systems including tropical and temperate water bodies. Cultural eutrophication of many water bodies has led to changes in the phytoplankton community with the development of greater biomass of algae, especially cyanobacterial populations. Mass developments, or blooms, of planktonic cyanobacteria frequently occur in fertile waters when warm stratified conditions permit (Paerl 1996). Many authors have concluded that the success of these organisms cannot be explained by a single characteristic but that several intrinsic and environmental factors work together to favour blooms (Paerl 1996; Hyenstrand et al. 1998; Oliver and Ganf 2000). Harmful algal blooms in a lake ecosystem are typically characterized by the heavy biomass accumulation of one or a few species. Roelke and Buyukates (2002) suggest that blooms represent a deviation from "normal" ecological systems, since they cause a disruption in the normal pattern of phytoplankton succession. In this situation diversity decreases, changing the interactions within the whole aquatic community from virus through zooplankton to fish (Romo and Miracle 1995; Zohary et al. 1996; Figueredo and Giani 2001). Cyanobacterial communities tend to create self-regulating populations (Hambright and Zohary 2000) characterized by a high degree of resilience that prevents ecosystem recovery to lower biomass levels.

Several species of cyanobacteria have the unfortunate ability to produce toxins, including hepatotoxins, neurotoxins, cytotoxins, and dermatotoxins. One major group of toxins is cyclic heptapeptides called microcystins, which are powerful hepatotoxins (Carmichael 1994). The general structure of microcystins is cyclo-(-D-Ala-*X*-D-MeAsp-Z-

Received 16 August 2004. Accepted 29 March 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 7 September 2005.
J18269

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 doi: 10.1139/F05-124

Adda-D-Glu-Mdha-), where *X* and *Z* are variable L-amino acids, D-MeAsp is D-*erythro*-β-methylaspartic acid, and Mdha is *N*-methyldehydroalanine. Adda is a 20-carbon nonprotein amino acid that is important for the toxicity of the compound. Microcystins inhibit protein phosphatase and are known to act as tumour promoters (Falconer and Humpage 1996). Acute animal and more rarely human poisonings have been reported worldwide, resulting from ingestion of water containing cyanobacterial cells (Lambert et al. 1994; Dow and Swoboda 2000).

The capacity of some cyanobacteria to produce toxins, and the presence/absence of toxicity in blooms, present both theoretical and practical challenges for aquatic scientists. What role do toxins perform for their producers? Are there special conditions that favour or control toxin production? It is possible that the toxicity of the community depends on the same environmental factors that favour cyanobacterial dominance (Jacoby et al. 2000; Kotak et al. 2000). Laboratory experiments performed on a variety of factors (Rapala et al. 1997; Lee et al. 2000; Oh et al. 2000) and several field studies (Vezie et al. 1998; Jacoby et al. 2000; Kotak et al. 2000) have focused on the effect of individual parameters on toxin production (for a review see Sivonen and Jones 1999). Production of cyanobacterial secondary metabolites, including toxins, may be regulated by environmental or endogenous factors affecting physiological state, growth stage, and biosynthesis (Paerl and Millie 1996). It is not entirely clear, however, whether the role of any or all toxins is primarily defensive; at least some seem to be constitutive and important for growth (Orr and Jones 1998; Lyck 2004).

In the present study, 22 water bodies in Quebec, Canada, of contrasting chemical and physical characteristics were surveyed to assess microcystin production and identify associated environmental factors. The variables examined in these systems included nutrient fractions (dissolved inorganic, dissolved organic, and total) for phosphorus and nitrogen, nutrient ratios, pH, temperature, dissolved free carbon dioxide, lake mean depth and water retention time, light-extinction coefficient, and the abundances of different cyanobacterial genera.

Material and methods

Sampling

Twenty-two lakes in the St. Lawrence lowlands, in the Eastern Townships (Appalachian geological province), on the Laurentian shield, and in Abitibi–Temiscamingue (western Quebec) were sampled between mid-June and mid-August 2001. A single integrated water sample was taken each time a lake was visited. Twenty-one of the lakes were sampled once and one was sampled (Lac St. Georges) twice. Lakes were chosen to represent a gradient of fertility, from oligotrophic (4.9 μg total phosphorus (TP)·L^{-1}) to eutrophic (130 μg TP·L^{-1}). Integrated water samples were collected at a central station by combining 1-m-long van Dorn bottle samples from all depths of the epilimnion in a clean polypropylene bottle, and subsampling after thorough mixing. Triplicate samples for measuring nutrients were taken from these samples. Samples for measuring dissolved nutrients were filtered immediately through 0.45-μm cellulose acetate filters and preserved with sulfuric acid for transport to the laboratory. TP and total dissolved phosphorus concentrations (0.2-mm-filtered water) were measured by the molybdenum-blue method after persulfate digestion. Total nitrogen (TN) and total dissolved nitrogen (0.2-mm-filtered water) concentrations were measured as nitrates after alkaline persulfate digestion. All colorimetric analyses were carried out on an Alpkem RFA300 or a Flow Solution IV autoanalyzer, for which detailed methodologies are given in Cattaneo and Prairie (1995). Concentrations of dissolved organic carbon in 0.2-mm-filtered sample water were measured by high-temperature oxidation on an OI Analytical TIC-TOC analyzer using persulfate oxidation after acidification. Temperature and oxygen profiles were obtained with a WTW Oxi 197 DO meter. Transparency (Z_{SD}) was measured with a 20-cm Secchi disk and the light-extinction coefficient (kd) was estimated using Poole and Atkins' (1929) formula: kd = $1.7/Z_{SD}$. This transformation provided information on the amount of light received by the plankton, and linearized the relationships based on light.

Watershed and morphometric variables were determined using GIS analysis of government digital maps and our digitization of lake bathymetric maps (Prairie and Soucisse 1999). Water-renewal times were calculated using mean annual precipitation minus evaporation, based on Quebec Ministry of the Environment data.

Chlorophyll *a* concentration was determined by filtration onto 47-mm GFF filters, extraction of pigments by hot 90% ethanol, and measurement of fluorescence (Nusch 1980; Riaux-Gobin and Klein 1993). Since many lakes contained a substantial fraction of chlorophytes, we did not correct chlorophyll *a* values for phaeopigments because of potential interference from chlorophyll *b*, which leads to underestimation of chlorophyll *a* and overestimation of phaeopigments (Gibbs 1979).

Phytoplankton samples for enumeration were collected at the same time as nutrient samples, and 100 mL was preserved with 0.5 mL of Lugol's iodine. Counts were done under an inverted microscope by Utermöhl's method (Utermöhl 1958). Algal biovolume was calculated from single cells according to Rott (1981) and converted to carbon biomass with the equations of Menden-Deuer and Lessard (2000). Concentrated plankton samples for microcystin determination were collected by a 35-μm-mesh plankton net. Water samples for estimating dry weight were filtered onto preweighed 47-mm GF/F glass-fiber filters.

Determination of microcystin concentration

Microcystin concentration was determined on lyophilized samples. Extraction was performed with 75% *v/v* methanol (Fastner et al. 1998). A weighed sample of freeze-dried material was extracted in a 2-mL microcentrifuge tube by adding 1.5 mL of solvent, sonicating, shaking for 10 min, and centrifuging at 3500 rpm. The extraction procedure was repeated twice and extracts were combined.

Analyses of microcystin content were carried out by the protein phosphatase inhibition assay and by high-performance liquid chromatography (HPLC). The inhibition assay measures the microcystin-dependent inhibition of the phosphatase enzyme in vitro. In the colorimetric assay (An and Carmichael 1994; Rivasseau and Hennion 1999), protein phosphatase-2A (PP2A) activity against *para*-nitrophenyl

phosphate (pNPP) is determined by quantitation of *para*-nitrophenol (pNP) hydrolyzed from pNPP. The pNP is estimated by absorbance at 405 nm using a microplate reader. The assay measures total PP2A inhibition without differentiating between different structural variants of microcystin and is calibrated with a microcystin-LR (MC-LR) standard curve, so microcystin content is expressed in MC-LR equivalents. Thus, in this paper microcystin concentrations represent the total toxicity of samples in MC-LR equivalents.

Phosphatase results were verified by HPLC analyses performed in the laboratories of Health Canada in Ottawa on a subset of 12 lakes. An immunoaffinity column was used for sample purification (Lawrence and Menard 2001). The use of antibodies for purification of the samples increased the sensitivity to the level of 1 $ng \cdot g^{-1}$. After extraction and dehydration, microcystin samples were diluted with acetonitrile and run on a reverse-phase C18 silica column with separation achieved over a gradient of water and acetonitrile. Results obtained by these two methods were positively correlated ($r^2 = 0.72$) and there were no major discrepancies.

An attempt was made to account for the presence and quantity of toxin in lake samples by correlation with the biomass of those species or genera that had previously been shown to have toxin-producing strains. The toxigenic genera included in the analysis included species of *Microcystis*, *Anabaena*, *Radiocystis*, *Aphanocapsa*, and *Oscillatoria*/*Planktothrix*. Total toxigenic biomass was estimated as a toxicity-weighted function of the biomasses of certain genera, as described in the Results. The variability of microcystin content per unit toxigenic biomass was calculated by dividing microcystin content per litre by total toxigenic biomass per litre. In a few lakes, it was possible to measure microcystin, although no known toxic species or very low concentrations of them were recorded in the phytoplankton samples. Several explanations are possible: some species of cyanobacteria, at present not known to be toxigenic, may also produce microcystin; the enzyme phosphatase may have suffered some inhibition other than by microcystin; or, more likely, the net sample we used for toxin analyses may have collected some colonial species that were not observed in the phytoplankton samples used for counting and biomass calculation.

Statistical analysis

To investigate the relationship between microcystin concentration and the independent variables, we performed correlation and univariate and multiple regression analyses on appropriately transformed data. Significant relationships were defined as $p < 0.05$. Transformations were chosen by Box–Cox maximum-likelihood optimization (Box and Cox 1964) followed by residual analysis (Draper and Smith 1980) in order to meet normality and linearity assumptions. The result was that all variables were log-transformed except pH and temperature, which were unchanged, and the light-extinction coefficient, which was transformed to the square root. Multiple regressions were performed by backwards stepwise procedures starting with all variables entered, using probability to leave of 0.10, but all coefficients of final models were significant at the $\alpha = 0.05$ level at least. Analysis of covariance (ANCOVA) was used when the goal was to compare regression slopes among groups. When log–log regression slopes greater than 1 were compared with a null hypothesis constant of 1 to test for arithmetic linearity, a *t* test was used. When log–log slopes less than 1 were similarly tested for linearity, in order to be conservative, *t* tests were based on model II (reduced major axis) slopes to account for attenuation biases due to error in *X* (Laws and Archie 1981). Analyses were run with the statistical package JMP® for Windows, version 5.1 (SAS Institute Inc. 2003).

Results

The physical and chemical data gathered during this study are summarized in Table 1. The lakes presented a wide range of trophy, with TP varying from 4.9 $\mu g \cdot L^{-1}$ (Brompton) to 130.0 $\mu g \cdot L^{-1}$ (Abitibi). The highest chlorophyll *a* concentrations were found in Tomcod Lake (31.0 $\mu g \cdot L^{-1}$) and the lowest in Truite Lake (1.4 $\mu g \cdot L^{-1}$). The lakes ranged from shallow (Z_{max} = 1.5 m) to moderately deep (Z_{max} = 57.9 m). Estimated water-retention time was relatively short, 0.04–8.6 years.

Three variables showed interesting relationships with lake plankton toxicity — TP, TN, and phytoplankton taxonomy — and these variables form the basis of this paper. When multiple regression was used to partial out nutrient effects by including TN and TP, there were no significant relationships, independent of TN and TP, between microcystin content and pH ($p = 0.38$), free carbon dioxide ($p = 0.26$), dissolved nutrient fractions (total dissolved phosphorus concentration: $p = 0.23$; total dissolved nitrogen concentration: $p = 0.79$; dissolved organic nitrogen: $p = 0.60$; NH_4: $p = 0.41$; NO_3: $p = 0.35$), lake morphometry (residence time: $p = 0.94$; mean depth: $p = 0.85$), or light-extinction coefficient ($p = 0.38$). Although microcystin content per litre was strongly related to TN and TP individually and together, it showed no relationship to the TN/TP ratio ($r^2 = 0.02$, $n = 23$, $p = 0.52$).

The relationship between TP in the studied lakes and the biomass of total phytoplankton, total cyanobacteria, and potential toxigenic species is illustrated in Fig. 1. In this instance, potential toxigenic biomass represents the unweighted sum of the biomasses of species of *Microcystis*, *Anabaena*, *Oscillatoria*, and *Aphanocapsa*. Comparing the slopes of the log–log relationships indicates that, with increasing TP, changes are much faster for cyanobacterial biomass (nearly quadratic; slope 1.96) than for total phytoplankton (nearly linear; slope = 1.16) and fastest for the potential microcystin-producing component of the community (slope = 2.17). The two cyanobacterial components increased significantly faster than did biomass (ANCOVA: $t = 2.56$, $p = 0.01$) but did not differ from each other ($t = 0.84$, $p = 0.40$). This implies that small increases in phosphorus concentration in the water may produce rapid changes in the toxicity of the resulting algal assemblage. At the same time, there was no indication in these data that microcystin producers increase as a fraction of total cyanobacteria along the trophic gradient.

We compared our data on changes in microcystin concentration per unit volume relative to TP with the data of Kotak et al. (2000) from several lakes in western Canada (ANCOVA; Fig. 2). Our results show that a similar relationship holds, in both slope ($p = 0.73$) and elevation ($p = 0.30$), in all these lakes without regard to geography or trophic sta-

Table 1. Physical and chemical characteristics of the studied lakes.

Lake	TP ($\mu g \cdot L^{-1}$)	TDP ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)	NO_3^- ($\mu g \cdot L^{-1}$)	NH_4^+ ($\mu g \cdot L^{-1}$)	DON ($\mu g \cdot L^{-1}$)	TN/TP (molar)	Chlorophyll *a* concentration ($\mu g \cdot L^{-1}$)	Z_{max} (m)	Z_{mix} (m)	Lake area (km^2)	Residence time (years)	MC-LR ($\mu g \cdot L^{-1}$)
À l'Ours	29.5	18.7	576	2.8	32.8	462	43.2	4.0	16.0	8.0	0.15	nd	0.009
Abitibi	130	nd	950	nd	nd	nd	16.2	60.0	15	nd	224	nd	0.41
Bowker	7.5	5.2	486	0	23.6	172	143.5	1.9	57.9	23.9	2.45	8.6	0.0017
Brompton	4.9	2.1	541	85.6	1.4	300	244.5	3.5	42.4	11.0	11.71	1.5	0.071
Brunet	41.5	23.9	588	2.8	100.0	331	31.4	9.8	1.5	1.0	0.24	0.32	0.013
Croche	6.9	5.2	755	8.4	8.7	298	242.3	1.5	11.0	5.3	0.18	nd	0.0031
Cromwell	9.8	7.5	588	2.5	9.4	458	132.9	6.3	7.5	2.0	0.09	nd	0.0008
Denison	47.1	20.5	691	0	40.9	417	32.5	21.3	5.0	1.6	0.3	0.04	0.054
Fraser	10.3	7.6	663	4.6	19.6	248	142.5	2.8	19.8	8.6	1.62	0.36	0.19
Heney	18.0	nd	410	nd	nd	nd	50.4	3.0	32		12.5	nd	0.0022
Libby	20.8	6.9	397	2.9	13.8	264	42.3	14.9	3.1	1.1	0.43	0.16	0.010
Lovering	16.5	10.9	437	33.3	19.5	332	58.6	3.9	25.0	9.8	4.9	1.6	0.0021
Montjoie	13.8	13.1	378	0	20.1	250	60.6	4.6	21.3	8.0	3.3	2.9	0.0022
Morency	24.2	12.2	356	3.2	30.0	321	32.6	2.4	20.0	7.5	0.26	1.4	0.0055
Parker	18.5	9.6	624	2.9	17.3	488	74.7	11.2	9.1	3.6	0.23	0.05	0.046
Simoneau	12.9	8.6	389	2.9	0	357	66.8	3.2	24.4	9.3	0.45	0.41	0.0010
St. Georges	46.1	14.0	901	3.6	21.5	469	43.3	41.8	4.3	1.8	0.51	0.18	0.23
Stukely	6.1	4.8	302	12.6	23.9	218	109.6	2.1	30.5	13.1	4.00	4.0	0.0032
Tomcod	58.5	39.6	834	2.8	37.5	592	31.6	31.0	1.8	0.9	0.81	0.06	1.91
Trois Lacs	36.9	16.0	681	34.6	30.5	458	40.9	19.3	10.1	1.3	2.4	0.01	0.076
Truite	31.7	8.5	449	3.5	50.9	256	31.4	1.4	12.2	3.3	2.4	0.30	0.046
Walfred	35.6	6.0	376	3.1	86.8	191	23.4	3.6	4.0	1.8	0.15	0.34	0.011

Note: Values for water-quality variables generally refer to July–August 2001 (nd, not determined). TP, total phosphorus concentration; TDP, total dissolved phosphorus concentration; TN, total nitrogen concentration; NO_3^-, nitrate; NH_4^+, ammonium; DON, dissolved organic nitrogen; TN:TP, ratio of total nitrogen to total phosphorus concentration; Z_{max}, lake maximum depth; Z_{mix}, epilimnion thickness; MC-LR, microcystin equivalents in the epilimnion.

Fig. 1. Relationships between log total phosphorus concentration (TP) and log biomass of phytoplankton (—●—), total cyanobacteria (- - -○- - -), and potentially microcystin-producing cyanobacteria (····■····). The respective regression equations are as follows: log phytoplankton biomass = –5.4 + 1.16 log TP, r^2 = 0.60; log cyanobacterial biomass = –7.3 + 1.96 log TP, r^2 = 0.64; log potential toxigenic biomass = –8.1 + 2.17 log TP, r^2 = 0.69 (biomass units for the equations are grams of carbon per litre).

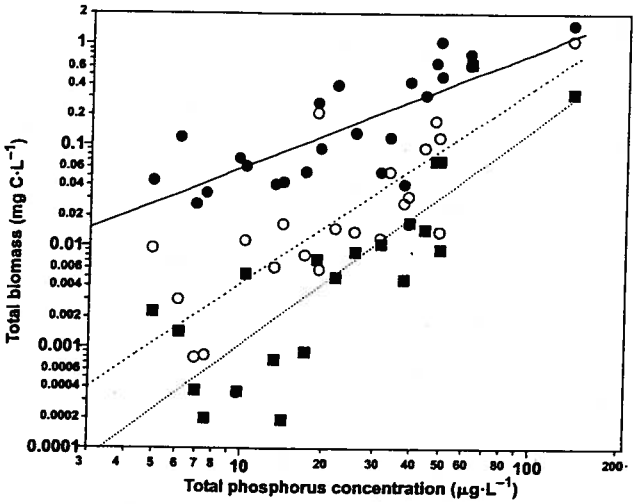
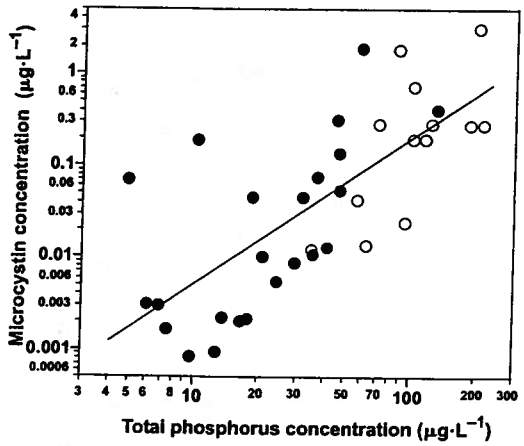


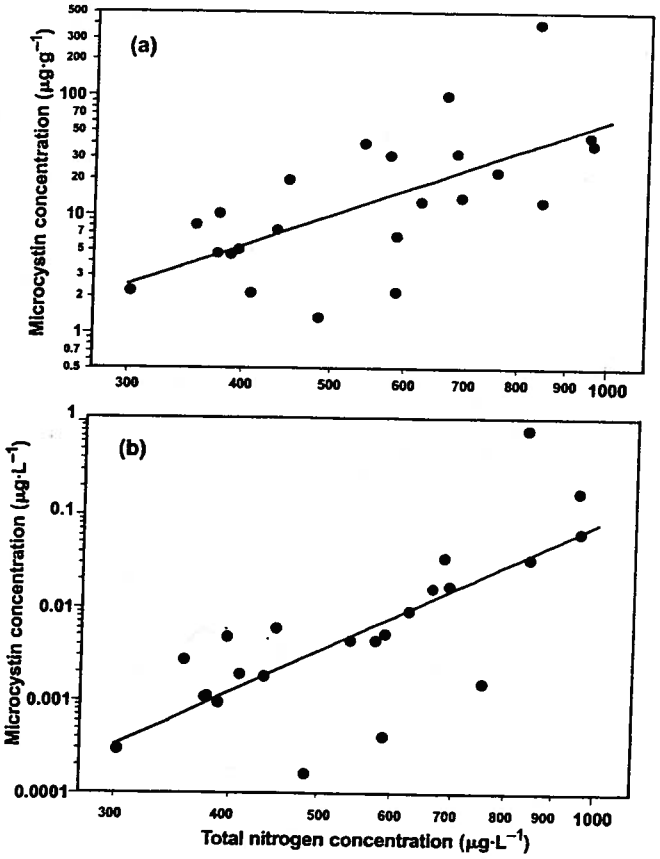
Fig. 2. Positive relationship between log total phosphorus concentration and log microcystin concentration in southern Quebec (this study; ●) and the summer mean for eutrophic lakes in Alberta (Kotak et al. 2000; ○). Lakes in both regions follow the same trend.



tus. The general equation was log(microcystin concentration (µg·L^{-1})) = –3.88 + 1.59 log TP (µg·L^{-1}), r^2 = 0.53, n = 36, $p < 0.0001$. The slope of this log–log relationship was significantly greater than linear (t test, $p < 0.02$), indicating the increasing relative importance of toxic species in richer lakes.

In our lakes, TN also showed positive significant relationships with microcystin concentration expressed in either micrograms per gram (Fig. 3*a*) or micrograms per litre (Fig. 3*b*). Increasing TN favours, or results from, a greater

Fig. 3. Scatter plots and regressions between log total nitrogen concentration (TN) and (*a*) sestonic log microcystin concentration and (*b*) epilimnetic log microcystin concentration in lakes of southern Quebec. Regression equations are log microcystin concentration (µg·g^{-1}) = –6.2 + 2.66 log TN, r^2 = 0.43, p = 0.0007 for *a* and log microcystin concentration (µg·L^{-1}) = –14.8 + 4.57 log TN, r^2 = 0.56, $p < 0.0001$ for *b*.



fraction of toxigenic species in the plankton, which increases the toxicity of the seston (Fig. 3*a*). Similarly, greater toxicity per unit volume of the water column is associated with more nitrogen (Fig. 3*b*).

To distinguish the relative importance of phosphorus and nitrogen for microcystin production, and to compare these results with trophic effects of an increase in phosphorus on the biomass of the phytoplankton, multiple regressions were calculated and F values compared. As expected, lake chlorophyll *a* content was best predicted by TP (p = 0.003) and secondarily by TN (p = 0.01). But unexpectedly, when predicting lake microcystin concentration, TP was relatively weakly significant in the multiple regression (p = 0.02), whereas TN was highly significant (p = 0.001). The equation was

$$\log(\text{microcystin concentration}) = -12.6 + 0.9 \log \text{TP} + 3.5 \log \text{TN}$$

where all variables are expressed in micrograms per litre (R^2 = 0.59, n = 23, $p < 0.0001$). Despite the moderately strong relationships between the absolute concentrations of

both nitrogen and phosphorus and microcystin concentration, no significant relationship was found here between microcystin concentration and the nitrogen:phosphorus ratio, as was noted above. This result is contrary to what has been seen periodically elsewhere (see Discussion). We should point out here that the range of TN/TP ratios in these Quebec lakes, starting at the Redfield molar ratio of 16 and going to 245 (Table 1), is within the range thought to indicate phosphorus, but not nitrogen, limitation.

The microcystin content of these lakes could be predicted on the basis of the biomass (g $C \cdot L^{-1}$) of only two genera, *Microcystis* and *Anabaena*. The regression equation

$$\text{microcystin concentration } (\mu g \cdot L^{-1}) = 3560(\textit{Microcystis}) + 2600(\textit{Anabaena})$$

explained 95% of the variation in toxin content ($F = 28.8$, $p < 0.0001$). *Microcystis* biomass had the greatest effect in the equation ($t = 13.15$ versus $t = 8.52$ for *Anabaena*). Using the molecular weight of microcystin (995.17) and its carbon content (49 atoms of carbon) we can estimate the average toxin fraction of *Microcystis* as 0.21% of its cell carbon. *Anabaena*'s toxin content was 0.15% of its cell carbon. Therefore, on average, *Anabaena* species were 70% as toxic as *Microcystis* species. There were eight lakes with apparent microcystin content but no evidence of *Microcystis* or *Anabaena*. These lakes had a low toxin content and contributed only to the error in the equation.

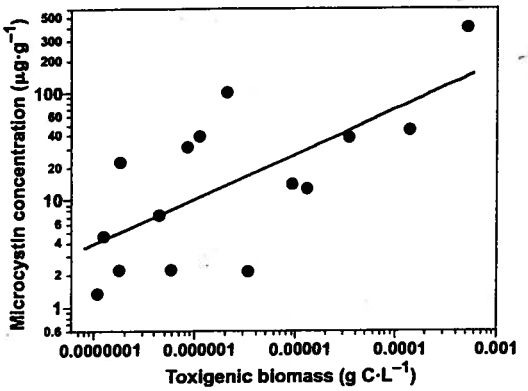
By weighting the biomass of *Anabaena* according to its toxin content relative to that of *Microcystis*, we could derive a measure of total toxigenic biomass in *Microcystis* equivalents. Using this weighted biomass, a positive and significant relationship was observed between microcystin concentration and biomass of toxic cyanobacteria (Fig. 4): microcystin concentration increased as the 0.8 power of biomass, a slope that is not different from 1 (model II, $p = 0.6$). In the lakes surveyed in this study, the microcystin concentration ($\mu g \cdot g^{-1}$) followed the same trend as the biomass of toxic cyanobacteria, depicted as a percentage relative to the total phytoplankton biomass (Fig. 5). Thus, the presence of known toxic species explained most of the toxicity.

The variability of microcystin concentration per unit toxigenic biomass was relatively constant, varying generally 10-fold among lakes, which is not markedly more variable than the carbon/chlorophyll *a* ratio. Much wider ranges were observed for microcystin concentration ($\mu g \cdot L^{-1}$), which varied more than 1000-fold, and for toxigenic cyanobacterial biomass (g $C \cdot L^{-1}$), which varied by four orders of magnitude among lakes.

Discussion

Our survey showed that all lakes contained detectable levels of toxic cyanobacteria, even the most oligotrophic systems, although usually at concentrations far below levels of concern (Health Canada 2003). This result is in accordance with the current hypothesis that microcystin-producing algae (primarily strains of *Microcystis* and *Anabaena*) contain this toxin as a constitutive physiological component (Hesse et al. 2001; Kaebernick and Neilan 2001). Along the trophic gradient, the only habitat factors that were significantly correlated with water-column toxicity were TP and TN. We will consider the role of each of these factors in turn.

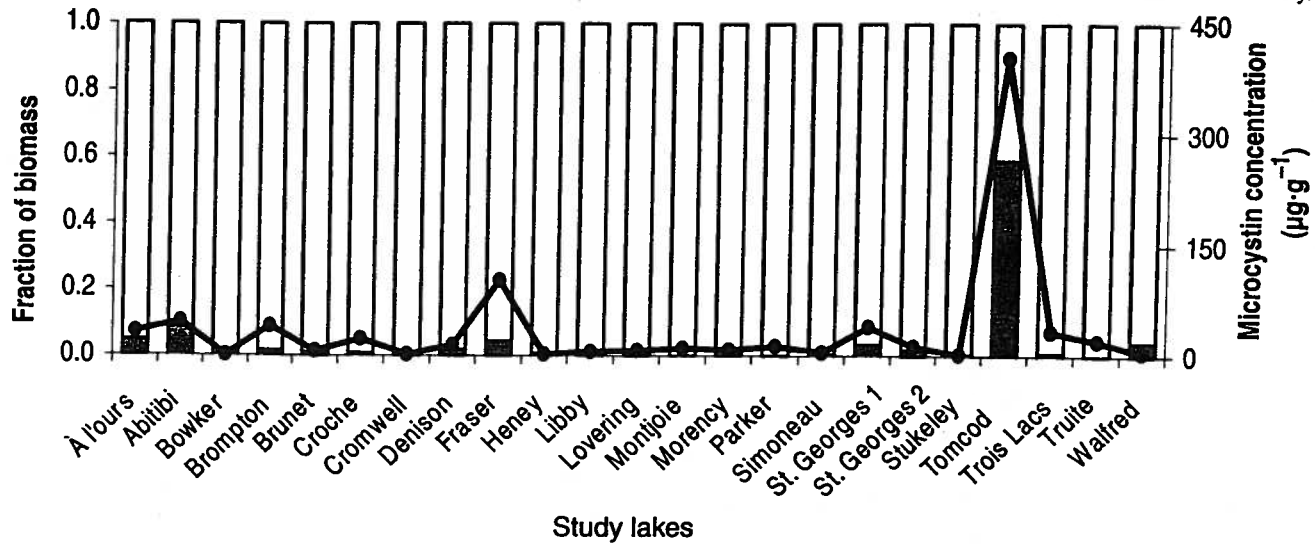
Fig. 4. Correlation plot and regression between log biomass of toxic cyanobacteria and log microcystin concentration in the seston: log microcystin concentration = 3.54 + 0.42 log toxigenic biomass, $r^2 = 0.46$, $p = 0.005$.



Phosphorus is the most limiting nutrient in these lakes (Prairie et al. 2002; Smith and Prairie 2004). In this study there were equally strong correlations between TP and the biomass of total phytoplankton, the biomass of total cyanobacteria alone, and the biomass of toxigenic cyanobacteria, where toxigenic implies microcystin-producing. What is most interesting is that the response to TP of these three variables became increasingly steeper, changing from linear (total phytoplankton) to quadratic (cyanobacteria) to greater than quadratic (toxigenic genera). But there are greater subtleties at work in the appearance of blooms and heavy dominance by cyanobacteria than those due to simple increases in nutrients. Cyanobacteria do not seem to possess affinities or uptake characteristics for phosphorus that are considerably different from those of the eukaryotic algae (Paerl 1996). Like the eukaryotes, they are able to store excess phosphorus in periods of excess supply (luxury consumption). But cyanobacteria are able to control their buoyancy via gas vacuoles, and through rapid vertical movements migrate to phosphate-rich aphotic waters, in this way avoiding phosphate-depleted environments. High TP has frequently been correlated with increased probability of cyanobacterial-bloom events (Canfield et al. 1989; Kotak et al. 2000; Downing et al. 2001). The greater rise in cyanobacterial share of biomass, especially the toxigenic fraction of biomass, relative to the increase in total phytoplankton with an increase in TP could be manifested as a sudden appearance of toxic blooms as lakes are slowly eutrophied by watershed development.

The literature on the effects of phosphorus on microcystin cellular production and content is contradictory. Watanabe and Oishi (1985) showed that under phosphorus-limited conditions, lower toxicity was found in *Microcystis aeruginosa*, probably related to different growth rates. Similarly, Sivonen (1990) found that toxin production in cultures of *Oscillatoria* responded positively to increasing phosphorus at levels between 0.1 and 0.4 mg phosphorus$\cdot L^{-1}$. However, higher phosphorus concentrations had no further effect on microcystin production. Contrary to the previous findings, Oh et al. (2000) observed that more microcystin per unit dry

Fig. 5. Percentages of toxic cyanobacteria in the total phytoplankton biomass and microcystin concentrations in the lakes in this study.



weight was produced by cultures of *M. aeruginosa* under more phosphorus-limited conditions. The same authors also found different production ratios of different microcystin variants according to the phosphorus concentrations. For instance, higher ratios of MC-LR to the variant microcystin-RR were found under phosphorus-limited conditions. In the present study, although the general trend was an increase of microcystin cellular content ($\mu g \cdot g^{-1}$ toxic species) with increasing phosphorus concentration, no significant correlation was found. Toxin-producing Cyanobacteria were present in both phosphorus-poor and phosphorus-rich lakes and contained similar cellular levels of toxins in all lakes. This seems to confirm the idea that control of cell-specific microcystin production by phosphorus is of negligible importance among lakes, but that increases in phosphorus act by enhancing the relative contribution of toxigenic Cyanobacteria to total phytoplanktonic biomass. Comparing the concentration of microcystin per litre ($\mu g \cdot L^{-1}$) obtained by Kotak et al. (2000) for a series of lakes in western Canada with our data shows that we detected toxicity at lower phosphorus concentrations, in a range where no observations were recorded in their study. Our results show that toxic cyanobacteria are present and produce toxins in lakes with lower nutrient concentrations. Because they are in low abundance in these environments, the total amount of microcystin is normally below the Canadian (1.5 $\mu g \cdot L^{-1}$) and World Health Organization (1 $\mu g \cdot L^{-1}$) guidelines for lifetime drinking-water exposure (Health Canada 2003).

Nitrogen is also an essential element for algal growth. Demands for exogenous nitrogen required for growth and reproduction are consistently high. Nitrogen attenuation and "retention" (often in the form of denitrification) are strongly influenced by hydraulic residence time in an analogous manner to that for phosphorus, and the role of nitrogen in controlling phytoplankton biomass is generally difficult to distinguish from that of phosphorus (Prairie et al. 1989). Because of the atmospheric component that contributes to nitrogen cycles and the possibility of nitrogen fixation by cyanobacteria, few models predicting biomass from nitrogen-loading exist. Yet in this study the observed responses of toxin concentration were stronger to nitrogen content than to TP. Significant correlations were found between TN and microcystin per litre ($\mu g \cdot L^{-1}$) ($r = 0.71$, $p < 0.001$) and per unit weight of net seston ($\mu g \cdot g^{-1}$ dry weight). The first illustrates the importance of nitrogen for the increase in biomass of toxin-producing cyanobacteria. Good correlations between nitrogen and cyanobacterial biomass were also found by Downing et al. (2001), who showed that TN was more strongly correlated with cyanobacterial dominance than TP. The second correlation, however, shows that TN was associated with changing seston content of microcystin, which increased by four orders of magnitude while TN was changing by less than one order of magnitude. In this case, nitrogen availability may be an important factor affecting cellular toxin production, as we might expect for peptides. As discussed below, we believe that when cells are not nutrient-limited, the microcystin content of producing cells does not vary significantly with changes in external nutrient supply. Previous culture studies have shown a consistently greater biomass-specific response to increased nitrogen than to increased phosphorus (Watanabe and Oishi 1985; Codd and Poon 1988; Utkilen and Gjølme 1995). Watanabe and Oishi (1985) reported a remarkable decrease in toxicity when the nitrogen concentration in the medium was reduced, but only minor changes when the phosphorus concentration was lower. Nutrient-ratio experiments by Lee et al. (2000) showed that the microcystin content of *M. aeruginosa* was best correlated with nitrogen content in the growth medium, whether the culture was phosphorus or nitrogen constant. Vezie et al. (2002) found experimentally that high nitrogen concentrations seemed to be necessary to foster growth of both toxic and nontoxic *Microcystis* strains.

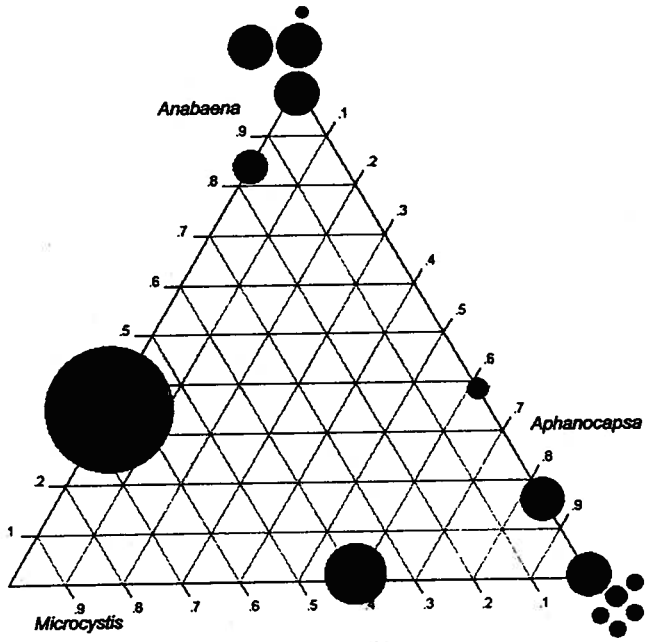
The importance of the TN/TP-ratio hypothesis for cyanobacterial dominance has been studied since Smith (1983) suggested that these organisms would be dominant at nitrogen/phosphorus (N/P) ratios below 29. The hypothesis was justified by the idea that cyanobacteria are better competitors for nitrogen than other phytoplankton species. They

show special features (such as the presence of heterocytes) that allow many taxa of this group to use atmospheric N_2 via nitrogen fixation, thus potentially avoiding situations of nitrogen limitation. They are able to use nitrogen in the form of ammonium, nitrate, and organic nitrogen (urea and a few amino acids). However, other studies have not upheld Smith's hypothesis (Canfield et al. 1989; Vincent 1989; Downing et al. 2001), although Kotak et al. (2000) found that the TN/TP ratio explained most of the variation in microcystin concentration ($\mu g \cdot L^{-1}$). Those authors suggested that a shift in the N/P ratio could increase the incidence of toxic blooms and the production of toxins. In our study no relation was observed between the N/P ratio and lake microcystin concentration, although N/P ratios observed in this study, from 16 to 245, were in a range completely apart from those recorded in the three Alberta lakes, from 1 to 24, that form the basis of Kotak et al.'s (2000) finding. The total amounts of both phosphorus and nitrogen, however, were important to explain the concentration of microcystin ($\mu g \cdot L^{-1}$). Similar to the conclusions of Vincent (1989) and Downing et al. (2001) when explaining the occurrence of cyanobacterial blooms in aquatic ecosystems, we found that the absolute amount of each nutrient, and not their ratio, determined the biomass and toxicity.

In these lakes, the presence and relative abundance of some cyanobacterial genera are the key variables that explain the toxicity of a bloom. The most commonly reported hepatotoxic genera are microplanktonic and include *Anabaena*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Oscillatoria*, *Nostoc*, and *Planktothrix* (Chorus and Bartram 1999), although this list is not exhaustive. Toxicity, or its lack, is not expressed at the generic level, nor at the species level, but varies among strains. Recent reports have also identified microcystin in unicellular and colonial picoplanktonic strains (Domingos et al. 1999). Microcystin is produced by cultured strains of *Aphanocapsa* and *Synechocystis*, for example (Oudra et al. 2002). The production of microcystin by picoplanktonic cyanobacteria opens a new challenge for water-treatment supplies and quality control because of the unsuspected risk of contamination. When we included *Aphanocapsa* as a potential toxic genus in our observations, for instance, we observed a stronger correlation between microcystin concentration ($\mu g \cdot g^{-1}$) and fraction of toxigenic biomass ($r^2 = 0.58$, $p < 0.0001$). In our survey of oligotrophic lakes, *Aphanocapsa* may have been associated with toxicity, especially in those lakes where *Microcystis* and *Anabaena* were not dominant (Fig. 6).

It is still unclear why some cyanobacteria produce toxins. Some may have a regulatory function within cell metabolism (Rapala et al. 1997; Jähnichen et al. 2001). Without knowing the role of the different toxins it is difficult or impossible to interpret the many conflicting reports of controlling environmental factors. Current understanding, however, seems to relate microcystin production to growth. Wicks and Thiel (1990) found a strong correlation between primary production per unit chlorophyll *a* and cell microcystin concentration. They suggest that with increasing light intensity and temperature, both growth rate and toxicity increase, although not at the same rate. Oh et al. (2000) showed a linear relationship between the microcystin-production rate and the cell growth rate, μ. They suggested that the microcystin-

Fig. 6. Ternary plot of the relative contributions of the genera *Microcystis* (bottom axis), *Anabaena* (left axis), and *Aphanocapsa* (right axis) to communities associated with a given amount of microcystin in our samples. The circles indicate the amount of toxin per microgram dry weight of seston (i.e., the radius of each circle is directly proportional to the square root of seston toxicity), and their position indicates the unweighted contribution of these three genera to total potential toxigenic biomass. The direction in which the axes should be read is indicated by the small extensions on each axis scale. For example, the largest circle indicates a toxigenic community made up of 65% *Microcystis*, 35% *Anabaena*, and 0% *Aphanocapsa*. Circles on the vertices or outside the triangle represent communities made up entirely of the cyanobacterial genus indicated at that vertex.



production rate in a water system is determined by μ. Orr and Jones (1998) believe that the effect of environmental parameters on microcystin production is the result of their influence on the rate of increase or decline of a strain, but not specific influences on microcystin biosynthetic or catabolic pathways. Lyck (2004) found that microcystin is constitutively produced in a way similar to protein and chlorophyll *a*, implying that just small changes in microcystin cell quota should be expected. Although the minimal and maximal cellular microcystin levels may be modulated by external factors within a toxigenic strain (Sivonen and Jones 1999), and although we may not have identified all toxigenic species and toxic compounds (Pietsch et al. 2001; Chorus 2002), the biomass-specific levels of microcystin are relatively constant and show the same 10-fold range found for carbon:chlorophyll *a* ratios in phytoplankton. This means that the microcystin concentration in all lakes was directly related to the biomass of toxigenic taxa. We conclude that the presence or absence of currently identified toxic species, and the population dynamics of these species, are the principal source of observed changes in bloom toxicity.

Acknowledgements

We thank C. Asselin and A. Rolland for help with sampling, C. Côté for nutrient determination, C. Ménard for HPLC work, and G. von Ruckert and M.C. Melo for microscopy. This study was supported by a fellowship to A.G. from the Coordenação de Aperfeiçoamento do Pessoal Docente, Brazil, a fellowship to D.F.B. from Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Brazil, by funds provided by the Groupe de Recherche Interuniversitaire en Limnologie, Quebec, and by grants from the Natural Sciences and Engineering Research Council of Canada. The manuscript benefited from comments made by anonymous reviewers.

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