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Characterization of phytoplankton seed banks in the sediments of a drinking water reservoir

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Abstract

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The presence, abundance, and composition of algal and cyanobacterial populations in lake sediments were evaluated in Lake St. Charles (Quebec City, Canada), an urban drinking water reservoir. This water body has recently experienced cyanobacterial blooms, and we tested the hypothesis that a seed population of noxious taxa that could potentially re-inoculate the water column was present in the lake sediments. Cores were obtained from 8 sites spanning a range of depth and sediment conditions in both basins of the lake; sampling was from May to October over 2 years. Three techniques were applied: observation of the surficial sediments by epifluorescence microscopy; pigment analysis by high-performance liquid chromatography (HPLC); and laboratory enrichment culture of sediment samples under different light, temperature, and nutrient conditions. These analyses revealed the presence of diverse phytoplankton pigments and fluorescent cells in the sediments, with a predominance of diatoms along with dinoflagellates, chrysophytes, chlorophytes, euglenophytes, and cryptophytes. Growth of benthic filamentous cyanobacteria was induced from the sediments during the incubations, but bloom-forming genera that occurred in the lake such as *Anabaena* and *Microcystis* were not detected in any of the sediments, either before or after incubation. These observations imply that the episodic blooms of cyanobacteria in Lake St. Charles were not derived from an abundant seed population distributed throughout the surficial sediments of the lake. Alternative inoculum sources may include localized populations in sediments at sites that were not sampled in the present study, cyanobacteria that may enter via the inflows, or holoplanktonic populations that persist in the water column at low cell concentrations.

Key words: Blooms, cyanobacteria, inocula, pigments, resting stages, sediments

Harmful algal blooms (HABs) are an increasing problem for lake and reservoir management (e.g., Lopez et al. 2008, Watson et al. 2008, Winter et al. 2011), and their detection, mitigation, and control requires an improved understanding of the ecological characteristics of bloom-forming species. HABs deteriorate water quality through effects on aesthetic, ecological, recreational, and industrial values (Lopez et al. 2008). Phytoplankton blooms cause a variety of specific problems for potable water supplies, including clogging filters and releasing taste and odor compounds (e.g., Watson et al. 2008, Zhao et al. 2013), and some taxa of bloom-forming cyanobacteria, such as *Microcystis* and *Anabaena*, produce toxins that create health risks for humans and animals (e.g., Codd et al. 2005).

The alternation of benthic–planktonic stages in phytoplankton in general and cyanobacteria specifically is an adaptive strategy that may allow bloom-forming species to survive unfavorable growth conditions during winter, such as cold temperatures, ice cover, and low irradiance (Barberio and Welch 1992, Head et al. 1999, Karlsson-Elfgren et al. 2004). When environmental conditions are optimal, resting stages contained in the surface layer of sediments may return to a more intense metabolic activity, differentiate, and then emerge in the water column (Latour et al. 2004, Holland and Walsby 2008). For example, *Anabaena flos-aquae* produces overwintering akinetes, which then germinate as gas-vacuolate vegetative cells that rise into the overlying water (Kaplan-Levy et al. 2010). Dormant stages of the

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genus *Microcystis* may occur as modified vegetative cells that remain in the sediments throughout winter, which then develop gas vacuoles and enter the water column during the subsequent growing season (Brunberg and Blomqvist 2002).

Recruitment from the sediments into the water column is determined by 2 essential conditions: (1) the presence of resting stage populations in the sediments that provide an inoculum; and (2) favorable abiotic conditions for the growth and release of these cells into the water column. Many studies have used traps of various designs to directly measure the vertical migration of algal flagellates or gas-vacuolate cyanobacteria (e.g., Hansson et al. 1994, Head et al. 1999, Brunberg and Blomqvist 2003, Karlsson-Elfgren et al. 2004, Rengefors et. al. 2004, Cao et al. 2005), while fewer studies have attempted to quantify the resting cell populations of bloom-forming cyanobacteria in lake sediments (e.g., Tsujimura et al. 2000, Tsujimura and Okubo 2003, Latour et al. 2007). Condition (1) above is a prerequisite for condition (2), and its analysis therefore provides an initial guide to the likelihood of recruitment.

The overall objective of the present study was to characterize the microalgal and cyanobacterial content of the sediments of an urban drinking water reservoir (Lake St. Charles, Quebec City, Canada) in which HABs have recently occurred. Over the last decade, this lake has experienced episodic blooms of noxious cyanobacteria, specifically *Anabaena flos-aquae* and *Microcystis aeruginosa*, and associated water quality problems have become of increasing concern (Rolland et al. 2013). Specifically, we aimed to test the hypothesis that an abundant population of bloom-forming cyanobacteria occurred throughout the sediments of Lake St. Charles, thereby fulfilling condition (1) for recruitment. We tested this hypothesis by using fluorescence microscopy, high-performance liquid chromatography (HPLC) pigment analysis, and laboratory incubations to detect and quantify phytoplankton cells in the lake sediments and to assess the ability of these potential seed populations to germinate and grow. To optimize coverage of the lake, we sampled at 8 sites over a range of depths, sediment types, and locations, including in a bay where cyanobacterial blooms have been especially prevalent. To ensure temporal coverage, we sampled these sites at 2-week intervals throughout summer (May to Oct), with additional sampling during winter to analyze the overwintering populations in the sediments.

Study area

Lake St. Charles (46°54′N, 71°22′W) is located 20 km north of Quebec City (QC, Canada), and provides the drinking water supply for 285,000 residents of the city and its surrounding region. It has a surface area of 3.6 km^2, a total water volume of 14.8×10^6 m^3, and is composed of 2 basins that differ in morphometry: the north basin is conical and reaches a maximum depth of 17.5 m, and the south basin has a maximum depth of 6 m (Fig. 1). The watershed of Lake St. Charles extends over 169 km^2, and the hydraulic residence time during summer ranges from 30 to 100 d. The trophic status of the lake is in the mesotrophic range, with summer average chlorophyll *a* (Chl-*a*) concentrations ~5 μg/L and total phosphorus (TP) concentrations ~10 μg/L. Since 2007, the proliferation of bloom-forming cyanobacteria such as *Microcystis aeruginosa* and *Anabaena flos-aquae* has become more pronounced (Rolland et al. 2013), implying a shift toward a more productive trophic status.

Materials and methods

Sediment sampling

We selected 8 sampling sites, including several bays as well as the open waters of both basins of the lake (Fig. 1), to cover a range of depths and sediment characteristics (Supplemental Table 1). The sampling was conducted at 2-week intervals in 2009 and 2010 from May (soon after the full ice-out that typically occurs in late Apr) throughout summer to early fall (Oct). Given the extensive coverage of the lake, each sampling extended over a 2-day period. Sediment cores were collected using a 75 mm diameter Kajac-Brinkhurst gravity corer (Aquatic Research Instruments, Hope, ID). Triplicate samples were taken at each site in 2009, and single cores were obtained per site in 2010 after the observation of low variation in total cell abundance between replicates (the mean coefficient of variation for triplicates in 2009 was 9.5%). Three additional cores were obtained at site N4 on January 2010 when the lake was covered by ice. All cores were covered with an opaque bag immediately after sampling to avoid exposure to light and were kept in a vertical position at 4 C for 24 h.

In the laboratory, the cores were subsampled in dim light using an extractor for expelling thin layers of sediment. Given the low net sediment accumulation rate in Lake St. Charles (~2.0 mm/yr; Tremblay et al. 2001), only the 2 uppermost 1 cm layers of sediment (likely encompassing the decade prior to sampling) and 20 mL of the overlying water were kept for further analysis. A subsample of sediment and the overlying water preserved with Lugol's iodine (5% final concentration) were stored in the dark at 4 C for microscopy analysis, and another sediment subsample was kept at −80 C for HPLC pigment analysis.

A subsample of the upper 1 cm layer was analyzed for granulometry by prescreening through a 2 mm mesh and then determination of particle size distribution in a laser diffraction LS13320 instrument (Beckman Coulter, Brea,

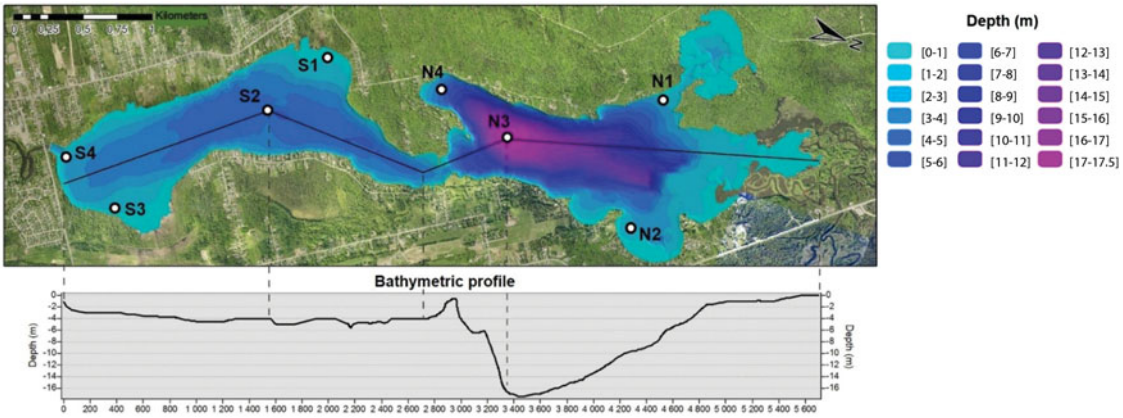

Figure 1. Bathymetry of Lake St. Charles and location of the sediment sampling sites. North basin: Talbot Bay (N1), Des Aigles Pêcheurs Bay (N2), deepest point of the lake (N3), Echo Bay (N4). South basin: Des Milans Bay (S1), middle of the south basin (S2), beach (S3), dam (S4). Modified from Sirois (2012).

CA). Additional subsamples were analyzed for organic content by combustion at 375 C for 16 h.

Epifluorescence microscopy

A 1 cm^3 volume of sediment was transferred to a glass test tube with a metallic spatula, and 1 mL of distilled water was added. The sample was agitated with a vortex shaker; large sand and silt particles were allowed to sediment, and then the supernatant was removed. This washing was repeated 3 times, and then the final aliquot (~3 mL) was observed under an inverted fluorescence microscope (Zeiss Axiovert 200 with N/HBO/103 illuminator, filter sets #05 and #14) in an Utermöhl (1958) counting chamber at 200×, 400×, and 1000× magnification. The results were expressed as cells/cm^3 of sediment. A test of this method was made by mixing a measured aliquot of a monospecific *Synechococcus* sp. culture into Lake St. Charles sediment, and the application of our protocol yielded a 95% recovery of cells.

Phytoplankton sampling

To compare the algal and cyanobacterial composition of the sediments with that in the overlying water column, phytoplankton samples were collected in the north basin (N3) and south basin (S2) of the lake during the same 2-day sampling visits every 2 weeks. Sampling was always done at the same approximate time each morning for both basins (8:00–10:00 h). Integrated water samples were collected with a 30 mm diameter plastic tube that extended from the surface to 2 m depth and were transferred into polyethylene bottles, fixed with Lugol's iodine (5% final concentration) and stored in the dark at 4 C. These were then examined by Utermöhl sedimentation and inverse microscopy (as in CEN 2006) for phytoplankton enumeration.

HPLC analysis

The frozen sediment samples were freeze-dried for 48 h and then stored frozen at −20 C. A dried subsample was weighed and transferred to a tube containing acetone (95%) and an aqueous solution of Sudan II (130 mg/L). The samples were sonicated and stored for 23 h in the dark at −20 C. After extraction, the tubes were centrifuged at 4 C and 4150 rpm for 15 min. The supernatant was then filtered (PTFE, 0.2 μm) and analyzed in a Thermo Scientific Accela 600 HPLC using the protocol of Zapata et al. (2000) for photosynthetic pigments. Chlorophylls were detected by fluorescence (excitation, 440 nm; emission, 650 nm), and carotenoids were detected by photodiode array (PDA) spectroscopy (350–750 nm) with a slit width of 2 nm. Absorbance chromatograms from the PDA were obtained at 450 nm. The results were calculated as μg/g of dried sediment.

Germination and growth experiments

Surficial sediment samples were collected from Lake St. Charles by sediment coring on 15 June and 16 September 2009 at deep (N3, 14.8 m depth), moderately deep (N4, 4.3 m depth), and shallow sites (S1 and S4, 1.1 and 1.0 m depth, respectively) to detect viable resting stages or akinetes by growth assays. The 2 uppermost 1 cm layers of sediment were transferred aseptically into sterilized 250 mL glass flasks containing liquid BG11 medium (Allen and Stanier 1968). The flasks were closed with a sterilized gauze stopper incubated at room temperature (20 C) and ambient light. At

1–2 week intervals, water subsamples of 10 mL were collected at the sediment interface, concentrated by Utermöhl sedimentation, and observed under the inverted microscope to determine the presence and growth of microalgae and cyanobacteria.

Additional sediment cores (24) were collected on 25 October 2010 at station N4, a site where cyanobacterial blooms have often occurred since 2006. These cores and their overlying water columns were maintained in their sampling tubes and installed in 4 programmable incubators under defined irradiance (150 μmol photons/m^2/s) and temperature (15–30 C) conditions, with and without phosphorus enrichment (10 μg P/L). Over a period of 60 d, the development of cyanobacteria was assessed each 3 d with a Trios MicroFluBlue in vivo probe to detect phycocyanin fluorescence, and every 7 d a sample of the overlying water was removed, preserved with Lugol's iodine (final concentration 5%), and later examined by Utermöhl sedimentation and inverted microscopy, as in CEN (2006). Full details of this experiment are provided in Supplemental Appendix 1.

Statistical analyses

Analyses were performed using SigmaStat (version 11.0). Initial Shapiro-Wilk tests indicated that the data for most variables were not normally distributed; thus Friedman ANOVA tests for matched groups were used to compare total cell densities, major taxonomic group densities, and pigment composition among dates or sampling sites. Pairwise multiple comparisons were then performed using Tukey's method to identify which pairs of groups were significantly different ($P < 0.05$).

Results

Sediment characteristics

The sites spanned a wide range of depths, and the sediment characteristics also varied (Supplemental Table 1). The sand content varied among sites from 17 to 75%, the silt content varied from 25 to 80%, and the small clay contribution ranged from 1.1 to 3.5%. Similarly, the organic content of the sediments varied by a factor of 3 among sites, from 4.4 to 12.8%.

Epifluorescence microscopy

Fluorescent microalgal and cyanobacterial cells were detected in all sediments on all dates of sampling, and there was little variation among sites (Fig. 2) or among dates of sampling. The overall mean values (±SD) for all cells were $17.6 \pm 2.8 \times 10^3$ cells/cm^3 in 2009 and $20.5 \pm 3.7 \times 10^3$ cells/cm^3 in 2010. The predominant taxa were diatoms, with detectable but much lower concentrations of chlorophytes, euglenophytes, dinoflagellates, chrysophytes, and cyanobacteria (Fig. 2). No taxa of bloom-forming cyanobacteria were observed in any of the sediment samples, either as vegetative cells or akinetes, including at site N4 sampled in winter.

The diatom populations were composed of planktonic species (*Aulacoseira ambigua*, *Fragilaria crotonensis*, *Asterionella formosa*, and *Tabellaria flocculosa*), including thick-walled resting cells, and the benthic taxa *Gomphonema* and *Navicula*. The euglenophytes included spherical resting cells of *Trachelomonas* and/or *Euglena,* accounting for ~50% of the populations in this phylum. The chlorophytes included the genera *Ankistrodesmus*, *Pediastrum*, *Scenedesmus*, *Coelastrum*, and *Chlorococcum*, while chrysophytes were dominated by *Dinobryon divergens*, *Ochromonas* sp., and *Mallomonas* sp. The dinoflagellate *Ceratium hirundinella* and unidentified small-cell cryptomonads were also conspicuous components of the sediment flora. Cyanobacteria were represented by filaments (overall average ± SD of $1.2 \pm 0.1 \times 10^3$ cells/cm^3) of typically benthic, oscillatorian cyanobacteria (*Oscillatoria* spp. and *Arthrospira* sp.).

Comparisons with the phytoplankton community

There were large variations in phytoplankton community structure throughout each year with a greater proportion of diatoms in spring (May) and fall (Oct). An increase in the proportional abundance of cyanobacteria from spring to midsummer was observed in the phytoplankton in both years (Fig. 3), with dominance of the colonial taxa *Aphanocapsa* sp., *Anabaena flos-aquae*, or *Microcystis aeruginosa* (details in Rolland et al. 2013). This planktonic community structure and its variation contrasted markedly with the sediment assemblages; the sediment assemblages varied to a much lesser extent than the planktonic communities in terms of the proportional abundance of different phyla. There was consistent dominance of the sediment populations by planktonic diatoms, while cyanobacteria made only a small contribution on all dates of sampling (Fig. 3).

Although resting cells of bloom-forming cyanobacteria were never observed in the sediments, low concentrations (<50 cells/mL) of akinetes of *Anabaena flos-aquae* were observed on 2 occasions, both in water samples from the Baie d'Echo (site N4). The first observation was in surface water sampled 10 June 2010, and the second was in water overlying the sediment of a core taken 10 October 2010. On both occasions the akinetes appeared to be in a state of germination (Fig. 4).

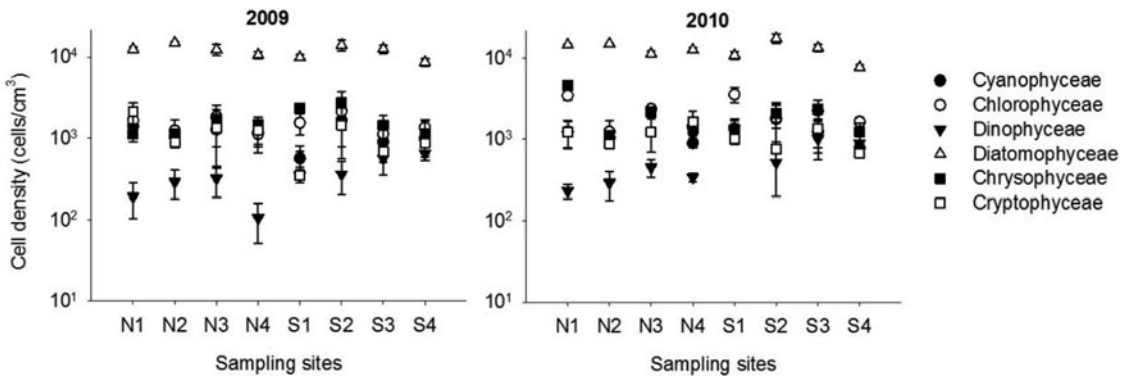

Figure 2. Spatial variation of the major taxonomic groups in the surficial sediments of Lake St. Charles. Plots are means for all sampling dates each year ± SD. Note the log scale.

Pigment analysis of the surficial sediments

The sediments of Lake St. Charles contained high concentrations of undegraded Chl-*a* at all sites, mostly in the range of 100–200 μg/g, with significantly lower values at S3 ($P < 0.05$). Consistent with the examinations by microscopy, there was a variety of accessory pigments, with the pigment fucoxanthin comprising a major fraction (>25%) of the total at most sites. The pigments included those found in chlorophytes and euglenophytes (Chl-*b*, lutein, neoxanthin, and violaxanthin), cryptophytes (alloxanthin), diatoms and dinoflagellates (fucoxanthin, diadinoxanthin, and diatoxanthin), and cyanobacteria (zeaxanthin and canthaxanthin), with many other degraded, unidentified carotenoids that had a lutein-like absorption spectrum. The seasonal (Jun to Sep) and interannual (between 2009 and 2010) variations

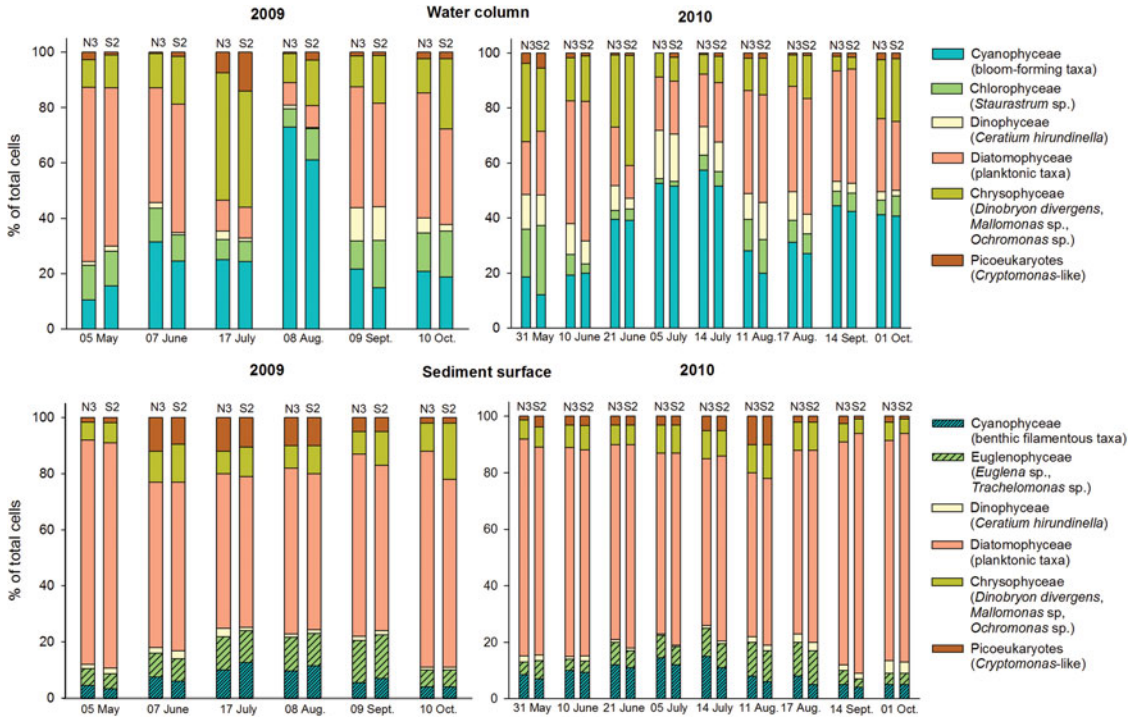

Figure 3. Relative proportion of major taxonomic groups in the water column and sediment surface in Lake St. Charles at N3 (middle of northern basin) and S2 (middle of southern basin) during summers 2009 and 2010. The dominant taxa for each group are given in parentheses.

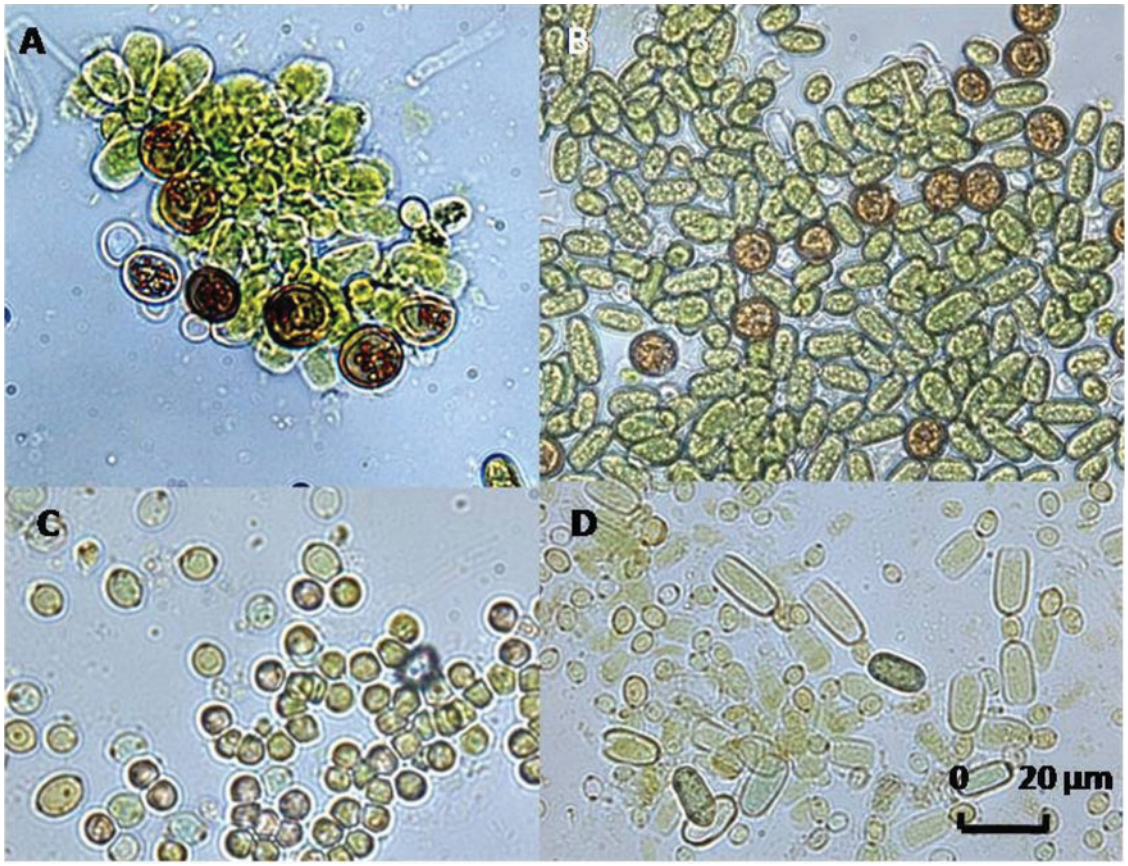

Figure 4. Photomicrographs of germination stages of *Anabaena flos-aquae* akinetes observed in surface lake water on 10 June 2010. (A) Opening of the akinetes and vegetative cells emerging from the envelope; (B) mature akinetes and vegetative cells; (C–D) germination of the vegetative cells.

in sediment pigment stocks were examined at 4 stations and showed no significant differences among sampling dates ($P = 0.20$). The spatial variation in sediment pigment stocks was examined in mid-July 2010 and showed significant differences among stations ($P = 0.034$; Fig. 5). The highest Chl-*a* concentrations were at N2 (184 μg/g), S2 (182 μg/g), and S4 (187 μg/g), and lowest abundance was at S3 (55 μg/g). Cyanobacterial pigments occurred in trace concentrations, at or near the detection limit; maximum concentrations of canthaxanthin, the most abundant cyanobacterial carotenoid, were ~0.6%.

Germination and growth experiments

Diatoms, chlorophytes, and cyanobacteria grew in the cultures of lake sediments (sampled 15 Jun and 16 Sep 2009 from sites N3, N4, S1, and S4) that were incubated at room temperature with the BG11 nutrient enrichment. At the end of 10 weeks, coccoid chlorophytes were among the most abundant cells (>20% of occurrence in 30 fields examined) in water overlying the sediment cultures from all sites except S1 and S4 sampled in June 2009, where they were not detected. Diatoms were also well represented, with 10–20% occurrence in 30 fields examined for all sites, again with the exception of S1 and S4 in June 2009, where only frustule fragments were detected. *Oscillatoria* spp. grew in the cultures from all sites and both dates of sampling (5–20% occurrence). *Chroococcus* sp. was similarly common (but not detected in the September S1 culture), while the filamentous taxon *Cylindrospermum* cf. *majus* was common (>20% occurrence) in the N3 and N4 cultures from both dates, but never in the S1 and S4 cultures. Other cyanobacterial taxa that were infrequently observed (5–10% occurrence in cultures from 1 to 3 sites) included: *Aphanocapsa* sp., *Woronichinia naegeliana*, *Lyngbya* cf. *birgei*, *Lyngbya* cf. *limnetica*, and *Merismopedia* sp.

The sediment samples from site N4 incubated under controlled light and temperature conditions, with and without P enrichment, yielded similar results. The P-enriched treatments at 20, 25, and 30 C showed substantial growth of

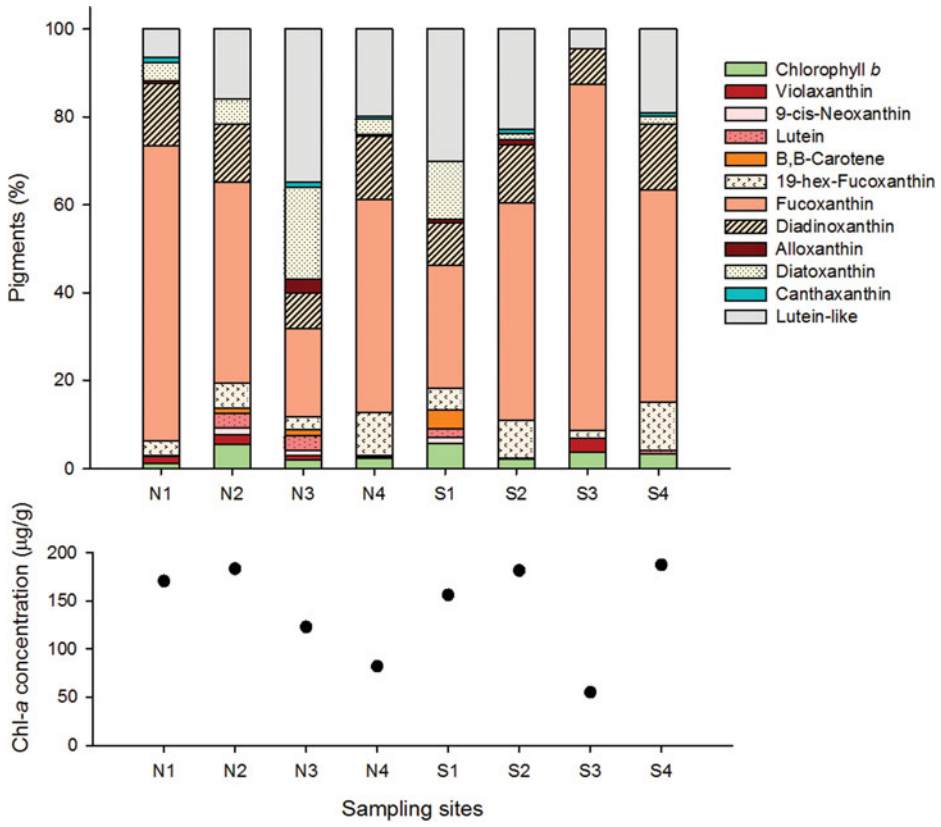

Figure 5. Accessory pigment composition (above) and chlorophyll *a* (Chl-*a*) concentrations (below) of the surficial sediments in Lake St. Charles, in mid-July 2010.

coccoid chlorophytes, to maximum concentrations of 4.6, 6.6, and 4.4 $\times$ 10^3 cells/mL (respectively) by the end of the experiment (60 d). The walls of the cylinders incubated at 30 C were colonized by attached filamentous cyanobacteria (*Oscillatoria* spp.) in both treatments with and without P additions; however, no phycocyanin fluorescence was registered in the water overlying the sediments in any of the treatments throughout the experiment, implying the absence of planktonic cyanobacteria, and this was confirmed by microscopy.

Discussion

Assemblages at the sediment surface

The combination of approaches used in the present study shows that the surficial sediments of Lake St. Charles contained pigment stocks and fluorescent algal and cyanobacterial cells that could be induced to grow in culture. The HPLC and microscopy confirmed the presence of diverse taxa that could potentially act as an inoculum to initiate phytoplankton growth in the overlying water column. The sediment community structure was strikingly different from that of the phytoplankton, however, with a continuous predominance of diatoms and low concentrations of cyanobacteria, as measured by cell counts and by HPLC pigment signatures.

In absolute terms, the diatom populations averaged 6 $\times$ 10^4 cells/cm^2 at the lake sediment surface. This density is low relative to mesotrophic to eutrophic shallow lakes that have been examined elsewhere in Quebec (e.g., 10^6 to 10^7 cells/cm^2 in the Abitibi region; Cattaneo et al. 2011), although that study included growing periphytic species. There was a correspondence between several diatom taxa in the Lake St. Charles sediments and water column, notably *Asterionella*, *Aulacoseira*, *Tabellaria*, and *Fragilaria*, which are known to thrive in the phytoplankton of temperate mesotrophic and eutrophic lakes (Bellinger and Sigee 2010). This observation and the responsiveness of the sediment diatoms to culture imply that the sediments act as an important reserve of cells for the planktonic life cycle phase of these organisms, as is well known for many diatom taxa (Reynolds 2006).

The chrysophyte flagellate *Dinobryon divergens* and the large dinoflagellate *Ceratium hirundinella* similarly co-occurred in sediments and water columns but in greater proportional abundance in phytoplankton. As noted by Hansson et al. (1994) in Lake Mendota (Wisconsin), these taxa may depend on initial inoculation from the sediments, but the bloom magnitude in the plankton may have little relationship to the abundance of the sediment populations. The euglenoid flagellates *Trachelomomas* and *Euglena* were a substantial component of the sediment flora but occurred only sporadically in the water column. These taxa are common in organic-rich environments (Reynolds 2006), and their growth may have been favored by association with the organic-containing sediments.

Several cyanobacterial taxa were detected in the sediments, but these were mostly filamentous benthic species, which subsequently grew in the sediment culture assays; however, bloom-forming taxa such as *Anabaena flos-aquae* and *Microcystis aeruginosa*, which were common and abundant in the phytoplankton at certain times of the year, were conspicuously absent from the sediments. *Microcystis* blooms are known to be initiated by dense overwintering mats on the sediment surface of many lakes (e.g., Hansson et al. 1994, Brunberg and Blomqvist 2002, Latour et al. 2007), but this does not seem to be the case in Lake St. Charles. Although the colonial planktonic cyanobacteria *Merismopedia* and *Woronichinia naegeliana* grew in some of the sediment enrichment cultures, these taxa occurred only sporadically and in low abundance in the phytoplankton of this lake. Studies elsewhere have shown the recruitment of *Woronichinia* from sediments into the water column, such as in Lake Guelph, Canada (Trimbee and Harris 1984), and Esthwaite Water, UK (Head et al. 1999), but this recruitment seems to be minimal in Lake St. Charles.

Limitations of the detection methods

Limitations of the present sampling and analytical methods must be considered to assess whether a sediment population of bloom-forming cyanobacterial resting cells could have in some way been undersampled or erroneously undetected. These limitations include sampling coverage of the lake, sample processing, and cyanobacterial detection.

Cyanobacterial cells could have been displaced to deeper than 2 cm in the sediments during the core collection or by bioturbation (Torres and Adámek 2013 and references therein). This displacement would also have affected the other taxonomic groups, however, and the presence of many other cell types and pigments in the surficial sediments suggests that a surface population would still be detectable. Previous studies on periphytic and benthic algal communities in temperate lakes (Cattaneo 1990, Kahlert et al. 2002) have shown that microalgae are patchily distributed at small (cm) and large (m) length scales, and a high intensity of sampling is therefore required to adequately define such communities. This effect of patchiness is a major challenge for sampling all sediment properties (Downing and Rath 1988). In the present study, however, we assessed small-scale patchiness via the use of triplicate core samples, and the coefficients of variation were small. Greater variation was observed among sites throughout the lake; however, 291 cores were examined in the present study, covering a wide range of sediment conditions and times of year, including winter at station N4, in the sheltered Baie d'Echo where summer blooms have occurred. Despite this extensive spatial and temporal coverage, the results were all contrary to our hypothesis that bloom-forming cyanobacteria were abundantly distributed throughout the lake.

We applied a microscopy method that has been previously used by many authors (e.g., Brunberg and Blomqvist 2002, Tsujimura and Okubo 2003, Latour et al. 2007), and our preliminary assays using *Synechococcus* cells mixed into sediments showed that these test populations could be readily detected. Cyanobacteria such as *Microcystis* colonies may aggregate with suspended particles and be obscured by inorganic detritus (Verspagen et al. 2005), and thus some colonies could have been lost during the treatment of samples prior to their observation by microscopy. This seems unlikely, however, given that other colonial cyanobacteria such as *Merismopedia* and *Woronichinia naegeliana* were detected by microscopy and in the germination experiment.

A differential loss of pigments may have contributed to the underrepresentation of taxonomic groups in the HPLC analysis; however, by comparison with the diatom marker pigment fucoxanthin, which was usually present in high concentrations, the cyanobacterial signature pigments zeaxanthin and canthaxanthin are chemically stable. For example, zeaxanthin is well preserved even in aerobic environments (Buchaca and Catalan 2007), yet was present in only trace amounts in the Lake St. Charles sediments.

Alternative source populations

Inocula can be highly localized in time and space, and it is possible that the sampling design of the present study was inadequate to detect a locally important source population of cyanobacteria in the Lake St Charles sediments. Given the spatial and temporal coverage discussed earlier, this explanation seems unlikely.

Terrestrial inputs of microbiota from river runoff can be substantial and could potentially be a contributing inoculum for cyanobacterial blooms (Caporaso et al. 2012). Most of the water entering Lake St. Charles comes from the Hurons

River, which drains 80% of the watershed, but the residence time in this river is likely to be short (<1 h), an insufficient time for the development of a substantial bloom-forming inoculum. A second inflow enters Lake St. Charles from upstream Lake Delage but with a discharge that is on average <5% of the Hurons River (Bourget 2011). Both inputs will require more detailed analysis in the future.

Another possibility for inocula in Lake St. Charles is that the bloom-forming species are holoplanktonic; in other words, resting cells including akinetes (as observed in water samples from the lake; Fig. 4) persist in the water column throughout winter, and these provide the starting population for a subsequent bloom (Lennon and Jones 2011). The observation of germinating akinetes of *Anabaena* in the water column at station N4 indicated the presence of resting stages in the plankton. These pelagic populations would be able to take immediate advantage of improved conditions early during the growing season.

Oliver and Ganf (2000) estimated that the probability of blooms developing is low in lakes where the water residence time is <10 d and the inoculum concentration is <15 cells/mL. The summer hydraulic residence time for Lake St. Charles is much longer than 10 d (30–100 d), and at concentrations below the detection limits of light microscopy, overwintering communities could still represent a substantial total biomass for inoculating the next season's growth. For example, we observed no *Microcystis* sp. or *Anabaena* sp. cells in May, but our detection limit of ~50 *Microcystis* cells/mL would be equivalent to a total lake-wide population (to a depth of 4 m) of $\sim 10^{15}$ *Microcystis* cells. Densities of an order of magnitude lower would not be detectable, yet would still represent a huge resident population to initiate summer growth.

Conclusions

The application of different methods to the sediments of Lake St. Charles provided a consistent set of results; the sediments contained a large, diverse seedbank of viable cells, but not of bloom-forming cyanobacteria. The sediment assemblages included diatoms, chrysophytes, and phytoflagellates that may be capable of recruitment and growth in the water column. Planktonic cyanobacteria, and notably the taxa *Anabaena flos-aquae* and *Microcystis aeruginosa* that have created water quality problems in the lake, were conspicuously absent (or undetected) in the sediments and could not be induced to grow from sediment samples, even under warm, P-enriched conditions that might be expected to favor these cyanobacteria (Reynolds 2006). Other potential inocula of noxious cyanobacteria include localized sediments, riverine inputs, and a low density population of cells that persist in the water column after the period of bloom collapse each year. The absence of an abundant population of noxious cyanobacteria in the sediments combined with the short residence time of this reservoir implies that Lake St. Charles may respond rapidly to nutrient abatement projects in the catchment.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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