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Sedimentary pigments as indicators of cyanobacterial dynamics in a hypereutrophic lake

**Bethany N. Deshpande · Roxane Tremblay ·
Reinhard Pienitz · Warwick F. Vincent**

Abstract Lac Saint-Augustin is an urban lake located on the outskirts of Quebec City, one of North America's oldest cities. Anthropogenic inputs from land clearing, agriculture, highway development and urbanization in the surrounding catchment have resulted in strong impacts on the limnology of the lake throughout the past three centuries. In recent years, this lake has experienced severe eutrophication, including persistent cyanobacterial blooms. In winter 2011, a sediment core was extracted from the deepest area of the lake. A detailed paleopigment analysis was used to assess eutrophication processes in the lake and to determine the timing and appearance of cyanobacterial blooms and their subsequent variability. Extracted chlorophyll *a*, its degradation products and 11 carotenoid pigments were identified and quantified via reverse-phase high performance liquid chromatography to examine relative changes in the phytoplankton. The results revealed large variations in the phytoplankton community structure of Lac Saint-Augustin over the past 356 years. Chlorophyll *a* concentrations per unit organic matter (OM) increased significantly from the base of the core to present day, rising more than 15-fold from 18.4 μg $(g\ OM)^{-1}$ at the base of the core to 287 μg $(g\ OM)^{-1}$ in the most recent strata. Biostratigraphical analysis revealed three major periods of enrichment, with episodes of cyanobacterial abundance from the 1890s onwards. The greatest changes occurred in the most recent period (from the 1960s to the present) relative to earlier periods, with pigment increases for all phytoplankton groups. The cyanobacterial pigments canthaxanthin, echinenone and zeaxanthin (also a marker for green algae) showed concentrations in the surface sediments that were significantly above values at the bottom of the core, and these differences were large, even giving consideration to the lesser pigment degradation near the surface. Overall, the results indicate that cyanobacterial blooms are not a recent feature of Lac Saint-Augustin but began to occur soon after catchment modification 150 years ago. The pigment records also imply that cyanobacterial and associated algal populations have risen to unprecedented levels over the last few decades of ongoing development of the Lac Saint-Augustin catchment. This study highlights the utility of multiple pigment analysis of lake sediments for identifying the timing and magnitude of anthropogenic impacts.

B. N. Deshpande (✉) · W. F. Vincent
Département de biologie, Centre d'Études Nordiques
(CEN), Université Laval, Quebec, QC G1V 0A6, Canada
e-mail: bethany.deshpande.1@ulaval.ca

R. Tremblay · R. Pienitz
Aquatic Paleoecology Laboratory, Département de
géographie, Centre d'Études Nordiques (CEN), Université
Laval, Quebec, QC G1V 0A6, Canada

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Introduction

Persistent cyanobacterial blooms are indicators of eutrophication caused by nutrient enrichment of lakes from agricultural run-off, industrial inputs or municipal wastes. Cyanobacterial blooms impact not only the access of local populations to recreational services but also have ecological and economic impacts on an aquatic ecosystem including decreased light penetration, depletion of oxygen, release of toxins, reduced local property values, and loss of ecosystem services such as drinking water and fishing (Chorus and Bartram 1999; Vincent 2009).

Quebec City is located in south-eastern Canada and was one of the first regions colonized by the Europeans upon their arrival in North America. This region has been inhabited for more than 400 years and is an important part of the cultural and environmental history of Canada. Today, the city and surrounding region has a total population of more than 750,000 inhabitants. As in urban areas elsewhere in the world, the growth of this population has resulted in greatly increased pressure on Quebec's environmental and limnological resources and has heightened the need for understanding the resultant changes.

Lac Saint-Augustin is an urban lake located 20 km west of Quebec City, in the municipality of Saint-Augustin-de-Desmaures. Over the past 60 years, this lake has experienced high levels of nutrient enrichment (Roberge et al. 2002), engendering persistent phytoplankton blooms and the degradation of lake water quality. Diatom, dinoflagellate and cyanobacterial blooms have been observed in recent years. As a result of cyanobacterial blooms and the presence of cyanotoxins, human and domestic animal contact with the water is prohibited during summer.

Lac Saint-Augustin is an excellent model system for pigment analysis. With a maximum depth of 6.1 m (average 3.6 m), pigments do not travel a great distance to the sediments and therefore encounter less obstacles to preservation (Hodgson et al. 2004), although shallow lakes are prone to resuspension which may confound paleolimnological interpretations (Scheffer 2004). The anoxic benthic zone also allows pigments to be protected from oxidation once they reach the sediments (Leavitt 1993; Vinebrooke et al. 1998; Hurley and Armstrong 1991). High productivity and diverse phytoplankton groups result in strong pigment signals. Additionally, historical

archives for the region and observations from past research provide complementary information about the ecology of this lake.

In a previous paleolimnological study on Lac Saint-Augustin, concentrations of total phosphorus were reconstructed using a diatom-based transfer function (Pienitz et al. 2006). This revealed the presence of three distinct periods of nutrient enrichment throughout the history of the lake: pre- and early settlement period (1670–1750), human settlement period (1750–1904), and agricultural, industrial and urban development (1904–2002). Nutrients (phosphorus, nitrogen), metals (Al, Fe, Mg, Mn, Zn, Pb, Cu), and contaminants (Cd, Cr, Ni, Pb, Hg, As) have been measured in high concentrations in the recent sediments of the lake (Galvez-Cloutier et al. 2003; Pienitz et al. 2006). However, the past dynamics of cyanobacteria in Lac Saint-Augustin have not been addressed.

Photosynthetic pigments have been used for several decades to identify changes in phytoplankton community dynamics (Borghini et al. 2010; Fietz et al. 2007; Leavitt et al. 1994; Vinebrooke et al. 1998; Züllig 1981). Long-term fossilization of these polar molecules provides a phylum-specific record of phytoplankton abundance that can be useful for determining periods of change within an aquatic environment. Fossil pigments remain preserved in sediments for thousands of years (Watts and Maxwell 1977), which makes them valuable bio-indicators for identifying ecologically significant events over historical time scales. Fossil pigment analysis has also be used to determine cyanobacterial episodes in the past (Bianchi et al. 2000; Hall et al. 1997; McGowan et al. 2012), and was the approach adopted here.

The present study aimed to obtain a high temporal resolution record of past phytoplankton community dynamics in Lac Saint-Augustin by the extraction and analysis of fossil pigments from a sediment core. We hypothesized that this lake has experienced large qualitative changes in phytoplankton composition over the past four decades, and that cyanobacterial blooms are a recent phenomenon associated with the last two decades of enrichment, corresponding to the period of reported water quality problems. Our specific objectives in the present study were to: (1) determine how the phytoplankton communities of Lac Saint-Augustin have changed over the past 350 years, and (2) identify the initial occurrence and subsequent dynamics of cyanobacterial blooms in this lake. This study extends

the knowledge base for sedimentary pigments as indicators of eutrophication and the impact of anthropogenic development on aquatic ecosystems.

Study site

Lac Saint-Augustin is located on the north shore of the Saint-Lawrence River near Quebec City, Canada (Fig. 1). This lake has a variety of anthropogenic activities within its 7.64 km^2 water basin, including residential homes, farming, and urban development. The lake is bordered on one side by a highway, constructed in 1977, as well as by newly developed and older homes. Most of these homes are suburban homes, inhabited all year around, while others serve as summer cottages.
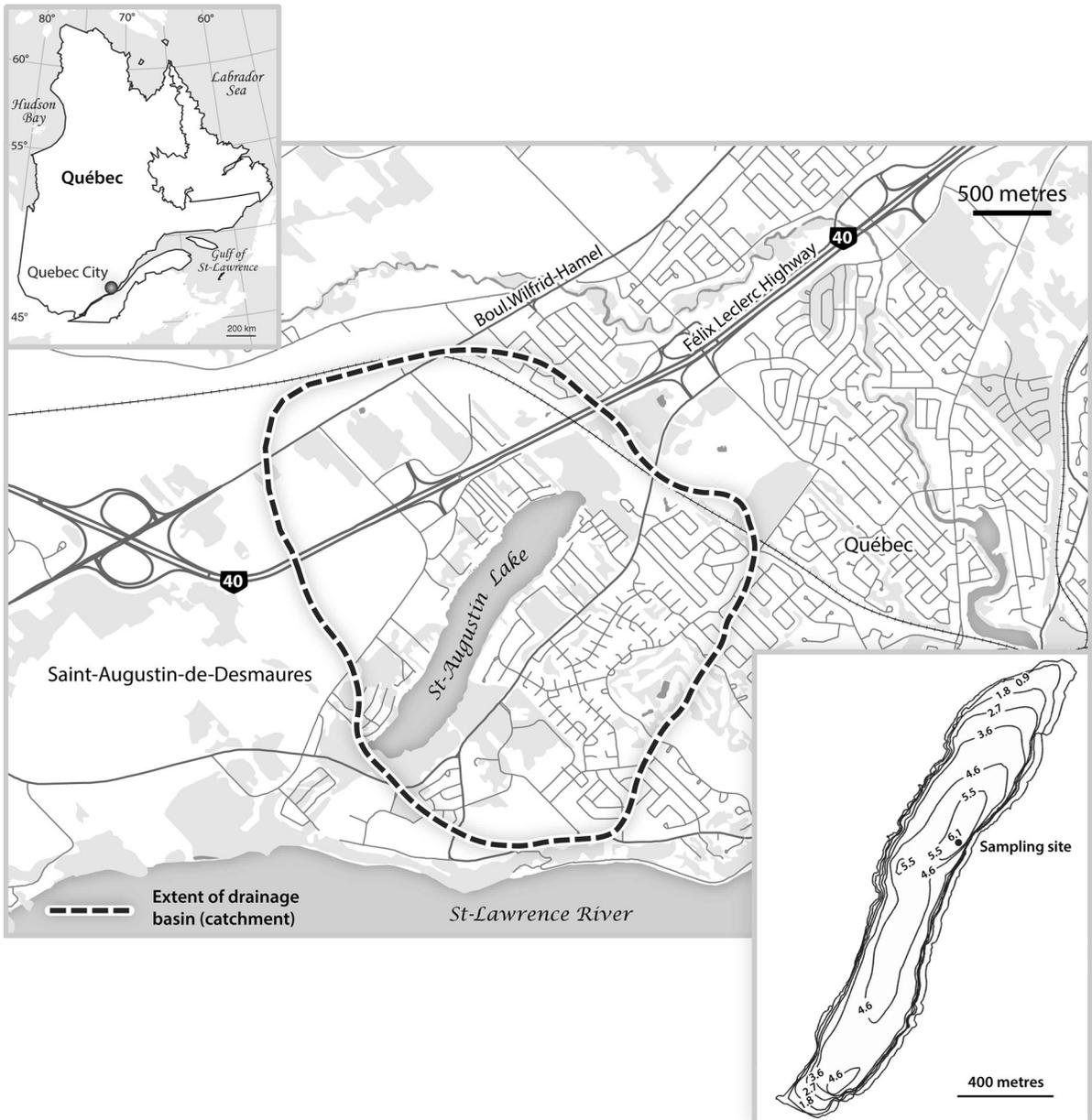

Fig. 1 Location of Lac Saint-Augustin (Quebec City, Canada) and lake basin bathymetry. The *dashed line* demarks the extent of its watershed (modified from Pienitz et al. 2006)

Table 1 Limnological characteristics of Lac Saint-Augustin during the summer of 2010

	June 21	July 29	August 23	Average (SD)
Secchi depth (m)	3.30	1.50	1.15	1.98 (1.15)
Temperature (°C)	20.9	23.8	21.7	22.1 (1.48)
Chlorophyll a (µg L^{-1})	11.5	80.0	74.0	55.2 (37.9)
Dissolved oxygen (mg L^{-1})	6.79	7.30	6.06	6.72 (0.62)
pH	8.71	9.38	7.97	8.69 (0.71)
Conductivity (mS cm^{-1})	0.692	0.733	0.712	0.712 (0.020)
Dissolved organic carbon (mg L^{-1})	3.92	4.92	5.52	4.79 (0.81)
Total nitrogen (mg L^{-1})	0.220	0.674	0.314	0.403 (0.240)
Total phosphorus (µg L^{-1})	16.1	81.8	108	68.6 (47.3)

The averages are means from the three sampling dates

This lake has a total area of 0.672 km^2, a maximum depth of 6.1 m, and an average depth of 3.6 m (Pienitz et al. 2006). During the summer of 2010, Lac Saint-Augustin was visited three times at monthly intervals in summer, giving averages (SD) for the following physical and chemical properties of the surface waters of the lake (Table 1). The Secchi disc was visible to a depth of 1.98 (1.15) m, or approximately a third of the maximum depth. The concentration of total phosphorus in the lake was 69 (47) µg L^{-1}, with a chlorophyll a concentration of 55 (38) µg L^{-1}, placing it in the hypereutrophic category. The concentration of total dissolved nitrogen was 0.40 (0.24) mg L^{-1}, while dissolved oxygen was 6.9 (1.2) mg L^{-1} and dissolved organic carbon was 4.8 (0.8) mg L^{-1}. The water column was alkaline with a pH of 8.7 (0.7), with a conductivity of 0.712 (0.020) mS cm^{-1} and an average summer temperature of 22 (1.2) °C. Further limnological information is given in Pienitz et al. (2006).

Materials and methods

Core sampling and sediment analysis

A sediment core was sampled on 27 January, 2011 via a hole through the lake ice using an open-barrel, manually driven corer and plexiglass tube. The 55-cm-long, 9-cm internal-diameter core was extracted from a depth of approximately 5.5 m, near the point of maximum lake depth (Fig. 1). The core was kept upright and immediately covered in opaque material to reduce displacement of sediments or degradation of fossil pigments (Leavitt and Hodgson 2001). The core was then returned to the Aquatic Paleoecology Laboratory, Laval University, and stored at 4 °C until it was sub-sectioned at 0.5-cm intervals between 0 and 20 cm and at 1-cm intervals between 20 and 55 cm. Two sub-samples were collected from each sediment level: one for pigment analysis and another for dating and loss on ignition analysis. Once the core had been sub-sectioned, each sample was weighed and stored at −20 °C.

Prior to sub-sectioning, the sediment lithography (sediment type and colour) was described. The uppermost part of the core (0–21 cm) was composed of highly organic material with a slurry-like consistency. The sediment was brown in colour and contained several dark-coloured gyttja laminations. Between 11 and 16 cm, a blackened, organic section was observed and density increased. This 5 cm segment was unique from the remainder of the core. The bottom segment of the core (21–55 cm) was composed of high-density, gray clay material. From 21 to 39 cm, it was clay-like and contained dark coloured laminations, though in lesser frequency than in the top segment of the core. From 39 to 55 cm, the clay was significantly denser and grey in colour.

Sediment dating

Core age and chronology was estimated using ^{210}Pb and ^{137}Cs analysis as well as ^{14}C accelerator mass spectrometry (AMS). At the Radiochronology Laboratory at the Centre for Northern Studies (CEN, Laval University), approximately 0.5 g of dry sediment was used from 15 core levels from the uppermost section of the core for analysis of ^{210}Pb and ^{137}Cs activity by gamma spectrometry. A constant rate of supply (CRS) dating model was used as of the 14.0–14.5 cm peak in ^{137}Cs activity to calibrate a ^{210}Pb date at depths of 27.0–28.0 and 32–33 cm (Appleby and Oldfield 1978; Sorgente et al. 1999). For ^{14}C-dating, a wood sample was taken from the 54–55 cm level. Extraction was performed at the Radiochronology Laboratory and the resulting combusted CO_2 gas was dated at the Keck Carbon Cycle AMS Facility (UC Irvine, California). The ^{14}C

result was calibrated using the CALIB 6.0 software (Stuiver and Reimer 1993; Reimer et al. 2009).

Loss on ignition

In order to determine the sediment organic matter (OM) content, loss-on-ignition (LOI) was measured using the method described by Dean (1974). Sediment samples were taken from each centimeter of the core and freeze-dried for 96 h. Sub-samples were oven-dried for 24 h at 95 °C and then weighed before and after combustion at 550 °C for 4 hours. OM results were used to determine sedimentation rates and for the calculation of pigment concentrations.

HPLC analysis

Frozen samples of sediment (about 6.5 g) for pigment analysis were freeze-dried for 48 h and kept frozen at −20 °C. Approximately 0.2 g of dried sediment was then extracted in pure acetone and argon gas was added to each tube. Extraction continued at −20 °C for 20 h, followed by centrifugation at 4,100 rpm for 10 min at +4 °C. The liquid was then filtered through 0.2 μm PTFE filters and argon was added to the vial. One hundred microlitres of each sample was injected into a ThermoFisher Scientific high performance liquid chromatography (HPLC) Accela 600, equipped with an Autosampler, an Accela 600 pump, a photo-diode array (PDA) and a fluorescence detector. The column used was a TheromoFisher Scientific brand Hypersil Gold C8 (150 × 4.6 mm, 3 μm). Sediment samples were immediately analysed using a reverse-phase protocol as described by Zapata et al. (2000). Pigments were identified by comparing signature peaks with those of laboratory standards (Sigma-Aldrich Canada Ltd and DHI Water and Environment). Pigment concentrations were calculated, considering the mass of the sediment sample and the average extinction coefficient of each pigment. All pigment concentrations are expressed as micrograms per gram of sediment organic matter [μg (g OM)$^{-1}$].

Chlorophyll *a* was used as an overall indicator of phytoplankton biomass along with β,β-carotene, which is known to be more stable over time (Leavitt and Carpenter 1990). Echinenone, canthaxanthin and zeaxanthin were used as indicators of cyanobacterial abundance (Borghini et al. 2010; Züllig 1981), however zeaxanthin is also found in green algae (Brown and Jeffrey 1992). Canthaxanthin originates from Nostocales cyanobacteria, an order of filamentous cyanobacteria (Patoine and Leavitt 2006) that includes *Anabaena* and *Aphanizomenon*, two nitrogen-fixing, potentially toxic genera that have been previously observed in the lake. Alloxanthin and crocoxanthin were used as signatures of cryptophytes (Pennington et al. 1985), lutein as a measure of chlorophytes, while diadinoxanthin and diatoxanthin were combined to represent diatom biomass, although potentially also influenced by dinoflagellates (Olaizola et al. 1994). Vaucheriaxanthin was used as an indicator for eustigmatophytes (Owens et al. 1987; Roy et al. 2011), which are small (2–32 μm), unicellular algae that are most commonly associated with nutrient-rich, non-stratified, cold waters (Salmaso and Tolotti 2009; Hegewald et al. 2007) and saline environments (Hu and Gao 2006).

Statistical analysis

Hierarchical constrained clustering was applied as a method to objectively delimit biostratigraphic zones within the sediment core. Pigment concentrations, treated as proxies for phytoplankton group abundance, were transformed using the Hellinger transformation and the Euclidean distance measure (Legendre and Gallagher 2001) prior to Constrained Incremental Sums of Squares Cluster Analysis (CONISS) using all identified pigments (Grimm 1987). This method constrains clusters by sample order, thereby identifying periods of major ecological transition based on pigment concentrations.

An analysis of covariance (ANCOVA) was used to determine the variability of pigments throughout periods of ecological transitions. The Hellinger transformation was applied to pigment concentrations prior to analysis to ensure normality (Legendre and Gallagher 2001). Periods, as determined by CONISS clustering, were used as the categorical independent variable, and chlorophyll *a* concentrations were used as a covariate, representing the net effect of photosynthetic pigment export to the sediments and degradation.

Results

Core chronology and organic content

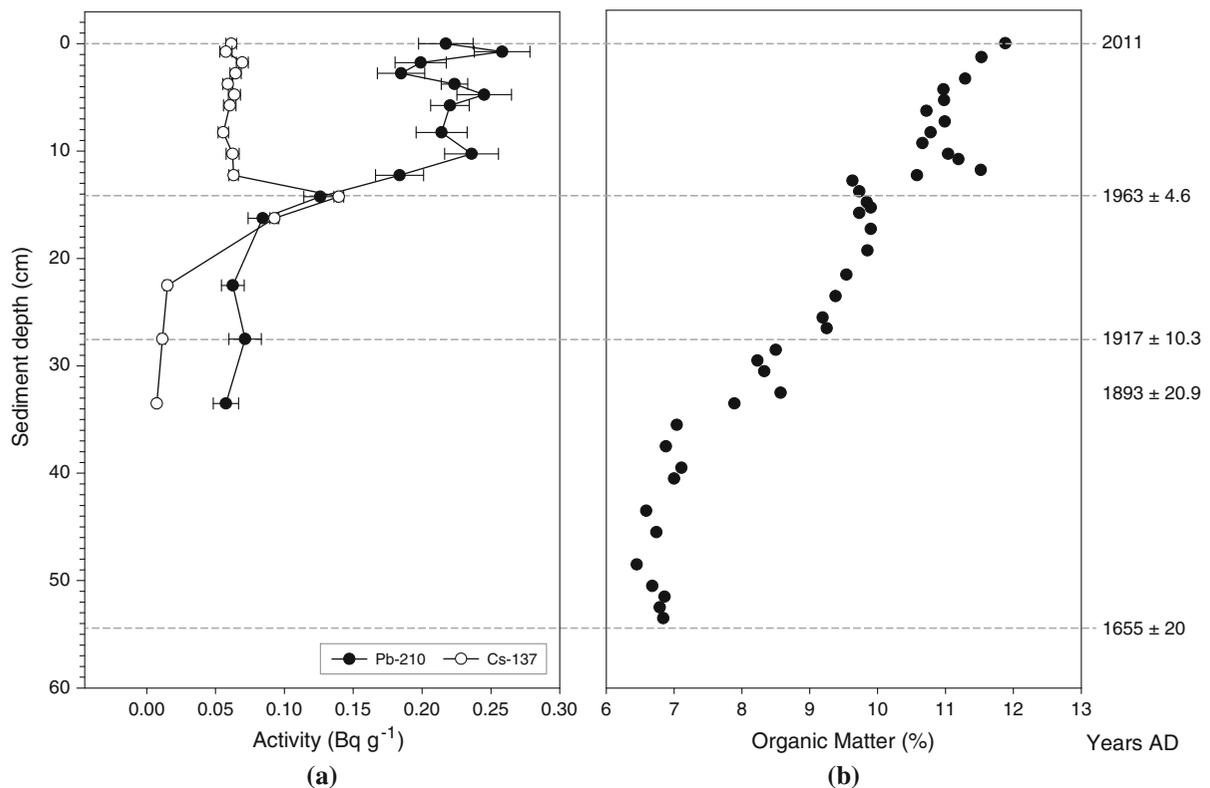Surface sediment mixing was apparent from the dating and OM analysis (Fig. 2), and the results from the

Fig. 2 **a** Dating results of Lac Saint-Augustin. The 15 uppermost samples were analysed by ^{210}Pb and ^{137}Cs. The lowermost sample was analysed by AMS ^{14}C and has been dated to 1655 ± 20 CAL AD, **b** organic matter (%) content of the sediment core

uppermost 14.5 cm were therefore considered together as "modern sediments". There was a ^{137}Cs peak at 14.0–14.5 cm, representing activity dating to 1963 ± 4.6 years AD (Appleby 2001). The 27.0–28.0 cm core level was identified as 1917 ± 10.3 years AD and the 32–33 cm core level was identified as 1893 ± 20.9 years AD. The deepest part of the core (54–55 cm) was ^{14}C-dated as 356 years before present (1655 ± 20 CAL AD). The percentage of organic matter in the sediments ranged from 6 to 12 %, with the maximum values observed within the modern sediments (Fig. 2). The organic matter content was relatively constant prior to the 40–41 cm core level and then increased steadily beyond that date to the present.

Pigments

We differentiated fourteen pigments within the 38 levels analyzed down the core (Fig. 3). Pigment

concentrations were minimal in the deepest part of the sediment core, and then increased toward the surface with peaks at 33.0–34.0, 27.0–28.0, 25.0–26.0, 21.0–22.0 cm, and within the modern sediments (Fig. 3).

There was a highly significant, order-of-magnitude (1,038 %) rise in chlorophyll a between the mean values at the bottom and top of the core (t = 3.51, n = 5, p = 0.008). The overall minimum for all core levels was 15.3 μg (g OM)$^{-1}$ in the 52.0–53.0 cm core level, and the maximum was 287.2 μg (g OM)$^{-1}$ at the surface of the core. A secondary chlorophyll a maximum of 243 μg (g OM)$^{-1}$ was observed in ~1930 (25–26 cm), with high associated values for several other pigments. Discernible pigment peaks were also observed at the 33.0–34.0 cm core level (chlorophyll a = 110 μg (g OM)$^{-1}$), at 21.0–22.0 cm (chlorophyll a = 157 μg (g OM)$^{-1}$), and at 10.0–10.5 cm (chlorophyll a = 284 μg (g OM)$^{-1}$). The 33.0–34.0 cm peak marked the first date of elevated cyanopigments
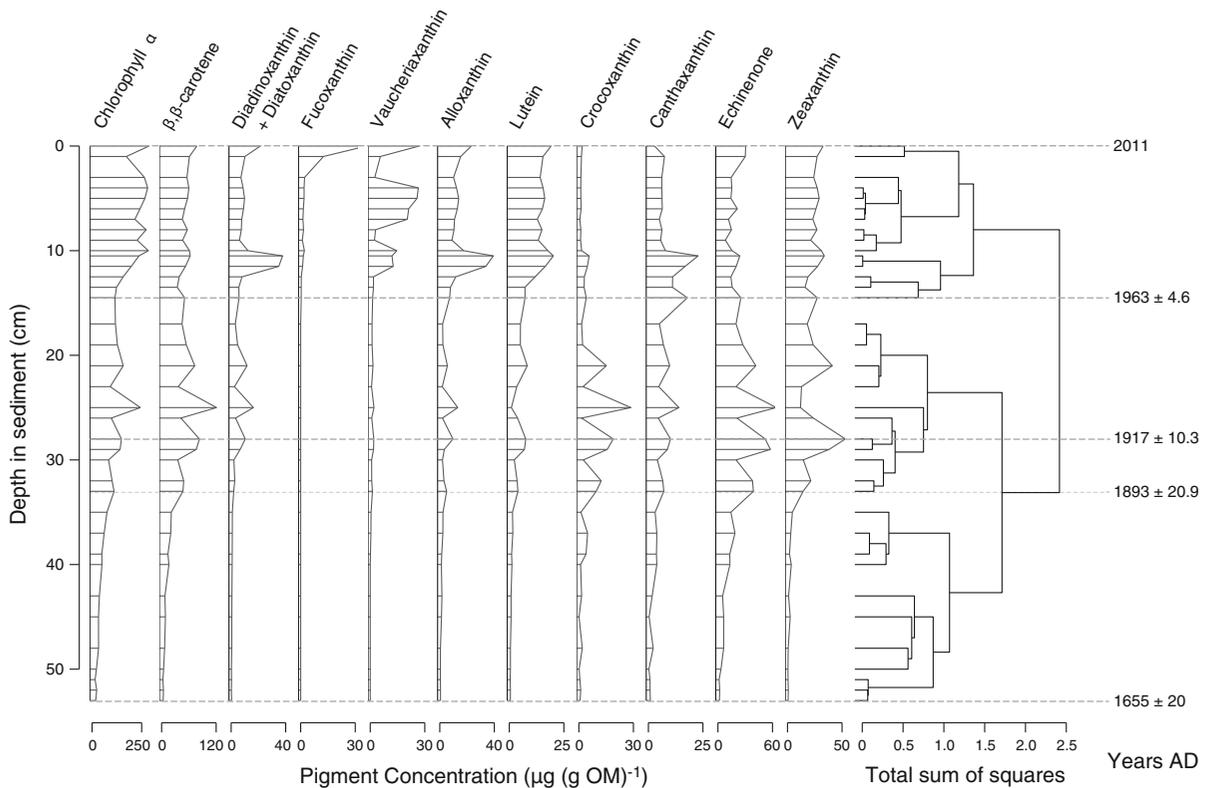
Fig. 3 Pigment stratigraphy of Lac Saint-Augustin showing concentrations of chlorophyll *a*, β,β-carotene, diadinoxanthin + diatoxanthin, fucoxanthin, vaucheriaxanthin, alloxanthin, lutein, crocoxanthin, canthaxanthin, echinenone, and zeaxanthin from 38 core levels. Hierarchical constrained clustering performed using the CONISS method is in the right-most column. This zonation identifies the areas of greatest difference between adjacent core levels

(zeaxanthin = 14.3 μg (g OM)$^{-1}$; echinenone = 39.0 μg (g OM)$^{-1}$; canthaxanthin = 7.06 μg (g OM)$^{-1}$). Peak values of cyanopigments were also observed at 10.5–11.0 cm (zeaxanthin = 33.7 μg (g OM)$^{-1}$; echinenone = 24.0 μg (g OM)$^{-1}$; canthaxanthin = 22.8 μg (g OM)$^{-1}$), at 21.0–22.0 cm (zeaxanthin = 41.1 μg (g OM)$^{-1}$; echinenone = 41.6 μg (g OM)$^{-1}$; canthaxanthin = 9.76 μg (g OM)$^{-1}$) where a peak of diadinoxanthin was also observed (11.7 μg (g OM)$^{-1}$), and at 28–29 cm (zeaxanthin = 53.0 μg (g OM)$^{-1}$; echinenone = 52.0 μg (g OM)$^{-1}$; canthaxanthin = 10.1 μg (g OM)$^{-1}$). The concentrations of all pigments increased within the modern sediments, with the exception of crocoxanthin. The concentration of chlorophyll *a* remained above 115 μg (g OM)$^{-1}$, with an overall average of 223 μg (g OM)$^{-1}$. Within the modern sediments, a peak was observed of canthaxanthin, indicating dominance by Nostocales cyanobacteria. All of the cyanobacterial markers were in significantly higher concentrations at the top versus

bottom of the core (mean of the uppermost and lowermost five levels), with a 5,273 % increase in zeaxanthin (t = 4.85, n = 5, *p* = 0.001), a 561 % increase in echinenone (t = 2.99, n = 5, *p* = 0.017), and a 500 % increase in canthaxanthin (t = 4.288, n = 5, *p* = 0.003). Vaucheriaxanthin was at relatively stable levels around 1.10 μg (g OM)$^{-1}$ from 39.0 to 12.5 cm, and then rose abruptly to 12.6 μg (g OM)$^{-1}$ at 11.5–12.0 cm. This pigment reached a maximum of 27.0 μg (g OM)$^{-1}$ in the surface of the core, and averaged 14.4 μg (g OM)$^{-1}$ from 12.5 to 13.0 cm to the surface. All modern sediment pigment concentrations observed were exceptionally high in comparison with the values observed at the base of the sediment core.

Correlations between pigments

There were significant correlations between many of the pigments, including between chlorophyll *a* and

Table 2 Pearson product moment correlations between phytoplankton indicator pigments and chlorophyll *a* and β,β-carotene

	Chlorophyll *a*	β,β-carotene
β,β-carotene	0.740***	
Alloxanthin	0.871**	0.443*
Diadinoxanthin	0.536***	NS
Lutein	0.926***	0.608***
Vaucheriaxanthin	0.406**	NS
Zeaxanthin	0.673***	0.904***
Canthaxanthin	0.314*	0.448**
Echinenone	0.725***	0.926***

* Indicates significant at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *NS* not significant ($p > 0.05$)

β,β-carotene ($r = 0.740$, $p < 0.001$), lutein and chlorophyll *a* ($r = 0.926$, $p < 0.0001$) and zeaxanthin and β,β-carotene ($r = 0.904$, $p < 0.0001$) (Table 2). There were weaker but significant correlations between canthaxanthin and chlorophyll *a* ($r = 0.314$, $p < 0.05$) and between vaucheriaxanthin and chlorophyll *a* ($r = 0.406$, $p < 0.01$). There was a strong correlation between vaucheriaxanthin and diadinoxanthin ($r = 0.964$, $p < 0.0001$), but no relationship between diadinoxanthin and β,β-carotene nor between vaucheriaxanthin and β,β-carotene.

The cyanopigments demonstrated statistically significant covariation. Echinenone and canthaxanthin were significantly correlated ($r = 0.575$, $p < 0.001$) and both also correlated with zeaxanthin (zeaxanthin and echinenone: 0.879, $p < 0.0001$; zeaxanthin and canthaxanthin: 0.721, $p < 0.0001$). Zeaxanthin and lutein were also highly significantly correlated ($r = 0.625$, $p < 0.0001$).

Biostratigraphic zonation

The hierarchical cluster analysis showed the zonation of core levels based on pigment composition (Fig. 3). The greatest difference was observed in core levels before and after the 14.5–15.0 cm core level, dividing modern sediments from the remainder of the core. Another division was apparent in the middle section of the core, clustering all core levels from 33.0 to 14.5 cm. The uppermost section of the core was further divided into two groups: 14.0–10.5, and 10.0 cm to the sediment surface.

Analysis of covariance

Analysis of covariance (ANCOVA) revealed that the covariate, chlorophyll *a* was a significant predictor of pigment concentrations (Table 3), and beyond this effect, there remained significant variations among the periods of anthropogenic development for pheophytin *a*, pyropheophytin *a*, β,β-carotene, diadinoxanthin + diatoxanthin, vaucheriaxanthin, alloxanthin, lutein, echinenone, and zeaxanthin.

Pigment degradation

The ratios of chlorophyll *a* to pheophytin *a* and pyropheophytin *a* were largely above 1.0 throughout most of the length of the core (Fig. 4). The chlorophyll *a*: pheophytin *a* ratio was >1.0 except for four core levels near the surface, from 4 to 7 cm, while chlorophyll *a*: pyropheophytin *a* was above 2.0 for the entire core. There was a statistically significant, strong relationship between chlorophyll *a* and pyropheophytin *a* ($r = 0.966$, $p < 0.001$, n = 38), but no significant correlation between chlorophyll *a* and pheophytin *a*. Comparison of five core levels from the surface and bottom sediments indicated significant differences between the ratios of chlorophyll *a* to pyropheophytin *a* (t = 2.496, n = 5, $p = 0.037$), where the mean ratio at the surface was 2.4 times greater than at the bottom of the sediment core, however there were no significant differences between the ratios of chlorophyll *a* to pheophytin *a* at the top and bottom of the core (t = 109, n = 5, $p = 0.791$).

Pigment and sediment accumulation

Average sediment accumulation rates were calculated for three periods (Fig. 5) and rose from 0.10 cm year^{-1} from 1655 to 1893 (32–54 cm core levels) to 0.17 cm year^{-1} from 1893 to 1963, and to a maximum of 0.43 cm year^{-1} in the modern sediments. Comparisons between sediment dry weight and pigment accumulation were used to identify differences between allochthonous and autochthonous inputs. Similar trends were observed in all pigment accumulation rates, including β,β-carotene that is known to be relatively well preserved (Leavitt and Carpenter 1990). Pigment accumulation rates for chlorophyll *a*, β,β-carotene, canthaxanthin and

Table 3 Analysis of covariance (ANCOVA) of pigment concentrations throughout the sediment core, with the covariate chlorophyll *a* and periods (as identified by CONISS clustering) as treatment variables

Pigment	Variance explained by chlorophyll *a*	Variance explained by period	R^2
Pheophytin *a*	0.040***	0.029***	0.922
Pyropheophytin *a*	0.010***	0.035***	0.899
β,β-carotene	0.095***	0.089***	0.821
Diadinoxanthin + diatoxanthin	0.029***	0.009***	0.686
Fucoxanthin	0.037***	NS	0.543
Vaucheriaxanthin	0.033***	0.010***	0.844
Alloxanthin	0.034***	0.010***	0.619
Lutein	0.019***	0.013***	0.595
Crocoxanthin	0.010***	NS	0.436
Canthaxanthin	0.028***	NS	0.607
Echinenone	0.034***	0.011***	0.665
Zeaxanthin	0.154***	0.015**	0.706

* Indicates significant at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *NS* not significant ($p > 0.05$)
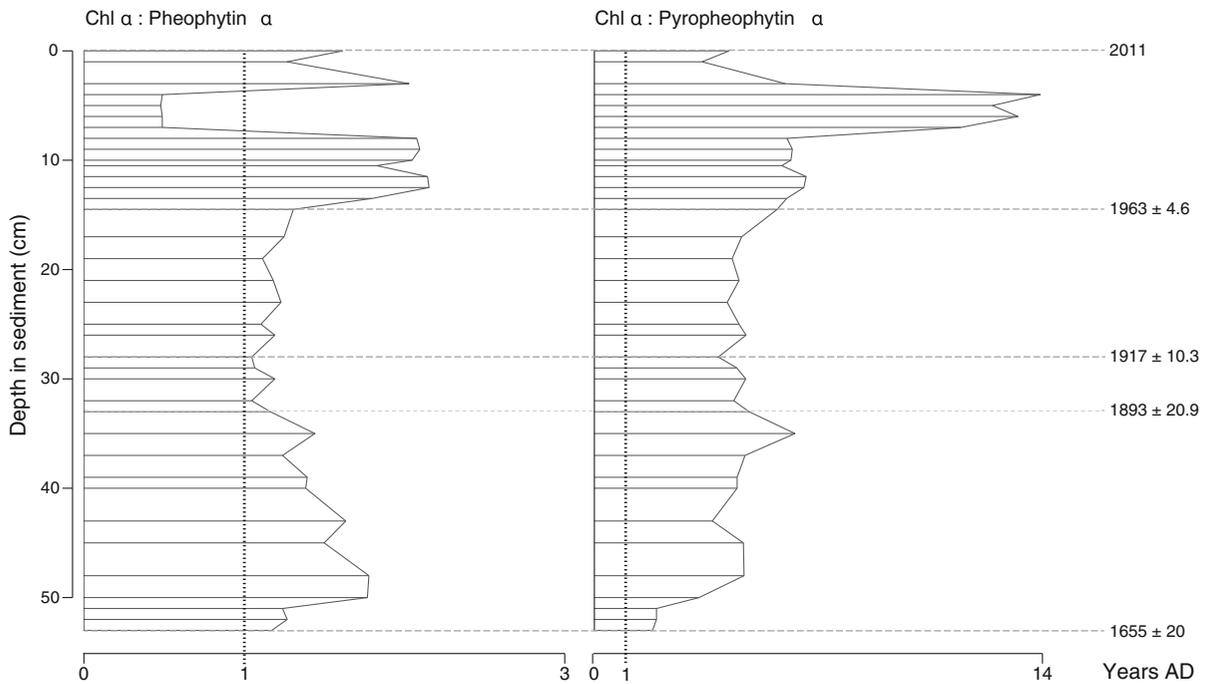

Fig. 4 Stratigraphic plot of chlorophyll *a* and its degradation products, pheophytin *a* and pyropheophytin *a*, throughout the sediment core

echinenone demonstrated peaks at 32–33, 29–30, 25–26 cm, and within the modern sediments (Fig. 5).

Discussion

Our analysis showed that the Lac Saint-Augustin sediments contained diverse phytoplankton pigments, and most of the pigments were present in high concentrations. The high ratios of chlorophyll *a* to its degradation products pheophytin *a* and pyropheophytin *a* imply a good level of pigment preservation throughout most of the core. An exception was the 4–7 cm stratum which had chlorophyll *a*: pheophytin *a* ratios <1.0, implying surface sediment mixing and bioturbation. The sediment chronology and organic
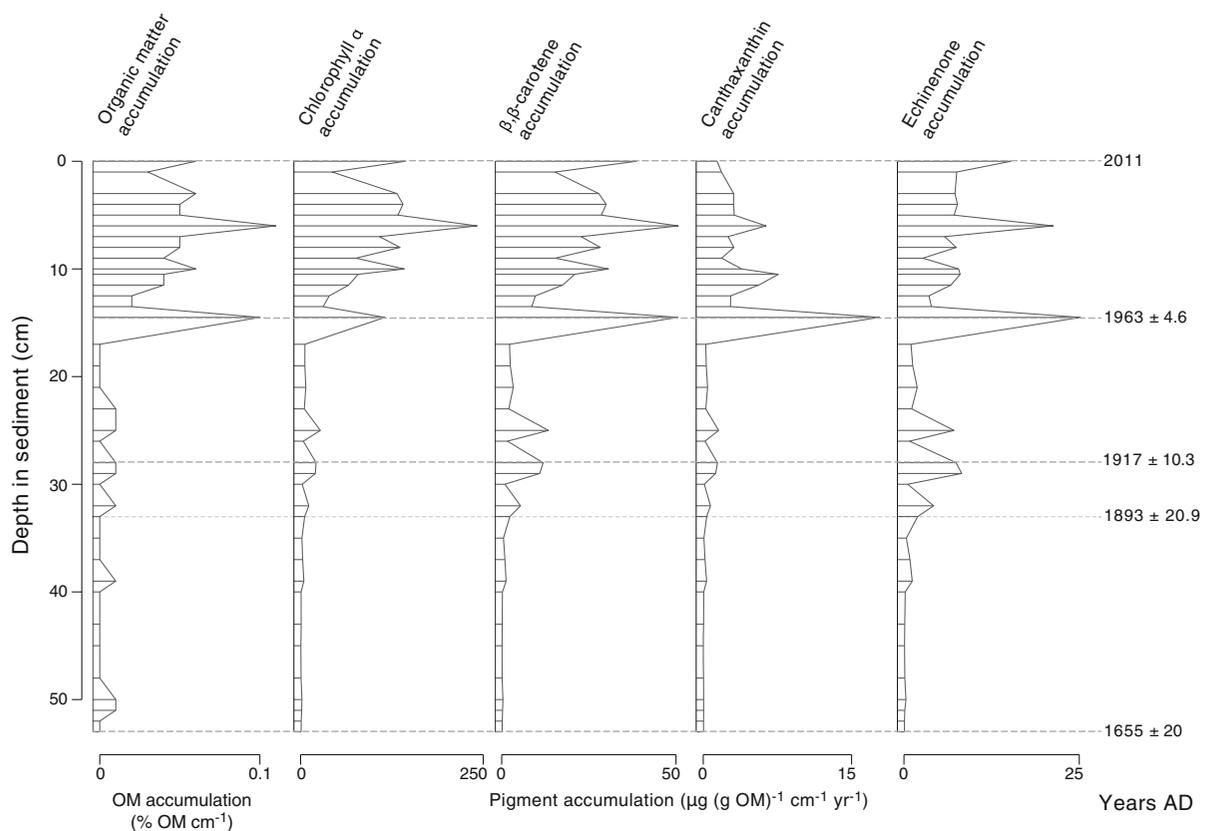
Fig. 5 Stratigraphic plot of organic matter and pigment accumulation rates

matter data also indicate the physical disruption of sediments within this stratum (Fig. 2). There was a significant (143 %) rise in the ratios of chlorophyll *a*: pyropheophytin *a* from the core bottom to surface, though no significant difference identified in the chlorophyll *a*: pheophytin *a* ratios. Overall, this indicates little change in the magnitude of pigment degradation and diagenesis throughout the sediment core.

The pigments preserved in lake sediments are derived from multiple sources: phytoplankton, benthic macrophytes, lake and inflow periphyton and terrestrial vegetation (Bianchi and Canuel 2011). It is possible that in the early period of the sediment core record analysed here, benthic plant and algal communities were favoured by clearer water conditions and contributed to the sediment pigment stocks. Diatom analysis has shown that benthic diatoms were present during this early period, although they were less abundant than planktonic diatoms (Pienitz et al. 2006). A major contribution of benthic communities seems

unlikely in the more recent part of the record given the hypereutrophic state of the lake, the high standing stocks of phytoplankton (up to 100 μg Chl *a* per litre) and the weak penetration of light to the lake floor.

Pigment concentrations (μg pigment per g OM of sediment) in Lac Saint-Augustin were higher than many paleopigment studies elsewhere (McGowan et al. 2012; Reuss et al. 2010; Hall et al. 1997; Engstrom et al. 2006), reflecting the hypereutrophic status of the lake. The maximum values were well above those observed in Williams Lake (Hall et al. 1997) and Lake Okeechobee (Engstrom et al. 2006). However, pigment concentrations remained below the levels observed in Lake Taihu, a hypereutrophic lake located in eastern China, which has more than double the sediment pigment values observed in Lac Saint-Augustin, and is characterized by continuous blooms of *Microcystis* (Xue et al. 2007).

Three pigments were analysed as potential proxies for cyanobacterial abundance. Canthaxanthin and echinenone were strongly correlated, and were both

correlated with zeaxanthin, consistent with their known co-occurrence in cyanobacteria. However, zeaxanthin was also correlated with lutein, and both are secondary pigments of chlorophytes. Thus green algae likely play a role in the dynamics of both pigments, and canthaxanthin and echinenone may be less ambiguous indicators of cyanobacterial dynamics than zeaxanthin. Similarly, other authors have found that sedimentary zeaxanthin concentrations are not always consistent with cyanobacterial markers, such as other cyanopigments (Patoine and Leavitt 2006) or microcystin toxin accumulation (Efting et al. 2011). However zeaxanthin appears to be a consistent marker of non-colonial cyanobacteria (Fietz et al. 2007; Steenbergen et al. 1994; Bianchi et al. 2000).

The pigment stratigraphy of Lac Saint-Augustin (Fig. 3) demonstrated an overall increase in phytoplankton biomass throughout the length of the core with four distinct zones that differed in terms of pigment quantity and composition. The most recent stratum (0–3.5 cm) at the very top of the core was likely to be still responding to recent sedimentation processes and rapid degradation of the most labile pigments (Leavitt and Hodgson 2001), and was therefore considered not comparable with the groupings deeper in the core. Changes throughout periods of anthropogenic development have been identified as significant predictors of many pigment concentrations, including some cyanopigments (Table 3). The ANCOVA indicated that periods of anthropogenic development in the area had a statistically significant influence on concentrations of many of the pigments, regardless of the magnitude of overall photosynthetic biomass as demonstrated by chlorophyll *a* concentration.

Three main periods can be identified in the core (Fig. 3), and these correspond well to the diatom zonation from a previous study (Pienitz et al. 2006). The ANCOVA also supported this separation of zones. The first zone at the base of the core extends from 1655 until ∼1893 (55–32 cm), and corresponds to the period of initial human settlement prior to major anthropogenic influence in this area (Pienitz et al. 2006). The second zone, from ∼1893 until 1963 (33–14.5 cm), corresponded to the period of intensified agricultural activities within the region and around the lake, as well as further human settlement and industrialization (Pienitz et al. 2006). This second zone marks a highly productive period within the lake

and catchment area, and the pigment data suggest that it was a period of cyanobacterial blooms. Finally, the third zone, from 1963 until the present day, corresponds with the period of construction of the highway bordering the lake (completed in 1977), the construction of new homes and greatly increased urbanization (Ministère des richesses naturelles 1979; Pienitz et al. 2006). This third zone marks the most continuous period of high productivity within the lake including major occurrences of cyanobacterial blooms. The average sediment accumulation rate was four times greater within the modern sediments than at the base of the core (Fig. 5).

Throughout the early colonization zone (1655 to ∼1893), there was a gradual increase in pigment concentrations. In the early 1900s, at core level 28–29 cm, there was an increase in chlorophyll *a*, β,β-carotene and the cyanopigments. This may correspond to significant nutrient inputs and a period of increased productivity. A corresponding peak between sediment and pigment accumulation rates indicates that this increase was not only due to allochthonous inputs of organic matter, but also to increased autochthonous phytoplankton production. It may indicate the effects of further deforestation, intensified agricultural activity, and increased fertilizer inputs known to occur at this time (Ministère des richesses naturelles 1979; Pienitz et al. 2006). The pigment record also suggests that cyanobacterial blooms first occurred in the lake at this time (Fig. 3). This would imply high cyanobacterial populations shortly after the establishment of human settlements, in contrast to many European lakes where the onset of eutrophic conditions was the result of several centuries of anthropogenic activity (Rose et al. 2010; McGowan et al. 2012).

The turn of the 20th century was marked by an increase of agriculture within the region as well as industrialization and further development of infrastructure in Quebec City (Ministère des richesses naturelles 1979; Pienitz et al. 2006). A large peak of sediment and pigment accumulation was observed at the 28–29 cm core level (dated as 1917 AD), with canthaxanthin and echinenone accumulation rates comparable to those within the modern sediments. The actual cyanobacterial abundance could potentially be higher throughout this period than in modern times taking into consideration the degradation of pigment signals over time. This implies a period of high

enrichment associated with major perturbation of the Lac Saint-Augustin catchment, and the occurrence of cyanobacteria-dominated phytoplankton communities. Throughout the period from 1963 until the present there was a further increase in overall productivity, demonstrated by steady increases in both chlorophyll *a* and β,β-carotene. This appears to have been a period of transition in the lake, with strongly increasing impacts of anthropogenic development in the catchment. A further period of heightened activity was detected around the 21–22 cm core level, as demonstrated by a simultaneous increase in chlorophyll *a*, β,β-carotene, canthaxanthin, echinenone, zeaxanthin and diadinoxanthin. This may represent another period of cyanobacterial blooms in the lake, and possibly the onset of high concentrations of diatoms during spring and fall mixing.

The modern sediments of Lac Saint-Augustin had elevated concentrations of all pigments, including the cyanopigments. The rise observed in pigment concentrations throughout this stratum is greater than the increase of ratios between chlorophyll *a* and its degradation pigments, suggesting that the increase in pigment concentrations is not solely related to diagenetic processes. The highest magnitudes of chlorophyll concentration were observed within the period 1963–2011, and the maximum sediment and pigment accumulation rates were also observed throughout this period. This period of sharp increase corresponds with the construction of the highway bordering the lake, which likely resulted in increased soil disturbance and mobilization of nutrients (Ministère des richesses naturelles 1979). For example, geochemical analysis of a previous sediment core demonstrated the highest values of phosphorus in the modern period (1,500 μg P g^{-1} dry mass, compared with an overall sediment core average of 850 μg P g^{-1} dry mass; Pienitz et al. 2006), and this large increase in phosphorus loading may have promoted the proliferation of cyanobacteria, as inferred from the sedimentary pigments. Furthermore, as a longer term impact of this highway, major ion inputs into the lake likely have increased, especially during the winter months when salt is distributed on the road for de-icing (Ministère des richesses naturelles 1979). Increases in salinity may have favoured cyanobacterial growth at the expense of other freshwater phytoplankton that have relatively low tolerances to rising salinity (Hart et al. 2003; Nielsen et al. 2003; Tonk et al. 2007;

Vinebrooke et al. 1998). It is possible that the road salt also contained nutrients such as phosphate, which would have further stimulated cyanobacterial growth (Ministère des richesses naturelles 1979). In Lac Saint-Augustin, construction of the highway and increased salt inputs also corresponded to the timing of appearance of vaucheriaxanthin. This major carotenoid of eustigmatophytes was at trace values through much of the core, but then increased abruptly as a prominent component of the modern pigment assemblage, further indicating pronounced phytoplankton community shifts in response to recent environmental change.

The pigment record from Lac Saint-Augustin indicates that activities in its surrounding catchment, including reduction in forest cover, increased agriculture production and urbanization have perturbed the ecosystem. Although there is evidence of cyanobacterial episodes in the past, the concentrations of pigments observed within the past 40 years are without precedent over the last three and a half centuries, and are significantly above the levels observed prior to the development of the highway or city.

Cyanopigment biomarkers have been previously used to determine the onset of eutrophication caused by increased nutrient input (Efting et al. 2011; Bianchi et al. 2000; Paerl et al. 2003) as well as the effects of climate change (Reuss et al. 2010; Squier et al. 2002). The results reported here for Lac Saint-Augustin underscore the utility of this approach, particularly with the use of canthaxanthin and echinenone as markers of cyanobacterial blooms. Such data are best used in combination with a broad range of other biological and geochemical markers, as were available for Lac Saint-Augustin from previous studies, in order to infer cause-and-effect patterns of environmental change.

Conclusions

This study illustrates the effectiveness of HPLC pigment analysis in determining phytoplankton community dynamics and periods of transition within Lac Saint-Augustin, and probably other hypereutrophic lakes. As a result of improved HPLC technology and methodology, identification and quantification of fossil pigment molecules can provide detailed

information about cyanobacterial and phytoplankton dynamics. Using the combination of echinenone, zeaxanthin and canthaxanthin as proxies for cyanobacterial biomass, it was possible to identify the first cyanobacterial bloom event of Lac Saint-Augustin in the 1890s, and major cyanobacterial blooms in the early 1900s. The cyanopigment records indicate that contrary to our initial hypothesis, such blooms are a long-standing feature of the lake and became apparent soon after colonization. This analysis is particularly useful for management strategies in which it is important to place current water quality issues in a long-term historical context.

Lac Saint-Augustin has changed substantially over the last 350 years. Although it had lower phytoplankton biomass three centuries ago, the pigment analyses of the present study indicate that this lake has experienced cyanobacterial blooms since before the 20th century, and diatom blooms from the mid-1900s onwards. The lake experienced its highest concentrations of phytoplankton since 1963, and this most recent period corresponds to a time of greatly increased pigment, nutrient and metal concentrations in the sediments. These observations imply that the current proliferation of cyanobacteria, as inferred by their high pigment concentrations in the sediments, is likely a result of the last half century of unabated catchment development. Paleopigment analysis of the type applied here provide insights into the history of urban lakes such as Lac Saint-Augustin, and will contribute to their environmental management.

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