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Picophytoplankton during the ice-free season in five temperate-zone rivers

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Although picophytoplankton (PP) (0.2–2 μm) are ubiquitous in lakes and oceans, their importance in rivers has rarely been studied. We examined PP assemblages during the ice-free period in five rivers of a temperate region varying in trophic state (9–107 μg/L total phosphorus) and water discharge (1–87 m^3/s). In these rivers, PP abundance reached concentrations as high as those observed in lakes and oceans (~10^4–10^5 cells/mL). The highest density of PP (4.9×10^5 cells/mL) was observed in the most eutrophic river when the water temperature (28°C) and total phosphorus (293 μg/L) were highest. For the most part, PP abundance was dominated by non-phycoerythrin-containing cyanobacteria; phycocyanin-rich cells accounted for ~75% of PP abundance in all the rivers. In multiple regression analyses, water temperature and nitrate concentrations explained about half of the variation in PP abundance across the rivers. Discharge had no effect on PP abundance or biomass, whereas it had a significant negative effect on total algal biomass among the rivers. The PP contribution to total chlorophyll-*a* averaged 27% (ranging 16–46%) and did not decline with increasing nutrients as found in lakes and oceans. The PP biomass from microscopic enumerations reached a maximum of 9% of total phytoplankton biomass, comparable with that observed in lakes. The results of this study demonstrate the importance of including picophytoplankton when analysing phytoplankton communities in rivers.

KEYWORDS: picophytoplankton; phycocyanin picocyanobacteria; chl-*a* size distribution; nutrients; temperate rivers

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INTRODUCTION

Phototrophic picoplankton (0.2–2.0 μm) were first discovered in the open ocean in the late 1970s and subsequently in lakes in the mid-1980s (Sieburth *et al.*, 1978; Caron *et al.*, 1985; Stockner and Antia, 1986). These pro- and eukaryotic cells account for 10–90% of biomass and/or production in oceans and freshwater ecosystems and represent an important component of the microbial food web (Stockner, 1991; Pick, 2000; Callieri, 2008). However, little is known about these small cells in river ecosystems. Photosynthetic picoplankton have been considered insignificant in rivers compared with lakes (Reynolds *et al.*, 1994) and most studies of rivers have focused on larger phytoplankton such as nanoplankton and microplankton (e.g. Rojo *et al.*, 1994; Reynolds and Descy, 1996).

Because of the challenges of taxonomically identifying cells at the limit of a light microscope, picophytoplankton (PP) have been classified into functional groups based on their photosynthetic pigments. Two main groups can be distinguished by their fluorescence characteristics: picocyanobacteria (Pcy) and picoeukaryote (PEuk) cell types. In freshwater systems there are two main Pcy groups to consider further: one group contains phycocyanin (PC) phycobiliprotein and the other contains phycoerythrin (PE) in addition to PC. Phycoerythrin picocyanobacteria (PE-Pcy) tend to dominate oligotrophic to mesotrophic systems, while phycocyanin picocyanobacteria (PC-Pcy) are more dominant in lakes with lower transparency resulting from either higher algal biomass or humic substances. This shift in dominance is linked to changes in the prevailing light quality where red light, which becomes more important in eutrophic or coloured systems, favours PC-Pcy (Pick, 1991; Vörös *et al.*, 1998; Stomp *et al.*, 2007). Generally, PEuk are much less abundant than Pcy. However, lower light conditions in eutrophic systems seem to favour PEuk; the contribution of PEuk to total PP appears to increase in more eutrophic lakes as a function of increasing light attenuation (Craig, 1987; Pick and Agbeti, 1991). These findings suggest niche differentiation of PP along the light spectrum.

PP are particularly important in terms of biomass and productivity in oligotrophic systems, as they have a high surface-to-volume ratio, which provides a competitive advantage under low nutrient conditions (e.g. Raven, 1986). When systems are nutrient enriched, larger cells tend to dominate algal biomass (Watson and Kalff, 1981; Riegman *et al.*, 1993). Several studies have shown that the relative PP biomass and productivity decline as a function of trophic state in lakes and oceans both empirically and experimentally (Perin *et al.*, 1996; Agawin *et al.*, 2000; Pick, 2000). However, recent studies have demonstrated higher than expected PP biomass in eutrophic estuaries, indicating that their abundance can also be significant in nutrient-rich systems (Murrell and Lores, 2004; Gaulke *et al.*, 2010). For example, in North Carolina's eutrophic Neuse River Estuary, PP (<3 μm) contributed between 35 and 44% of the total chlorophyll-*a* (chl-*a*) (Gaulke *et al.*, 2010).

Seasonal distributions of PP communities typically show strong positive correlations with temperature (e.g. Caron *et al.*, 1985; Agawin *et al.*, 2000). In Lake Ontario, the dominant PE-Pcy peaked in abundance (6.5×10^5 cells/mL) and biomass when temperature was highest (Caron *et al.*, 1985; Pick and Caron, 1987). Similarly, in an annual study of phytoplankton communities along the salinity gradient of the York River Estuary in Virginia, small cells (pico- and nanoplankton) were responsible for much of the chl-*a* in the warmer summer months (Sin *et al.*, 2000). Seasonality can also cause variations in abundance for the different PP functional groups. A study of five oligotrophic to mesotrophic lakes in Ontario showed that while peaks of picocyanobacteria abundance occurred in midsummer, the picoeukaryotic community represented on average ~50% of the picoplankton biomass in spring and early summer (Pick and Agbeti, 1991).

In contrast to lakes, rivers are characterized by the unidirectional flow of water. This can have a negative effect on planktonic abundance under conditions of high discharge or low water residence time, because algal growth rates cannot keep pace with advective losses (Reynolds, 1994). In general, river phytoplankton communities tend to be dominated by small nanoplanktonic (2–20 μm) cells that reproduce more rapidly than netplankton (>64 μm) regardless of river trophic state (Chételat *et al.*, 2006). However, the importance of cells <2 μm in addition to the nanoplankton has not been comprehensively examined yet. Another physical constraint to algal growth in rivers can be low light levels, which arise particularly in turbid systems and in large (deep) rivers when the ratio of euphotic zone to mixing depth approaches 0.2 (Cole *et al.*, 1992).

The goal of this study was to determine the ecological significance of PP in selected temperate rivers varying in trophic state and discharge. The first objective was to analyse seasonal variations of PP abundance and composition based on the photosynthetic pigment groups (PC-Pcy, PE-Pcy and PEuk). We hypothesized that seasonal patterns are linked to changes in temperature such that increases in water temperature would lead to increases in overall PP abundance, as observed in lakes. The second goal was to compare PP in relation

to the total algal community to test the hypothesis that total PP abundance in rivers is related to trophic state. Based on findings in lakes and oceans, we expected that systems with lower nutrient levels would have a higher relative biomass of PP than more eutrophic rivers. Given the high growth rates of PP along with their capacity for photosynthesis under very low light conditions (Stockner and Antia, 1986), we also anticipated that river discharge would have a minimal effect on PP.

METHOD

Study sites

Samples were collected from five temperate lowland rivers in central Canada: four in Ontario and one in Quebec (Table I). The rivers chosen varied in nutrient concentrations because of different geology and land use ranging from undisturbed forest to agricultural and urbanized areas. The most eutrophic system, the South Nation River, is situated in a largely agricultural area rising near the St Lawrence River and flowing in a north-easterly direction discharging into the Ottawa River near Plantagenet, Ontario. The Castor River is a tributary of the South Nation River and one of its four major sub-watersheds. The Raisin River is also surrounded mainly by agricultural lands, flowing into the St Lawrence River near Lancaster, Ontario, upstream of Montreal. Some of the first-order streams of the Raisin arise in peatland areas, which contribute to more coloured waters than found in the other rivers. Both the Castor and Raisin rivers have fairly low discharge and are relatively nutrient enriched (Basu and Pick, 1996). The Rideau River is a lake-fed medium-size lowland river that flows from its headwaters, the Lower Rideau Lake, and empties out into the Ottawa River (Basu and Pick, 1995). This mesoeutrophic system is primarily used for recreation as part of the Rideau Canal system and is surrounded by residential development as well as some agricultural lands. Lastly, the Gatineau River (Quebec) is the least impacted by agriculture and urbanization and the most oligotrophic system (Basu and Pick, 1996). The Gatineau flows south from the Canadian Shield into the Ottawa River. With the exception of the Raisin River, all rivers discharge to the Ottawa River.

Table I: Sampling location, drainage basin area, average annual historical discharge and average 2009 discharge for rivers in Ontario (Water Survey of Canada) and Quebec (Le Centre d'expertise hydrique du Québec), Canada

River	Lat (N)	Long (W)	Drainage basin (km^2)	Annual historical discharge (m^3/s)	2009 discharge (m^3/s)
South Nation	45.5594	75.0631	3810	44	50
Castor	45.2861	75.2257	433	5.5	7.6
Raisin	45.1332	74.5440	404	5.2	4.9
Rideau	45.1063	75.6186	3830	41	48
Gatineau	45.6457	75.9192	6840	126	127

All the rivers sampled have gauging stations monitored by the Water Survey of Canada (2011) in Ontario and Le Centre d'expertise hydrique du Québec (2011) in Quebec. Information on the size of the upstream watershed and the historical and daily river discharge were obtained from these agencies (Table I). The sampling sites were chosen upstream of these gauging station with no major tributaries in close proximity. To examine the potential effect of discharge on PP, we calculated the average daily discharges of the 7 days prior to and including the water collection dates (Pace *et al.*, 1992; Basu and Pick, 1996).

River sampling and laboratory analyses

Each river was sampled every 2 weeks during the ice-free period from late May to early November 2009. On occasion, sampling was postponed for a minimum of 2 days following major rain events. A total of 11 samples were collected per river to describe the seasonal variability of the water-column characteristics and algal abundance (Chételat and Pick, 2001). Water was collected in Nalgene bottles mid-channel where rivers are typically deepest. The bottles were triple rinsed with river water before collecting subsurface grab samples. *In situ* water measurements of temperature, dissolved oxygen (DO), DO percent saturation (%DO), pH and conductivity (SPC) were taken with a Hydrolab Minisonde Multiprobe 4a. Light measurements were occasionally taken using a LI-COR light meter and turbidity was determined in the laboratory using a LaMotte 2020 turbidimeter following every sampling event. Turbidity readings represent the ratio between the scattered light at 90° and 180° from the light source and are given in nephelometric turbidity units (NTU).

Subsamples of the water collected from each site were preserved with a 10% paraformaldehyde solution for a final 1% concentration in order to maintain, for ~1 month, the natural fluorescence of PP (Stockner *et al.*, 2000) and separately preserved in Lugol's iodine solution for phytoplankton (>2 μm) identification. Water samples were also brought to the City of Ottawa's

Robert O. Pickard Environmental Centre for nutrient analysis using standard methods (Basu and Pick, 1995). The nutrients analysed were total phosphorus (TP), reactive phosphorus (RP), total Kjeldahl nitrogen (TKN), nitrate + nitrite ($N0_3 + N0_2$) and ammonia + ammonium ($NH_3 + NH_4^+$). Total nitrogen (TN) was calculated by adding TKN to $N0_3 + N0_2$.

Because chl-*a* is widely used as a measure of phytoplankton biomass, chlorophyll concentrations of the algal and PP communities were determined separately by parallel filtration: individual 250 mL aliquots of water were filtered through 0.2 and 2 μm polycarbonate membranes. When filtering the water, vacuum pressure was set <15 mm Hg to avoid cell breakage. Following filtration, filters were stored at −25°C until they were processed. Chl-*a* was extracted by adding 15 mL of ethanol to each sample for a minimum of 24 h (Jespersen and Christoffersen, 1987), and concentrations were estimated with a Cary® 100 BIO UV-Visible Spectrophotometer, Varian, Inc. On the dates when analyses of the 2 μm fraction were taken in duplicate, the coefficient of variation ranged from 7 to 21% and averaged 14% across the rivers.

Chl-*a* collected on the 0.2-μm membranes represented the total algal biomass, while chl-*a* collected on the 2 μm represented the biomass in the >2-μm size fraction. PP chl-*a* (<2 μm) was calculated by subtracting the total chl-*a* from the >2 μm chl-*a*. The contribution of PP chl-*a* to total algal chl-*a* was expressed as a percentage of the total algal chl-*a*.

Enumeration of phytoplankton

Fluorescence microscopy was used to quantify the abundance of PE-Pcy, PC-Pcy and PEuk populations (Caron *et al.*, 1985; Pick and Agbeti, 1991). From preserved samples, an aliquot of 20 mL was filtered at low pressure (<200 mmHg) on Irgalan Black pre-stained polycarbonate 0.2-μm membranes. Following filtration, the filters (~16–17 mm in diameter) were placed on a microscope slide, followed by a drop of low-fluorescence oil and a glass cover placed over the filter. The microscope slides were then stored at −20°C to preserve the autofluorescence of cells until PP counts were processed.

The enumerations were obtained with a Zeiss AXIO A1 inverted microscope equipped with a green excitation band pass (BP) of 546/12 nm and a red emission range of 575 to 640 nm. A second set of filters was used for blue excitation with a BP of 540–490 nm and an emission long pass of 515 nm (yellow/orange emission). The total abundance of Pcy was obtained based on enumeration of small cells fluorescing bright red under green excitation. Under blue excitation, PE-Pcy appear as yellow/orange cells and PEuk emission is red. As a result, the abundance of PC-Pcy was obtained by subtracting the total Pcy count from the PE-Pcy count. Although PP have traditionally been counted by epifluorescence microscopy, a fluorescence inverted microscope is also suitable. Comparisons between the two methods yielded similar results.

A minimum of 30 randomly chosen fields of view for each cell type were counted at ×1000 magnification. Cell counts included both rod and cocci type cells. Colonial forms of PP assemblages were also counted; however, very few were seen in the river systems.

PP biomass (μg/L) based on enumerations was calculated by converting cell volume (assuming a sphere with an average diameter of 1 μm for Pcy and 2 μm for PEuk, as estimated on random cells using an Empix camera and an Eclipse image analysis system) into biomass assuming a specific density of 1 g/cm^3, used by convention for phytoplankton biovolume to biomass conversions. Total algal biomass was calculated from phytoplankton counts of cells >2 μm using a Zeiss AXIO A1 inverted microscope at ×200, ×400 and ×630 magnifications. Ten millilitres of preserved phytoplankton samples were settled overnight in 26-mm diameter chambers and enumeration of a minimum of 300 cells per sample was made following the Utermöhl method (Lund *et al.*, 1958). Counts and cell dimensions were recorded using the computer counting program, Algamica, version 4.0 (Gosselain and Hamilton, 2000). From this program, the total volumetric biomass (mg/m^3) for cells >2 μm was obtained for each sample. The total biomass was then calculated by adding the PP biomass to >2 μm biomass values.

Data analysis

Statistical analyses consisted of parametric correlations and regressions. Bonferroni-adjusted Pearson correlations coefficients were calculated to determine the relationship between physical and chemical variables and algal biomass from chl-*a* and microscope counts for the PP and >2-μm size fractions. Linear regressions were used to determine the relationship between chl-*a* in the <2-μm size fraction and relative PP concentrations from the total chl-*a*. Multiple regression analyses, including forward and backward procedures, were performed to provide the best model predicting PP abundance as a function of environmental conditions. Variables were log transformed to satisfy normality when necessary; all statistical analyses were done with S-Plus® version 8.0.

RESULTS

River physical and chemical characteristics

The largest river, the Gatineau, had the highest annual average discharge (127 m^3/s) and the Raisin River had the lowest annual average discharge (4.9 m^3/s) (Fig. 1A; Table II). Water discharge varied seasonally with high discharge recorded mid-summer in 2009, mostly in July (Fig. 1A). High discharge values were also seen in late autumn, following several days of heavy rain. The South Nation and Castor rivers had the highest turbidity values (Fig. 1F). Water clarity was lowest following periods of high water discharge, as seen with the high turbidity reported in July and in late autumn.

Water temperature varied similarly in all the rivers with low values recorded in late May and in autumn and high temperatures reported in the late summer months (Fig. 1B). A maximum temperature of 28°C was recorded in the South Nation (24 June 2009). Water pH and conductivity varied the least seasonally, but showed more pronounced differences between rivers (Fig. 1C and D). The Gatineau River had the most neutral pH, whereas the other rivers were more alkaline. Conductivity varied from as low as 20 μS/cm in the Gatineau River to a maximum of 818 μS/cm in the Castor River. As expected, DO also varied throughout the season. The highest value, 14 mg/L (exceeding levels of saturation at 170%), was recorded in the South Nation River (25 August 2009).

The Castor, South Nation and Raisin rivers had the highest nutrient concentrations, the South Nation River being the most nutrient enriched (Fig. 1G–J). Seasonal variations in RP concentrations were observed for each river, with more pronounced differences observed in the South Nation River (Fig. 1G). The highest TP concentration of the study was noted in the South Nation River on August 25 (293 μg/L). The Rideau River had the second to lowest average annual concentrations for TP (26 μg/L) and total nitrogen (TN, 712 μg/L), whereas the Gatineau River had the lowest average TP (10 μg/L) and TN (376 μg/L) concentrations. Nitrate concentrations varied similar to TN throughout the study period in all the rivers (Fig. 1I).

The average TN:TP ratio for all the rivers was 33, indicating that phosphorus was more likely to be limiting than nitrogen although the presence of significant levels of dissolved inorganic nutrients suggests a lack of strong nutrient limitation overall. The Raisin River had the lowest ratio (27), followed by the Rideau River, the South Nation River and the Castor River, and the highest ratio (41) was observed in the Gatineau River.

Seasonal patterns of PP density

Seasonal patterns of abundance for each pigment group of PP were observed in the five rivers (Fig. 2A–E). The South Nation River showed the highest peak of PC-Pcy abundance on June 24 (4.89×10^5 cells/mL), the date and location where the highest water temperature was also recorded, 28°C (Fig. 2A). A second, but less pronounced, peak of PC-Pcy was observed in early August. The PEuk community reached high density values in late June and early July ($\sim 10^2$–10^3 cells/mL). The PE-Pcy community showed consistently low and essentially negligible abundance.

In the Castor River (Fig. 2B), the highest peak (2.07×10^4 cells/mL) of PC-Pcy occurred in early September. Although much lower in abundance, there were two peaks noted for the PEuk community, in late June and early September. From May to November, the PE-Pcy contribution to PP abundance was negligible. As in the Castor River, the highest PP abundance recorded in the Raisin River occurred in early September (3.66×10^4 PC-Pcy cells/mL) (Fig. 2C). Two weeks prior and following that sampling event, the abundance of PC-Pcy was also high compared with the other sampling dates. PEuk abundance in the Raisin also showed two peaks, the first in late June and the second and highest (3.24×10^3 cells/mL) in early September. The PE-Pcy community was negligible, as found in both the South Nation and Castor.

In the mesoeutrophic Rideau River, PP seasonal abundance patterns were different from those described in the more eutrophic systems (Fig. 2D). PC-Pcy abundance was high throughout the summer months, except in early August, following a major rain event. The highest abundance of PC-Pcy recorded was 4.54×10^4 cells/mL in late August. For the most part, the PEuk community was less abundant than PE-Pcy cells. PE-rich Pcy reached higher densities than in the eutrophic rivers and showed a continuous increase in abundance through the summer, leading to a maximum abundance in mid-September (1.56×10^3 cells/mL).

The Gatineau River had high abundances of PC-Pcy throughout the summer months (Fig. 2E), with the highest recorded in mid-July (1.32×10^4 cells/mL). PEuk and PE-Pcy had similar seasonal abundance patterns with high density ($\sim 1.5 \times 10^3$ cells/mL) in early summer followed by continuous decreases in abundance.

For all the rivers, PC-Pcy was the most important PP pigment group; on average, 75% of total PP abundance corresponded to PC-rich Pcy, whereas PEuk cells contributed 13%, and ~11% was represented by PE-Pcy. The median relative abundance of PE-Pcy was low for

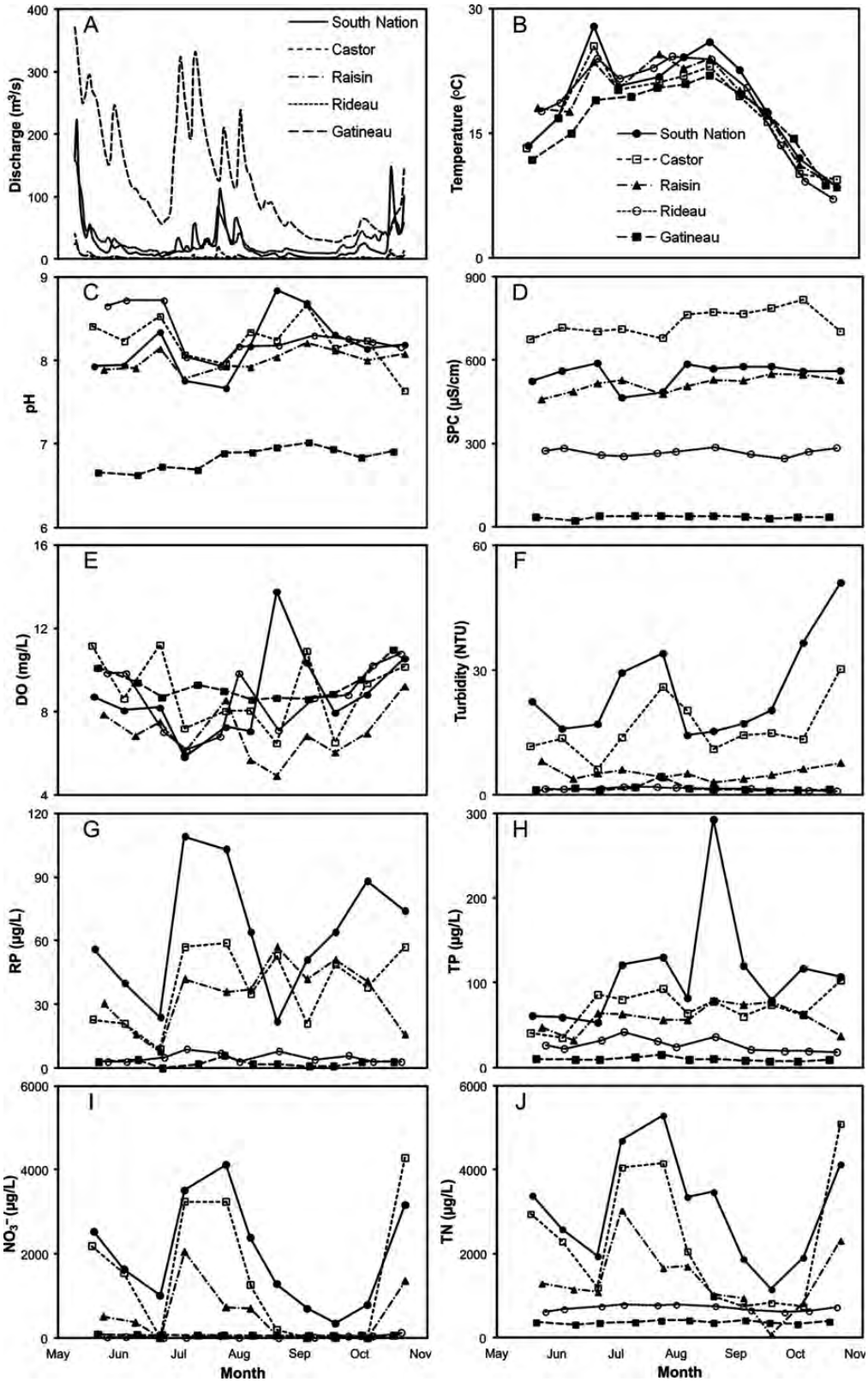

Fig. 1. (**A–J**) Physical and chemical variables of rivers, 2009. Daily water discharge from the Water Survey of Canada and Le centre d'expertise hydrique du Québec (A), temperature (B), pH (C), conductivity (D), dissolved oxygen (E), turbidity (F), reactive phosphorus (G), total phosphorus (H), nitrate (I) and total nitrogen (J). Figure legend in (B) applies to (B–J).

Table II: Seasonal median and ranges (n = 11, 2009) for physical and chemical properties [pH, dissolved oxygen (DO), temperature, conductivity (SPC), turbidity, extinction coefficient and water discharge], for nutrient concentrations [reactive phosphorus (RP), total phosphorus (TP), ammonia + ammonium ($NH_3 + NH_4^+$), nitrate (NO_3^-) and total nitrogen (TN)] and for picophytoplankton densities [phycocyanin-rich picocyanobacteria (PC-Pcy), phycoerythrin-rich picocyanobacteria (PE-Pcy) and picoeukaryotes (PEuk)] in Ontario and Quebec rivers

	South Nation	Castor	Raisin	Rideau	Gatineau
Physical properties					
pH	8.17 (7.67–8.84)	8.24 (7.64–8.67)	8 (7.78–8.21)	8.22 (7.93–8.72)	6.89 (6.63–7.02)
DO (mg/L)	8.19 (5.82–13.8)	8.62 (6.49–11.2)	6.84 (4.92–9.22)	8.8 (6.15–10.8)	8.99 (8.57–11)
Temperature (°C)	20.58 (8.49–27.8)	19.5 (9.38–25.5)	20.1 (8.47–24.5)	20.5 (7.04–24.2)	18.9 (8.7–21.2)
SPC (μS/cm)	561 (464–589)	718 (675–818)	526 (458–550)	269 (245–285)	34.5 (20.1–37.6)
Turbidity (NTU)	20.4 (14.4–51.0)	13.8 (6.22–30.3)	5.17 (3.12–8.14)	1.53 (0.97–1.93)	1.31 (0.97–4.46)
Extinction coefficient[a]	3.69 (2.56–6.98)	4.06 (1.61–5.19)	2.29 (1.87–5.59)	0.78 (0.70–1.14)	1.66 (1.51–2.43)
Water discharge (m^3/s)	20.77 (1.87–64.6)	2.64 (0.49–9.76)	1.4 (0.11–4.23)	20.6 (10.7–52.1)	87.1 (29.8–272.2)
Water chemistry (μg/L)					
RP	64 (22–109)	38 (9–59)	37 (8–57)	4 (3–9)	2 (0–6)
TP	107 (53–293)	74 (35–103)	63 (32–79)	24 (18–42)	9 (7–15)
$NH_3 + NH_4^+$	65 (7–124)	63 (23–92)	43 (3–80)	27 (2–53)	15 (8–26)
NO_3^-	1640 (346–4123)	1266 (0–4285)	367 (0–2051)	21 (0–127)	60 (52.3–82.5)
TN	3355 (1162–5295)	2066 (753–5099)	1157 (82.4–3035)	732 (611–795)	374 (323–423)
Algal parameters (cells/mL)					
PC-Pcy	2.53×10^3 (0–4.89×10^5)	1.18×10^3 (2.94×10^1–2.07×10^4)	1.34×10^3 (1.67×10^2–3.66×10^4)	4.38×10^3 (8.34×10^2–4.54×10^4)	7.84×10^3 (1.03×10^3–1.32×10^4)
PE-Pcy	9.8×10^1 (2.94×10^1–4.60×10^2)	9.8×10^1 (9.80–5.00×10^2)	1.47×10^2 (4.9×10^1–5.49×10^2)	1.76×10^2 (0–1.56×10^3)	5.58×10^2 (2.16×10^2–1.59×10^3)
PEuk	1.18×10^2 (3.92×10^1–1.13×10^3)	3.43×10^2 (2.94×10^1–3.35×10^3)	4.7×10^2 (5.88×10^1–3.24×10^3)	2.25×10^2 (4.9×10^1–1.16×10^3)	3.23×10^2 (7.84×10^1–1.88×10^3)

[a] $n = 5$–9.

all the rivers. However, on May 20, when PC-rich cells were not present in the sample, PE-Pcy relative abundance in the South Nation River was 85% (although their absolute abundance was very low at 2.25×10^2 cells/mL). PEuk were relatively more important in the Castor and Raisin rivers with high contributions to total PP density recorded in early July (73 and 53%, respectively).

PP biomass from chl-*a* and microscope enumerations

Chl-*a* concentrations and biomass from microscope counts showed variations in the distribution of phytoplankton biomass in the >2 and <2-μm size fractions within and among the rivers (Table III). The highest percent PP of total chl-*a* was recorded in the South Nation River at 85% on June 5 and the highest relative PP biomass from microscope counts was 9.18% on June 24 in the same river. On three separate sampling occasions in the South Nation River, the PP contribution to total chl-*a* was >50%. The South Nation River also had the highest total chl-*a* concentration on August 25 (126 μg/L) when the total algal biomass, based on microscope counts, was also the highest (38 222 μg/L). Taxonomic identification revealed that this high algal biomass was mostly caused by a bloom of the colonial green alga, *Pandorina morum*, but PP were also abundant (5.71×10^3 cells/mL).

The Rideau River had the second highest relative contribution of PP to chl-*a* followed by the Castor River, the Raisin River and the Gatineau River (Table III). For the total algal chl-*a* concentrations, values were consistent with river trophic state as the most eutrophic systems had the highest values and the most oligotrophic system, the Gatineau River, had the lowest total median chl-*a* (0.82 μg/L).

The total algal biomass based on microscopic enumerations was also consistent with trophic state with the exception of the South Nation River, which had the second lowest median value (Table III). The relative contribution of PP to the total biomass was much lower than the PP contribution to the total chl-*a*. The median

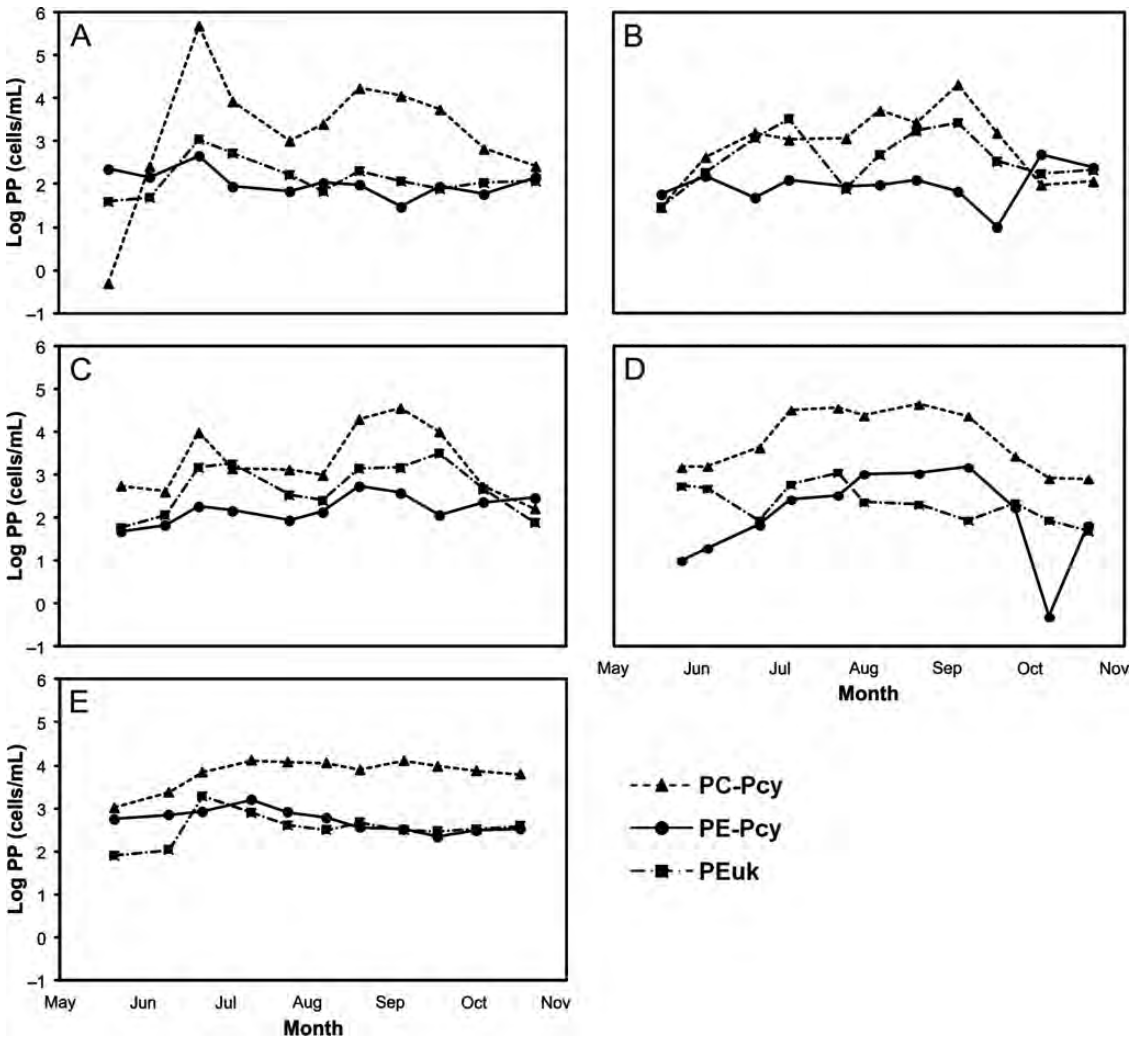

Fig. 2. (**A**–**E**) PP abundance separated by functional group: PC-Pcy, PE-Pcy and PEuk in the South Nation (A), the Castor (B), the Raisin (C), the Rideau (D) and the Gatineau (E) rivers.

*Table III: Median and range of chl-*a *and algal biomass based on microscope enumerations during 2009* (n = *8–11)*

	South Nation	Castor	Raisin	Rideau	Gatineau
Chl-*a*					
Total	2.06 (1.14–126)	5.71 (1.51–19.3)	2.63 (1.22–14.1)	3.91 (1.97–9.89)	0.82 (0.45–1.14)
>2 μm	1.55 (0.22–84.1)	2.44 (1.97–17.1)	2.19 (0.87–9.96)	3.17 (0.96–6.41)	0.66 (0.47–1.13)
<2 μm	0.86 (0.15–42.4)	1.12 (0–6.24)	0.44 (0–4.11)	1.07 (0–3.48)	0.13 (0–0.38)
% PP	41.8 (8.82–85.1)	17.8 (0–75)	15.6 (0–55.6)	28.49 (0–57.3)	15.43 (0–40)
Biomass					
Total	689 (271–38 222)	1813 (1103–5401)	867 (305–3551)	2315 (744–26 549)	275 (121–420)
>2 μm	687 (270–38 211)	1811 (1102–5396)	866 (304–3543)	2301 (743–26 529)	269 (114–408)
<2 μm	1.67 (0.28–261)	2.26 (0.17–22.2)	2.37 (0.57–25.5)	3.03 (0.68–25.2)	6.31 (1.16–12.0)
% PP	0.24 (0.09–9.18)	0.11 (0.01–0.82)	0.24 (0.07–2.03)	0.15 (0.07–1.00)	2.85 (0.39–6.25)

The percent contribution of PP to total biomass (% PP) is also presented. Chl-*a* and biomass are both measured in μg/L.

relative contribution of PP to the total algal biomass ranged from 0.11% in the Castor River to 2.85% in the Gatineau River (Table III). The Gatineau River had the highest median contribution of PP to the biomass but the lowest to chl-*a*. However, the overall biomass in this river was very low.

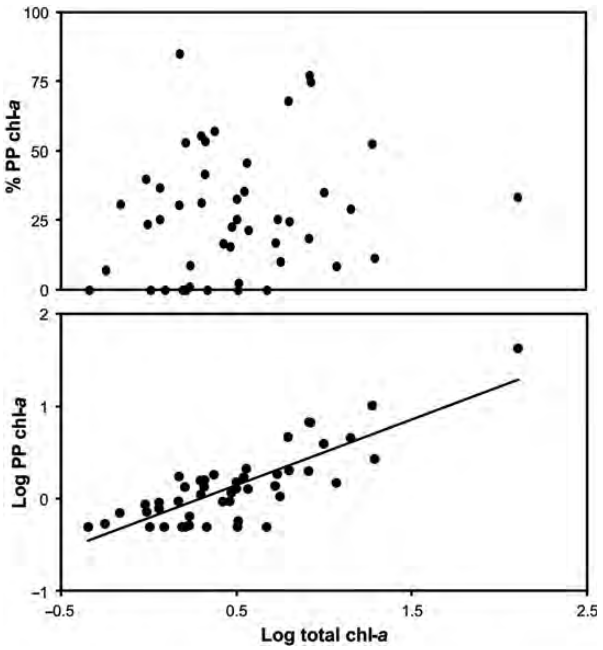

Fig. 3. Relationship between relative (upper panel: $r^2 = 0.050$, $P = 0.126$) and log absolute (lower panel: $r^2 = 0.675$, $P < 0.0001$) PP chl-*a* as a function of log total chl-*a*. Chl-*a* values are in μg/L ($n = 48$).

Across rivers, the relative PP contribution to total chl-*a* showed no relationship with chl-*a* (Fig. 3, upper panel: $r^2 = 0.050$, $P = 0.126$), even following arcsin transformation of the proportional data. The absolute PP chl-*a* showed a statistically significant positive relationship with the total chl-*a* biomass (Fig. 3, lower panel: $r^2 = 0.675$, $P < 0.0001$) and corresponds to the following equation: log (PP chl-*a*) = 0.71−0.21 log (total chl-*a*).

PP response to environmental variables

Chl-*a* concentrations and biomass from microscope counts in the pico fraction and in the >2-μm size class showed few statistically significant relationships with environmental variables (Table IV). Positive correlations arose with pH in both size fractions for chl-*a* and in the >2 μm fraction for biomass based on biovolume. For the larger size fraction, the only other statistically significant correlations were a negative response to water discharge for both chl-*a* and biomass and a positive response to SPC for the >2 μm cells based on microscope counts. The only statistically significant response of PP biomass from cell counts was a positive correlation with temperature. For both the pico and larger cell sizes, no significant response to nutrient concentrations was observed. TN and nitrate values were both

*Table IV: Pearson correlations and statistical significance based on Bonferroni-adjusted probabilities (*P < 0.05; **P < 0.01) for chl-*a (n = 47) and biomass based on microscope enumerations (n = 54) of the PP size fraction (<2 μm) and phytoplankton greater than 2 μm in relation to the following variables: pH, %DO, temperature, SPC, turbidity, water discharge, RP, TP, $NH_3 + NH_4^+$, NO_3^- and TN*

	Chl-*a* (μg/L)		Biomass (μg/L)	
	<2 μm	>2 μm	<2 μm	>2 μm
pH	0.483*	0.655**	−0.060	0.711**
% DO	0.184	0.233	0.056	0.038
Temperature (°C)	0.109	0.381	0.620**	0.356
SPC (μS/cm)	0.363	0.466	−0.182	0.512**
Turbidity (NTU)	0.182	−0.035	−0.315	−0.008
Water discharge (m^3/s)[a]	−0.241	−0.506*	−0.167	−0.520**
RP (μg/L)	0.138	−0.034	−0.201	0.032
TP (μg/L)	0.332	0.290	−0.091	0.302
$NH_3 + NH_4^+$ (μg/L)[a]	0.107	−0.158	−0.290	0.106
NO_3^- (μg/L)[a]	0.009	−0.268	−0.406	−0.195
TN (μg/L)[a]	0.136	0.168	−0.360	0.230

%DO was arcsin transformed for normality and variables marked by[a] were log transformed.

negatively correlated with the PP biomass from microscope counts ($r = 0.36$−0.41), but due to the conservative Bonferroni correction, the relationships were not statistically significant.

With respect to PP abundance, individual simple regressions indicated a positive response to increasing water temperature (Fig. 4, upper panel) and negative response to nitrate concentrations (Fig. 4, lower panel). Multiple regressions were used to evaluate the response of PP abundance to the environmental variables measured concurrently (DO, pH, temperature, SPC, turbidity, discharge, RP, TP, nitrate, TN, ammonia + ammonium and extinction coefficient). Since DO is in part a product of algal activity and is not likely controlling PP abundance, it was not included as an independent variable. Light extinction was also excluded due to missing values. When multicollinearity among the independent factors arose (based on the results of a Pearson's cross-correlation analysis, Table V), the most biologically significant variables were selected. For the nutrients, because TP was highly correlated with RP and TN with nitrate and ammonium, the more bioavailable forms (i.e. RP and nitrate) were retained for the multiple regression analysis. Furthermore, since turbidity and SPC were highly correlated with all nutrient measurements and since pH was highly correlated with

discharge, pH, turbidity and SPC were also excluded from the regressions. When temperature, discharge, RP and nitrate were considered in a multiple regression analysis, the variables that were retained and found to be significant (whether using forward or backward stepwise regression analyses) were temperature and nitrate. These two variables provided the best multiple regression model to predict PP abundance and together explained 51% of the variation ($P < 0.0001$) across rivers [log (PP abundance) = 2.61 + 0.08 (Temperature) −0.21 log (Nitrate)].

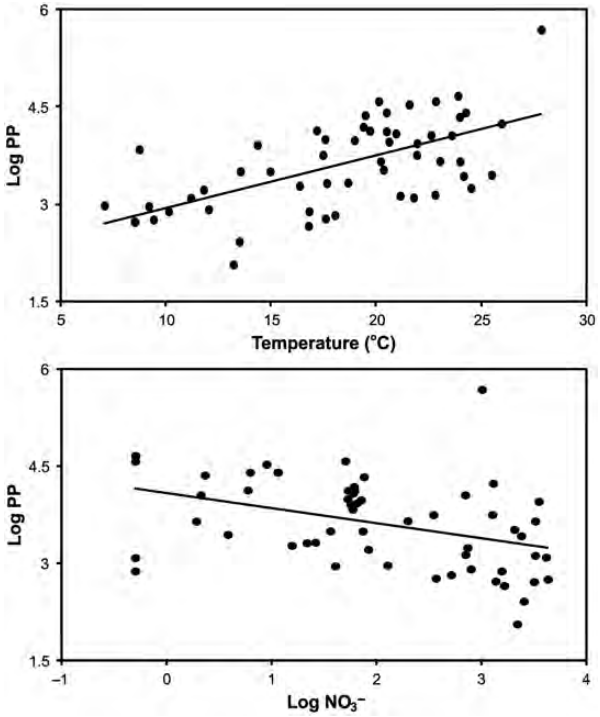

Fig. 4. Linear regression of log PP abundance from fluorescence counts versus temperature (upper panel: $r^2 = 0.384$, $P < 0.0001$) and versus log of total nitrate (NO_3^-, μg/L) (lower panel: $r^2 = 0.155$, $P = 0.003$) ($n = 55$).

DISCUSSION

In contrast to earlier assumptions (Reynolds *et al.*, 1994), this study of five lowland rivers in central Canada demonstrates that PP can reach significant densities and are important contributors to the phytoplankton biomass in the systems. Densities were as high as those reported from lakes and oceans (Partensky *et al.*, 1996; Pick, 2000) and ranged from 10^2 to 10^5 cells/mL.

Of the different pigment groups, PC-rich cyanobacteria dominated PP abundance and comprised approximately three quarters of the total abundance. The strong dominance of PC-Pcy type was anticipated, given the empirical and experimental evidence showing the numerical dominance of PC-rich picocyanobacteria in turbid waters (Pick, 1991; Vörös *et al.*, 1998; Stomp *et al.*, 2007). Collectively, the rivers in this study had a high average light extinction coefficient (2.87/m) with values ranging from 0.70 to 6.9/m. The models of Pick (1991) and Stomp *et al.*, (2007) predict a decline in the PE-rich cyanobacteria and a rise in the dominance

*Table V: Pearson correlations and statistical significance based on Bonferroni-adjusted probabilities (*P < 0.05; **P < 0.01; ***P < 0.001) for the following river variables: pH, dissolved oxygen percent saturation (DO, %), temperature (Temp, °C), conductivity (SPC, μS/cm), turbidity (Turb, NTU), water discharge (Dish, m^3/s), reactive phosphorus (RP, μg/L), total phosphorus (TP, μg/L), nitrate ($NO3^-$, μg/L), total nitrogen (TN, μg/L), ammonia + ammonium (Amm, μg/L), extinction coefficient and total chlorophyll-a (Chl-a, μg/L)*

	pH	DO	Temp	SPC	Turb	Disch[a]	RP	TP	NO_3^-[a]	TN[a]	Amm[a]	Ext coef
DO	−0.075											
Temp	0.164	0.163										
SPC	0.715***	−0.218	0.040									
Turb	0.258	−0.089	−0.186	0.568***								
Disch[a]	−0.587***	0.382	−0.187	−0.728***	−0.043							
RP	0.281	−0.444	−0.008	0.623***	0.815***	−0.295						
TP	0.482*	−0.271	0.111	0.747***	0.790***	−0.455*	0.910***					
NO_3^-[a]	−0.080	0.012	−0.092	0.232	0.567***	0.214	0.498**	0.347				
TN[a]	0.418	−0.077	0.040	0.646***	0.716***	−0.149	0.665***	0.681***	0.670***			
Amm[a]	0.124	−0.217	−0.104	0.508**	0.533**	−0.172	0.542**	0.507**	0.507**	0.563***		
Ext coef	0.227	−0.223	−0.132	0.571*	0.795***	−0.133	0.650***	0.639**	0.440	0.529	0.501	
Chl-*a*[a]	0.751***	0.196	0.336	0.560**	0.083	−0.508*	0.087	0.396	−0.189	0.217	−0.012	0.035

%DO was arcsin transformed for normality and variables marked by[a] were log transformed.

(>50%) of PC-cyanobacteria above extinction coefficients of 0.5/m. On average, PE-Pcy represented only 11% of the total PP abundance, but the highest average PE-Pcy abundance was found in the clearest and more oligotrophic rivers, the Gatineau and the Rideau rivers. PEuk were more abundant in early summer and sometimes had two peaks in abundance, one in early summer and the other occurring in late August. Similar patterns have been described in lakes where PEuk were generally one order of magnitude less abundant than PC and often showed peaks in spring and mid-summer, whereas PC peak in mid-summer (Pick and Agbeti, 1991; Stockner, 1991; Callieri and Stockner, 2002).

In the rivers studied, the contribution of PP to the total chl-*a* (~27% on average) was significant but was not a simple function of river trophic state (Table III, Fig. 3). In contrast, in lakes, the percent contribution of PP to the total biomass clearly decreases with increasing trophic status in regional surveys (Søndergaard, 1991; Vörös *et al.*, 1998; Callieri *et al.*, 2007). Here, even in the most eutrophic river (the South Nation), PP still contributed to almost half of the total chl-*a*. However, more rivers should be examined over a wider range of nutrient concentrations as the results here fall within the variability observed in very large data sets for lakes and oceans (Bell and Kalff, 2001; Callieri *et al.*, 2012). These findings are consistent with recent studies reporting a high contribution of PP to total planktonic chlorophyll in a eutrophic estuary (Gaulke *et al.*, 2010) and reports of increasing PP abundance with chl-*a* in saline (soda) lakes (Keresztes *et al.*, 2010). It could be that all these systems have low abundances of PP grazers and that these high PP values are the result of less top-down control of PP abundance, which is typically strong in lakes (Lavallée and Pick, 2002). The average contribution of PP biomass to algal biomass estimated from microscope counts was <1% and much lower than the average contribution to chl-*a* (27%). However, the highest values for PP biomass contribution (~9%) compare with estimates from oligo-mesotrophic Ontario lakes (Pick and Agbeti, 1991). It should be noted that the PP abundance and biomass values could be slightly underestimated as slides were not prepared (and frozen) immediately following collection and some fading of auto fluorescence may have occurred despite preservation and refrigeration.

The most important factor explaining variation in PP abundance was temperature. In this study, high abundance of PP was linked to high temperatures, observed throughout the summer months similarly across the rivers. This is consistent with previous studies linking high PP biomass with optimal temperatures >20°C (Agawin *et al.*, 2000; Gaulke *et al.*, 2010). In Lake Maggiore, maximal PP abundance was noted when surface water reached 18–20°C (Callieri and Piscia, 2002). Similarly, in a study of five oligotrophic to mesotrophic Ontario Lakes, PC abundance was highest in the late summer months when temperature was >20°C (Pick and Agbeti, 1991). In this study, the highest PP abundance (4.89×10^5 cells/mL) occurred on the same day as the highest water temperature was recorded (28°C) in the most eutrophic and turbid river (South Nation).

PP abundance was not related to river discharge, in contrast to the significant negative relationship observed between larger phytoplankton and discharge for both chl-*a* and biomass from cell counts (Table IV). This is likely because the growth rates of PP are very high (doubling times <1–2 days, Lavallée and Pick, 2002), particularly at higher temperatures, such that losses from advection downstream would be insignificant. Several river studies have reported negative relationships between algal biomass and discharge (Reynolds, 1984) because relatively long water residence times may be required to enable accumulation of slower growing algae (i.e. larger taxa).

PP abundance was also not positively related to nutrient concentrations, as might be expected if nutrients were limiting this community. PP are likely rarely nutrient limited in rivers, given their high affinity for nutrients at low concentrations and the generally higher nutrient supply rates. In the more eutrophic rivers, RP was above detection and at times quite high, which is indicative of a surplus of bioavailable phosphorus. Interestingly, PP abundance exhibited a negative relationship with nitrate. This could reflect either strong consumption of nitrate or competition for nitrate with large phytoplankton or some indirect effect of top-down factors operating in the more eutrophic rivers. Negative effects of nutrient additions on PP abundance have been demonstrated experimentally in lakes (Tzaras *et al.*, 1999). While the higher phytoplankton biomass was not correlated with nutrients (phosphorus and nitrogen fractions), a significant correlation with conductivity was observed and conductivity has been considered to be a surrogate of productivity in rivers (Biggs, 1988).

In summary, this study demonstrates the importance of PP to the plankton of five lowland temperate rivers varying in trophic state and size. These results suggest that PP should be studied further in river systems. Given their high turnover rates, the community also likely plays an important role in carbon and nutrient cycling in rivers that has yet to be recognized.

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