Phytoplankton communities in temperate rivers

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To Mom, Dad, and
Rhys

Abstract

The structure of phytoplankton communities was examined seasonally across five rivers with a focus on small cells and their relative importance. Picophytoplankton (0.2-2 μm), previously considered insignificant in rivers, reached densities as high as those observed in lakes and oceans (~ 10^4-10^5 cells/mL). Their relative importance was not a function of trophic state with the highest contribution to algal biomass found in the most eutrophic river. Body size distributions were analyzed from both chlorophyll-*a* size fractions and taxonomic enumerations; no significant effect of river or season was detected, suggesting that phytoplankton size distribution is not a useful metric of change in rivers. Unlike lake ecosystems, the rivers were uniformly dominated by small cells (< 20 μm). Taxonomic analyses of the seasonal succession did not reveal a common periodicity of particular divisions (e.g. diatoms). However, strong dominance was more typical of eutrophic rivers even though taxa richness was similar.

Résumé

La structure des communautés phytoplanctoniques a été examinée de mai à novembre dans cinq rivières tempérées avec une attention particulière portée aux petites cellules et à leur importance relative. Le picophytoplancton (0,2-2 μm), auparavant considéré sans importance dans les cours d'eau, a atteint des densités aussi élevées que celles observées dans les lacs et les océans (~ 10^4-10^5 cellules/mL). Leur importance relative n'était pas reliée à l'état trophique des rivières car la contribution la plus élevée fut notée dans la rivière la plus eutrophe. La répartition en taille des cellules a été analysée avec des fractions de chlorophylle-*a* et des énumérations taxonomiques. Qu'aucun effet significatif de rivière ou de saison n'ait été décelé suggère que la répartition en taille n'est pas un paramètre utile de changements environnementaux dans les rivières. Contrairement à ce qui a été retrouvé dans les lacs, les rivières étaient uniformément dominées par des cellules de petite taille (< 20 μm). Les analyses taxonomiques de succession saisonnière dans les rivières n'ont pas révélé une périodicité commune par divisions particulières (ex. diatomées). Toutefois, la dominance taxonomique était plus forte dans les systèmes eutrophes même si la diversité des espèces était assez similaire.

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Introduction

1.1 Introduction to river phytoplankton

Rivers are important freshwater resources for human use, and in addition harbour significant biodiversity. In fact, many of the world's great cities have been built along rivers. However, over the last century, humans have caused extensive changes to the health of these lotic ecosystems (e.g. Karr, 1999).

For rivers, as for other ecosystems, a healthy state has been defined as the system's ability to maintain structure and function against external stressors (Costanza and Mageau, 1999; Reynolds, 2003). For example, when the loading of organic wastes becomes excessive, the assimilative capacity of the system is exceeded and the outcome can result in the loss of fish habitat and the accumulation of undesirable taxa, such as toxic blooms of cyanobacteria (blue-green algae) (Hötzel and Croome, 1994). Under these conditions, the stress and strain exerted on the system leads to a loss of function (e.g. reductions in fish yields). Since rivers are important ecosystems to maintain and manage, understanding their structure and function is crucial. However, rivers, in comparison to lakes and oceans, have been studied far less, particularly with respect to the ecology of primary producers (e.g. Rojo *et al.*, 1994).

There are many different ways to assess the state of a river. Of these, the analysis of benthic diatom communities has been successfully applied to assess water quality and ecosystem health for several decades (Patrick, 1965). However, phytoplankton communities have received much less attention, yet they are present in almost all major rivers that have been studied (Greenberg, 1964; Lewis, 1988; Rojo *et al.*, 1994; Train and Rodrigues, 1997) and are present throughout the year (Sze, 1981).

Suspended algae in rivers can arise from several sources. Those that originate from lakes are considered limnoplankton. Those that grow primarily on substrates but are then entrained into the water column are in fact benthic algae, and true river phytoplankton that

grow in the river are termed potamoplankton (Reynolds, 2006). Early river research, such as the River Continuum Concept (RCC), suggested that suspended algae were only abundant in large rivers (Vannote *et al.*, 1980); however, subsequent studies have demonstrated the importance of phytoplankton in small to medium-sized temperate rivers (e.g. Søballe and Kimmel, 1987; Basu and Pick 1996). More recent models of river systems suggest suspended algae are important sources of carbon to river food webs (Thorp and Delong, 1994).

1.2 Factors influencing phytoplankton growth and seasonality

The ecological factors that influence phytoplankton growth in rivers are the same as those regulating algal growth in lakes. These include light, nutrient concentrations, and herbivore grazing. However, in rivers, water discharge (or flow) is a significant factor that can constrain algal growth as advection downstream can lead to reductions in standing crop (Søballe and Kimmel, 1987). Several studies have demonstrated water residence time as an important factor for algal growth in lotic systems; longer water residence times tend to be associated with increases in algal abundance (Søballe and Kimmel, 1987; Reynolds, 1988).

Light availability is an important variable regulating phytoplankton populations. Throughout the growing season, the depth of the photic zone (depth of 1% of incident radiation) varies in response to sediment loads in rivers and decreases when self-shading occurs with high phytoplankton abundance. For example, in the Lot River in France, the photic zone varies from 0.7 to 5.3 m throughout the year (Décamps *et al.*, 1984). Generally, when light becomes limiting, this leads to a reduction in phytoplankton abundance. For example, in the highly turbid Ohio River, low light availability frequently limits phytoplankton growth (Koch *et al.*, 2004). The RCC considered light to be to be the most important factor controlling autotrophic production in rivers (Vannote *et al.*, 1980); however,

more recent studies have demonstrated the significant impact that nutrient concentrations have on river phytoplankton (Søballe and Kimmel, 1987). For example, a study of 31 rivers in eastern Canada reported a positive correlation between chlorophyll-*a* (chl-*a*) and total phosphorus concentrations analogous to the well known relationship in lakes (Basu and Pick, 1996).

Both light and nutrients can be altered by the flow regime. Fast flowing water increases turbulence and may affect light availability. In general, as rivers get deeper with increasing flow, the mixed layer deepens such that suspended algae are entrained below the euphotic zone. A ratio of euphotic zone to mixed layer depth less than 0.2 m is indicative of light limitation and constrains phytoplankton growth (Cole *et al*., 1992). Changes in water discharge caused by increased runoff (from precipitation, snow, or glacial melt) can also alter transport and cycling of nutrients in the water column. In instances of high rainfall, nutrient concentrations can rapidly increase or decrease several fold (e.g. Perkins and Jones, 1994). There tends to be an inverse relationship between water discharge and river phytoplankton abundance (Filardo and Dunstan, 1985; Sullivan *et al.*, 2001). However, some taxa can benefit from these conditions. For example, diatom abundance can increase in periods of high rainfall and turbulence (Train and Rodrigues, 1997).

Grazing pressure can also play an important role in regulating phytoplankton abundance. However, zooplankton grazing does not seem to significantly decrease algal biomass in small to medium rivers (Pace *et al.*, 1992; Reynolds and Descy, 1996). In contrast, benthic grazers can considerably reduce phytoplankton populations (Basu and Pick, 1997; Caraco *et al.,* 1997). For example, in the Hudson River (New York) the zebra mussel, *Dreissena polymorpha*, was capable of filtering the entire water column every one to four days in the summer. This decreased phytoplankton biomass by 85% and affected nutrient

concentrations, water clarity, and other variables at the ecosystem level (Caraco *et al.*, 1997). However, since grazing rates are normally low in medium to small rivers, the majority of the primary production is usually carried downstream (Allan and Castillo, 2007).

Longitudinal and seasonal patterns are important to consider when assessing algal communities in rivers. Significant changes in phytoplankton abundance can occur longitudinally; early studies reported increases in phytoplankton abundance with downstream travel (Greenburg, 1964; Hynes, 1970). In contrast, seasonal changes in phytoplankton communities have not been as extensively studied (Hudon *et al.*, 1996). There are no river models of seasonal succession analogous to the Plankton Ecology Group-model that describes the seasonal patterns and interactions of phytoplankton and zooplankton in temperate lakes (Sommer *et al.*, 1986).

The factors that regulate phytoplankton growth (nutrient concentrations, temperature, light availability and discharge) vary seasonally in temperate rivers (Basu and Pick, 1997). In the Rideau River (Ontario), a lowland mesotrophic system, nutrient concentrations are higher in summer months (Basu and Pick, 1997). These coincide with increases in phytoplankton abundance, seen as a positive relation between chl-*a* and TP (Basu and Pick, 1995 and 1997). However, in the Ottawa River (Ontario), which is much larger and oligotrophic, phytoplankton biomass varied little between spring, summer, and fall (Hudon *et al.*, 1996), although it tended to be highest during the low-discharge mid-summer period. In the St. Johns River, Florida, fluctuating light availability influenced by seasonality strongly affected phytoplankton abundance: less coloured water recorded in spring and summer led to lower light extinction, thus favouring algal growth (Phlips *et al.*, 2000).

1.3 Size distribution of phytoplankton

The size of an organism determines rate processes such as metabolism, production, and excretion (White *et al.*, 2007). Body size is also related to higher level attributes of ecological systems such as population abundance, productivity, competitive and facilitative relationships (Woodward *et al.*, 2005). The relationship between body size and abundance can determine resource allocation and shapes food web dynamics (Brown *et al.*, 2004; Woodward *et al.*, 2005).

Body size analysis of phytoplankton communities is possible because individual taxa range four orders of magnitude in size from small picoplankton (0.2-2 μm) to macroscopic species with colonies visible to the naked eye (> 1000 μm) (Reynolds, 1984). The first mention of phytoplankton distribution into size categories was in 1892 (Schütt, referenced in Callieri, 2008). In the 1950s, screens, nets, and filters varying in pore size were used to further separate plankton into size fractions. Upon the discovery of significant populations of increasingly smaller phytoplankton cells, the following terms were introduced to describe plankton size categories: micro-, nano-, ultra- and pico-plankton (Sicko-Goad and Stoermer, 1984).

Significant differences in size distribution can be observed in phytoplankton communities because of the strong size dependence of growth rates, nutrient uptake, light acquisition and sinking from the euphotic zone. Particularly in the oceans, the size distribution of plankton has a major effect on biogeochemical cycles, in particular the carbon cycle (Finkel *et al.*, 2009).

The first examination of the size distribution or spectrum for plankton summarized annual aggregations of the mean biomass of size classes of organisms in marine systems (Sheldon *et al.*, 1972). Since then, this approach has proven useful in marine systems

(Tremblay *et al.*, 1997; Li, 2002) and in lakes (Sprules and Munawar, 1986). In marine environments, positive correlations have been reported between average phytoplankton size and chl-*a* (Li, 2002), nitrate (Vidal and Duarte, 2000), and iron (Tsuda *et al.*, 2003) concentrations. In lakes, phytoplankton average cell size tends to increase with nutrient concentrations (Watson and Kalff, 1981). Generally, higher nutrient concentrations stimulate phytoplankton growth and create a lag in the response of large predators to nutrient increases (Armstrong, 2003). This creates more microzooplankton grazers that feed on the smaller cells, thus favouring an increase in larger phytoplankton cell volume (Armstrong, 2003). However, in shallow non-stratified lakes, small cells benefit from higher nutrient concentrations (Reynolds, 1994). This occurs because they are more efficient at absorbing light and have lower sinking rates and thus proliferate by remaining in the photic zone (Reynolds, 1994; Finkel *et al.*, 2009).

Few studies have investigated the link between size structure and nutrient concentrations in rivers and streams (Rojo *et al.*, 1994). One study of 46 temperate rivers suggested that there was no significant variation in plankton size distribution conducted during summer base flow conditions; nanoplankton (2-20 μm) dominated phytoplankton biomass and no significant correlations were detected with nutrient concentrations (Chételat *et al.*, 2006). On the other hand, within one river (Rideau River), longitudinal differences were observed where larger size classes (22-64 μm and > 64 μm fractions) consistently increased in the downstream reaches from July to September. These patterns were concomitant with longitudinal increases in nutrient concentrations and water residence times (Yang *et al.*, 1997).

In rivers, the different hydrological and hydrodynamic factors such as discharge and water residence times may alter the phytoplankton community size structure (Chételat *et al.*,

2006). Because growth rate is inversely related to cell size (Reynolds, 1984), small cells in theory should proliferate in river reaches and/or at times of short water residence times whereas larger cells would be more abundant at longer water residence times. In the Rideau River, increases in chl-*a* concentrations were found in reaches where water retention times were 72 hours or longer (Basu and Pick, 1995). However, in rivers, phytoplankton community size structure might be more affected by light availability than by the hydrological regime because of high turbidity (Chételat *et al.*, 2006). If light is limiting, smaller cell sizes would be selected for because of how efficient they are at absorbing low light (Reynolds, 1994).

1.4 Taxonomic composition of phytoplankton

Phytoplankton taxa present in rivers can reflect specific ecological conditions and changes in species composition and distribution can be linked to the impact of pollution (e.g. Giorgio *et al.*, 1991). At highly impacted sites, broad-tolerance species may outcompete more sensitive organisms and increase in abundance. For example, the presence of planktonic diatom species such as *Aulacoseira granulata*, *Stephanodiscus parvus*, and *Stephanodiscus hantzschii* indicate eutrophic conditions (Yang *et al.*, 1997). With the observation that certain species are abundant in specific environmental settings, the idea of accessing river conditions based on community composition was developed. When changes occur in species composition, this affects the functioning of the entire system (Karr, 1999). Studying community species composition rather than the presence or absence of a particular species is a more dependable way of assessing habitat conditions and several indices have been developed to assess river conditions based on this knowledge (Karr, 1999; Reynolds *et al.*, 2002). In rivers, benthic diatoms are frequently used as bioindicators as they are

usually the most abundant type of algae and the composition of these communities is particularly sensitive to environmental change (Kelly and Whitton, 1995). Recently, a biotic index based on periphytic diatoms has been established for rivers in Canada (Lavoie *et al.*, 2008). These types of indices are based on the community composition of diatoms, which reflect certain types of habitat and chemical conditions that indicate the overall state of the river (e.g. Szczepocka and Szulc, 2009).

Most river studies have focused on diatoms and not the entire phytoplankton community (Kelly, 1998; Fore and Grafe, 2002; Szczepocka and Szulc, 2009). In temperate rivers, while diatoms appear to dominate in species abundance and biomass, the diversity of river phytoplankton can be highly variable and other phytoplankton groups are also important (Rojo *et al.*, 1994). In a survey of 67 rivers, Rojo *et al.* (1994) found that diatoms represented 40% of the average number of species in temperate rivers, yet green algae contributed 28%, cyanobacteria represented 10%, and euglenoids averaged 7% of species abundance. In a study of 31 temperate rivers in Ontario and Quebec, diatoms also dominated with the largest percent of the total phytoplankton biomass (34%), however, other divisions also contributed to overall biomass: cryptophytes (24%); chlorophytes and chrysophytes (15%); and cyanobacteria (3%) (Chételat *et al.*, 2006). Therefore, it is important to consider other phytoplankton groups besides diatoms.

1.5 Thesis rationale and objectives

The overall objective of this thesis was to determine whether river phytoplankton can be used to evaluate the state of rivers by analyzing communities over a growing season in systems varying in physical and chemical conditions.

The first component of this research examined the contribution of the very smallest phytoplankton to algal biomass. Picophytoplankton (0.2- 2 µm in diameter) have not been well studied in rivers. Most studies describe phytoplankton in rivers without assessing picophytoplankton because separate techniques are required to determine their abundance. In Chapter 2, I hypothesized that picophytoplankton would not be significant in rivers relative to larger phytoplankton. Since lake and ocean studies have demonstrated that these small organisms are better competitors under low nutrient conditions (Stockner, 1988; Søndergaard, 1991), I predicted that picophytoplankton abundance would only be significant in the most oligotrophic rivers.

The second part of this thesis (Chapter 3) examined the use of phytoplankton size distribution as an indicator of river condition, an approach which is widely used in marine and lake ecosystems. The size distribution of chl-*a* and algal biomass from microscope measurements were compared between seasons and rivers. I hypothesized that phytoplankton size would differ mainly because of different nutrient concentrations such that higher nutrient concentrations would lead to dominance of larger cells.

The last part of this research focused on the phytoplankton taxonomic community composition of rivers (Chapter 4) and in particular the value of assessing all phytoplankton taxa as opposed to only diatoms. The hypothesis that phytoplankton taxonomic composition varies between rivers as a function of environmental variables was tested, and, in particular that nutrient concentrations would be a significant driver of taxonomic composition.

- Chapter 2 -

Picophytoplankton in temperate rivers: seasonal patterns and contribution to algal biomass

Abstract

Although picophytoplankton (0.2-2 µm) are ubiquitous in lake and oceans, their abundance in rivers is considered insignificant to biomass and trophic dynamics. This study revisited this assumption and examined picophytoplankton (PP) assemblages during the ice-free period in five temperate rivers ranging in trophic state (9-107 µg/L total phosphorus). PP abundance reached concentrations as high as those observed in lakes and oceans (~ 10^4-10^5 cells/mL). For the most part, PP abundance was dominated by non-phycoerythrin containing cyanobacteria; phycocyanin-rich cells accounted for approximately 75% of PP abundance in all rivers. Multiple regression analysis determined that water temperature and nitrate concentrations could explain about half of the variation in PP abundance across the rivers ($p<0.0001$). The PP contribution to total chlorophyll-*a* averaged 27% (ranging 16-46%) and did not decline with trophic state as in lakes. PP biomass from fluorescence enumerations reached a maximum of 9% of total phytoplankton biomass, as high as observed in lakes. The results of this study demonstrate the importance of including picophytoplankton when analyzing phytoplankton communities in rivers.

2.1 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 early 1980s (Sieburth *et al.,* 1978; Caron *et al.*, 1985). 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; Bell and Kalff, 2001). However, little is known about these small cells in river systems. Photosynthetic picoplankton are considered insignificant in rivers compared to lakes (Reynolds, 1994) and most studies of rivers have focused on larger phytoplankton organisms such as nanoplankton and microplankton (e.g. Szelag-Wasielewska, 2004; Szelag-Wasielewska *et al.*, 2009).

Picophytoplankton (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 (Raven, 1986; Søndergaard, 1991). When systems are nutrient enriched, larger cells tend to dominate (Watson and Kalff, 1981; Riegman *et al.*, 1993). Several studies have shown that PP biomass and productivity decreases in eutrophic 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).

Studying PP abundance has proven useful for assessing environmental changes because of their high growth rates (Stockner *et al.*, 2000). PP respond rapidly to changing environmental conditions and seasonality (Stockner *et al.*, 2000; Wang *et al.*, 2008). In lakes

and oceans, long water residence times coupled with warm temperatures represent favourable conditions for high PP abundance (Phlips *et al.*, 1999; Wakabayashi and Ichise, 2004). Seasonal distributions of PP communities show strong positive correlations with temperature (e.g. Caron *et al.*, 1985; Agawin *et al.*, 2000). In Lake Ontario, the dominant phycoerythrin picocyanobacteria peaked in abundance ($6.5x10^5$ 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 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 about 50% of the picoplankton biomass in spring and early summer (Pick and Agbeti, 1991).

Because of the challenges of taxonomically identifying cells at the limit of the light microscope, PP have been classified into functional groups based on their photosynthetic pigments. Two main groups can be distinguished by their fluorescence characteristics: picocyanobacteria (PC) and picoeukaryote (PEuk) cell types. In freshwater systems there are two main PC groups to consider: the first group contains phycocyanin (PY) phycobiliprotein, whereas the other group contains phycoerythrin (PE) in addition to PY.

Phycoerythrin picocyanobacteria (PE-PC) tend to dominate oligotrophic to mesotrophic systems while phycocyanin picocyanobacteria (PY-PC) are more dominant in lakes with lower transparency resulting from either higher algal biomass or humic substances (Pick, 1991). A parameterized competition model can be used to predict PC coexistence in lakes and oceans; PE-PC tend to dominate in clear waters and PY-PC dominate in highly

turbid waters, while coexistence of both PC tend to occur at intermediate turbidity (Stomp *et al.*, 2007). Furthermore, 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 picophytoplankton along the light spectrum.

The goal of this study was to determine the ecological significance of PP in temperate rivers. The first objective was to analyze seasonal variations of PP abundance and composition based on the photosynthetic pigment groups (PY-PC, PE-PC, and PEuk). I 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 in rivers of different trophic status along environmental gradients. I hypothesized that total PP abundance in rivers is related to trophic state. Based on findings in lakes and oceans, I expected that systems with lower nutrient levels would have higher overall PP abundance than eutrophic rivers.

2.2 Methods

2.2.1 Study sites

Samples were collected from five temperate rivers in central Canada (Table 2-1). 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 where it rises near the St. Lawrence River and flows 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 a low-land river that is also mainly surrounded by agricultural lands, flowing into the St. Lawrence River near Lancaster, Ontario, upstream of Montreal. Both the Castor and Raisin rivers have relatively low discharge and are 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 represents the system least impacted by agriculture and urbanization and the most oligotrophic (Basu and Pick, 1996). The Gatineau flows south from the Canadian Shield into the Ottawa River. With the exception of the Raisin River, that discharges directly to the St. Lawrence River, all rivers discharge to the Ottawa River.

All rivers sampled have gauging stations monitored by the Water Survey of Canada, in Ontario and Le Centre d'expertise hydrique du Québec, in Quebec. Sites were chosen upstream of these gauging station with no major tributaries in close proximity. These gauging stations report the size of the upstream drainage basin as well as the historical and daily river discharge (Table 2-1). For this study, discharge values were obtained by calculating the average daily discharges seven days prior to and including the water collection dates (Pace *et al.*, 1992; Basu and Pick, 1996).

2.2.2 River sampling and laboratory analyses

Each river was sampled every two weeks from late May to early November 2009. On occasion, sampling was postponed for a minimum of two 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), dissolved oxygen 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 degrees from the light source and are given in Nephelometric Turbidity Units (NTU).

The water collected from each site was preserved in a 10% paraformaldehyde solution in order to maintain, for approximately one month, the natural fluorescence of PP (Stocker *et al.*, 2000) and in Lugol's iodine solution for phytoplankton 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 analyzed 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 μm and 2 μm polycarbonate membranes. When filtering the water, vacuum pressure was set below 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 hours, and concentrations were estimated with a Cary® 100 BIO UV-Visible Spectrophotometer, Varian Inc (Jespersen and Christoffersen, 1987).

On the dates when analyses of the 2 μm fraction were taken in duplicate, the average was calculated. For these duplicates, the coefficient of variation (CV) ranged from 7-21% and averaged 14% across the rivers.

Chl-*a* collected on the 0.2 μm membranes represented 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 greater than 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*.

2.2.3 Enumeration of phytoplankton

Fluorescence microscopy was used to quantify the abundance of PE-PC, PY-PC, and of PEuk populations (Caron *et al.*, 1985; Pick and Agbeti, 1991). From preserved samples, an aliquot of 20 mL was vacuumed at low pressure (< 200 mm Hg) 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 counts were made using 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 nm 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 (LP) of 515 nm (yellow/orange emission). PY-PC were identified as red fluorescing cells and enumerated using green excitation (Appendix 3) while the other cells

were counted using blue excitation. When excited with blue light, PE-PC appear as yellow/orange cells and PEuk emission is red. Although PP have traditionally been counted by epifluorescence microscopy (e.g. Pick and Caron, 1987), a fluorescence inverted microscope is also suitable. Comparisons between the two methods yield similar results.

Enumerations were made for 30 randomly chosen fields of view for each cell type at X1000 magnification. Cell counts included both rods 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 average radius of 0.5 µm) to biomass assuming a specific density of 1 g/cm^3, used by convention for all phytoplankton taxa. Total algal biomass was calculated from phytoplankton counts of cells greater than 2 µm using a Zeiss AXIO A1 inverted microscope at X200, X400, and X630 magnifications. Ten mL of preserved phytoplankton samples were settled overnight in 26 mm diameter chambers and enumeration of a minimum of 300 cells per sample were 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, total volumetric biomass (mg/m^3) for cells > 2 µm were obtained for each sample. Total biomass was then calculated by adding PP biomass to > 2 µm biomass values.

2.2.4 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 greater than 2 µm size fractions. Linear regressions were used to

determine the relationship between chl-*a* in < 2 μm size fraction and relative PP concentrations from 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 and all statistical analyses were done with S-Plus® version 8.0.

2.3 Results

2.3.1 Physical and chemical variables

The largest river, the Gatineau River, 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 2.1, A; Appendix 1). Water discharge varied seasonally with high discharge recorded midsummer in 2009, mostly in July (Fig 2.1, A). High discharge values were also seen in late fall, following many days of heavy rain. The South Nation and Castor rivers had the highest turbidity values (Fig 2.1, F). Water clarity was lowest following periods of high water discharge, as seen with the high turbidity reported in July and in the late fall.

Water temperature varied similarly in all rivers with low values recorded in late May and in the fall, and high temperatures reported in the late summer months (Fig 2.1, B). A maximum temperature of 28°C was recorded in the South Nation (June 24, 2009). Water pH and conductivity varied the least seasonally, but had more pronounced differences between rivers (Fig 2.1, C and D). The Gatineau River had the most neutral pH, while the other rivers represented more alkaline conditions. Conductivity varied from as low as 20 μS/cm in the Gatineau River to a maximum of 818 μS/cm in the Castor River. Dissolved oxygen (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 (August 25, 2009).

The Castor, South Nation, and Raisin rivers had the highest nutrient concentrations with the South Nation River being the most nutrient enriched (Fig 2.1, G to J). Seasonal variations in reactive phosphorus (RP) concentrations were observed for each river, with more pronounced differences observed in the South Nation River (Fig 2.1, G). The highest total phosphorus (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) while the Gatineau River had the lowest average TP (10 µg/L) and TN (376 µg/L) concentrations. Nitrate concentrations varied similarly to TN throughout the study period in all rivers (Fig 2.1, I).

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

2.3.2 Seasonal patterns in density

Seasonal patterns of abundance for each pigment group of PP were observed in the five rivers (Fig 2.2, A-J). The South Nation River showed the highest peak of PY-PC 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 2.2, A). A second, but less pronounced peak of PY-PC was observed in early August. The PEuk community reached high density values in late June and early July (~ 10^2-10^3 cells/mL). The PE-PC community showed consistently low and essentially negligible abundance (Fig 2.2, B).

In the Castor River (Fig 2.2, C and D), the highest peak ($2.07x10^4$ cells/mL) of PY-PC 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, PE-PC contribution to PP abundance was also negligible. As in the Castor River, the highest PP abundance recorded in the Raisin River occurred in early September ($3.66x10^4$ PY-PC cells/mL) (Fig 2.2, E). Two weeks prior and following that sampling event, the abundance of PY-PC was also high compared to the other sampling dates. In addition as in the Castor, PEuk abundance in the Raisin showed two peaks, the first in late June, and the second and highest ($3.24x10^3$ cells/mL) in early September. In comparison, the PE-PC community was negligible, as found in both the South Nation and Castor (Fig 2.2, F).

In the mesoeutrophic Rideau River, PP seasonal abundance patterns were different than those described in the more eutrophic systems (Fig 2.2, G and H). PY-PC abundance was high throughout the summer months, except in early August, following a major rain event. The highest abundance of PY-PC recorded was $4.54x10^4$ cells/mL in late August. For the most part, the PEuk community was less abundant than PE-PC cells. Phycoerythrin-rich PC 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 ($1.56x10^3$ cells/mL).

The Gatineau River had high abundances of PY-PC throughout the summer months (Fig 2.2, I), with the highest recorded in mid-July ($1.32x10^4$ cells/mL). PEuk and PE-PC had similar seasonal abundance patterns with high density (~ $1.5x10^3$ cells/mL) in early summer followed by continuous decreases in abundance (Fig 2.2, J).

For all the rivers, PY-PC was the most important PP pigment group (Table 2-2); on average, 75% of total PP abundance corresponded to phycocyanin-rich PC while PEuk cells

contributed 13%, and approximately 11% was represented by PE-PC. The median relative abundance of PE-PC was low for all rivers. However, on May 20, when phycocyanin-rich cells were not present in the sample, PE-PC 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).

2.3.3 PP biomass from chl-a and microscope counts

Chl-*a* concentrations and biomass from microscope counts showed variations in the distribution of phytoplankton biomass in the > 2 μm and < 2 μm size fractions within and among the rivers (Table 2-3). 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 9.05% 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 greater than 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 (38,220 μg/L). Taxonomic identification revealed that this high algal biomass was mostly caused by a bloom of the colonial green alga, *Pandorina morum* (Appendix 6), 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 2-3). 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).

Total algal biomass based on enumerations was also consistent with trophic state with the exception of the South Nation River that had the second lowest median value (Table 2-3). The relative contribution of PP to total biomass was much lower than the PP contribution to total chl-*a*. The median relative contribution of PP to total algal biomass ranged from 0.06% in the Castor River to 1.91% in the Gatineau River (Table 2-3).

Across rivers, the relative PP contribution to total chl-*a* showed no relationship with chl-*a* (Fig 2.3, upper panel: r^2= 0.050, p=0.126); whereas, the absolute PP chl-*a* showed a statistically significant positive relationship with total chl-*a* biomass (Fig 2.3, lower panel: r^2= 0.675, p<0.0001).

2.3.4 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 2-4). Positive correlations were associated with pH in both size fractions for chl-*a* and in the > 2 μm 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 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 negatively correlated to PP biomass from microscope counts (~ r = 0.40), 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 2.4, upper panel) and negative response to

nitrate concentrations (Fig 2.4, lower panel). Multiple regressions were used to evaluate the response of PP abundance to the environmental variables measured concurrently (DO, pH, temperature, conductivity (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 results of a Pearson's cross-correlation analysis, Appendix 2), 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 to all nutrient measurements and since pH was highly correlated to 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)).

2.4 Discussion

In contrast to earlier assumptions from the literature (Reynolds, 1994), the results of this study demonstrated that picophytoplankton can reach significant densities and are important contributors to phytoplankton biomass in rivers ranging in trophic state. 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 pigments groups, phycocyanin-rich cyanobacteria dominated PP abundance and comprised approximately three quarters of the total density. The strong dominance of PY-PC type was anticipated given the empirical and experimental evidence showing the numerical dominance of phycocyanin-rich picocyanobacteria in turbid waters (Pick, 1991; 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 (> 50%) of PY-cyanobacteria above extinction coefficients of 0.5/m. The river results are also consistent with reports of PY-PC dominance in shallow eutrophic lakes (Silvoso *et al.*, 2010). PE-PC were not significant contributors to overall PP abundance (~ 11%), but the highest average PE-PC abundance was found in the clearest river 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 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 midsummer (Pick and Agbeti, 1991; Stockner, 1991; Callieri and Stockner, 2002).

In the rivers of this study, the contribution of PP to total chl-*a* and biomass was significant (~ 27% on average) but was not a simple function of river trophic state (Table 2-3, Fig 2.2). In contrast, in lakes, the percent contribution of PP to total biomass clearly decreases with increasing trophic status (Søndergaard *et al.*, 1991, Vörös *et al.*, 1998; Callieri *et al.*, 2007). In this study, even in the most eutrophic system (i.e. South Nation River), PP still contributed to almost half of the total chl-*a*. These findings are consistent

with a recent study reporting a high contribution of PP to total planktonic chlorophyll in a eutrophic estuary (Gaulke *et al.*, 2010). PP biomass from microscope counts also accounted for as much as 9% of total biomass, which is consistent with estimates from Ontario lakes (Pick and Abgeti, 1991).

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 above 20°C (Agawin *et al.*, 2000; Gaulke *et al.*, 2010). In Lake Maggiore, maximal PP abundances were noted when surface water reached 18°C to 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 greater than 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 enumerations (Table 2.4). This is likely because the growth rates of PP are very fast (doubling times < 1- 2 days, Lavallée and Pick, 2002), particularly at higher temperatures, such that losses from advection downstream are 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. However, 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, reactive phosphorus was above detection and at times quite high, which is indicative of a surplus of bioavailable phosphorus. Interestingly enough, 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 nutrients on PP have been demonstrated experimentally in lakes (Tzaras *et al.*, 1999). While the larger phytoplankton biomass was not correlated with nutrients (phosphorus and nitrogen fraction), a significant correlation with conductivity was observed and conductivity has been considered a surrogate of productivity in rivers (Biggs, 1988). However, large phytoplankton in this river study were highly sensitive to discharge; this is in contrast to broader regional studies and larger data sets that suggest that nutrients are the primary factor controlling algal biomass in rivers as in lakes (Basu and Pick, 1996; Van Nieuwenhuyse and Jones, 1996).

In summary, this study demonstrated the importance of picophytoplankton to the plankton of temperate rivers, regardless of trophic state and river size. Given their high turnover rates, the community also likely plays an important role in river carbon and nutrient cycling that has yet to be recognized.

Table 2-1 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	3,810	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	3,830	41	48
Gatineau	45.6457	75.9192	6,840	126	127

Table 2-2 Median and range of the relative picophytoplankton (PP) abundance from fluorescence counts of each functional group: phycocyanin-picocyanobacteria (PY-PC), phycoerythrin-picocyanobacteria (PE-PC), and picoeukaryotes (PEuk) during 2009 (n=11).

	Relative abundance per functional group (%)		
River	PY-PC	PE-PC	PEuk
South Nation	93 (0-100)	4 (0-85)	6 (0-22)
Castor	56 (13-90)	3 (0-65)	24 (6-73)
Raisin	75 (31-95)	5 (1-54)	18 (4-53)
Rideau	94 (73-97)	2 (0-7)	3 (0-27)
Gatineau	90 (62-95)	5 (2-34)	4 (2-19)

Table 2-3 Median and range of chlorophyll-*a* (Chl-*a*) and algal biomass based on microscope enumerations during 2009 (n=8-11). The percent contribution of PP to total biomass (% PP) is also presented. Chl-*a* and biomass are both measured in µg/L.

		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 (271-38,220)	1,812 (1,103-5,397)	867 (305-3,545)	2,313 (744-26,547)	274 (120-413)
	> 2 µm	687 (270-38,211)	1,811 (1,102-5,396)	866 (304-3,543)	2,301 (743-26,529)	269 (114-408)
	< 2 µm	1.42 (0.13-257)	1.01 (0.06-12.3)	0.93 (0.28-20.2)	2.03 (0.49-24.5)	5.06 (0.87-8.14)
	% PP	0.16 (0.02-9.05)	0.06 (0-0.25)	0.10 (0.04-1.61)	0.09 (0.03-0.89)	1.91 (0.29-5.36)

Table 2-4 Pearson correlations and statistical significance based on Bonferroni adjusted probabilities (*p<0.05; ** p<0.01) for chlorophyll-*a* (chl-*a*) (n=47) and biomass based on microscope enumerations (n=54) of the picophytoplankton size fraction (< 2 µm) and phytoplankton greater than 2 µm in relation to the following variables: pH, dissolved oxygen percent saturation (%DO), temperature, conductivity (SPC), turbidity, water discharge, reactive phosphorus (RP), total phosphorus (TP), ammonia+ammonium (NH_3+NH_4^+), nitrate (NO_3^-), and total nitrogen (TN). %DO was arcsin transformed for normality and variables marked by † were log transformed.

	Chl-*a* (µg/L)		Biomass (µg/L)	
	< 2 µm	> 2µm	< 2 µm	> 2µm
pH	0.483*	0.655**	-0.125	0.711**
% DO	0.184	0.233	0.083	0.038
Temperature (°C)	0.109	0.381	0.613**	0.356
SPC (µS/cm)	0.363	0.466	-0.304	0.512**
Turbidity (NTU)	0.182	-0.035	-0.335	-0.008
Water discharge (m^3/s) †	-0.241	-0.506*	-0.030	-0.520**
RP (µg/L)	0.138	-0.034	-0.248	0.032
TP (µg/L)	0.332	0.290	-0.164	0.302
$NH_3 + NH_4^+$ (µg/L) †	0.107	-0.158	-0.386	0.106
NO_3^- (µg/L) †	0.009	-0.268	-0.408	-0.195
TN (µg/L) †	0.136	0.168	-0.406	0.230

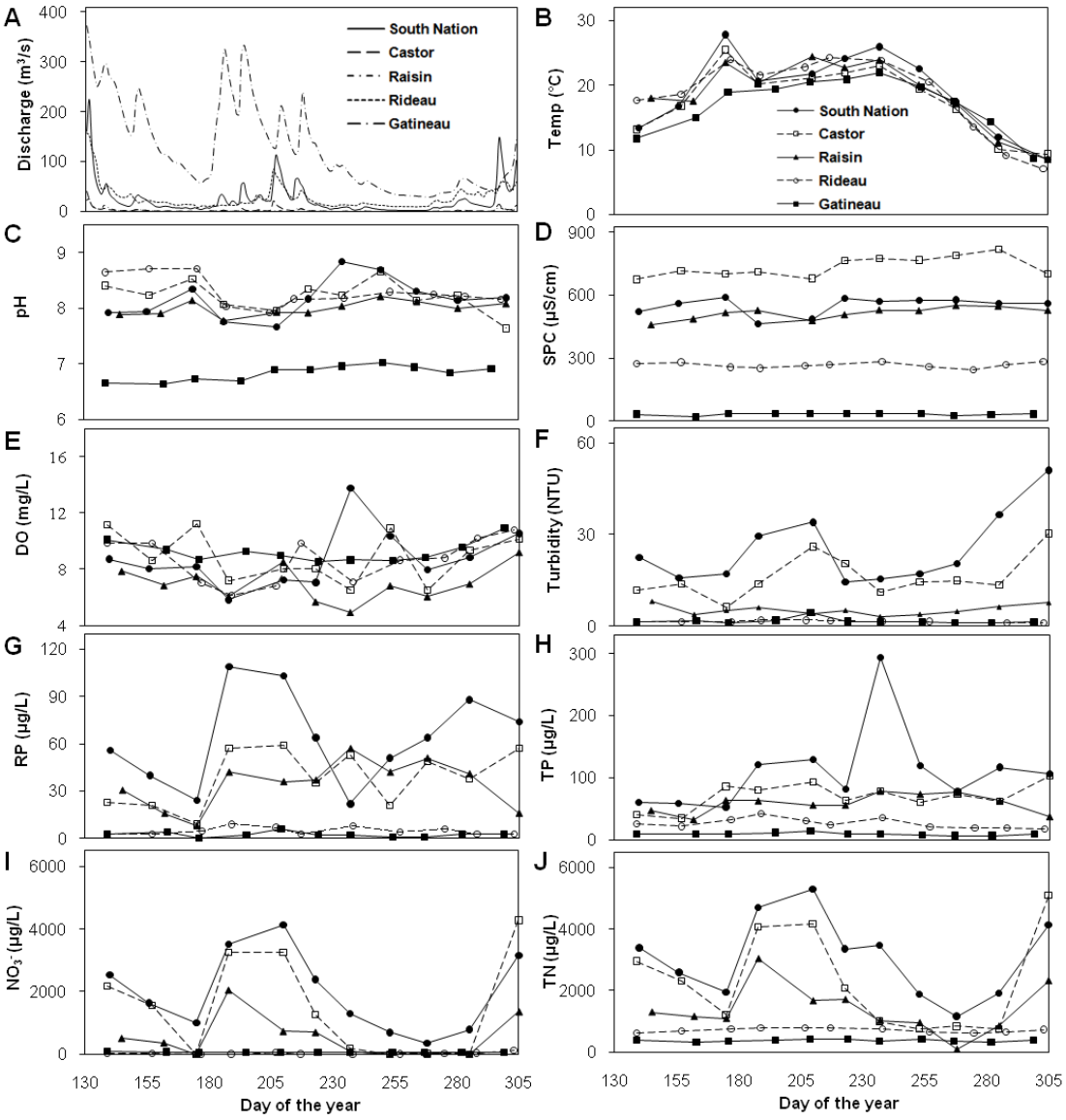

Fig 2.1 A-J: Physical and chemical variables of rivers, 2009. Daily water discharge from the Water Survey of Canada and Le centre d'expertise hydrique du Québec (A), temperature (B), pH (C), conductivity (D), dissolved oxygen (E), turbidity (F), reactive phosphorus (G), total phosphorus (H), nitrate (I), and total nitrogen (J). Figure legend in (B) applies to panels B-J.

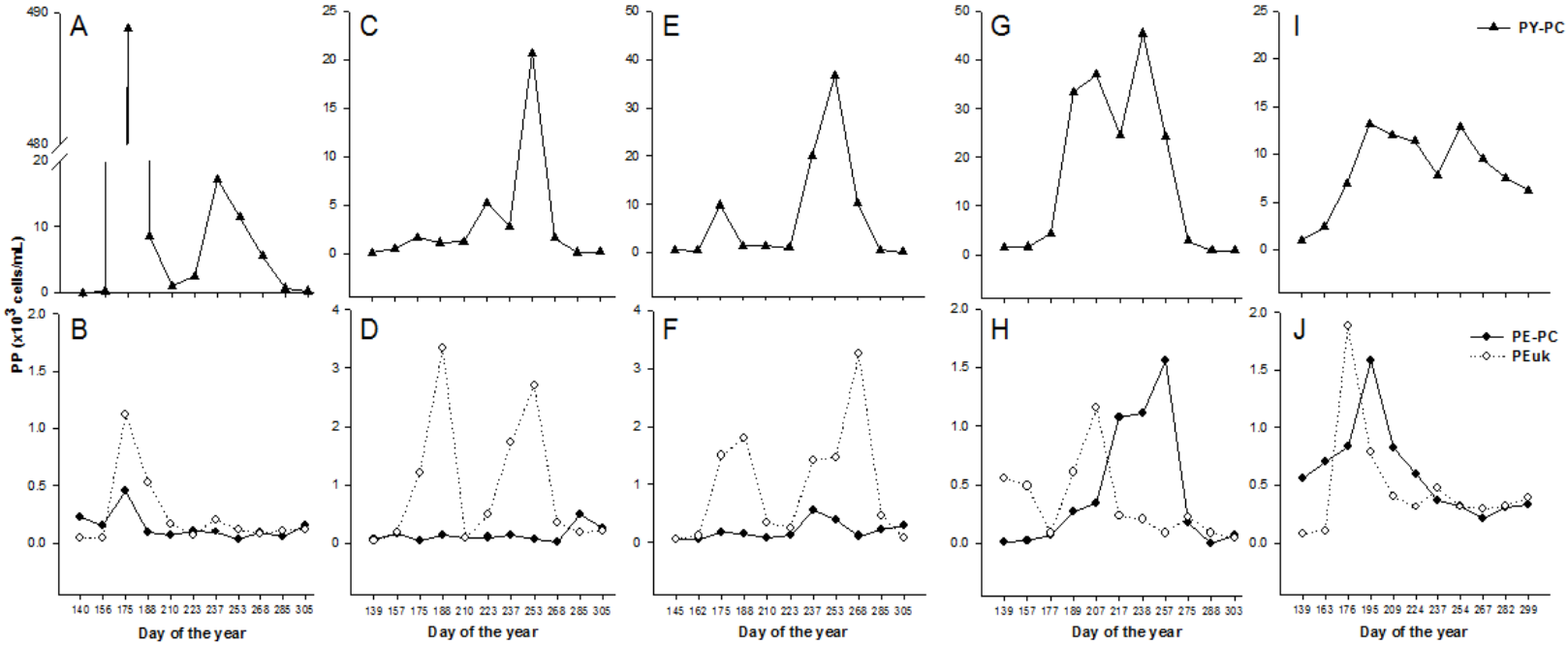

Fig 2.2 A-J: Picophytoplankton (PP) abundance separated by functional group: phycocyanin-rich picocyanobacteria (PY-PC), phycoerythrin-rich picocyanobacteria (PE-PC), and picoeukaryotes (PEuk) in the South Nation (A-B), the Castor (C-D), the Raisin (E-F), the Rideau (G-H), and the Gatineau (I-J) rivers. Figure legend in (I-J) applies for all panels. Note the change in scale between rivers and pigment groups.

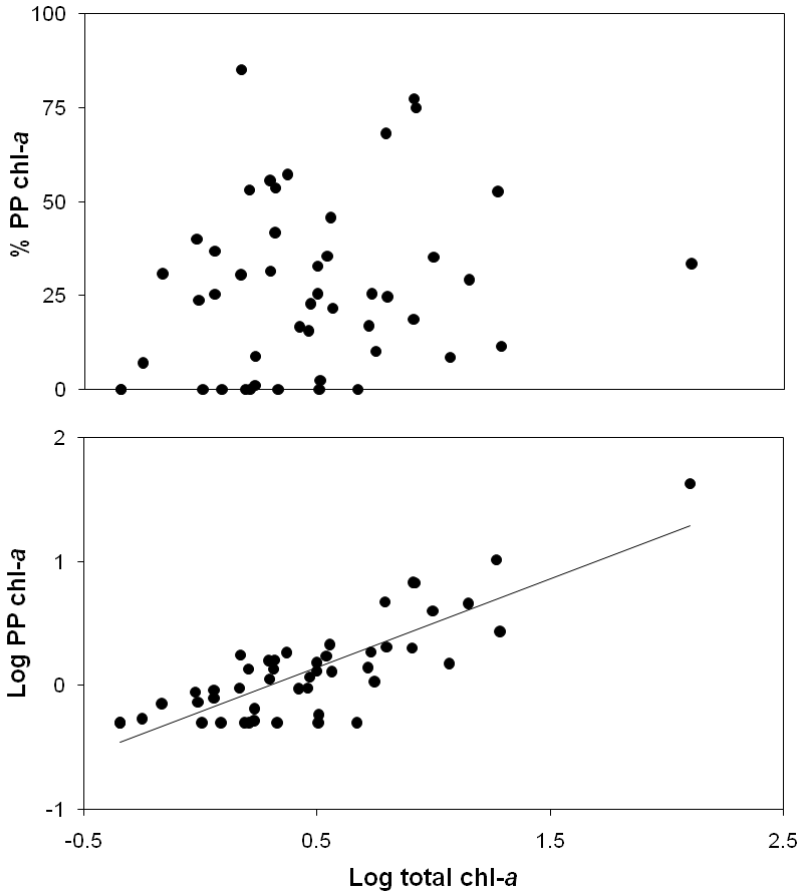

Fig 2.3 Relationship between relative (upper panel, r^2 =0.050, p=0.126) and log absolute (lower panel, r^2 =0.675, p<0.0001) picophytoplankton (PP) chlorophyll-*a* (chl-*a*) as a function of log total chl-*a*. Chl-*a* values are in µg/L (n=48).

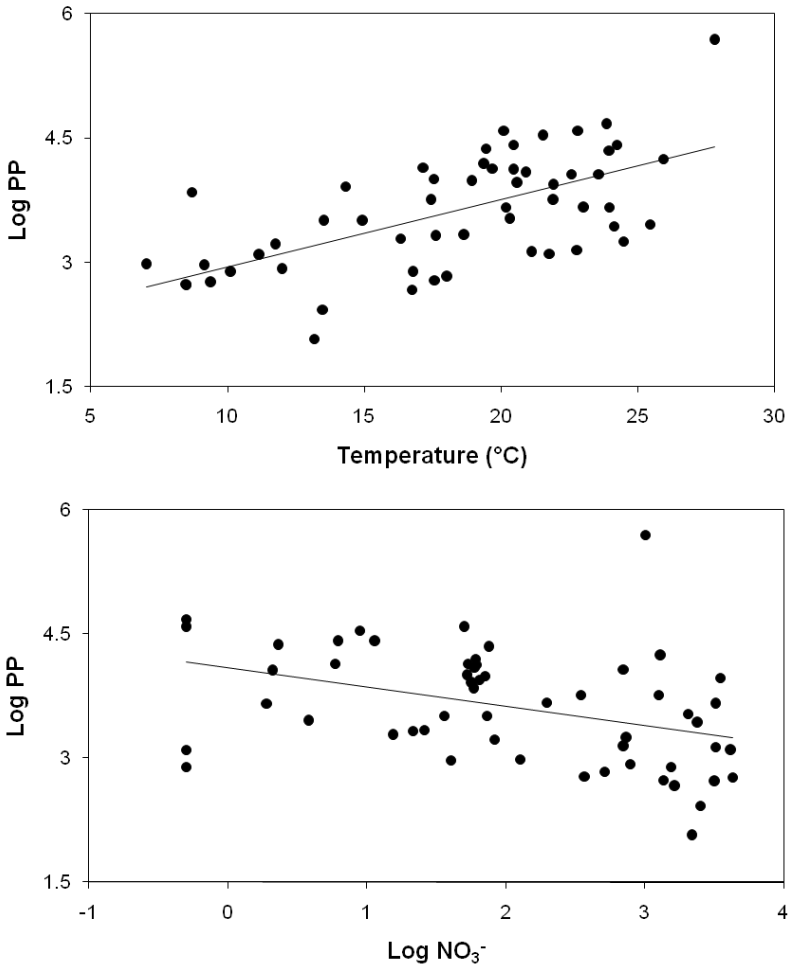

Fig 2.4 Linear regression of log PP abundance from fluorescence counts versus temperature (upper panel): r^2 =0.384, p<0.0001; and versus log of total nitrate (NO_3^-, µg/L) (lower panel): r^2 =0.155, p=0.003 (n=55).

- Chapter 3 -

The influence of season and trophic state on phytoplankton size distribution in temperate rivers

Abstract

The size structure of communities has been used as an alternative to taxonomic analyses to assess anthropogenic stresses in aquatic environments. This study examined phytoplankton size distribution using two methods in five temperate rivers ranging in trophic state throughout the ice-free season. Seasons were separated into four categories: spring, early summer, base flow, and fall. The size distribution of suspended chl-*a* was analyzed by linear regression as the proportion of chl-*a* retained on the filter versus filter size. Taxon-abundance spectra were analyzed by linear regression as phytoplankton density vs. the mean greatest axial linear dimension (GALD) of each taxon in the sample. The regression slopes for these analyses were used as a measure of change in the relative importance of small versus large cells. There was no significant effect of river or season on the slopes of both estimates of size distribution. However, the slopes of the suspended chl-*a* size distribution were positively correlated with total nitrogen (TN) concentrations, but the latter only explained 20% of the variance. Slopes for the taxon-abundance size spectra did not significantly vary with TN concentrations and neither size distribution was related to total phosphorus (TP) concentrations. The microplankton (20-64 μm) was the only size fraction to significantly respond to nutrients. The results of the study demonstrate the stability of the phytoplankton size distribution slopes across different rivers throughout the ice-free period and the lack of response to changing nutrient concentrations. The phytoplankton size distribution in temperate rivers appears relatively invariant and is thus not a useful indicator of river condition.

3.1 Introduction

In aquatic ecosystems, body size distributions have been used as a measure of community structure instead of taxonomic based descriptions (Cattaneo *et al.,* 1997; DeNicola *et al.,* 2006; Finkel *et al.,* 2009). This approach is particularly useful when analyzing planktonic communities since many small taxa are difficult to identify or remain unknown. Body size drives fundamental rate processes (e.g. metabolism, grazing, and sedimentation) that collectively affect ecosystem functions. Sheldon *et al.* (1972) was the first to describe a biomass distribution model of marine plankton communities and noted that the biomass found in logarithmically equal size intervals was roughly constant. Since Sheldon *et al.* (1972), studies have demonstrated the ecological significance and predictability of size biomass or abundance models in aquatic systems (Sprules *et al.*, 1983; Sprules and Munawar, 1986). Changes in cell size distribution can be used to provide early indications of stressors such as nutrient enrichment (Sprules and Munawar, 1986; Wunsam *et al*., 2002). However, in rivers, few studies have assessed phytoplankton size distribution (Rojo *et al.*, 1994; Chételat *et al.*, 2006).

Factors such as nutrients, temperature, and light can influence body size distribution in lakes and oceans (Cyr *et al.*, 1997). In general, algal biomass is usually controlled by nutrient concentrations but shifts in phytoplankton size distribution with nutrient enrichment can also occur because of different taxon size resource requirements or grazing pressure (Malone, 1980; Vanni, 1987). When nutrients are limiting, small cells often dominate larger cells because their high surface area to volume ratio makes them better at acquiring nutrients (Wen *et al.*, 1997). In lakes, nanoplankton often dominate oligotrophic systems, while larger cells are more abundant in eutrophic environments (Watson and Kalff, 1981; Kalff, 2002). However, studies of diatom size distribution have reported variables results. For example, a

study of intermediate-sized alkaline lakes reported negative responses of planktonic diatom cell size with increasing phosphorus and nitrogen concentrations (Finkel *et al.*, 2009).

Similar patterns have been observed for benthic algae in lakes and streams: *in situ* and experimental studies have demonstrated a positive response of periphyton cell size to increasing phosphorus concentrations in these systems (Cattaneo, 1987; Cattaneo *et al.*, 1997; DeNicola *et al.*, 2006). In Quebec streams, Lavoie *et al.* (2006, 2010) demonstrated that benthic diatom size distributions did not respond to environmental change, in particular phosphorus concentrations, and concluded that diatom body size should not be used as a proxy for nutrient status in environmental monitoring.

Trends observed in lakes might differ for rivers due to the physical differences related to discharge and light availability between the two types of ecosystems. In rivers, discharge may impact cell size distributions as longer residence times might allow for larger cells to increase their biomass. In a mesoeutrophic river, the Rideau River (Ontario), larger size classes (20-64 µm and > 64 µm) were observed more frequently in downstream reaches where water residence times were longer (Yang *et al.*, 1997). In rivers, the presence of suspended materials, combined with the lack of stratification, can lead to highly turbid environments. Under light limiting conditions, small cells are favoured since they can out-compete larger cells due to their greater light capturing abilities (Cole *et al*., 1992). This was observed in a study of 46 rivers in Ontario and western Quebec where the smallest cells, the nanoplankton (2-20 µm), dominated biomass (Chételat *et al.*, 2006). In temperate rivers, light limiting conditions would be more pronounced during periods of high flow such as the spring.

The goal of this study was to determine if phytoplankton size distribution can be used as an alternative method to taxonomic resolution when assessing the state of rivers. Since

previous studies have focused on slope analyses of size distributions (or size spectra) (Shin *et al.*, 2005, Petchey and Belgrano, 2010), I examined seasonal patterns of the slopes of both phytoplankton abundance and biomass versus cell size among different rivers ranging in physical and chemical conditions. I hypothesized that phytoplankton size distribution patterns are linked to trophy such that increases in nutrient concentrations would lead to the dominance of larger cell sizes. As found in lakes, smaller cells were expected to dominate under more oligotrophic conditions.

3.2 Methods

3.2.1 Study sites

Samples were collected from five temperate Ontario and Quebec rivers ranging in land use and nutrient concentrations. River descriptions and hydrological regimes are summarized in Chapter 2 (Table 2-1).

3.2.2 River sampling and laboratory analyses

Each river was sampled every two weeks from late May until early November 2009. On occasion, sampling was postponed for a minimum of two days following major rain events. A total of 11 samples were collected per river to represent phytoplankton communities and river conditions over the growing season (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), dissolved oxygen 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 is a measure of the degree of light scattering, related to particle concentration.

The water collected from each site was preserved in a 10% paraformaldehyde solution in order to maintain, for approximately one month, the naturally occurring fluorescence in picophytoplankton (Stocker *et al.*, 2000) and 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 analyzed 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 $N0_3$+$N0_2$.

3.2.3 Phytoplankton chl-a fractions and microscope enumerations

Chlorophyll-*a* (chl-*a*) concentrations in different size fractions were determined by parallel filtration the same day sampling occurred. Separate aliquots of water were filtered through 0.2 µm, 2 µm, 10 µm and 20 µm polycarbonate filters, whereas 35 µm and 64 µm were filtered through Nitex mesh. For the 0.2 µm and 2 µm filters, 250 mL of water was used whereas 500 mL was filtered through the larger pore size filters. A separate aliquot of 500 mL of water was also filtered through glass microfiber Whatman 934-AH 47 mm filters (~ 1.5 µm pore size). When filtering the water, the vacuum pressure was set below 15 mm Hg to avoid cell breakage. The polycarbonate membranes for the small pore sizes came from different providers (GE Polycarbonate from Water & Process Technologies for the 0.2 µm and 10 µm filters, and Poretics for the 2 µm and 20 µm filters).

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 hours, and concentrations were estimated with a Cary® 100 BIO UV-Visible Spectrophotometer, Varian Inc (Jespersen and Christoffersen, 1987). The 0.2 μm and 10 μm filters were less resistant to more aggressive solvents such as DMSO therefore ethanol was used to extract chl-*a* (Burnison, 1980; Jespersen and Christoffersen, 1987) from all filters. On the dates when analyses of the 2 μm and 10 μm fractions were taken in duplicate, the average and the coefficient of variation (CV) were calculated. Across rivers, the CV of the 2 μm filters averaged 14% (range 7-21%) and 11% for the 10 μm filters (range 6-15%).

Phytoplankton identification and enumerations were made using a Zeiss AXIO A1 inverted microscope. Picophytoplankton (0.2-2 μm) counts were made for 30 randomly chosen fields of view at X1000 magnification under fluorescence (Chapter 2). An aliquot of 20 mL was vacuumed at low pressure (< 200 mm Hg) on Irgalan Black pre-stained polycarbonate 0.2 μm membranes. Phytoplankton greater than 2 μm preserved in Lugol's were counted and measured under light microscopy at X200, X400, and X630 magnifications. Ten mL of Lugol's preserved water samples were settled overnight in 26 mm diameter chambers and the enumeration of a minimum of 300 cells per sample were 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). Algamica calculates cell density and the mean greatest axial linear dimension (GALD) based on dimensions measured for each taxon per sample. The program also computes total density for the following size fractions: picoplankton (< 2 μm), ultraplankton (2-10 μm), nanoplankton (10-20 μm), microplankton (20-64 μm), and

netplankton (> 64 µm). Picophytoplankton abundance from fluorescence counts were added to the light microscope counts using a GALD of 1 µm.

3.2.4 Data analysis

Seasonal data were pooled into four time periods: spring, early summer, base flow, and fall. Spring was based on three sampling occurrences from late May until end of June, representing the period of declining discharge. The early summer category consisted of three sampling dates covering July to early August. Since during 2009 heavy rain was recorded throughout the month of July, base flow conditions occurred later during the ice-free season; late August and September (n=3) represented base flow conditions. Fall samples were represented by two sampling dates that took place in October and early November when the flows tend to rise again.

The relationship between the proportion of chl-*a* retained on the filter and filter size was used to describe the size distribution of suspended chl-*a* for each river according to season. The slope of this relationship was then used to quantify in one metric the relative importance of small versus large cells. This approach is similar to the one used by Mazumder *et al.* (1988) to examine the size distribution of particulate phosphorus in aquatic systems. The slopes of the relationship between phytoplankton density and the GALD of each taxon across the rivers and seasons were also analyzed, and included all phytoplankton taxa. This is similar to mass density analyses developed for other taxonomic groups (e.g. Currie and Fritz, 1993) and relates to metabolic scaling, an active area of research in ecology (e.g. White *et al.*, 2007).

Statistical analyses consisted of correlations, regressions, and analysis of variance (ANOVA). Pearson correlation analysis was used to determine the response of

phytoplankton density separated into size fractions to TP and TN. Linear regressions were used for the analysis of the size distribution (slope data) of suspended chl-*a* and taxon-abundance, and to determine the relationship between size distribution and environmental variables. Two-way ANOVAs were carried out to determine the effect of river and season on the phytoplankton size distributions based on chl-*a* and taxonomic enumerations. One-way ANOVAs were used to determine the effect of different size fractions and the effect of seasonal periods on phytoplankton biomass from chl-*a* and density. For these analyses, the assumption of normality, homoscedasticity, and independence were tested using the residuals and statistical significance was considered when $p<0.05$. All statistical analyses were done with S-Plus® version 8.0; certain variables required log transformations to satisfy normality.

3.3 Results

3.3.1 Physical and chemical variables

Physical and chemical parameters varied throughout the study period and across the different rivers. A summary of these variables can be found in Chapter 2 (Fig 2.1, Appendix 1).

3.3.2 Size distribution of suspended chl-a

The size distribution of suspended chl-*a* was examined across the five rivers and seasons (Fig 3.1 and Table 3-1). The relationships between filter size and the proportion of chl-*a* observed in size fractions ranging from picoplankton to net plankton were all strong and highly significant ($r^2 > 0.80$, $p<0.05$). The regression slopes ranged from -0.683 to -0.284: more negative slopes mean a greater proportion or dominance of small cells vs. large cells. The average slope was -0.455 and ranged from an average of -0.504 in the Gatineau to

-0.417 in the South Nation. Seasonally, the shallowest slope was observed in the Castor during spring (-0.284) and the steepest slope in the Gatineau during fall (-0.683). However, overall there was no significant effect of river or season on the slopes of the size distribution of chl-*a* and there were no significant interactions (two-way ANOVA, $p>0.05$).

A one-way ANOVA determined a significant difference between relative chl-*a* biomass in three size fractions ($p<0.05$) (Fig 3.2). On average, the smallest size fraction (2-20 μm) accounted for almost two-thirds of total biomass, cells between 20 and 64 μm represented 32% of biomass, and the largest cells (> 64 μm) contained the least biomass (4%). The dominance of small cells was consistent throughout the study period.

3.3.3 Phytoplankton density size distribution

Phytoplankton taxon abundance in relation to the GALD of each taxon was also analyzed seasonally within and between the rivers (Fig 3.3 and Table 3-2). GALD generally explained less than half of the variance ($r^2 \approx 0.40$) in phytoplankton density, but all relationships were significant, with the exception of two sampling dates. The average regression slope was -1.061 and ranged from an average of -1.168 in the Rideau to an average of -0.967 in the Gatineau. The South Nation had the steepest individual slope in spring time (-1.941) and the Raisin the shallowest individual slope (-0.495) in early summer (Table 3-2). Overall, the steepest slopes tended to be associated with the base flow period (-1.179) and the shallowest in the fall (-0.911). However, as for the chl-*a* size distributions, there were no significant effects of river and season on the taxon-abundance spectra (two-way ANOVA, $p>0.05$) and no interactions detected.

Phytoplankton density differed significantly among the five size fractions and with respect to season (one-way ANOVA, $p<0.05$) (Fig 3.4). As expected, the picoplankton size

fraction had the highest median density ($3.42x10^6$ cells/L), followed by the ultraplankton, nanoplankton, and microplankton. The least abundant size fraction was netplankton with a median density of $1.63x10^4$ cells/L. Across seasons, base flow had the highest median density ($1.41x10^7$ cells/L) and this was statistically different (t-test; $p<0.05$) than the lowest median density observed in the fall ($2.03x10^6$ cells/L).

3.3.4 Nutrients and phytoplankton cell size

There was no relationship between the slopes of the size distribution of suspended chl-*a* and TP (r^2 =0.061, p=0.071) but there was a significant positive relationship with TN (r^2 =0.196, p<0.001) (Fig 3.5). The chl-*a* based slopes were less steep in samples with higher TN indicating that larger taxa were more important under higher nitrogen concentrations. No statistically significant relationships between the slopes of the size-abundance spectra from density vs. GALD measurements (Fig 3.6) were observed between TP concentrations (r^2 =0.005, p=0.610) and TN (r^2 =0.056, p=0.083).

Pearson coefficients correlating phytoplankton abundance in varying size fractions to TP and TN were mostly non significant (Table 3-3). The only statistically significant result was a positive response of microplankton (20-64 µm) to TP. Picoplankton and netplankton (> 64 µm) showed negative trends with respect to both TP and TN while the other size fractions showed slight positive tendencies with nutrients.

3.4 Discussion

Previous lake studies have observed changes in size spectra slopes that indicate an increase of larger cells in perturbed systems (e.g. Sprules and Manuwar, 1986). However, in this river study, the size distribution of the phytoplankton community whether derived from

chl-*a* estimates of biomass or taxon-abundance did not change significantly between rivers, despite the large nutrient gradient (~ 2.5 orders of magnitude). The slopes also did not vary significantly between spring, early summer, base flow, and fall conditions for both types of analyses. The stability of the slopes observed is consistent with recent studies of benthic diatom communities in streams, where body size distribution did not change across 29 streams in eastern Canada throughout the ice-free season (Lavoie *et al.*, 2006, 2010).

In this study, the across river variation in size distribution was still greater than the within river seasonal variation for both types of analyses. For chl-*a*, the average difference in slope within rivers seasonally was 0.26 and the difference was almost double this across the rivers (0.40). For taxon-abundance analyses, the slopes varied an average of 0.96 within each river and 1.73 between the rivers. Other environmental variables such as discharge might have affected the slopes since flow is more variable across than within rivers.

As opposed to all the chl-*a* based analyses, the relationship between density and GALD was not statistically significant on two sampling dates (Table 3-2). Both cases were unusual. In the South Nation, under the base flow conditions of August 25, a very steep slope resulted from the high abundance of small cells from a bloom of the colonial green alga, *Pandorina morum* (Appendix 6). The colonies had an approximate GALD of 30 μm. However, the Lugol's iodine preservative caused the colonies to separate into individuals and the taxon was mostly counted as having a much smaller size (~ 5-6 μm). The second non-significant regression was found in the Castor River during spring. The variation was mostly due to the May 19 sampling when three large diatoms, likely of benthic origin, were very abundant (~ 10^4-10^5 cell/L): *Diatoma* spp., *Navicula* spp., and *Nitzschia* spp. had GALD values ranging from 48 to 62 μm. On the two other spring dates, such large and mostly benthic species were either not observed or were much less abundant in the Castor. In

general, the shallowest rivers tended to have a few benthic species entrained into the plankton, especially during spring and fall when discharge was highest (Chapter 4).

Based on the linear regression slopes, the only significant response to nutrients was an increase in chl-*a* biomass of the larger size fraction with increasing TN concentrations. An increase in larger phytoplankton is typically observed in lakes with increasing nutrients (Kalff, 2002). However, here only a small amount of variation in size distribution (i.e. slopes) was explained by TN (~ 20%). Furthermore, no significant response was observed between the size distribution of phytoplankton density with TP or TN concentrations. The only size fraction that was significantly correlated to nutrients was the microplankton (20-64 µm), where an increase in TP lead to higher abundances in this size fraction. The relationship of microplankton with TN was not quite significant, perhaps because of the conservative nature of Bonferroni corrections. Similarly, the relationship between picoplankton and TN was almost significant, consistent with the results of Chapter 2.

The size distribution analysis for both chl-*a* and phytoplankton density found little variation across the seasons, suggesting that the size distribution of river phytoplankton remained essentially constant throughout the ice-free period. However, the highest phytoplankton densities were observed during base flow and the lowest densities in the fall (Fig 3.4), largely reflecting the changes in picophytoplankton abundance (Chapter 2). Small cells (2-20 µm) represented the majority (64%) of total phytoplankton chl-*a*. This is comparable to studies that have observed dominance of nanoplankton (< 20 µm) in the summer months of rivers (Gosselain *et al.*, 1988; Chételat *et al.*, 2006).

The lack of variation in slopes regardless of changing environmental conditions and taxonomic composition indicate that phytoplankton size distribution is not a reliable metric

of change in nutrient concentrations in rivers and would not be a useful tool for monitoring these ecosystems, as has been widely advocated for marine and lake ecosystems. The results point to fundamental differences in body size relationships between lakes or oceans and flowing waters.

Table 3-1 Regression analyses of slopes for phytoplankton size distribution based on chl-*a* (log proportion of chl-*a* retained vs. log filter size). Average slope and standard deviation, as well as average r^2 values of three sampling occasions during spring, early summer, and base flow and two sampling events represent fall values. Sample size (n) represents the number of filters analyzed per sample. All regressions were significant ($p<0.05$).

River	Season	Slope	Std dev	r^2	n
South Nation	spring	-0.466	0.086	0.908	6-7
	early summer	-0.396	0.053	0.789	6-7
	base flow	-0.393	0.014	0.843	6-7
	fall	-0.407	0.030	0.883	7
Castor	spring	-0.384	0.088	0.731	6-7
	early summer	-0.474	0.135	0.819	6-7
	base flow	-0.446	0.051	0.831	7
	fall	-0.427	0.105	0.644	7
Raisin	spring	-0.419	0.079	0.848	7
	early summer	-0.465	0.038	0.798	7
	base flow	-0.563	0.040	0.736	5-7
	fall	-0.416	0.003	0.735	7
Rideau	spring	-0.475	0.078	0.858	7
	early summer	-0.462	0.117	0.951	6-7
	base flow	-0.458	0.037	0.843	7
	fall	-0.406	0.049	0.921	7
Gatineau	spring	-0.457	0.046	0.698	7
	early summer	-0.471	0.013	0.833	7
	base flow	-0.520	0.056	0.748	7
	fall	-0.577	0.150	0.922	6-7

Table 3-2 Summary of the size density regression analyses of log density vs. log mean greatest axial linear dimension (GALD). Average slope and standard deviation, as well as average r^2 values of three sampling occasions represent spring, early summer, and base flow and two sampling events represent fall values. Sample size (n) represents the number of phytoplankton taxa in each sample. All regressions were statistically significant ($p<0.05$) with the exception of the South Nation during base flow and the Castor in the spring.

River	Season	Slope	Std dev	r^2	n
South Nation	spring	-1.268	0.587	0.404	34-39
	early summer	-1.239	0.203	0.434	31-44
	base flow	-0.921	0.318	0.235	21-31
	fall	-0.958	0.094	0.484	37-43
Castor	spring	-0.795	0.503	0.255	28-40
	early summer	-1.005	0.096	0.366	33-47
	base flow	-1.269	0.048	0.447	39-47
	fall	-0.774	0.303	0.389	36-48
Raisin	spring	-1.116	0.330	0.516	37-41
	early summer	-0.729	0.282	0.291	38-45
	base flow	-1.492	0.286	0.552	37-45
	fall	-0.976	0.267	0.481	35-37
Rideau	spring	-1.087	0.202	0.360	27-34
	early summer	-1.317	0.147	0.473	26-57
	base flow	-1.270	0.156	0.385	34-47
	fall	-0.911	0.077	0.401	26-36
Gatineau	spring	-0.897	0.122	0.404	38-42
	early summer	-1.064	0.125	0.450	38-49
	base flow	-0.945	0.084	0.399	46-48
	fall	-0.937	0.032	0.267	46-47

Table 3-3 Bonferroni corrected Pearson correlation coefficients (*p<0.01) relating phytoplankton density of different size classes to total phosphorus (TP, µg/L) and total nitrogen (TN, µg/L). Data were pooled from all five rivers throughout the sampling season (n=55). All variables were log transformed.

Density (cell/L)	TP	TN
Picoplankton (< 2 µm)	-0.143	-0.369
Ultraplankton (2-10 µm)	0.196	-0.177
Nanoplankton (10-20 µm)	0.304	0.004
Microplankton (20-64 µm)	0.506*	0.367
Netplankton (> 64 µm)	-0.131	-0.236

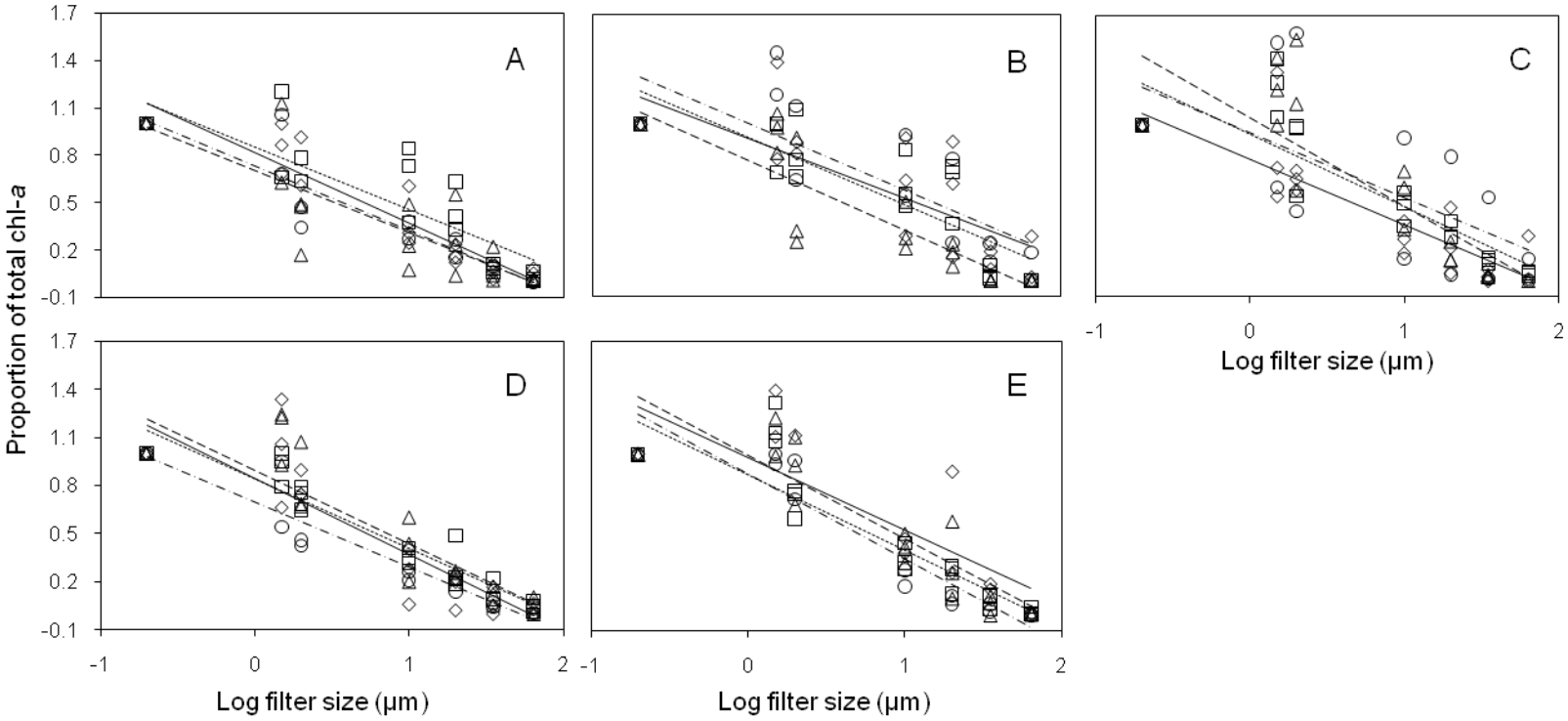

Fig 3.1 The size distribution of chl-*a* from the proportion of total chl-*a* (µg/L) retained on the filter and the log value of the filter size in the South Nation (A), the Castor (B), the Raisin (C), the Rideau (D), and the Gatineau (E) rivers. Symbols and regression lines represent the following seasonal categories: spring (◊, ———); early summer (□, ---------); base flow (Δ, – – –); and fall (○, – · – · –). A summary of the results are presented in Table 3-1.

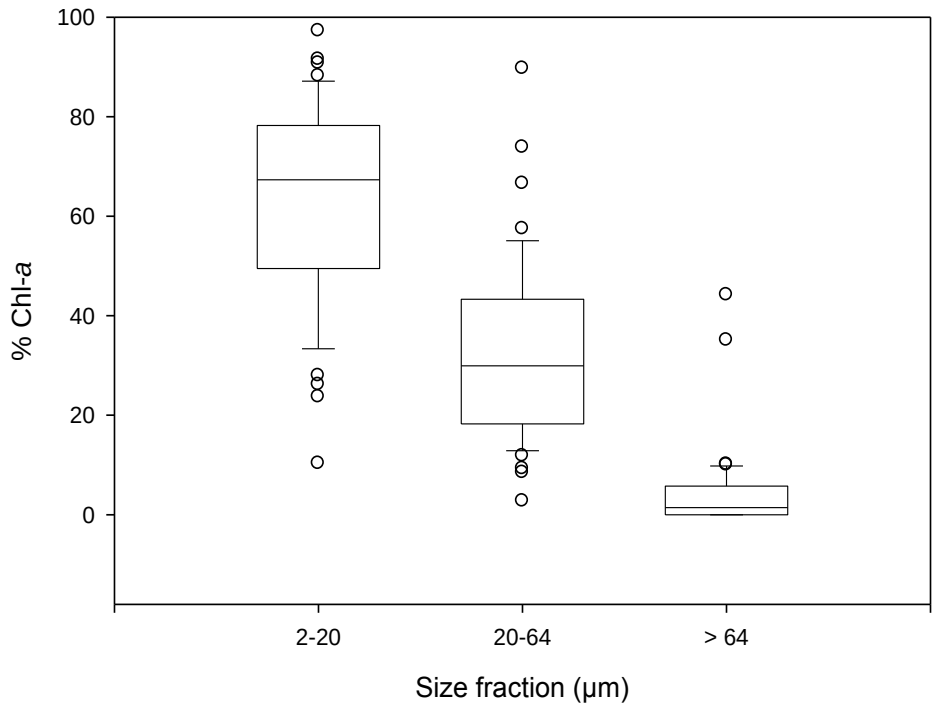

Fig 3.2 Box plots of the relative phytoplankton chl-*a* in three size fractions (2-20, 20-64, and > 64 µm). Each box represent the pooled data of the five rivers throughout the sampling season (n=46). The central line of each box represents the median, the outer edges are the 25th and the 75th percentiles, and the limits of error bars are at the 10th and 90th percentiles. Points display the mean of outlying values.

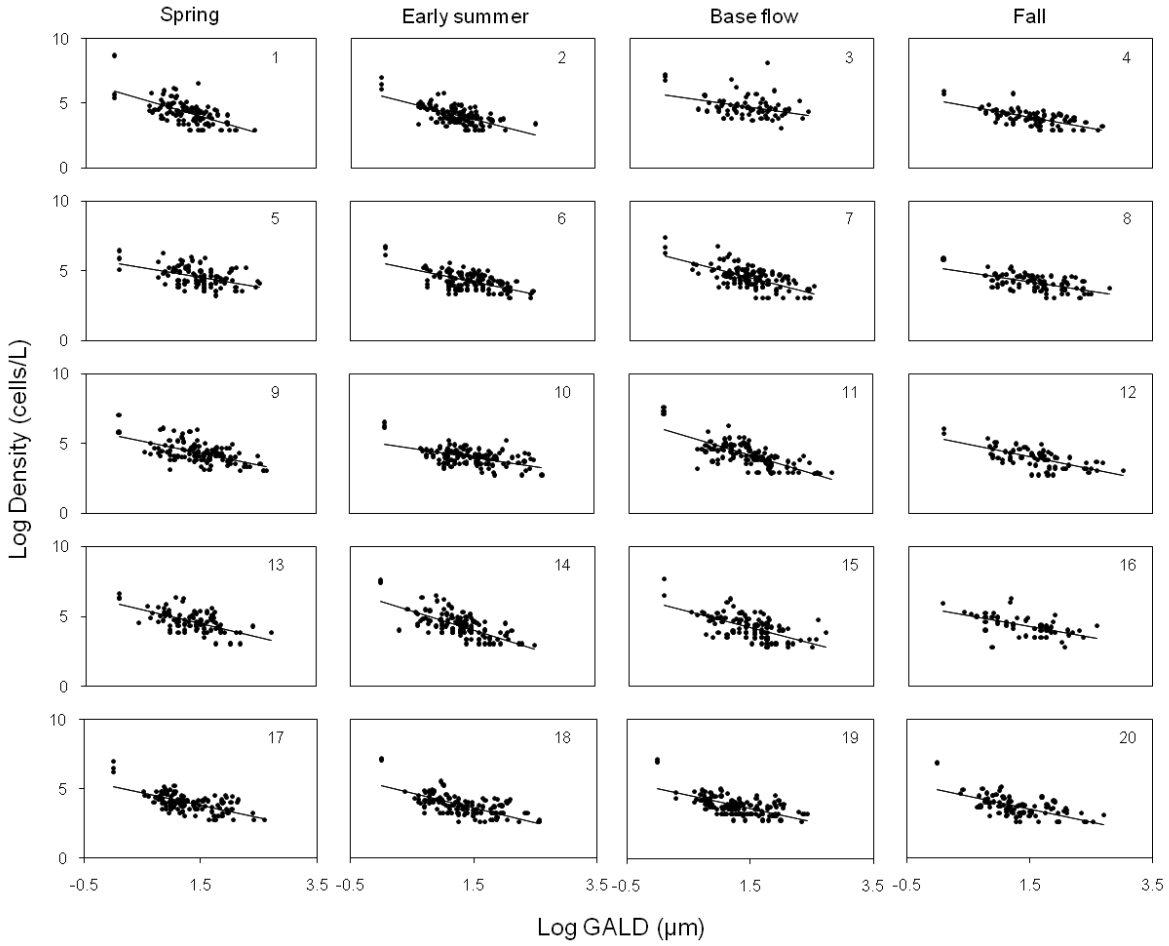

Fig 3.3 The size-abundance spectra of each river (South Nation: 1-4; Castor: 5-8; Raisin; 9-12; Rideau: 13-16; Gatineau: 17-20) presented as the log density of phytoplankton as a function of the log mean greatest axial linear dimension (GALD) of each taxon per seasonal category (spring, early summer, base flow, and fall). A summary of the results are presented in Table 3-2.

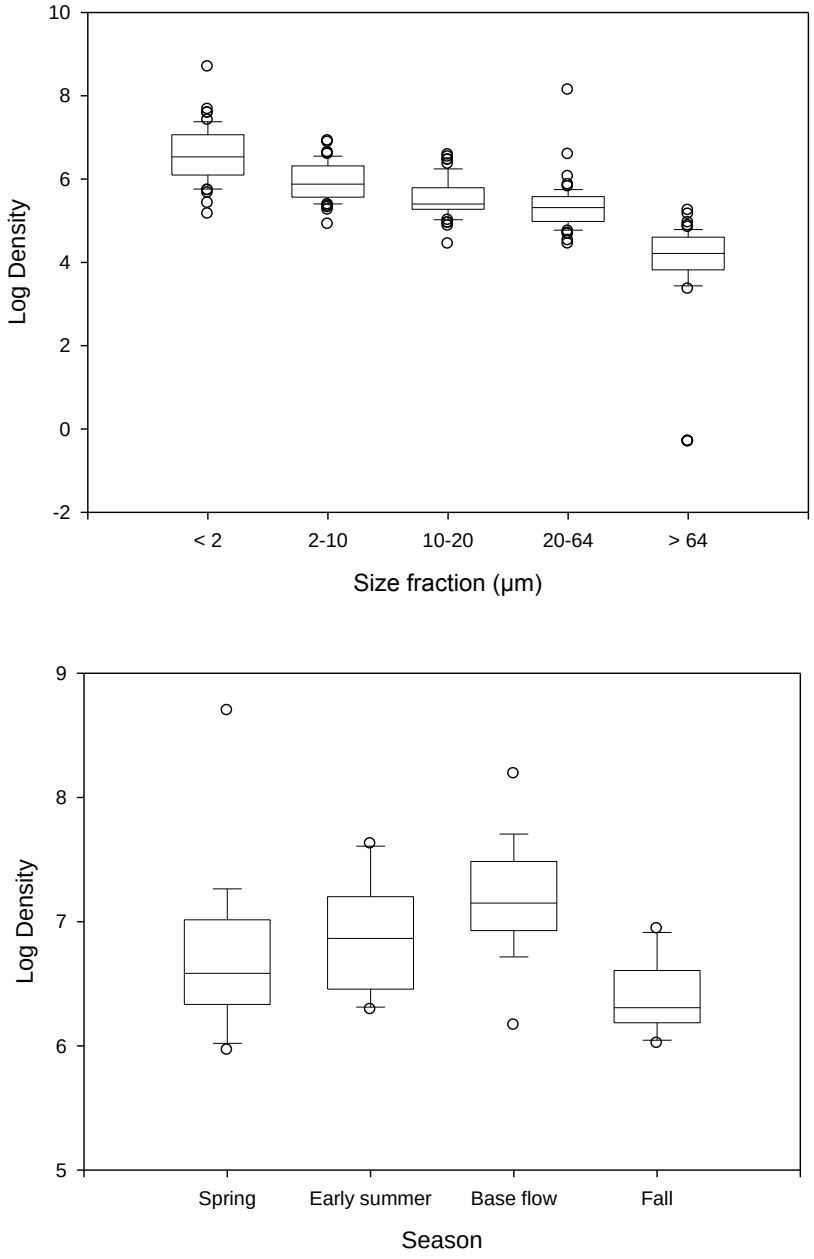

Fig 3.4 Box plots of the density (cells/L) of phytoplankton in five size fractions: picoplankton (< 2 µm), ultraplankton (2-10 µm), nanoplankton (10-20 µm), microplankton (20-64 µm), and netplankton (> 64 µm) (upper panel), and per seasonal category: spring, early summer, base flow, and fall (lower panel). Each box represent the pooled data of the five rivers throughout the sampling season (n=55) (upper panel) and the total density per sampling event, pooled for the five rivers in spring, early summer and base flow (n=15), and fall (n=10) (lower panel). The central line of each box represents the median, the outer edges are the 25th and the 75th percentiles, and the limits of error bars are at the 10th and 90th percentiles. Points display the mean of outlying values.

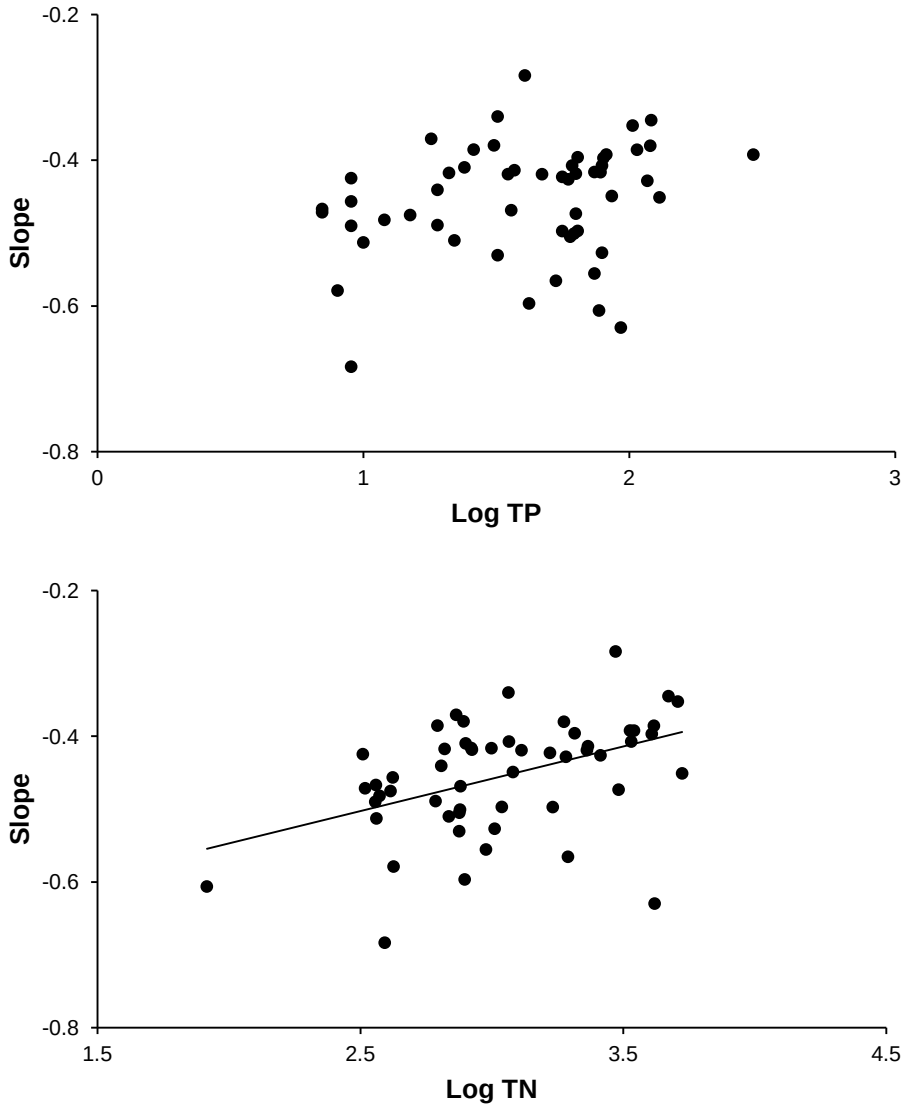

Fig 3.5 The size distribution of chl-*a* (represented as the slope of relationships of Fig 3.1) in relation to total phosphorus (upper panel; TP, µg/L) and total nitrogen (lower panel; TN, µg/L). Each point represents an individual river sample (n=54). The relationship was not significant with TP concentrations (r^2=0.061, p=0.071), but was significant with TN concentrations (r^2 = 0.196, p<0.001).

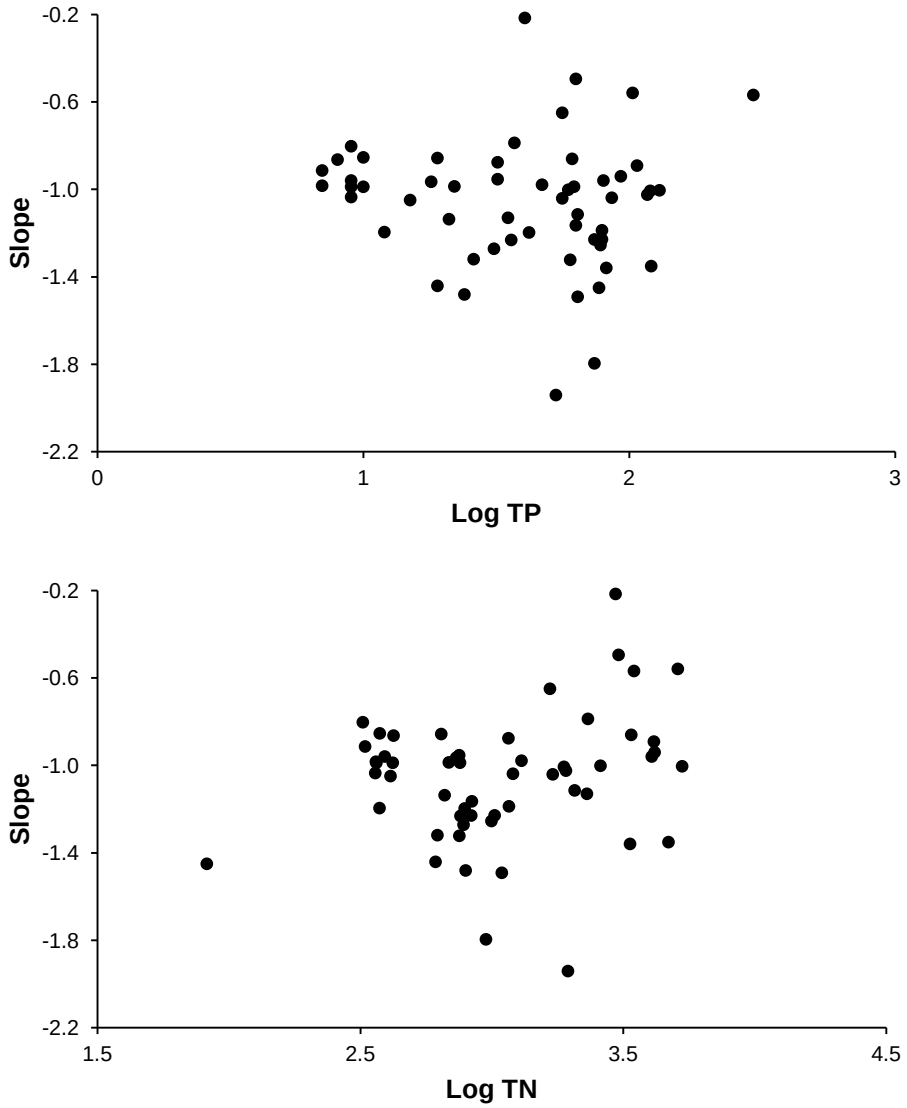

Fig 3.6 The taxon-abundance spectra (represented as the slope of relationships of Fig 3.3) in relation to total phosphorus (upper panel; TP, µg/L) and total nitrogen (lower panel; TN, µg/L). Each point represents an individual river sample (5 rivers on 11 sampling dates, n=55). The relationship was not significant for TP concentrations (r^2=0.005, p=0.610) or TN concentrations (r^2 = 0.056, p=0.083).

- Chapter 4 -

Seasonal succession of phytoplankton taxa in temperate rivers and response to environmental factors

Abstract

The taxonomic composition of phytoplankton communities was assessed throughout a growing season in five temperate rivers ranging in trophic state (9-107 µg/L total phosphorus) to determine the impact of major environmental factors such as nutrients and flow on community structure. Even though diatoms were on average the most important division (38% of total biomass) in four of the five rivers, other taxonomic divisions were also important. Cryptophytes (mainly *Cryptomonas*) rather than diatoms dominated biomass in the mesoeutrophic Rideau River. More eutrophic rivers showed stronger overall dominance of particular taxa. A bloom of colonial green alga (*Pandorina morum*) accounted for as much as 90% of total biomass in the more nutrient enriched system compared to the highest dominance (34%) found in the oligotrophic Gatineau River. Despite differences in dominance patterns, the overall species richness was independent of trophic state and fairly constant (the median number of taxa in individual samples ranged from 30 to 39). Seasonal succession differed across the five rivers and no consistent pattern emerged such as described for lakes. Collectively, a negative response to water discharge was found for diatoms, cryptophytes, chlorophytes, and euglenoids while reactive and total phosphorus were positively associated with euglenoids. Chrysophytes biomass decreased with increasing nitrate concentrations and diatoms were positively correlated with total nitrogen.

4.1 Introduction

The study of phytoplankton taxonomic composition provides more valuable information than aggregate measures of community structure such as algal biomass (chlorophyll-*a*) and size distribution analyses (e.g. Sabater *et al.*, 2008). Changes in environmental conditions can shift phytoplankton species composition and alter ecosystem processes, function, and stability which makes understanding taxonomic changes important for ecosystem management (e.g. Suikkanen *et al.*, 2007). In lakes, it has been well established that phytoplankton community composition and diversity change with increasing nutrients (Reynolds, 1984). These changes occur because of the different nutrient uptake, storage, growth, and loss rates among different phytoplankton taxa (Kalff and Knoechel, 1978; Reynolds, 1984). However, in rivers, phytoplankton communities have not been as intensively studied and are still poorly understood (Basu and Pick, 1996; Piirsoo *et al.*, 2008; Wu *et al.*, 2011).

In temperate rivers, diatoms are considered to be the most important taxonomic group both in terms of abundance and biomass (Rojo *et al.*, 1994). Diatom based studies have observed that shifts in species composition can provide evidence of anthropogenic impacts (e.g. Lowe, 1974; Yang *et al.*, 1996) and the presence of certain species can be indicative of the state of the ecosystem. For example, *Aulacoseira granulata* and *Stephanodiscus parvus* are diatom species representative of nutrient enriched lakes and typically bloom in highly eutrophic European rivers, dominating total algal biomass (Moss and Balls, 1989; Rojo *et al.*, 1994; Cumming *et al.*, 1995). However, other phytoplankton taxonomic groups can constitute an important component of total abundance and biomass. In a survey of 67 rivers, Rojo *et al.* (1994) found that diatoms represented 40% of the average number of species in temperate rivers, yet green algae contributed 28%, cyanobacteria 10%, and euglenoids 7%. A

study of 31 temperate rivers in Ontario and Quebec observed that diatoms also dominated phytoplankton biomass (34% of total); however, other divisions were also significant to total biomass (Chételat *et al.*, 2006). These divisions were cryptophytes (24%), chlorophytes and chrysophytes (15%); in contrast, cyanobacteria were not well represented (3%). Therefore, it is important to consider other lesser known phytoplankton groups besides diatoms.

In lakes, phytoplankton distribution patterns are strongly correlated with environmental variables (Reynolds, 1984; Kalff, 2002). However, in rivers, it can be difficult to determine if hydrological, physical, chemical, or biological factors are most important in controlling community composition (Basu and Pick, 1995). Some studies have indicated that nutrients such as phosphorus concentration control phytoplankton biomass (e.g. Søballe and Kimmel, 1987; Basu and Pick, 1996; Van Nieuwenhuyse and Jones, 1996) while other studies have indicated that hydrological conditions are more important to phytoplankton development (e.g. Pace *et al.*, 1992; Reynolds *et al.*, 1994). In rivers as in lakes, dissolved silicate is particularly important to sustain diatom growth as it is needed to build their cell walls (Bormans and Webster, 1999). When concentrations of silica are depleted in rivers, this can lead to a collapse in diatom populations (De Ruyter van Steveninck *et al.*, 1992). Although this does not affect algal biomass, it can explain shifts in phytoplankton community abundance.

Classic lake phytoplankton seasonal succession models such as the Plankton Ecology Group-model for temperate lakes (Sommer *et al.*, 1986) may not be readily applied to rivers as different factors may be controlling phytoplankton development (e.g. short water residence times and/or weak top-down control from zooplankton). However, early river studies have observed patterns such as diatom blooms in the spring, followed by shifts of dominance between green algae and cyanobacteria in the summer months, and then

dominance of diatoms and/or dinoflagellates in the fall (Lewis, 1978). In the Rideau River, phytoplankton seasonal succession was observed with high diatom biomass in early summer followed by cryptophytes and chlorophyte dominance in the late summer (Yang *et al.*, 1997; Ley and Hamilton, 1995; Ley *et al.*, 1997). However, very few studies have analyzed seasonal patterns of phytoplankton species composition in rivers (Reynolds and Descy, 1996; Gosselain and Descy, 2001) and more studies are needed to determine seasonal patterns across rivers ranging in environmental conditions.

The objectives of this study were to: (i) describe the seasonal succession of phytoplankton in five temperate rivers ranging in environmental conditions and (ii) to determine the main drivers of community structure across the rivers. I hypothesized that phytoplankton seasonal succession will vary per river because of different flow and nutrient dynamics and that dominance patterns will be related to nutrient concentrations across the rivers.

4.2 Methods

4.2.1 Study sites

Samples were collected from five temperate Ontario and Quebec rivers ranging in land use and nutrient concentrations. River descriptions and hydrological regimes are summarized in Chapter 2 (Table 2-1). For this study, discharge values were obtained by calculating the average daily discharges seven days prior to and including water collection dates (Pace *et al.*, 1992; Basu and Pick, 1996).

4.2.2 River sampling and laboratory analyses

Each river was sampled every two weeks from late May until early November 2009. On occasion, sampling was postponed for a minimum of two days following major rain events. A total of 11 samples were collected per river to represent phytoplankton communities and river conditions over the growing season (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), dissolved oxygen percent saturation (%DO), pH, and conductivity (SPC) were taken with a Hydrolab Minisonde Multiprobe 4a. 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 degrees from the light source and are given in Nephelometric Turbidity Units (NTU).

The water collected from each site was preserved in Lugol's iodine solution for phytoplankton 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 analyzed 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 $N0_3$+$N0_2$. In late May and late August samples were also analyzed for silica concentrations.

As a measure of total algal biomass, chlorophyll-*a* (chl-*a*) concentrations were determined by filtering 250 mL of water through 0.2 μm polycarbonate membranes. When filtering the water, vacuum pressure was set below 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 hours, and concentrations were estimated with a Cary® 100 BIO UV-Visible Spectrophotometer, Varian Inc (Jespersen and Christoffersen, 1987).

4.2.3 Phytoplankton identification and enumeration

Phytoplankton identification and enumerations were made using a Zeiss AXIO A1 inverted microscope equipped with an Empix camera and an Eclipse image analysis system. Phytoplankton preserved in Lugol's that were greater than 2 μm were identified, counted and measured under light microscopy at X200, X400, and X630 magnifications. Ten mL of Lugol's preserved water samples were settled overnight in 26 mm diameter chambers and the enumeration of a minimum of 300 cells per sample were 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). Algamica calculates cell density (cells/L) and biomass (mg/m^3) based on dimensions measured for each taxon per sample. Phytoplankton were identified as much as possible to the highest resolution (~ genus/species). When species identification was not possible, cells were counted in the correct division as unknown species. Therefore, different taxa were accounted for but the species name could not always be confirmed. For example, the *Chryptomonas* genus of the cryptophyte division was separated into species one through four based on size and shape (Appendix 4). Similarly, centric diatoms were identified as *Stephanodiscus* or *Cyclotella* based on size (Appendix 5). Species identification of these genera would have required separate observations with a Scanning Electron Microscope (SEM) as many of them were quite small. Algamica also computes total phytoplankton density, biomass, and number of taxa for 12 algal divisions. However, half were dismissed due to low abundance and

biomass and only the following divisions were considered in the analyses: Bacillariophyta (diatoms), Cryptophyta (cryptophytes), Chlorophyta (geen-algae), Euglenophyta (euglenoids), Chrysophyta (chrysophytes), and Pyrrhophyta (dinoflagellates).

4.2.4 Data analysis

Seasonal data were pooled into four time periods: spring, early summer, base flow, and fall. Spring was based on three sampling occurrences from late May until end of June, representing the period of declining discharge. The early summer category consisted of three sampling dates covering July to early August. Since during 2009 heavy rain was recorded throughout the month of July, base flow conditions occurred later during the ice-free season; late August and September (n=3) represented base flow conditions. Fall samples were represented by two sampling dates that took place in October and early November when the flows tend to rise again.

Taxa dominance occurs when a significant amount of the total biomass (> 30%) in a sample is represented by one taxa. However, when only one or two cells were identified in a sample and accounted for a high percentage of biomass (~ 10%), these rare (yet very large) taxa were removed from the analysis. To further reduce the impact of extreme values, biomass estimates were log transformed.

Bonferroni adjusted Pearson correlations coefficients were calculated to determine the response of phytoplankton biomass to physical, chemical, and biological variables in the six taxonomic divisions. Variables were log transformed to satisfy normality and all statistical analyses were done with S-Plus® version 8.0.

4.3 Results

4.3.1 Physical and chemical variables

Physical and chemical parameters varied throughout the study period and across the different rivers. A summary of these variables can be found in Chapter 2 (Fig 2.1, Appendix 1).

4.3.2 Phytoplankton seasonal succession

South Nation River

In the South Nation River, diatoms had the highest median biomass of all the divisions encountered (Table 4-2). However, seasonally, dominance shifted between diatoms, chlorophytes, and cryptophytes (Fig 4.2). On August 25, chlorophytes dominated at 96% of total biomass (36,522 mg/m^3) caused by a bloom of the colonial motile species *Pandorina morum* (Fig 4.2; Appendix 6). In the weeks prior to and following the bloom, its abundance increased from $6.8x10^3$ cells/L on August 11 to $135x10^6$ cells/L at the peak and declined to $143x10^3$ cells/L on September 10. This was the only occurrence of green alga dominance found in this study. Throughout the ice-free period, cryptophytes (mainly *Cryptomonas*) consistently represented more than 15% of total biomass (Table 4-1). A colonial centric diatom, *Melosira* was also significant contributing 19 to 28% of the phytoplankton biomass in spring, early summer, and fall (Table 4-1).

Castor River

On average, diatoms dominated phytoplankton biomass in the Castor River (Table 4-2) and also on most dates during the ice-free season (Fig 4.2). Very high diatom dominance was observed in the spring and fall (93%). The centric diatom *Stephanodiscus*

was present in all seasonal categories and represented as much as 55% of total biomass during base flow (Table 4-1; Appendix 7). One decline in diatoms occurred during spring and the other occurred during base flow when cryptophytes (mostly *Cryptomonas*) represented 42% of the total biomass. The Castor was the only river with very high euglenoid biomass: during base flow and fall, *Euglena* represented 23% and 13% of total phytoplankton biomass (Table 4-1).

Raisin River

Diatoms had yet again the highest biomass on average compared to the other taxonomic groups (Table 4-2). Seasonally, the Raisin River was a very dynamic system with six major taxonomic divisions fluctuating in their relative importance (Fig. 4.1). Cryptophytes (*Cryptomonas*) was the most important division and represented often more than 40% of total phytoplankton biomass, dominating in all seasonal categories except early summer when the diatom *Melosira* dominated (78%) (Fig 4.2; Table 4-1; Appendix 8).

Rideau River

In contrast to the other four rivers, the Rideau River was on average dominated by cryptophytes (Fig 4.2; Table 4-2). In the spring, *Cryptomonas* showed high dominance (83%) and continuously dominated total biomass across the seasons (Table 4-1). Diatoms had high percent biomass on three sampling events (early summer and base flow) with *Fragilaria* and *Aulacoseira* as the taxa representing the most biomass (Fig 4.2 and Appendix 9). Chrysophytes had high individual contribution to total biomass during spring (*Dinobrynon divergens*) and fall (*Synura uvella*) (Table 4-1). The Rideau River was the only

system where pyrrhophyta (mainly *Peridinium*) represented more than 15% biomass (three sampling dates, Table 4-1).

Gatineau River

Overall, biomass was much lower in the Gatineau River than in the other rivers (Table 4-2). Diatoms had the highest median biomass, then cryptophytes followed closely by chrysophytes and chlorophytes. The Gatineau River had lower dominance of individual taxa compared to the other rivers (Fig 4.2). The highest taxa dominance observed was a colonial chrysophyte (*Dinobryon divergens*), that reached 34% of total phytoplankton biomass (Table 4-1) in spring. All other taxa represented individually less than 30% of total biomass. This was different from the other rivers of this study where at least once, a specific taxon accounted for greater than 50% of total biomass. The main diatom taxa in the Gatineau were *Tabellaria, Melosira* and *Cocconeis* while the cryptophytes were mainly represented by *Cryptomonas* (Appendix 10).

Taxa diversity

The main algal divisions varied in species diversity across the different rivers (Table 4-2). The number of taxa was highest for diatoms (7-11) and green algae (9-13). Cryptophyte diversity ranged from 4 to 6 taxa while chrysophytes included 4 and 11. Chrysophyte taxa diversity was highest in the most oligotrophic system: the Gatineau River had 8 to 12 taxa. Across all rivers, the median value of total taxa in a given sample varied from 30 to 39, regardless of system trophy. Both a eutrophic (Castor) and an oligotrophic river (Gatineau) had the highest median number of taxa (39). The maximum species diversity was observed in the Rideau River (51) and the second highest (46) was found in the Castor

River. The lowest number of taxa (20) was observed in the South Nation River during the *Pandorina morum* bloom.

4.3.3 Phytoplankton taxonomic divisions and response to environmental variables

The biomass of the main algal divisions responded differently to physical, chemical, and biological factors (Table 4-3). When all the rivers and samples were considered, positive and significant correlations were observed between pH and diatoms, cryptophytes, and green algae. Green algae were the only phytoplankton division to show a positive association with temperature while diatoms and euglenoids responded positively to conductivity. Water discharge lead to negative correlations for the majority of the phytoplankton divisions: diatoms, cryptophytes, chlorophytes, and euglenoids. In contrast, the response of the divisions to nutrients varied from positive to negative and was often not statistically significant. However, euglenoids responded positively and significantly to phosphorus (both as RP and TP). Chrysophytes had a negative association with nitrate, and diatoms tended to increase with total nitrogen concentrations. Total algal chl-*a* was positively correlated with diatoms, chlorophytes, chrysophyes, and euglenoids.

4.4 Discussion

No common pattern in the phytoplankton communities was observed across the different rivers (e.g. Fig 4.1). There was a lack of a common periodicity in seasonal succession as has been described for decades in lakes and now modeled (Sommer *et al.*, 1986). For example, while diatoms were often most prominent in the spring and fall as in lakes, this was not the case for all rivers. Diatoms were also important during summer and during base flow.

The most striking difference between the rivers was in the relative dominance of taxa. High taxa dominance (> 50%) was observed in the more eutrophic systems such as the South Nation and Castor in comparison to the more oligotrophic Gatineau River. In the South Nation, during the *Pandorina morum* bloom (> 90% of total biomass) in August, the water was visibly green. This species has bloomed in previous years in this highly eutrophic river. In lakes, high biomass combined with strong dominance by a few species is very typical of highly eutrophic systems (Watson *et al.*, 1997). A more diverse phytoplankton taxonomic composition with most algal groups represented over the growing season is seen in mesoeutrophic lakes (Eloranta, 1986), and low phytoplankton biomass with high species diversity in oligotrophic lakes (Reynolds, 1984).

In the past, the majority of the river studies have focused on diatoms since they appear to dominate suspended algal biomass in temperate rivers (Rojo *et al.*, 1994). In this study, diatoms also tended to dominate algal biomass but some benthic diatoms were included in the phytoplankton enumerations and this could have lead to an over-representation of the planktonic diatom community. However, planktonic diatoms are usually more abundant and benthic algae occur at relatively low abundance in large rivers compared to smaller systems. For example, in the mesoeutrophic Rideau River, benthic diatoms never exceeded 20% of total diatom abundance (Yang *et al.*, 1997).

In rivers, low diatom biomass can sometimes be attributed to silica limitation (< 0.40 mg/L) (Bormans and Webster, 1999). In this study, the lowest concentration of silica was observed in late May in the Rideau River at 0.56 mg/L which then increased to 1.9 mg/L in late August. All of the other silica concentrations were much higher than the limitation threshold, indicating that silica was not limiting diatom growth in any of the rivers.

In rivers, there also tends to be high biomass of centric compared to pennate diatoms as they have higher growth rates and lower sinking losses (Corbelas and Rojo, 1994). In the Warnow River, high centric diatom biomass has been observed in the spring and in autumn (Bahnwart *et al.,* 1998). Similarly, this study observed high biomass of the centric diatoms, *Stephanodiscus* and *Cyclotella*. In particular, high *Stephanodicus* biomass was noted throughout the growing season with the highest dominance occurring from summer to early fall in the Castor River. Scanning Electron Microscope (SEM) studies would confirm whether the species were the same as typically found in eutrophic European rivers (e.g. *S. hantzschii*) (Hasle and Fryxell, 1970).

The only river that did not show diatom biomass dominance was the Rideau River where cryptophytes accounted for almost half of the total biomass. Cryptophytes are known to proliferate in slow flowing water and are able to control their sinking rates through flagella motion which give them an advantage over other species under light limiting conditions (Bahnwart *et al.,* 1998). The Rideau River is a more regulated river than the others (as part of the Rideau Canal system operated by Parks Canada) and water residence times at the Baxter sampling site may have represented more lake-like conditions than the other river sites. However, the Rideau River had the lowest light extinction coefficients and lowest turbidity (Appendix 1) compared to the other rivers and was thus the least likely to be light limited.

The strong negative response of diatoms, cryptophytes, chlorophytes, and euglenoids to water discharge observed in this study is in agreement with past studies indicating the strong impact that hydrological regimes have on phytoplankton development in rivers (Descy, 1987; Piirsoo *et al.,* 2008). In this study, cyanobacteria were observed occasionally; however, they were not important contributors to total biomass, as found in previous studies, likely because they are less competitive under the high water turbulence of rivers

(Moss *et al.*, 1984; Chételat *et al.*, 2006). During this study period of 2009, the river discharge was generally higher than the historical average due to high precipitation, especially during July. Significant rain events (> 30 mm) can increase discharge and decrease algal biomass as much as 100 fold over short periods of time in the Rideau, however, phytoplankton communities can recover in less than seven days (Ley *et al.*, 1997).

While some studies have reported positive response of all taxonomic phytoplankton group biomass to TP in lakes (Watson *et al.*, 1997), the results of this study only found a significant positive correlation between euglenoids and phosphorus concentrations. Results also indicated a negative response of the chrysophyte group to nitrate concentrations and a positive association between diatoms and nitrogen concentrations was observed. The diatom response to TN corresponds to results of previous studies that have observed changes in diatom biomass with total nitrogen concentrations (Yang *et al.*, 1997).

Taxonomic resolution to species level would provide additional information on the state of rivers and allow for more complex multivariate statistical analyses. However, more sophisticated laboratory analyses such as SEM are needed to confirm identification, especially for diatoms. It is important to keep in mind that this study provided correlations between phytoplankton communities and environmental variables that may have some predictive value; however these correlations may not reflect direct causal associations. Establishing causal relationships in rivers is difficult since *in situ* manipulations and experimentation are usually not feasible at the relevant scales.

Table 4-1 Significant algal genera (≥ 15% of total biomass) in each river per sampling period. Spring, early summer, and base flow periods are represented by three sampling events and fall by two. Numbers in brackets refer to the maximum contribution to total biomass observed in each season. Genera marked by * indicate possible benthic or attached origin.

	Season			
River	Spring	Early summer	Base flow	Fall
South Nation	*Cryptomonas* (26%) *Euglena* (21%) *Melosira* (19%) *Skeletonema* (19%) *Stephanodiscus* (14%)	*Peridinium* (33%) *Cryptomonas* (28%) *Melosira* (26%)	*Pandorina* (90%) *Plagioselmis* (60%) *Carteria* (17%) *Cryptomonas* (16%)	*Melosira* (28%) *Cryptomonas* (25%) *Stephanodiscus* (21%) *Plagioselmis* (20%)
Castor	*Stephanodiscus* (37%) **Nitzschia* (25%) *Cryptomonas* (23%) *Diatoma* (22%) *Synedra* (20%) *Carteria* (19%)	*Stephanodiscus* (54%) *Cryptomonas* (18%) *Melosira* (12%)	*Stephanodiscus* (55%) *Cryptomonas* (42%) *Cyclotella* (32%) *Euglena* (23%)	**Diatoma* (30%) **Nitzschia* (22%) *Cryptomonas* (15%) *Euglena* (13%) *Stephanodiscus* (12%)
Raisin	*Cryptomonas* (46%)	*Melosira* (78%) *Cryptomonas* (56%) *Euglena* (21%)	*Cryptomonas* (50%) *Cyclotella* (37%) *Trachelomonas* (17%)	*Cryptomonas* (44%) *Fragilaria* (21%) *Synura* (13%) **Nitzschia* (13%)
Rideau	*Cryptomonas* (83%) *Dinobyron* (35%) *Peridinium* (18%) *Plagioselmis* (14%)	*Cryptomonas* (40%) *Fragilaria* (38%) *Peridinium* (15%)	*Cryptomonas* (66%) *Aulacoseira* (36%)	*Cryptomonas* (32%) *Synura* (29%) *Plagioselmis* (26%) *Peridinium* (19%)
Gatineau	*Dinobyron* (34%) *Peridinium* (11%)	*Cryptomonas* (16%) *Tabellaria* (14%) *Plagioselmis* (12%) *Melosira* (12%)	*Cryptomonas* (24%) *Tabellaria* (18%) *Melosira* (18%) *Chromulina* (14%)	*Melosira* (25%) *Cryptomonas* (14%) **Cocconeis* (13%)

Table 4-2 Median and range of both biomass (mg/m^3) and number of taxa per algal division in each river (n=11) and the total per river (n=55) during the ice-free period of 2009.

		South Nation	Castor	Raisin	Rideau	Gatineau
Bacillariophyta	Biomass	211 (91-540)	1190 (543-2,425)	304 (76-3,020)	191 (15-1,085)	55 (29-112)
	Taxa	9 (4-17)	11 (7-21)	11 (7-14)	7 (4-14)	9 (6-13)
Cryptophyta	Biomass	165 (0-737)	264 (3-1,296)	172 (45-1,610)	739 (139-2,011)	48 (14-114)
	Taxa	5 (0-6)	5 (1-6)	6 (3-7)	6 (5-7)	4 (2-10)
Chlorophyta	Biomass	37 (7-36,522)	40 (21-1,541)	31 (6-349)	52 (14-309)	22 (8-32)
	Taxa	11 (9-15)	15 (4-22)	11 (6-14)	9 (4-21)	13 (10-17)
Pyrrhophyta	Biomass	28 (0-577)	26 (0-255)	54 (0-346)	1 (0-357)	5 (0-71)
	Taxa	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-2)	2 (0-2)
Chrystophyta	Biomass	33 (9-268)	60 (3-337)	62 (13-501)	238 (37-583)	28 (19-190)
	Taxa	4 (3-10)	5 (1-8)	7 (3-8)	7 (3-12)	11 (8-12)
Euglenophyta	Biomass	21 (0-497)	68 (0-1,191)	45 (0-270)	0 (0-80)	0 (0-2)
	Taxa	1 (0-3)	1 (0-3)	3 (0-5)	0 (0-1)	0 (0-1)
Total	Biomass	654 (258-38,212)	1,811 (1,045-5396)	757 (304-3,543)	1,431 (291-2,598)	173 (100-394)
	Taxa	30 (20-41)	39 (24-46)	36 (33-44)	32 (23-51)	39 (34-44)

Table 4-3 Pearson correlations and statistical significance based on Bonferroni adjusted probabilities (*p<0.05; ** p<0.01; ***p<0.001) for the following variables: pH, dissolved oxygen percent saturation (% DO), temperature, conductivity (SPC), turbidity, water discharge, reactive phosphorus (RP), total phosphorus (TP), ammonia+ammonium ($NH_3 + NH_4^+$), nitrate ($NO3^-$), total nitrogen (TN), total chlorophyll-*a* (Chl-*a*) and total biomass (mg/m^3) for each main phytoplankton taxonomic divisions (n=55). %DO was arcsin transformed and variables marked by † were log transformed for normality**.**

	Bacillariophyta†	Cryptophyta†	Chlorophyta†	Pyrrhophyta†	Chrysophyta†	Euglenophyta†
pH	0.489*	0.738***	0.470*	0.115	0.226	0.367
% DO	-0.033	-0.051	0.230	0.015	0.172	-0.114
Temperature (°C)	0.162	0.327	0.544**	0.183	0.093	0.327
SPC (µS/cm)	0.702***	0.405	0.324	0.142	-0.073	0.633***
Turbidity (NTU)	0.285	-0.087	-0.096	-0.043	-0.294	0.234
Water discharge (m^3/s) †	-0.495*	-0.497*	-0.478*	-0.216	-0.136	-0.602***
RP (µg/L)	0.265	0.018	-0.064	0.124	-0.314	0.485*
TP (µg/L)	0.451	0.236	0.242	0.198	-0.079	0.562*
NH_3+NH_4^+ (µg/L) †	0.356	0.029	-0.119	0.007	-0.267	0.213
NO_3^- (µg/L) †	0.117	-0.326	-0.338	-0.095	-0.569**	0.065
TN (µg/L) †	0.541**	0.046	-0.061	-0.092	-0.352	0.294
Chl-a†	0.531**	0.221	0.819***	0.363	0.458*	0.468*

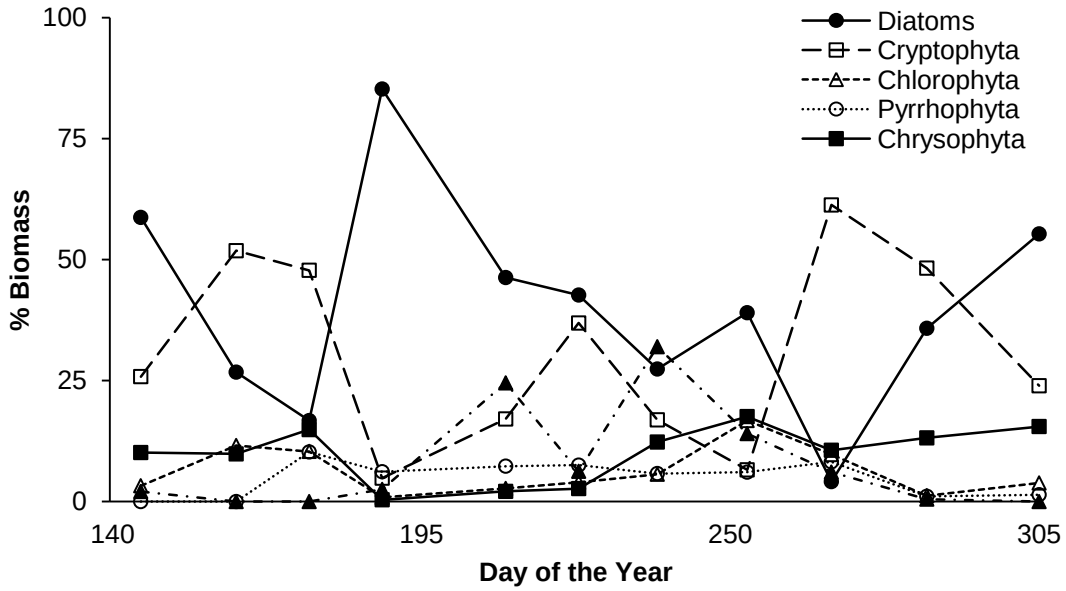

Fig 4.1 Percent phytoplankton biomass in major algal divisions throughout the study period (May-November, 2009) in the Raisin River. Each symbol represents a sampling event. Cyanobacteria, xanthophyta, haptophyta, and rhodophyta were not included because of low biomass.

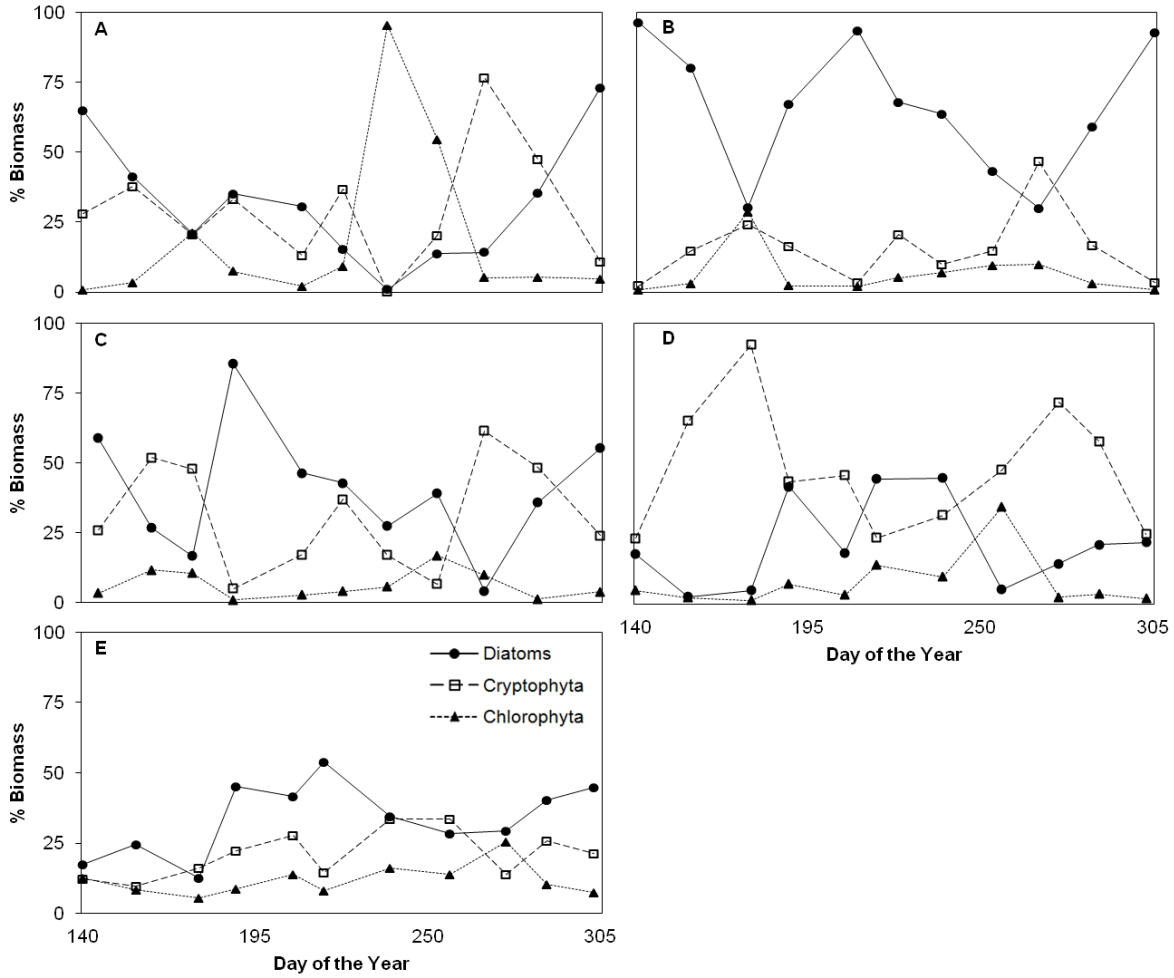

Fig 4.2 Percent phytoplankton biomass in the three most important algal divisions throughout the study period (May-November, 2009) in the following rivers: South Nation (A), Castor (B), Raisin (C), Rideau (D), Gatineau (E). Each symbol represents a sampling event. Pyrrhophyta, chrysophyta, euglenophya, cyanobacteria, xanthophyta, haptophyta, and rhodophyta were not included because of low biomass.

- Chapter 5 -

General Conclusion

The aim of this thesis was to examine the structure of phytoplankton communities in rivers with a particular focus on the relative importance of small cells (< 20 μm) to biomass and seasonal dynamics. While the smallest size fraction of the plankton typically dominates oligotrophic lakes and oceans, picophytoplankton also contributed significantly to chlorophyll-*a* (chl-*a*) and biomass in rivers, even in the most eutrophic systems. The analysis of the size distribution of planktonic chl-*a* and taxa based abundance size spectra both showed that phytoplankton communities were uniformly dominated by small cells throughout the ice-free period. This dominance is perhaps a general response to constant turbulence and lower average light climate compared to lakes. These typical river conditions might also represent a favourable environment for phycocyanin-rich picocyanobacteria, which could explain their dominance of the picophytoplankton assemblage. Higher nutrient concentrations did not lead to the usual shift to large cells observed in lakes and oceans. However, high nitrate concentrations coupled with high temperature represented optimal conditions for picophytoplankton. The presence of small cells implies a high metabolic turnover and as a result the productivity and nutrient cycling in rivers is also likely very high, which might explain the higher fish yields observed in rivers compared to lakes (Randall *et al.*, 1994).

In contrast to the analysis of structure based on size, the taxonomic composition of the five rivers was much more variable. Across the rivers, no consistent seasonal succession pattern was observed. Diatoms dominated in all rivers with the exception of the Rideau River where cryptophytes were the most important phytoplankton group. High taxa dominance (> 50% of biomass) was common in the more eutrophic rivers but the number of taxa observed was similar across all rivers, regardless of trophy. While some taxonomic divisions responded positively to nutrients, water discharge negatively affected most phytoplankton

taxonomic divisions. However, the picophytoplankton community was not affected by discharge.

Many new questions arise from this thesis requiring further investigation. Phytoplankton identification to the species level would have allowed for more sophisticated data analyses but involve more detailed work, in particular the use of a Scanning Electron Microscope (SEM) for diatoms, which was beyond the scope of this study. With a higher level of taxonomic resolution, the results could be analyzed with multivariate statistics to identify indicator species. Principle component analyses (PCA) and cluster analyses could also be used to find associations between phytoplankton taxa and environmental variables. Since taxa of benthic origin can be brought to the surface in periods of high flow and turbulence and can increase the biomass of phytoplankton divisions (e.g. large pennate diatoms), identifying and removing these species from analyses could reduce biomass variability. Furthermore, based on the results of this study, more rivers will need to be examined to establish seasonal patterns. The Plankton Ecology Group-model was developed based on many seasonal studies of numerous lakes (e.g. Sommer *et al.*, 1986). Lastly, 2009 was an unusually high flow year and this might help explain the importance of discharge for the phytoplankton communities. The patterns observed in this study might have been different under a much lower discharge scenario. Rivers should be studied over many years to capture different hydrological regimes (high and low flow years) in order to determine the relative importance of flow vs. nutrients on phytoplankton communities.

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Appendices

Appendix 1

Seasonal median and ranges (n=11, 2009) for physical properties (pH, dissolved oxygen (DO), temperature, conductivity (SPC), turbidity, extinction coefficient, and water discharge), for water chemistry (reactive phosphorus (RP), total phosphorus (TP), ammonia+ammonium (NH_3+NH_4^+), nitrate ($NO3^-$), and total nitrogen (TN)), and for algal parameters (phycocyanin-rich picocyanobacteria (PY-PC), phycoerythrin-rich picocyanobacteria (PE-PC), and picoeukaryotes (PEuk)) in Ontario and Quebec rivers.

Physical properties	**South Nation**	**Castor**	**Raisin**	**Rideau**	**Gatineau**
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*	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^-	1,640 (346-4,123)	1,266 (0-4,285)	367 (0-2,051)	21 (0-127)	60 (52.3-82.5)
TN	3,355 (1,162-5,295)	2,066 (753-5,099)	1,157 (82.4-3,035)	732 (611-795)	374 (323-423)
Algal parameters (cells/mL)					
PY-PC	$2.53x10^3$ (0-$4.89x10^5$)	$1.18x10^3$ ($2.94x10^1$-$2.07x10^4$)	$1.34x10^3$ ($1.67x10^2$-$3.66x10^4$)	$4.38x10^3$ ($8.34x10^2$-$4.54x10^4$)	$7.84x10^3$ ($1.03x10^3$-$1.32x10^4$)
PE-PC	$9.8\ x10^1$ ($2.94x10^1$-$4.60x10^2$)	$9.8x10^1$ (9.80-$5.00x10^2$)	$1.47x10^2$ ($4.9x10^1$-$5.49x10^2$)	$1.76x10^2$ (0-$1.56x10^3$)	$5.58x10^2$ ($2.16x10^2$-$1.59x10^3$)
Peuk	$1.18x10^2$ ($3.92x10^1$-$1.13x10^3$)	$3.43x10^2$ ($2.94x10^1$-$3.35x10^3$)	$4.7x10^2$ ($5.88x10^1$-$3.24x10^3$)	$2.25x10^2$ ($4.9x10^1$-$1.16x10^3$)	$3.23x10^2$ ($7.84x10^1$-$1.88x10^3$)

*n=5-9

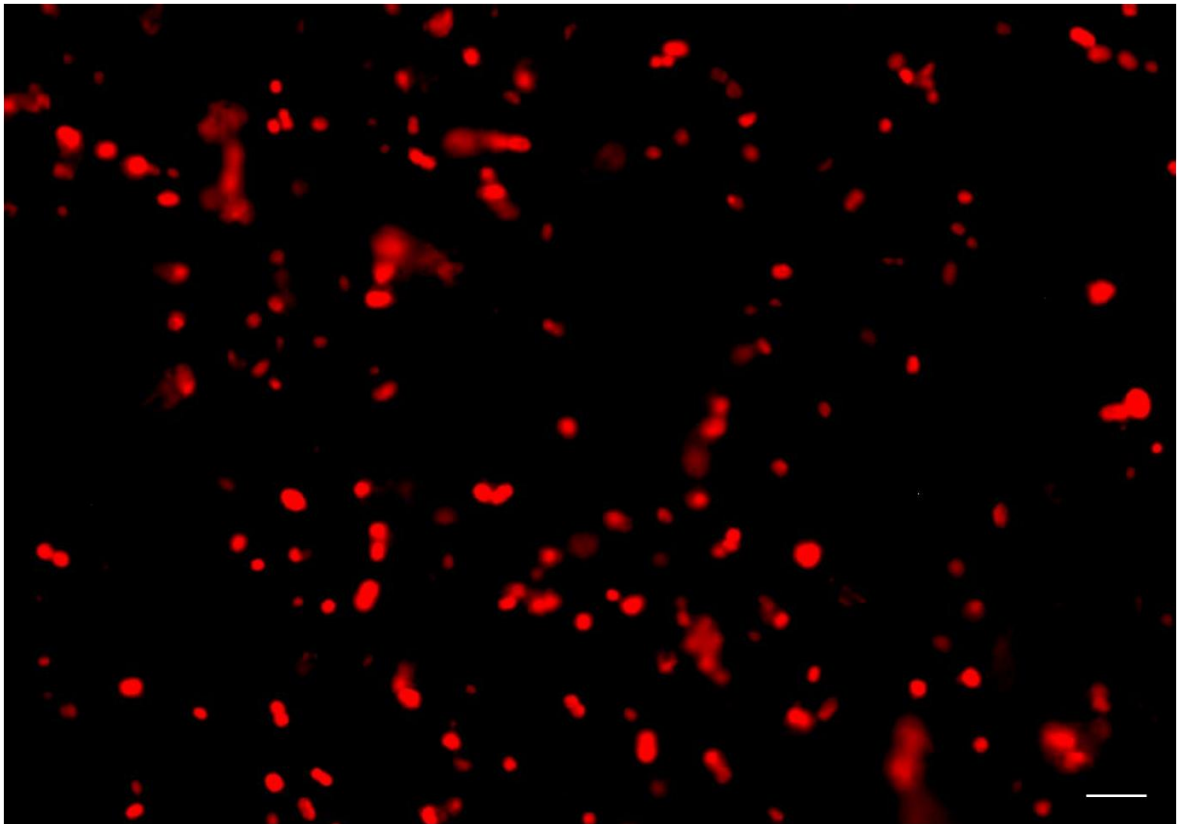
Appendix 2

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). %DO was arcsin transformed for normality and variables marked by † were log transformed.

	pH	DO	Temp	SPC	Turb	Disch†	RP	TP	NO_3^-†	TN†	Amm†	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†	-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^-†	-0.080	0.012	-0.092	0.232	0.567***	0.214	0.498**	0.347				
TN†	0.418	-0.077	0.040	0.646***	0.716***	-0.149	0.665***	0.681***	0.670***			
Amm†	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*†	0.751***	0.196	0.336	0.560**	0.083	-0.508*	0.087	0.396	-0.189	0.217	-0.012	0.035

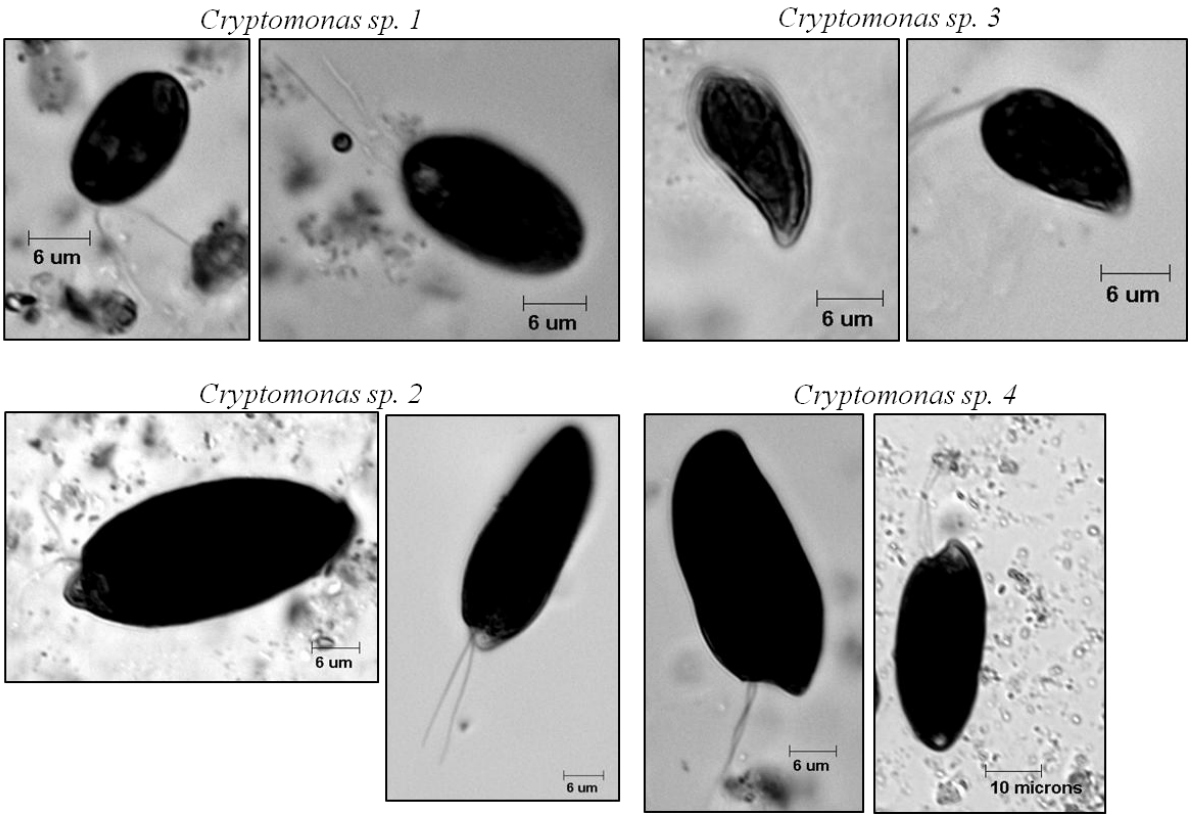
Appendix 3

Phycocyanin-rich picocyanobacteria fluorescing red under green excitation in the South Nation River (June 24, 2009). The image represents an overlay of several focus points of the same field of view and was taken at X1000 magnification with a Zeiss AXIO A1 inverted microscope equipped with an Empix camera and an Eclipse image analysis system. Pictures were taken at 8-bit grayscale and the red colour was added using ImageJ. Scale bar, 2 μm.



Appendix 4

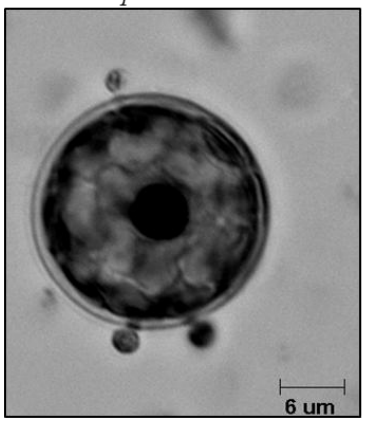
For the *Cryptomonas* genus, identification was classified into four categories. *Cryptomonas sp. 1* were counted as rounded cells < 20µm; *Cryptomonas sp. 2* were rounded cells > 20µm; *Cryptomonas sp. 3* were counted as pointed cells < 20µm; and *Cryptomonas sp. 2* were pointed cells > 20µm.



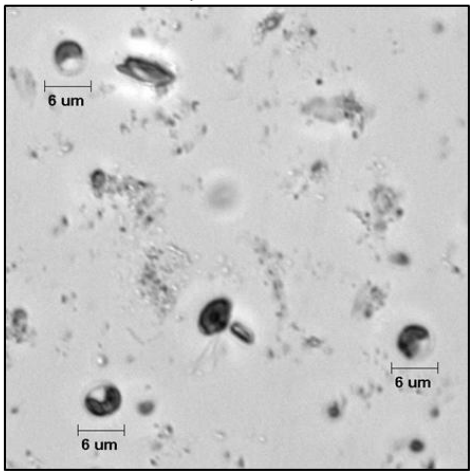
Appendix 5

For the centric diatoms, genus identification was *Stephanodiscus* when cell size was > 6 μm and *Cyclotella* when cells were < 6 μm.

Stephanodiscus

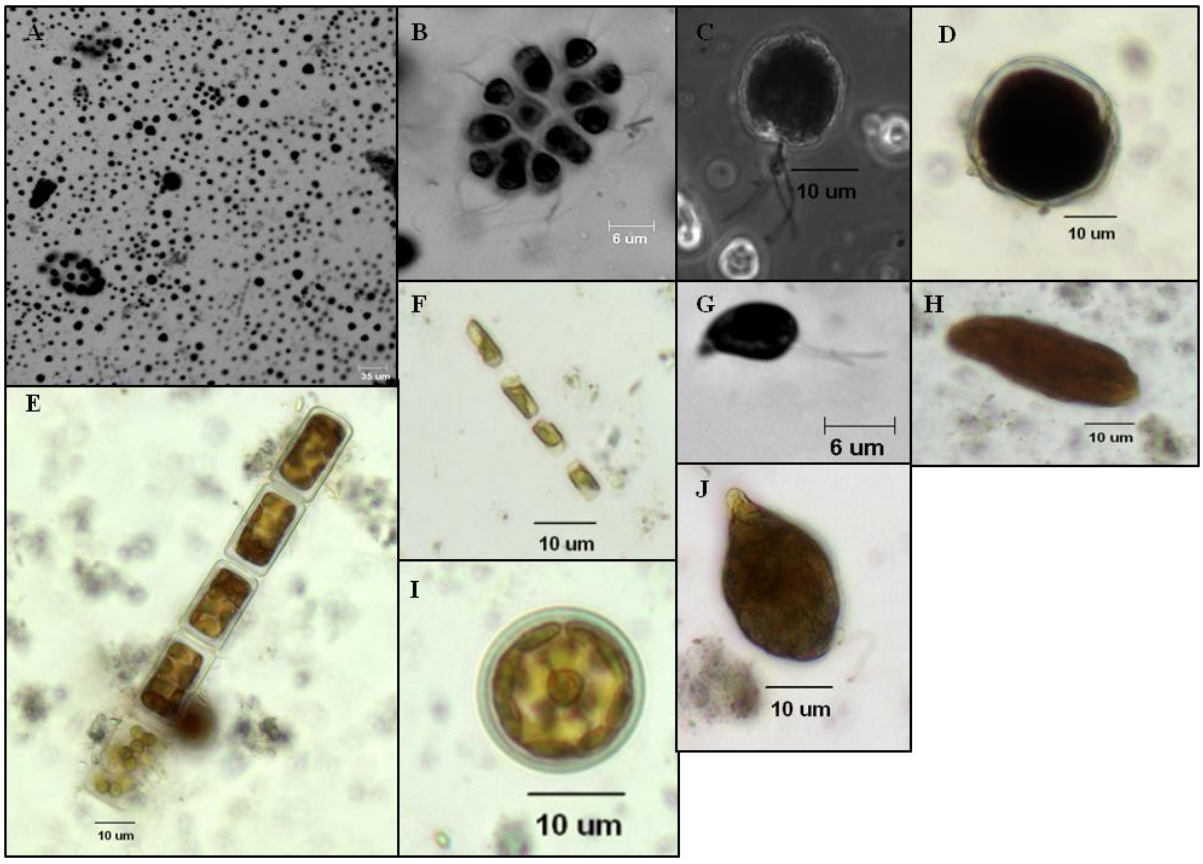


Cyclotella



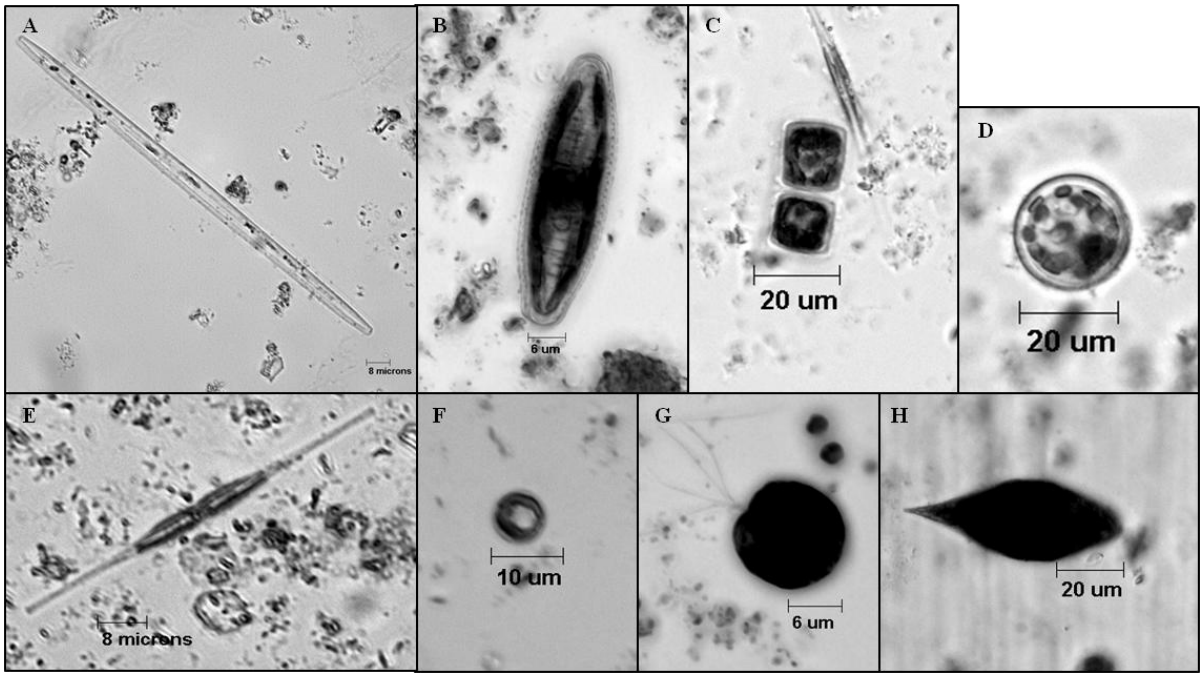
Appendix 6

Dominant genus/species of the South Nation River. (A) *Pandorina morum* broken to single cells following preservation with Lugol's solution; (B) colonial form of *Pandorina morum*; (C) *Carteria sp.*; (D) *Peridinium sp.*; (E) *Melosira varians*; (F) *Skeletonema potamos*; (G) *Plagioselmis nannoplanctica*; (H) *Cryptomonas*; (I) *Stephanodiscus*; and (J) *Euglena*.



Appendix 7

Dominant genus/species of the Castor River. (A) *Synedra*; (B) *Diatoma*; (C) *Melosira varians*; (D) *Stephanodiscus*; (E) *Nitzschia*; (F) *Cyclotella*; (G) *Carteria*; and (H) *Euglena*.

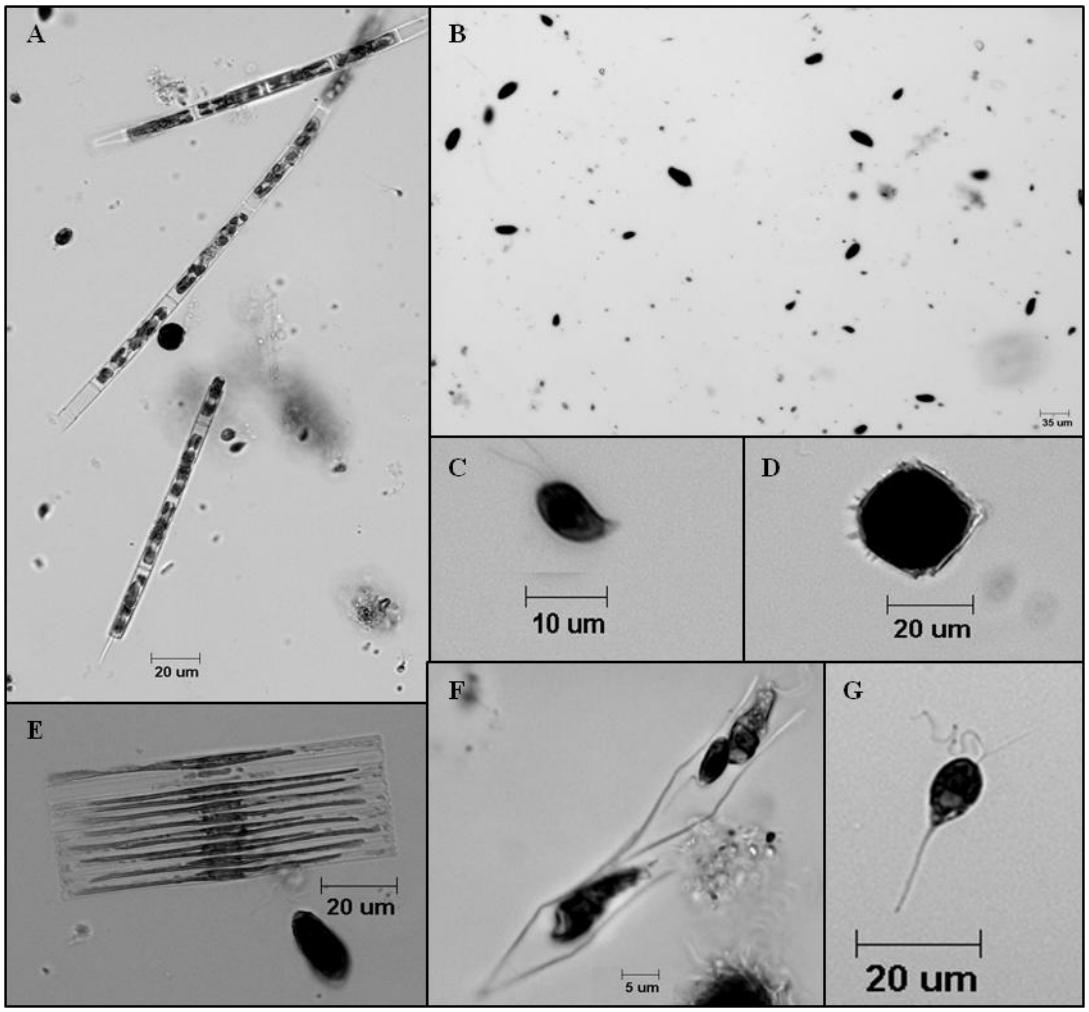


Appendix 8

Dominant genus/species of the Raisin River. (A) *Fragilaria*; (B) *Melosira varians*; (C) *Nitzschia*; (D) *Euglena*; (E) *Cryptomonas*; (F) *Cyclotella;* (G) *Synura*; and (H) *Trachelomonas*.

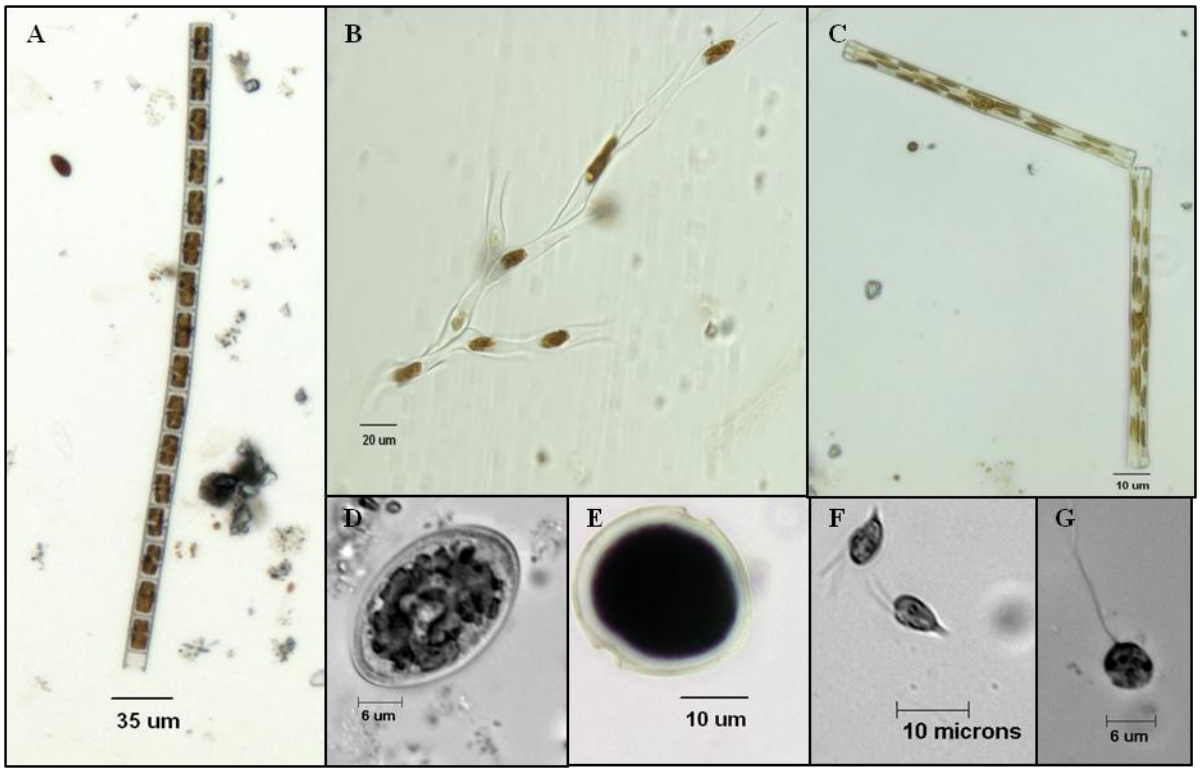
Appendix 9

Dominant genus/species of the Rideau River. (A) *Aulacoseira granulata;* (B) high *Cryptomonas* abundance; (C) *Plagioselmis nanoplanktica*; (D) *Peridinium*; (E) *Fragilaria*; (F) *Dinobryon divergens;* and (G) *Synura*.



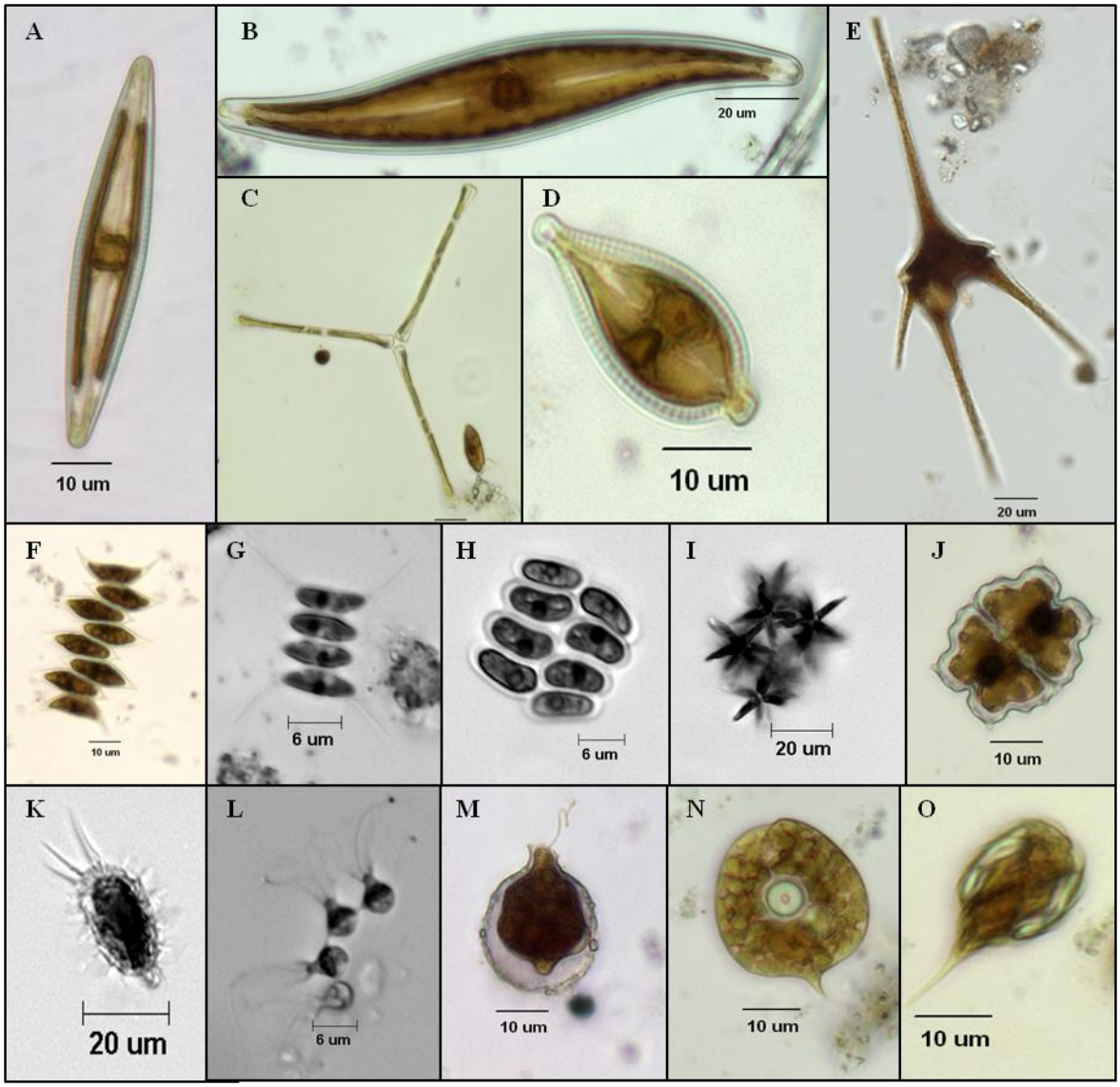
Appendix 10

Dominant genus/species of the Gatineau River. (A) *Melosira varians;* (B) *Dinobryon divergens*; (C) *Tabellaria flocculosa var. linearis;* (D) *Cocconeis placentula*; (E) *Peridinium*; (F) *Plagioselmis nanoplanktica;* and (G) *Chromulina*.



Appendix 11

Some of the genus/species diversity observed across the South Nation, Castor, Raisin, Rideau, and Gatineau rivers. (A) *Navicula cryptocephala;* (B) *Gyrosigma sp*; (C) *Asterionella formosa;* (D) *Gomphonema*; (E) *Ceratium hirundinella;* (F) *Scenedesmus;* (G) *Scenedesmus quadrispina*; (H) *Scenedesmus obtusus;* (I) *Actinastrum hantzschii*; (J) Desmid; (K) *Mallomonas*; (L) *Desmarella*; (M) *Trachelomonas*; and (N-O) *Phacus*.



Appendix 12(a)
Physical properties in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period.

River	Date	pH	DO%	DO (mg/L)	Temp (°C)	SpC (µS/cm)	Ext coef.	Turbidity (NTU)	Water Discharge (m^3/s)
S. N.	May 20	7.9	92	8.72	13.5	524	2.56	22.45	43.13
	Jun 5	7.9	91	8.06	16.8	561	3.35	15.90	23.16
	Jun 24	8.3	114	8.19	27.8	589	3.69	17.07	6.59
	Jul 7	7.8	69	5.82	20.6	464	3.76	29.37	20.77
	Jul 29	7.7	85	7.26	21.8	485	4.50	34.00	64.60
	Aug 11	8.2	92	7.06	24.1	585	-	14.37	29.14
	Aug 25	8.8	169	13.8	25.9	569	3.66	15.33	7.53
	Sep 10	8.7	120	10.4	22.6	577	-	17.17	4.51
	Sep 25	8.3	83	7.95	17.4	577	3.55	20.37	1.87
	Oct 12	8.1	81	8.82	12	559	5.13	36.57	19.80
	Nov 1	8.2	90	10.6	8.49	561	6.98	51.03	60.87
Castor	May 19	8.4	94	11.2	13.2	675	-	11.75	5.97
	Jun 5	8.2	97	8.62	16.8	718	3.21	13.70	2.80
	Jun 24	8.5	151	11.2	25.5	703	1.61	6.22	0.86
	Jul 7	8.1	87	7.2	20.2	712	2.44	13.83	1.98
	Jul 29	8	91	8.03	21.1	680	4.14	26.00	9.76
	Aug 11	8.3	100	8.05	21.9	764	3.03	20.37	3.37
	Aug 25	8.2	76	6.49	23	773	4.73	11.04	0.89
	Sep 10	8.7	119	10.9	19.5	767	-	14.43	0.58
	Sep 25	8.2	66	6.53	16.3	788	4.73	14.93	0.49
	Oct 12	8.2	84	9.36	10.1	818	4.06	13.43	2.64
	Nov 1	7.6	88	10.2	9.38	703	5.19	30.33	5.97

Appendix 12(b)

River	Date	pH	DO%	DO (mg/L)	Temp (°C)	SpC (µS/c)	Ext coef.	Turbidiy (NTU)	Water Discharge (m^3/s)
Raisin	May 25	7.9	91	7.87	18.0	458	1.87	8.14	3.84
	Jun 11	7.9	79	6.84	17.6	486	5.58	3.88	1.40
	Jun 24	8.1	95	7.51	23.6	516	2.03	5.20	0.61
	Jul 7	7.8	72	6.05	20.3	527	2.46	6.11	1.76
	Jul 29	7.9	103	8.55	24.5	478	2.02	4.24	1.69
	Aug 11	7.9	71	5.68	22.8	506	-	5.17	3.26
	Aug 25	8.0	58	4.92	23.9	529	2.03	3.12	0.59
	Sep 10	8.2	75	6.83	20.1	526	-	3.91	0.18
	Sep 25	8.1	63	6.05	17.1	550	2.29	4.83	0.11
	Oct 12	8.0	63	6.95	11.2	547	2.37	6.29	0.81
	Nov 1	8.1	78	9.22	8.5	528	3.15	7.73	4.23
Rideau	May 27	8.7	113	9.84	17.6	273	-	1.47	31.99
	Jun 6	8.7	116	9.84	18.6	281	-	1.43	20.63
	Jun 26	8.7	91	7.02	24.0	258	1.06	1.50	11.98
	Jul 8	8.0	76	6.15	21.5	252	1.14	1.93	12.43
	Jul 26	7.9	87	6.81	22.8	264	-	1.92	45.19
	Aug 5	8.2	90	9.84	24.2	269	-	1.75	33.14
	Aug 26	8.2	84	7.1	23.9	285	-	1.57	12.90
	Sep 14	8.3	89	8.65	20.5	261	-	1.56	10.66
	Oct 2	8.3	85	8.8	13.5	245	0.72		17.97
	Oct 15	8.2	88	10.22	9.2	269	0.78	1.07	38.13
	Oct 30	8.2	89	10.78	7.0	283	0.70	0.97	52.10
Gatineau	May 22	6.7	102	10.1	11.7	33.1	-	1.25	272.17
	Jun 12	6.6	102	9.39	14.9	20.1	1.51	1.66	123.70
	Jun 25	6.7	102	8.69	18.9	37	1.59	1.16	69.72
	Jul 14	6.7	113	9.29	19.4	37.6	1.78	1.88	245.41
	Jul 28	6.9	104	8.99	20.5	37.6	2.43	4.46	152.19
	Aug 12	6.9	97	8.57	20.9	36.4	-	1.57	165.01
	Aug 25	7.0	99	8.66	21.9	37	-	1.30	87.11
	Sep 11	7.0	95	8.6	19.7	34.5	-	1.31	41.51
	Sep 24	6.9	93	8.85	17.5	27.9	-	0.97	29.78
	Oct 9	6.8	94	9.54	14.3	32.8	1.51	1.20	43.96
	Oct 26	6.9	94	10.97	8.7	33.7	1.73	1.35	45.99

Appendix 13(a)
Water chemistry in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period. Ammonia+ammonium (Amm+Amm), nitrite, nitrate, total Kjeldahl nitrogen (TKN)**,** total nitrogen (TN), reactive phosphorus (RP), and total phosphorus (TP) concentrations are in µg/L.

River	Date	Amm + Amm	Nitrite	Nitrate	TKN	TN	RP	TP
S. N.	May 20	76	11.4	2,530	850	3,392	56	61
	Jun 5	65	40.0	1,640	910	2,590	40	59
	Jun 24	27	0.0	1,008	940	1,948	24	53
	Jul 7	106	34.7	3,514	1,150	4,698	109	121
	Jul 29	71	21.8	4,123	1,150	5,295	103	130
	Aug 11	20	18.3	2,387	950	3,355	64	82
	Aug 25	7	16.0	1,288	2,170	3,474	22	293
	Sep 10	19	3.1	697	1,180	1,881	51	120
	Sep 25	22	5.5	346	810	1,162	64	79
	Oct 12	124	5.0	789	1,120	1,914	88	117
	Nov 1	66	9.6	3,166	960	4,136	74	107
Castor	May 19	24	1.3	2,190	765	2,956	23	41
	Jun 5	81	40.0	1,550	710	2,300	21	35
	Jun 24	57	0.0	3	1,200	1,203	9	86
	Jul 7	92	41.1	3,248	770	4,059	57	80
	Jul 29	79	9.0	3,242	910	4,161	59	93
	Aug 11	33	0.0	1,266	800	2,066	35	64
	Aug 25	84	0.0	197	800	997	53	78
	Sep 10	24	0.7	2	750	753	21	60
	Sep 25	67	0.1	15	820	835	49	74
	Oct 12	39	86.9	0	670	757	38	62
	Nov 1	63	13.7	4,285	800	5,099	57	103

Appendix 13(b)

River	Date	Amm + Amm	Nitrite	Nitrate	TKN	TN	RP	TP
Raisin	May 25	73	4.5	512	780	1,297	31	47
	Jun 11	55	0.0	367	790	1,157	16	32
	Jun 24	38	0.0	2	1,090	1,092	8	64
	Jul 7	80	34.3	2051	950	3,035	42	63
	Jul 29	43	11.9	732	920	1,664	36	56
	Aug 11	46	14.1	701	990	1,705	37	56
	Aug 25	45	0.0	75	950	1,025	57	79
	Sep 10	3	0.0	0	950	950	42	74
	Sep 25	16	0.0	5	77	82	51	77
	Oct 12	36	0.0	0	840	840	41	63
	Nov 1	19	9.9	1361	950	2,321	16	37
Rideau	May 27	7	0.0	21	600	621	3	26
	Jun 6	27	0.9	25	660	686	3	22
	Jun 26	2	0.0	1	750	751	5	32
	Jul 8	53	0.0	8	780	788	9	42
	Jul 26	25	1.1	50	730	781	7	31
	Aug 5	8	79.5	6	710	795	3	24
	Aug 26	27	0.0	0	760	760	8	36
	Sep 14	38	0.5	11	650	661	4	21
	Oct 2	34	5.4	36	570	611	6	19
	Oct 15	22	2.5	40	600	642	3	19
	Oct 30	36	5.3	127	600	732	3	18
Gatineau	May 22	15	2.3	83	290	375	3	10
	Jun 12	21	0.0	73	250	323	4	9
	Jun 25	12	0.0	70	290	360	0	9
	Jul 14	22	4.0	60	310	374	2	12
	Jul 28	13	1.3	61	350	412	6	15
	Aug 12	26	1.1	59	360	420	2	9
	Aug 25	15	0.0	64	300	364	2	10
	Sep 11	15	0.0	53	370	423	1	8
	Sep 24	8	0.0	52	310	362	1	7
	Oct 9	19	3.7	56	270	330	3	7
	Oct 26	21	3.2	58	330	391	3	9

Appendix 14(a)
Total picophytoplankton abundance for each pigment group in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period. Phycocyanin-rich picocyanobacteria (PY-PC), phycoerythrin-rich picocyanobacteria (PE-PC), and picoeukaryotes (PEuk) values are in cells/mL.

River	Date	PY-PC	PE-PC	PEuk
S. N.	May 20	0.00E+00	2.25E+02	3.92E+01
	Jun 5	2.65E+02	1.47E+02	4.90E+01
	Jun 24	4.89E+05	4.60E+02	1.13E+03
	Jul 7	8.56E+03	8.82E+01	5.29E+02
	Jul 29	1.03E+03	6.86E+01	1.67E+02
	Aug 11	2.53E+03	1.08E+02	6.86E+01
	Aug 25	1.72E+04	9.80E+01	2.06E+02
	Sep 10	1.14E+04	2.94E+01	1.18E+02
	Sep 25	5.54E+03	8.82E+01	7.84E+01
	Oct 12	6.66E+02	5.88E+01	1.08E+02
	Nov 1	2.65E+02	1.47E+02	1.18E+02
Castor	May 19	2.94E+01	5.88E+01	2.94E+01
	Jun 5	4.31E+02	1.57E+02	1.86E+02
	Jun 24	1.58E+03	4.90E+01	1.20E+03
	Jul 7	1.09E+03	1.27E+02	3.35E+03
	Jul 29	1.18E+03	8.82E+01	7.84E+01
	Aug 11	5.15E+03	9.80E+01	4.90E+02
	Aug 25	2.75E+03	1.27E+02	1.73E+03
	Sep 10	2.07E+04	6.86E+01	2.70E+03
	Sep 25	1.57E+03	9.80E+00	3.43E+02
	Oct 12	9.80E+01	5.00E+02	1.76E+02
	Nov 1	1.18E+02	2.45E+02	2.16E+02

Appendix 14(b)

River	Date	PY-PC	PE-PC	PEuk
Raisin	May 25	5.68E+02	4.90E+01	5.88E+01
	Jun 11	4.11E+02	6.86E+01	1.18E+02
	Jun 24	9.82E+03	1.86E+02	1.50E+03
	Jul 7	1.43E+03	1.47E+02	1.80E+03
	Jul 29	1.34E+03	8.82E+01	3.43E+02
	Aug 11	9.99E+02	1.37E+02	2.55E+02
	Aug 25	2.01E+04	5.49E+02	1.42E+03
	Sep 10	3.66E+04	3.82E+02	1.47E+03
	Sep 25	1.03E+04	1.18E+02	3.24E+03
	Oct 12	5.39E+02	2.35E+02	4.70E+02
	Nov 1	1.67E+02	2.94E+02	7.84E+01
Rideau	May 27	1.54E+03	9.80E+00	5.58E+02
	Jun 6	1.64E+03	1.96E+01	4.90E+02
	Jun 26	4.38E+03	6.86E+01	8.82E+01
	Jul 8	3.34E+04	2.74E+02	6.07E+02
	Jul 26	3.70E+04	3.43E+02	1.16E+03
	Aug 5	2.45E+04	1.08E+03	2.35E+02
	Aug 26	4.54E+04	1.11E+03	2.06E+02
	Sep 14	2.42E+04	1.56E+03	8.82E+01
	Oct 2	2.80E+03	1.76E+02	2.25E+02
	Oct 15	8.41E+02	0.00E+00	8.82E+01
	Oct 30	8.34E+02	6.86E+01	4.90E+01
Gatineau	May 22	1.03E+03	5.58E+02	7.84E+01
	Jun 12	2.38E+03	7.05E+02	1.08E+02
	Jun 25	6.96E+03	8.33E+02	1.88E+03
	Jul 14	1.32E+04	1.59E+03	7.84E+02
	Jul 28	1.20E+04	8.23E+02	4.02E+02
	Aug 12	1.14E+04	5.98E+02	3.13E+02
	Aug 25	7.84E+03	3.62E+02	4.70E+02
	Sep 11	1.29E+04	3.23E+02	3.13E+02
	Sep 24	9.49E+03	2.16E+02	2.94E+02
	Oct 9	7.55E+03	3.04E+02	3.23E+02
	Oct 26	6.25E+03	3.33E+02	3.92E+02

Appendix 15(a)
Chlorophyll-*a* (Chl-*a*) and biomass from microscope enumerations in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period. Total values and those for larger cells (> 2 μm) and picophytoplankton (< 2 μm) are in μg/L. The relative abundance of picophytoplankton is also represented (%PP) for both chl-*a* and biomass from enumerations.

		Chl-*a*				Biomass			
River	Date	Total	> 2 μm	< 2 μm	% PP	Total	> 2 μm	< 2 μm	% PP
S. N.	May 20	1.70	1.55	0.15	8.82	831	831	0.14	0.02
	Jun 5	1.48	0.22	1.26	85.14	609	608	0.24	0.04
	Jun 24	-	-	-	-	2,838	2,582	256	9.05
	Jul 7	1.14	0.72	0.42	36.84	502	498	4.81	0.96
	Jul 29	-	-	-	-	490	489	0.66	0.14
	Aug 11	3.66	2.87	0.79	21.58	689	687	1.42	0.21
	Aug 25	126.49	84.13	42.36	33.49	38,221	38,212	9.15	0.02
	Sep 10	18.64	8.82	9.82	52.68	3,653	3,647	6.05	0.17
	Sep 25	8.17	1.85	6.32	77.36	964	961	2.99	0.31
	Oct 12	1.60	0.75	0.85	53.13	271	271	0.44	0.16
	Nov 1	2.06	1.20	0.86	41.75	348	347	0.28	0.08
Castor	May 19	8.10	6.59	1.51	18.64	2,523	2,523	0.06	0.00
	Jun 5	-	-	-	-	1,141	1,141	0.41	0.04
	Jun 24	19.28	17.06	2.22	11.51	5,397	5,396	1.48	0.03
	Jul 7	2.94	2.27	0.67	22.79	1,774	1,772	2.39	0.13
	Jul 29	1.54	2.61	0.00	0.00	1,146	1,146	0.70	0.06
	Aug 11	5.23	4.34	0.89	17.02	1,358	1,355	3.01	0.22
	Aug 25	6.18	1.97	4.21	68.12	3,837	3,835	2.42	0.06
	Sep 10	11.66	10.66	1.00	8.58	4,965	4,952	12.26	0.25
	Sep 25	8.32	2.08	6.24	75.00	1,812	1,811	1.01	0.06
	Oct 12	1.61	2.05	0.00	0.00	1,103	1,102	0.41	0.04
	Nov 1	3.46	2.23	1.23	35.55	2,506	2,506	0.30	0.01

Appendix 15(b)

		Chl-*a*				Biomass			
River	Date	Total	> 2 µm	< 2 µm	% PP	Total	> 2 µm	< 2 µm	% PP
Raisin	May 25	1.47	1.02	0.45	30.61	867	866	0.35	0.04
	Jun 11	2.63	2.19	0.44	16.73	305	304	0.31	0.10
	Jun 24	14.07	9.96	4.11	29.21	3,377	3,371	6.02	0.18
	Jul 7	3.22	3.14	0.08	2.48	3,545	3,543	1.77	0.05
	Jul 29	3.58	1.94	1.64	45.81	1,105	1,104	0.93	0.08
	Aug 11	1.69	1.67	0.02	1.18	714	713	0.73	0.10
	Aug 25	2.13	2.42	0.00	0.00	849	838	11.54	1.36
	Sep 10	3.19	4.94	0.00	0.00	1,255	1,235	20.15	1.61
	Sep 25	2.88	2.43	0.45	15.63	1,877	1,870	7.18	0.38
	Oct 12	1.96	0.87	1.09	55.61	474	473	0.65	0.14
	Nov 1	1.22	1.93	0.00	0.00	589	589	0.28	0.05
Rideau	May 27	5.37	4.00	1.37	25.51	1,549	1,548	1.10	0.07
	Jun 6	3.14	2.34	0.80	25.48	3,435	3,434	1.12	0.03
	Jun 26	5.58	5.01	0.57	10.22	2,190	2,187	2.38	0.11
	Jul 8	-	-	-	-	26,547	26,529	17.97	0.07
	Jul 26	6.28	4.73	1.55	24.68	2,435	2,415	20.17	0.83
	Aug 5	9.89	6.41	3.48	35.19	2,592	2,579	13.52	0.52
	Aug 26	4.67	5.00	0.00	0.00	2,735	2,711	24.47	0.89
	Sep 14	1.97	1.35	0.62	31.47	-	-	-	-
	Oct 2	3.14	2.11	1.03	32.80	1,433	1,431	1.68	0.12
	Oct 15	2.07	0.96	1.11	53.62	1,150	1,150	0.49	0.04
	Oct 30	2.34	1.00	1.34	57.26	744	743	0.50	0.07
Gatineau	May 22	-	-	-	-	296	295	0.87	0.29
	Jun 12	-	-	-	-	342	340	1.67	0.49
	Jun 25	1.01	1.13	0.00	0.00	413	408	5.06	1.23
	Jul 14	1.14	0.85	0.29	25.44	303	295	8.14	2.69
	Jul 28	0.97	0.74	0.23	23.71	182	175	6.95	3.81
	Aug 12	0.95	0.57	0.38	40.00	120	114	6.44	5.36
	Aug 25	0.56	0.52	0.04	7.14	274	269	4.54	1.66
	Sep 11	0.45	0.50	0.00	0.00	371	364	7.07	1.91
	Sep 24	0.68	0.47	0.21	30.88	205	199	5.24	2.56
	Oct 9	-	-	-	-	143	139	4.28	2.99
	Oct 26	0.76	0.00	0.00	3.65	191	188	3.65	1.91

Appendix 16(a)
Slope and regression statistics for chlorophyll-*a* (Chl-*a*) and microscope enumerations in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period. Size distribution slope analyses for chl-*a* represent the proportion of chl-*a* retained on the filter versus filter size. Slopes for enumerations represent the taxon-abundance size spectra with the analyses of phytoplankton density vs. the mean greatest axial linear dimension (GALD) of each taxon in the sample.

		Chl-*a*				Enumerations			
River	Season	Slope	r^2	p-value	n	Slope	r^2	p-value	n
S. N.	spring	-0.407	0.871	0.002	7	-0.861	0.392	0.000	39
		-0.426	0.944	0.001	6	-1.002	0.346	0.000	34
		-0.566	0.908	0.003	6	-1.941	0.474	0.000	33
	early summer	-0.345	0.648	0.051	6	-1.351	0.423	0.000	31
		-0.451	0.762	0.023	6	-1.005	0.392	0.000	44
		-0.392	0.958	0.000	7	-1.360	0.486	0.000	36
	base flow	-0.392	0.974	0.000	6	-0.569	0.081	0.211	21
		-0.380	0.722	0.016	7	-1.007	0.297	0.003	28
		-0.407	0.834	0.004	7	-1.188	0.326	0.001	31
	fall	-0.428	0.840	0.004	7	-1.024	0.458	0.000	37
		-0.386	0.925	0.001	7	-0.892	0.510	0.000	43
Castor	spring	-0.284	0.688	0.041	6	-0.216	0.041	0.303	28
		-0.419	0.581	0.053	6	-1.130	0.389	0.000	32
		-0.449	0.925	0.001	7	-1.039	0.335	0.000	40
	early summer	-0.397	0.666	0.025	7	-0.960	0.274	0.001	40
		-0.630	0.851	0.009	6	-0.941	0.381	0.000	33
		-0.396	0.941	0.000	7	-1.115	0.442	0.000	47
	base flow	-0.417	0.782	0.008	7	-1.255	0.459	0.000	47
		-0.505	0.865	0.002	7	-1.323	0.405	0.000	45
		-0.416	0.845	0.003	7	-1.230	0.478	0.000	39
	fall	-0.501	0.795	0.007	7	-0.988	0.546	0.000	36
		-0.353	0.493	0.054	7	-0.559	0.233	0.001	48

Appendix 16(b)

		Chl-*a*				Enumerations			
River	Season	Slope	r^2	p-value	n	Slope	r^2	p-value	n
Raisin	spring	-0.419	0.977	0.000	7	-0.979	0.582	0.000	38
		-0.340	0.794	0.007	7	-0.876	0.398	0.000	37
		-0.497	0.772	0.009	7	-1.492	0.568	0.000	41
	early summer	-0.473	0.791	0.007	7	-0.495	0.132	0.014	45
		-0.423	0.864	0.002	7	-0.650	0.219	0.003	38
		-0.497	0.740	0.013	7	-1.041	0.522	0.000	40
	base flow	-0.527	0.751	0.012	7	-1.229	0.568	0.000	47
		-0.556	0.558	0.053	6	-1.796	0.594	0.000	40
		-0.606	0.898	0.014	5	-1.451	0.494	0.000	37
	fall	-0.419	0.960	0.000	7	-1.165	0.521	0.000	37
		-0.414	0.509	0.054	7	-0.788	0.442	0.000	35
Rideau	spring	-0.386	0.975	0.000	7	-1.320	0.462	0.000	34
		-0.510	0.773	0.009	7	-0.987	0.347	0.000	31
		-0.530	0.824	0.005	7	-0.954	0.270	0.006	27
	early summer	-0.597	0.966	0.000	6	-1.197	0.424	0.000	26
		-0.380	0.905	0.001	7	-1.272	0.439	0.000	52
		-0.410	0.982	0.000	7	-1.481	0.557	0.000	57
	base flow	-0.469	0.781	0.008	7	-1.232	0.395	0.000	47
		-0.418	0.943	0.000	7	-1.137	0.299	0.001	34
		-0.489	0.806	0.006	7	-1.442	0.460	0.000	36
	fall	-0.441	0.886	0.002	7	-0.857	0.287	0.001	36
		-0.371	0.956	0.000	7	-0.966	0.515	0.000	26
Gatineau	spring	-	-	-		-0.854	0.453	0.000	38
		-0.425	0.579	0.053	7	-0.803	0.348	0.000	41
		-0.490	0.817	0.005	7	-1.035	0.411	0.000	42
	early summer	-0.482	0.860	0.003	7	-1.196	0.512	0.000	38
		-0.475	0.780	0.008	7	-1.049	0.430	0.000	44
		-0.457	0.859	0.003	7	-0.948	0.408	0.000	49
	base flow	-0.513	0.817	0.005	7	-0.988	0.446	0.000	48
		-0.579	0.515	0.054	7	-0.864	0.294	0.000	46
		-0.467	0.912	0.001	7	-0.984	0.456	0.000	48
	fall	-0.472	0.922	0.001	7	-0.914	0.395	0.000	46
		-0.683	0.923	0.002	6	-0.960	0.406	0.000	47

Appendix 17(a)
Total abundance for each size fraction in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period. Picoplankton (pico), ultraplankton (ultra), nanoplankton (nano), microplankton (micro), and netplankton (net) values are in cells/L.

River	Season	Pico (< 2 µm)	Ultra (2-10µm)	Nano (10-20 µm)	Micro (20-64 µm)	Net (> 64 µm)
S. N.	spring	2.65E+05	3.59E+05	1.92E+05	2.16E+05	1.45E+04
		4.60E+05	1.09E+06	4.28E+05	1.39E+05	6.52E+03
		4.90E+08	1.91E+06	3.39E+06	3.89E+06	0.00E+00
	early summer	9.18E+06	1.10E+06	2.20E+05	8.17E+04	5.96E+03
		1.26E+06	4.32E+05	2.21E+05	1.26E+05	6.81E+03
		2.70E+06	3.81E+05	8.02E+05	2.35E+05	5.96E+03
	base flow	1.75E+07	6.82E+05	2.27E+05	1.37E+08	2.27E+04
		1.15E+07	3.42E+05	2.86E+06	5.59E+05	1.43E+05
		5.71E+06	7.64E+06	1.35E+05	1.27E+05	6.81E+03
	fall	8.33E+05	7.54E+05	7.47E+04	7.76E+04	1.08E+04
		5.29E+05	2.78E+05	8.87E+04	1.38E+05	1.45E+04
Castor	spring	1.45E+05	8.18E+04	1.78E+05	4.91E+05	2.72E+04
		7.74E+05	9.96E+05	2.39E+05	2.47E+05	0.00E+00
		2.83E+06	3.49E+06	3.79E+06	1.14E+06	1.77E+05
	early summer	4.57E+06	9.67E+05	5.21E+05	3.52E+05	0.00E+00
		1.34E+06	3.37E+05	2.27E+05	2.98E+05	2.04E+04
		5.74E+06	9.59E+05	3.63E+05	2.56E+05	5.67E+03
	base flow	4.61E+06	2.23E+06	8.61E+05	5.07E+05	2.27E+03
		2.34E+07	8.26E+06	1.43E+06	4.00E+05	3.74E+04
		1.92E+06	2.25E+06	8.06E+05	2.17E+05	1.47E+04
	fall	7.74E+05	6.84E+05	2.70E+05	1.10E+05	4.31E+04
		5.78E+05	4.07E+05	2.21E+05	2.39E+05	8.96E+04

Appendix 17(b)

River	Season	Pico (< 2 µm)	Ultra (2-10 µm)	Nano (10-20 µm)	Micro (20-64 µm)	Net (> 64 µm)
Raisin	spring	6.75E+05	1.83E+06	2.28E+05	2.89E+05	1.25E+04
		5.98E+05	3.11E+05	1.99E+05	9.15E+04	5.84E+03
		1.15E+07	3.47E+06	2.86E+06	4.74E+05	3.40E+04
	early summer	3.38E+06	4.67E+05	1.71E+05	3.83E+05	2.41E+04
		1.77E+06	2.54E+05	3.39E+05	1.13E+05	5.28E+04
		1.39E+06	2.23E+05	2.51E+05	7.66E+04	1.59E+04
	base flow	2.20E+07	4.68E+05	2.73E+05	1.12E+05	1.63E+04
		3.85E+07	1.83E+06	5.73E+05	9.70E+04	5.10E+03
		1.37E+07	2.52E+06	9.08E+05	1.32E+05	1.53E+04
	fall	1.24E+06	6.16E+05	1.88E+05	1.30E+05	8.51E+03
		5.39E+05	2.42E+05	1.62E+05	2.06E+05	2.41E+04
Rideau	spring	2.11E+06	4.32E+06	6.02E+05	6.54E+05	2.84E+04
		2.15E+06	2.12E+06	2.51E+05	3.14E+05	6.81E+03
		4.54E+06	3.55E+06	6.21E+05	7.28E+05	0.00E+00
	early summer	3.45E+06	3.92E+06	1.76E+06	4.93E+05	8.45E+03
		3.85E+07	8.94E+05	2.27E+06	5.31E+05	9.07E+03
		2.58E+07	4.09E+06	6.87E+05	2.66E+05	5.90E+04
	base flow	4.67E+07	2.76E+06	7.27E+05	3.74E+05	6.92E+04
		-	1.03E+06	3.21E+05	1.16E+05	2.72E+03
		3.21E+06	1.59E+06	2.81E+05	4.70E+05	3.40E+03
	fall	9.94E+05	2.69E+06	1.89E+05	1.34E+05	3.85E+04
		1.15E+06	1.71E+06	1.02E+05	2.79E+05	3.40E+03
Gatineau	spring	1.67E+06	4.48E+05	8.66E+04	6.03E+04	5.56E+04
		3.19E+06	3.83E+05	1.53E+05	2.78E+04	7.60E+04
		9.67E+06	4.09E+05	4.00E+05	1.09E+05	6.13E+04
	early summer	1.55E+07	7.79E+05	1.06E+05	5.55E+04	4.48E+04
		1.33E+07	3.82E+05	3.06E+05	4.93E+04	4.78E+04
		1.23E+07	5.68E+05	2.75E+04	3.33E+04	2.81E+04
	base flow	8.69E+06	2.71E+05	1.73E+05	6.11E+04	4.08E+04
		1.35E+07	2.06E+05	2.35E+05	9.61E+04	6.01E+04
		1.01E+07	2.95E+05	1.29E+05	4.76E+04	2.13E+04
	fall	8.18E+06	1.80E+05	3.21E+05	5.91E+04	2.42E+04
		6.97E+06	3.62E+05	1.92E+05	6.49E+04	2.68E+04

Appendix 18(a)
Total biomass for each taxonomic division in the South Nation (S. N.), Castor, Raisin, Rideau, and Gatineau rivers over the 2009 study period. Bacillariophyta (diatoms), cryptophyta (crypto), chlorophyta (chloro), pyrrhophyta (pyrr), chrysophyta (chryso), and euglenophyta (euglenoid) values are in mg/m^3. The number of taxa (# taxa) represents the total number of species observed per sampling event.

River	Date	Diatoms	Crypto	Chloro	Pyrr	Chryso	Euglenoid	# taxa
S. N.	May 20	540	234	7	0	39	11	37
	Jun 5	211	193	17	0	53	38	30
	Jun 24	500	494	508	307	87	497	30
	Jul 7	175	165	37	53	9	58	30
	Jul 29	147	63	10	164	73	21	41
	Aug 11	101	241	60	229	12	11	34
	Aug 25	379	1	36,522	577	254	480	20
	Sep 10	499	737	1990	28	268	125	27
	Sep 25	137	737	50	12	20	6	30
	Oct 12	91	122	14	3	26	2	34
	Nov 1	246	36	16	6	33	0	40
Castor	May 19	2,187	51	21	0	3	5	24
	Jun 5	837	154	33	0	20	0	29
	Jun 24	1,631	1,296	1,541	255	243	430	39
	Jul 7	1,190	292	40	0	50	200	39
	Jul 29	1,053	37	24	0	10	0	28
	Aug 11	866	264	66	38	34	8	44
	Aug 25	2,425	377	267	228	64	450	46
	Sep 10	2,134	725	477	87	337	1,191	43
	Sep 25	543	845	178	71	105	68	38
	Oct 12	652	183	33	26	67	140	34
	Nov 1	2,302	86	21	0	60	13	45

Appendix 18(b)

River	Date	Diatoms	Crypto	Chloro	Pyrr	Chryso	Euglenoid	# taxa
Raisin	May 25	261	115	15	0	45	9	33
	Jun 11	81	158	35	0	30	0	36
	Jun 24	564	1,610	349	346	501	0	39
	Jul 7	3,020	172	31	218	13	89	44
	Jul 29	511	188	30	81	23	270	36
	Aug 11	304	263	28	54	19	45	38
	Aug 25	207	128	43	44	93	242	42
	Sep 10	481	81	207	75	217	173	38
	Sep 25	76	1,131	179	151	196	112	35
	Oct 12	168	227	6	5	62	2	35
	Nov 1	326	141	23	8	91	0	34
Rideau	May 27	191	252	52	1	583	0	28
	Jun 6	34	899	32	255	161	0	29
	Jun 26	104	2,011	26	0	41	0	24
	Jul 8	1,085	1,131	181	0	201	0	23
	Jul 26	431	1082	75	357	421	0	46
	Aug 5	985	522	309	0	325	80	51
	Aug 26	1,047	739	225	0	324	0	39
	Sep 14	15	139	100	0	37	0	34
	Oct 2	204	1,025	34	93	73	0	35
	Oct 15	146	401	24	44	76	2	32
	Oct 30	163	184	14	145	238	0	25
Gatineau	May 22	45	32	32	35	114	0	34
	Jun 12	72	28	25	34	134	2	36
	Jun 25	49	63	21	71	190	0	39
	Jul 14	112	55	22	10	49	0	36
	Jul 28	72	48	24	3	27	0	39
	Aug 12	56	15	8	5	19	0	44
	Aug 25	55	54	26	4	22	0	41
	Sep 11	40	48	20	0	34	0	39
	Sep 24	29	14	26	8	21	2	39
	Oct 9	49	31	13	0	28	0	39
	Oct 26	80	38	14	5	42	0	41