ORIGINAL ARTICLE

Water chemistry and periphyton biomass in the Rideau River: Have conditions changed after 24 years?

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ABSTRACT

Over the past three decades, the Rideau River (Ontario, Canada) watershed has experienced a land-use change from rural and agricultural land towards urban land, and the introduction and expansion of invasive species. This study examined spatial and temporal patterns of periphyton biomass (chlorophyll-*a* and ash-free dry mass) collected from riffle zones in 1995 and 2019 along a 66-km stretch of the Rideau River. This study also examined long-term changes in water nutrient and chloride concentrations collected through the Provincial Water Quality Monitoring Network between 2000 and 2018. Declines in total nitrogen between 2000 and 2018 occurred in areas that have not experienced urbanization since the 2000s. In contrast, declines in total phosphorus and increases in chloride between 2000 and 2018 occurred at midstream and downstream sites that have undergone urbanization during the same time period. Conductivity also showed an increase with distance downstream similarly in both 1995 and 2019. Average total phosphorus and total Kjeldahl nitrogen concentrations were lower in 2019 compared to 1995 but did not increase with distance downstream as seen in many river systems, including the Rideau River, in earlier years. Periphyton biomass did not change along the length of the river between 1995 and 2019, despite the declines in nutrient concentrations. These findings highlight the persistence of riverine periphyton in a multi-use watershed experiencing ongoing anthropogenic changes.

INTRODUCTION

Globally, riverine environments are heavily impacted by human activities including changes in water quantity and flow rates, invasive species, land-use change, excessive nutrients, pollution, and climate change (Reid *et al.,* 2019). Periphyton biomass is in part related to the biological, chemical, and physical properties of a river (Larned, 2010), and is also an important primary producer supporting riverine ecosystems and providing benthic habitat for other organisms (Vadeboncoeur and Power, 2017). Therefore, changes in periphyton biomass can be a key indicator of how human activities are altering riverine ecosystems (Biggs and Close, 1989; Hill *et al.*, 2000; Slavik *et al.*, 2004). Specifically, increased periphyton biomass has been linked to eutrophication (Biggs, 2000) and human-altered flow regimes which reduce the frequency of disturbance events (Biggs *et al.*, 1998).

Rivers are influenced by watershed land-use patterns that can alter the biological, physical, and chemical properties of the system (O'Brien and Wehr, 2010). For example, at over 200 long-term monitoring sites across Canada, agricultural land-use has been consistently associated with increased levels of nitrogen and phosphorus compared to forested landscapes (Chambers *et al.*, 2012). When agricultural land is converted to urban land, declines in total phosphorus (TP) are observed as non-point sources of phosphorus from fertilizers are reduced (Carpenter *et al.*, 1998); however, this pattern is not uniform across all study sites and may depend on the type of agricultural land-use before conversion (Raney and Eimers, 2014). Further, rivers globally have shown declines in inputs of phosphorus and nitrogen as a result

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of improved wastewater treatment practices (Schindler, 2012), and the impacts of such reductions have wide-ranging ecological consequences in freshwater ecosystems (Ibáñez and Peñuelas, 2019).

The Rideau River is one of two rivers that comprises the Rideau Canal system which extends from the city of Kingston to the city of Ottawa in eastern Ontario, Canada. The Rideau River has experienced a series of environmental changes over the last few decades, including land-use changes associated with urban sprawl around the city of Ottawa, climate change, and the introduction of invasive species, including the invasion of zebra mussels (*Dreissena polymorpha*) in the fall of 1990 (Martel, 1995; Vidal *et al.*, 2004; Martel and Madill, 2018). However, paleolimnological research has shown that lacustrine ecosystems located within the Rideau Canal waterway have been resilient in the face of environmental change; with the invasion of zebra mussels and nutrient enrichment having little impact on diatom assemblages over the last few decades (Karst and Smol, 2000).

The Rideau River has undergone moderate eutrophication, as indicated by water nutrient concentrations and periphyton biomass, compared with other rivers systems in the region (Chételat *et al.,* 1999; RVCA, 2012). Chételat *et al.* (2000) examined periphyton biomass collected at seven shallow riffle zones, where periphyton growth is not limited by light availability, in 1995 in the mid-section of the Rideau River to examine how periphyton biomass is related to water chemistry. The first objective of the present study was to revisit these sites to quantify any discrete changes in water chemistry and periphyton biomass in the Rideau River between 1995 and 2019 (a period of 24 years). The second objective of this study was to identify long-term trends in continuous water chemistry in the Rideau River over a sub-period of 18 years (2000-2018).

METHODS

Study area

The Rideau River runs for 110 km from Upper Rideau Lake to the Ottawa River and drains almost 3900 km^2 of land. Construction of the Rideau Canal began in the early-1800s with the intention of being a military and commercial route (Charron *et al.*, 1982). Because of its historical significance, the Rideau Canal is designated as a Canadian National Historic Site, a Canadian Heritage River and a UNESCO World Heritage Site (UNESCO, 2007; Canadian Heritage Rivers System, 2021; Parks Canada, 2021). Although the Rideau Canal no longer serves its intended industrial, military, and commercial transportation uses, it is an important economic driver of natural capital in the region, attracting more than a million visitors a year for recreation and tourism activities (Parks Canada, 2020), which rely on good water quality and a healthy ecosystem (Butler, 1980).

As of 2014, approximately 45% of the land in the Lower Rideau sub-watershed was forested or wetland areas, 35% was agricultural or rural land, and 20% was urban or residential (RVCA, 2019). Land-use change from agricultural to urban/suburban land between 2000-2015 has occurred along the Rideau River as the suburban area around the City of Ottawa has expanded (OMNR, 2019), and the population has increased from approximately 720,000 in 1996 to over one million today (Statistics Canada, 1996; 2017). Within the study area, upstream land use is primarily forest, wetland, agricultural, and rural land use, with midstream land use being a combination of agricultural, rural, and urban land, and downstream land use becoming increasingly urban as the river approaches the City of Ottawa.

Data collection

To assess changes in periphyton biomass in the Rideau River, seven sites were selected to match the seven sites sampled in 1995 (Chételat *et al.*, 2000). The sites were located along a 66 km stretch of the Rideau River (Fig. 1). Efforts were made to sample near the original locations sampled in 1995 by Chételat *et al.* (2000), but some of the exact locations sampled in 1995 were no longer publicly accessible via the shore in 2019 due to development in the area, so samples were collected from the nearest point to the previously sampled locations. Summer field sampling followed closely the methods of Chételat *et al.* (2000). Briefly, eight fist-sized rocks were collected from transects in riffle zones perpendicular to the shore at each of the seven periphyton sites on June 4 and August 2, 2019. Similarly, the samples collected in 1995 were collected at the end of May and end of July. The water depth (m) of each rock was measured before being collected, and mean water current velocity (m s^{-1}) was measured at 0.4 times the depth of the rock using a Global FP111 Flow Probe Current Meter. The average water discharge rate in the Rideau River in Ottawa (hydrometric station 02LA004) from May to August 1995 was 13.67 m^3 s^{-1} (range = 4.79-18.4 m^3 s^{-1}), whereas average water discharge rate from May to August in 2019 was 34.26 m^3 s^{-1} (range = 6.74-81.0 m^3 s^{-1}; ECCC, 2020). Water discharge rates from May to July in 2019 were consistently higher than those of 1995, whereas the water discharge rate in August 2019 was lower than that of 1995. The temporal variation in water discharge rates is likely due to the total precipitation from November 2018 to August 2019 (847.6 mm) being much higher than the total precipitation from November 1994 to August 1995 (754.7 mm), and the 1981-2010 climate normal for November to August (740.9 mm). Here, total precipitation from November to August takes into account both the total snowfall during the winter preceding the sampling season and the total rainfall during the sampling season.

Water samples were collected on the two dates periphyton were collected (June 4 and August 2, 2019), as well as three more dates (June 23, July 28, and August 22, 2019) for a total of five sampling dates. These additional water samples were collected to increase the sample size and thus the strength of statistical analyses of the water chemistry parameters. Water samples were collected at the upstream edge of each transect to avoid disturbance of the sediment during water sample collection. All rocks and water samples were stored in the dark at 4°C until processed. A YSI ProPlus Multiparameter Instrument was used to measure physical and chemical parameters (conductivity (μS cm^{-1}), water temperature (°C), dissolved oxygen concentration (mg L^{-1}), and pH) at each site on the same five dates the water samples were collected.

Laboratory analyses

In the laboratory, each rock was scrubbed with a nylon brush to remove all periphyton from their surface with a known volume of deionized water. This mixture was homogenized in a blender on high for 30 seconds and used to calculate periphyton biomass per unit area of the rock. The surface area of each rock was estimated by weighing the aluminum foil required to cover the surface of the rock in a single layer. The mass of the aluminum foil was used in a weight-to-area function derived from a linear regression which estimates surface area of the rock based off the mass of aluminum foil ($y = 0.0025x + 0.030$, $r^2 = 0.99$, $p<0.001$; Dudley *et al.*, 2001). Periphyton biomass

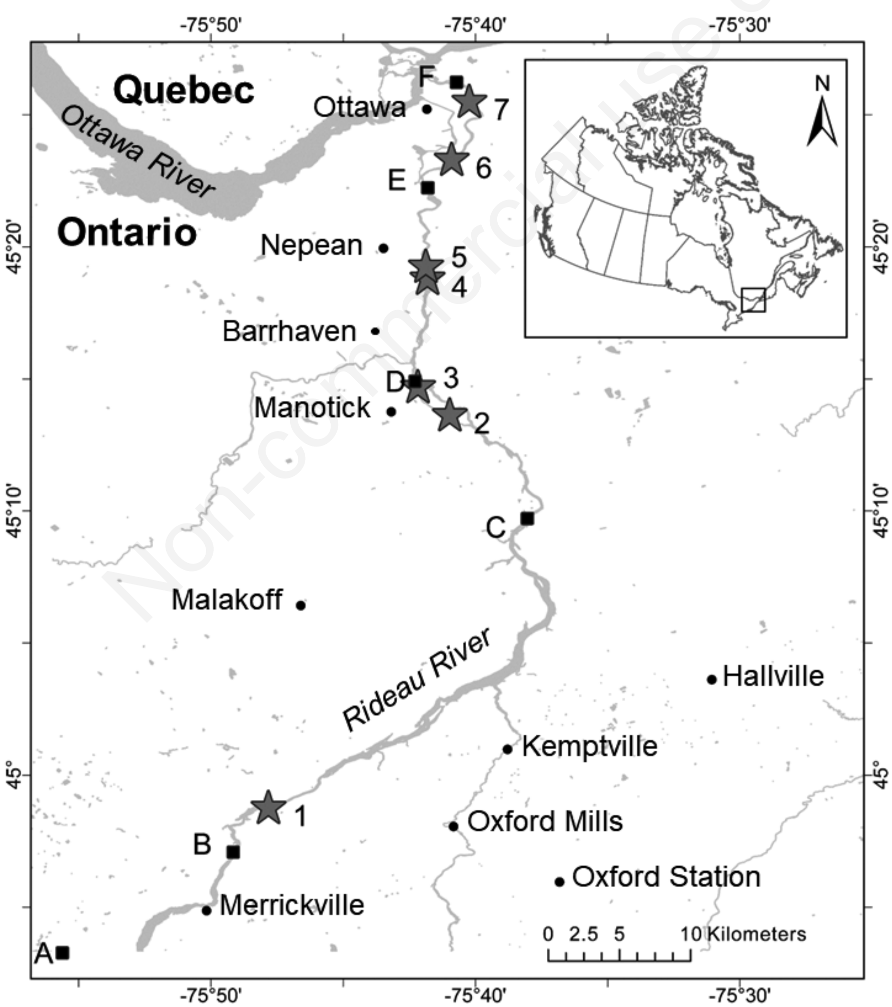

Fig. 1. Map showing the seven sample sites (stars, 1-7) and the six Provincial Water Quality Monitoring Network stations (squares, A-F) along the Rideau River in Ontario, Canada. Map created in ArcGIS Pro 2.1.0 using geospatial data acquired from Natural Earth (2018), Natural Resources Canada (2017), and Ontario Ministry of Natural Resources and Forestry (OMNR, 2019).

was quantified in two ways, chlorophyll-*a* (mg m^{-2}), and ash-free dry mass (g m^{-2}). Both measures of periphyton biomass are presented per unit area of each rock with the assumption that periphyton growth only takes place on 60% of the surface of the rock (Biggs and Close, 1989). This assumption was also made by Chételat *et al.* (2000).

Chlorophyll-*a* was measured by filtering 10 to 50 mL of the periphyton homogenate through Whatman grade 934-AH glass fibre filters using a vacuum filtration system. The filters were wrapped in aluminum foil and frozen until chlorophyll-*a* extraction. In a dark room, chlorophyll-*a* was extracted from each filter in test tubes using 15 mL of 95% ethanol heated in a water bath to 79°C for 5 min (ISO, 1992). Test tubes were placed on a rack, wrapped in aluminum foil, and stored in a refrigerator overnight at 4°C. Approximately 5 mL of this chlorophyll-*a* extract solution was filtered through a 0.45 µm filter into a glass cuvette (1 cm path length) and analysed in a UV-VIS spectrophotometer (Shimadzu UV-1280) at 665 and 750 nm wavelengths. The absorbance readings of each sample were recorded at each wavelength before subtracting the absorbance at 750 nm from the absorbance at 665 nm to correct the chlorophyll-*a* reading for turbidity. The concentration of chlorophyll-*a* was standardized according to the volume of deionized water used in the periphyton homogenate and for the volume of homogenate filtered, per unit area of rock (mg m^{-2}; 60% of total rock surface area).

In 1995, chlorophyll-*a* was extracted by Chételat *et al.* (2000) using a dimethyl sulfoxide (DMSO)-acetone solvent, as described in Burnison (1980) and Jeffrey and Humphrey (1975). In 2019, 95% ethanol was used to extract chlorophyll-*a* as the extraction method is simple and safer than that of DMSO-acetone, and the difference in the efficiency of chlorophyll-*a* extraction between methods is negligible (Sartory and Grobbelaar, 1984; Webb *et al.*, 1992). The main difference between the chlorophyll-*a* analysis method used in this study and the method used by Chételat *et al.* (2000), is in the equation used to estimate chlorophyll-*a* from the absorbance readings. The trichromatic equation described by Jefferey and Humphrey (1975; chlorophyll-*a* = $11.85E_{664} - 15.4E_{647} - 0.08E_{630}$) was used by Chételat *et al.* (2000), whereas a monochromatic equation described by Lorenzen (1967) and Wintermans and De Mots (1965; chlorophyll-*a* = $29.5E_{665}$) was used in this study. To account for any discrepancy caused by the use of different spectrophotometric equations, a correction factor was calculated by measuring chlorophyll-*a* in six samples using both the trichromatic and monochromatic equations described ($y = 0.3832x - 0.0045$, $r^2 = 0.998$, $n = 6$; see Supplementary Fig. S1). This correction factor (0.38) was multiplied by the 2019 concentrations of chlorophyll-*a* calculated using the monochromatic equation above.

Ash-free dry mass (AFDM) was determined by filtering 10 to 50 mL of the periphyton homogenate through pre-ashed Whatman grade 934-AH glass fibre filters. The mass of each pre-ashed filter was measured and recorded before being used to filter the periphyton material. The filters with the retained periphyton material were then dried overnight at 60°C in an oven and weighed again to obtain the dry mass of the periphyton and filter. The mass of each filter was subtracted from the dry mass of the periphyton and filter to obtain the mass of the dried periphyton alone. The dry filters and periphyton were then frozen until being ashed at 500°C for 4 hours in a muffle furnace and weighed to obtain the ash mass of the periphyton and filter. To calculate AFDM of the periphyton per unit area of rock, the ashed mass of periphyton was subtracted from the corresponding dry mass of periphyton and divided by 60% of the surface area of the rock (Chételat *et al.*, 2000).

To further characterize periphyton biomass, percent organic matter of the periphyton sample was calculated as the percent of AFDM relative to the total dry mass, and percent chlorophyll-*a* was calculated as the mass of chlorophyll-*a* relative to the AFDM of the sample. Percent organic matter indicates the proportion of the periphyton matrix that is composed of organic material, relative to inorganic material such as sediment. Percent chlorophyll-*a* provides an indication of the proportion of photosynthetic algae found in the periphyton matrix; if this percentage is low, this suggests a high proportion of heterotrophic organisms or detritus in the periphyton sample (Chételat *et al.*, 2000).

Water samples were sent to the City of Ottawa Environmental Services Department Water Chemistry Laboratory for nutrient analyses (TP and total Kjeldahl nitrogen; TKN). This is the same laboratory where water samples were analysed in Chételat *et al.* (2000). The methods used to analyse water samples were confirmed to be the same as in 1995 (COESD, 2018). The local Rideau Valley Conservation Authority (RVCA) uses TP and TKN concentrations as primary and secondary indicators of nutrient loading, respectively (RVCA, 2012). As such, the RVCA compares TP concentrations against a guideline of 0.03 mg L^{-1} and TKN concentrations against a guideline of 0.50 mg L^{-1} to determine instances of excessive nutrient loading. Total Kjeldahl nitrogen is the sum of organic nitrogen and total ammonia (un-ionized ammonia + ammonium). Anthropogenic inputs of nitrogen into surface waters via municipal effluent and agricultural run-off are largely comprised of the forms of nitrogen accounted for by TKN, plus nitrate (CCME, 2016). While TKN does not account for oxidized forms of nitrogen (i.e., nitrate, nitrite), TKN does account for the vast majority (mean = 80%; median = 86%) of the total concentration of total nitrogen in this river compared to nitrate (mean = 18%; median = 13%) and nitrite (mean = 0.9%; median = 0.8%; OMECC, 2018).

Continuous water chemistry data

In addition to the water quality data collected for this study, water chemistry data from six water quality monitoring stations from the Ontario Provincial (Stream) Water Quality Monitoring Network (PWQMN; OMECC, 2018) that best cover the stretch of the river where Chételat *et al.* (2000) sampled were selected to examine continuous trends in water chemistry indicators between 2000-2018. The stations IDs were 18003302602, 18003303502, 18003302902, 18003303702, 18003303102, and 18003303402, which are herein referred to as stations A, B, C, D, E, and F, respectively. Data from these PWQMN stations were analysed for temporal trends in the TP, total nitrogen (TN), and chloride concentrations of unfiltered water samples between 2000 and 2018. All three water chemistry parameters were quantified in the Ontario Ministry of Environment, Conservation, and Parks laboratory using colorimetric methods. To determine TP concentration, water samples first underwent acid digestion using sulfuric acid, mercuric oxide, and potassium sulfate. To determine TN concentration, water samples first underwent UV-assisted acid digestion using sulfuric acid and peroxodisulfate, while mercuric thiocyanate was used to treat water samples being analysed for chloride. Although some PWQMN data stretching back to the 1960s exists for some regions, samples were not collected consistently in this study region until the year 2000. The frequency of data collection through the PWQMN between 2000 and 2018 was variable, but most stations were sampled monthly between April and November of each year. Since the discrete analysis of water chemistry did not account for inorganic forms of nitrogen (i.e., nitrate and nitrite), continuous temporal trends in TN were analyzed to capture changes in nitrate and nitrite as well as organic forms of nitrogen.

Statistical analyses

All statistical analyses were conducted using the software R (R Core Team, 2019), all graphs were produced in R using the ggplot2 package (Wickham, 2016), and the map was created using ArcGIS Pro (version 2.1.0). To assess long-term trends in water quality indicators in the Rideau River, a linear regression model was used to examine if there was a significant change in the PWQMN TP, chloride, and TN concentrations between 2000 and 2018 for each of the six PWQMN stations (A-F). Linear regression models were also used to quantify the 2019 spatial trends in nutrient levels (TP) and periphyton biomass (chlorophyll-*a* and AFDM) in relation to distance downstream. Total phosphorus, chlorophyll-*a*, and AFDM data were $\log_{10}$ transformed to meet the assumptions of linear regressions.

To examine temporal variation of nutrient levels (TP), conductivity, and periphyton biomass (chlorophyll-*a* and AFDM) in relation to distance downstream, an analysis of covariance (ANCOVA) was carried out using year (1995 and 2019) as the covariate. To characterize the relationship between chlorophyll-*a* and AFDM with respect to water current velocity, a linear regression model was used for both the 1995 and 2019 datasets. Finally, linear regression models were used to assess if chlorophyll-*a* and AFDM were related to TP and TKN for the 2019 dataset to determine if nutrient levels influenced the measures of periphyton biomass in the Rideau River.

RESULTS

Discrete spatial and temporal variation in water chemistry and periphyton biomass

When data from all periphyton sampling sites were combined, chemical and physical variables measured in 2019 exhibited modest declines compared with those observed by Chételat *et al.* (2000) in 1995 (Tab. 1). In both 1995 and 2019, no relationship between TP and distance downstream was observed in the Rideau River (Fig. 2A). The largest variation in water TP concentration between years occurred at sites 2 and 3 (Fig. 2A). Similar to TP, TKN concentrations in the Rideau River showed no linear relationship with distance downstream in 2019. In contrast, conductivity had a significant increasing trend with distance downstream in both 1995 and 2019 (Fig. 2B). An ANCOVA indicated a significant difference in the intercept between conductivity and distance downstream in 1995 compared to 2019, while the slope did not significantly differ (Tab. 2).

With data from all sampling sites combined, chlorophyll-*a*, AFDM, and the percentage of chlorophyll-*a* in organic matter showed very little change between 1995 and 2019 (Tab. 1). There was no significant relationship between water TP concentration and periphyton chlorophyll-*a* concentrations, nor between TP and AFDM (Fig. 3). Similarly, there were no significant relationships between TKN and chlorophyll-*a* ($r^2 = 0.094$, $p=0.29$, $n=14$), nor between TKN and AFDM ($r^2 = 0.18$, $p=0.13$, $n=14$).

In 1995, there was a significant increase in periphyton chlorophyll-*a* concentration with distance downstream in the Rideau River, however this trend was no longer significant in 2019 (Fig. 2C), suggesting a slight homogenization of periphyton biomass over this stretch of the river. An ANCOVA indicated a significant difference in the intercept of the relationship between chlorophyll-*a*

Tab. 1. Comparison of biological, chemical, and physical parameters (mean ± SE) and sample sizes (n) between data collected in 1995 (Chételat *et al.*, 2000) and 2019.

	1995	n	2019	n
pH	8.4 ± 0.08	14	8.3 ± 0.02	28
Conductivity (µS cm^{-1})	368 ± 11	14	314 ± 7	28
Current velocity (m s^{-1})	0.42 ± 0.02	112	0.30 ± 0.03	112
Total phosphorus (µg L^{-1})	39 ± 2	33	33 ± 2	28
Total Kjeldahl nitrogen (mg L^{-1})	0.85 ± 0.41	888*	0.61 ± 0.01	28
Ash-free dry mass (g m^{-2})	12 ± 0.6	112	13 ± 0.6	112
Chlorophyll-*a* (mg m^{-2})	49 ± 4	112	53 ± 3	112
% Chlorophyll-*a* in organic matter	0.39 ± 0.02	112	0.43 ± 0.01	112
% Organic matter	34 ± 2	112	30 ± 1	112

**TKN concentrations were obtained directly from the City of Ottawa which resulted in a much higher sample size compared to measurements of TP by Chételat et al. (2000) in 1995.*

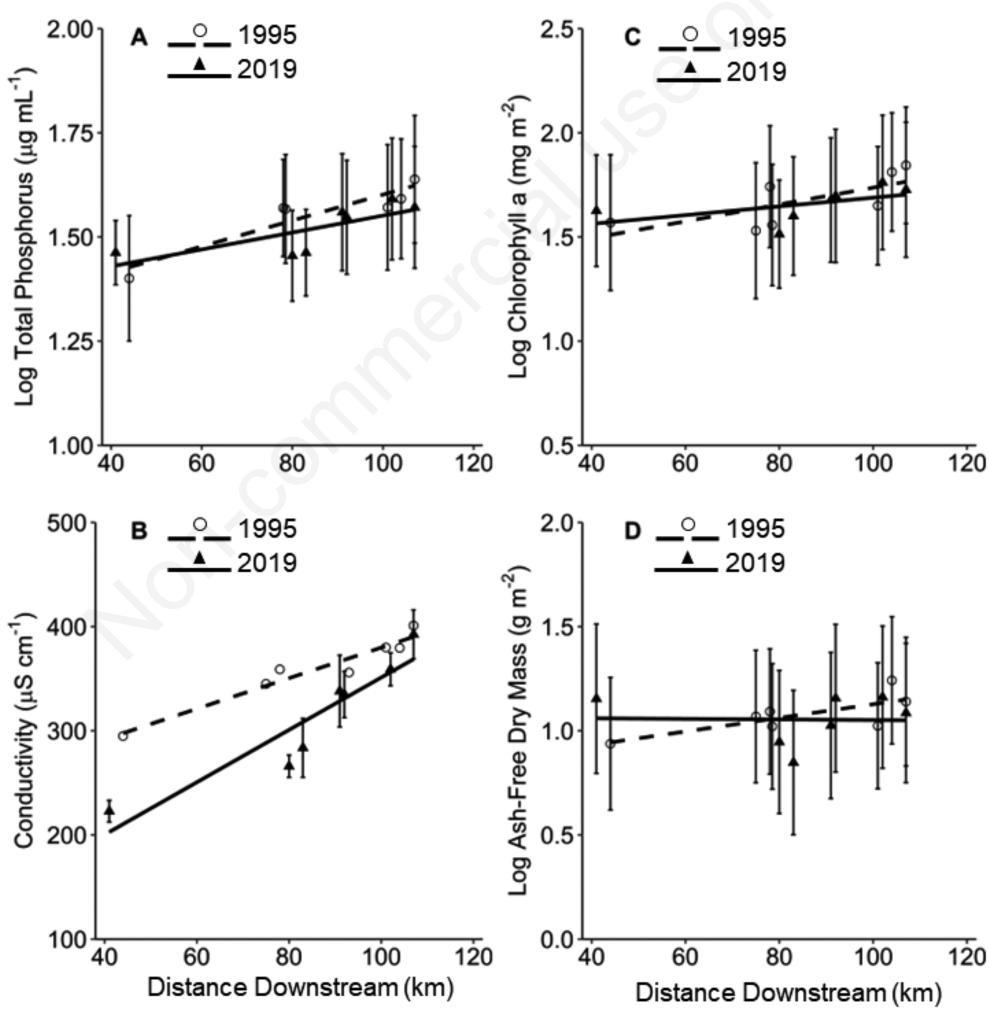

Fig. 2. The log concentration of total phosphorus (A), conductivity (B), log concentration of chlorophyll-*a* (C), and log ash-free dry mass (D) with trendlines in 1995 (open circles, dashed line; Chételat *et al.*, 2000) and 2019 (black triangles, solid line) with respect to distance downstream in the Rideau River in Ontario, Canada. A) 1995: $r^2 = 0.12$, p=0.065; 2019: $r^2 = 0.082$, p=0.14. B) 1995: $r^2 = 0.93$, p=0.00045; 2019; $r^2 = 0.86$, p=0.0026. C) 1995: $r^2 = 0.071$, p=0.0046; 2019: $r^2 = 0.028$, p=0.078. D) 1995: $r^2 = 0.086$, p = 0.0017, 2019: $r^2 = 0.00011$, p=0.91.

and distance downstream in 1995 compared to 2019, however the slope did not differ between years (Tab. 2).

Similarly, there was a significant increasing trend in AFDM with distance downstream in 1995, however this spatial trend was not evident in 2019 (Fig. 2D). In addition, an ANCOVA indicated a significant difference in the slope of the relationship between AFDM and distance downstream in 1995 compared to 2019, however the intercept did not significantly differ between years (Tab. 2). The changes in both chlorophyll-*a* and AFDM from 1995 to 2019 were most apparent at the upstream and midstream sites (sites 1, 2 and 3) compared to downstream sites, which corresponds with the stretch of the river that has experienced significant changes in PWQMN data for TP and chloride.

Continuous temporal trends in water chemistry

From 2000 to 2018 there were significant declines in TP concentration at the two midstream PWQMN stations C and D (Fig. 4a) whereas the other four stations showed no significant change in TP. In contrast to TP, chloride concentrations significantly increased at three of the six PWQMN stations between 2000 and 2018 (Fig. 4b). Meanwhile, the rural, upstream stations and Station E did not show significant changes to chloride concentration between 2000 and 2018. Lastly, TN concentrations significantly declined at 3 PWQMN stations from 2000 to 2018 (Fig. 4c), while the midstream stations, where there was a significant decline in TP, experienced no significant change in TN over the monitoring period.

DISCUSSION

This study found that there were no spatial relationships for AFDM nor chlorophyll-*a* along the study reach of the Rideau River in 2019, whereas periphyton biomass showed a significant increase with distance downstream in 1995 (Chételat *et al.*, 2000), suggesting that periphyton biomass in the Rideau River has become more spatially uniform. This study also suggests that midstream areas in the Rideau River have experienced declines in TP and increases in chloride over the past 18 years. Finally, no significant relationships between water TP concentration and periphyton biomass (neither chlorophyll-*a* nor AFDM) were observed in 2019, whereas both measures of periphyton biomass were

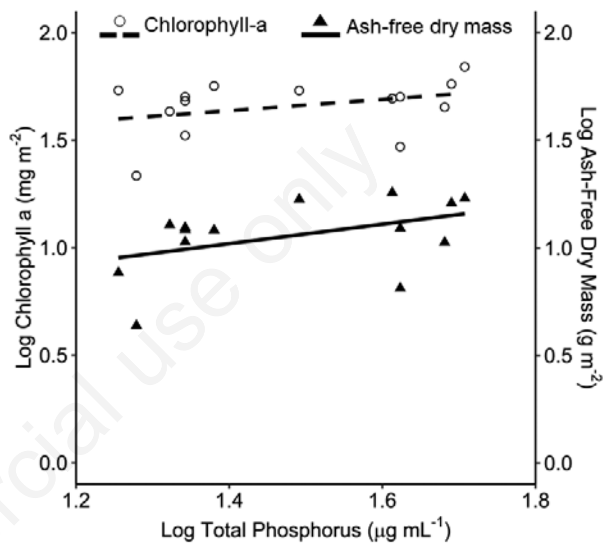

Fig. 3. The log concentration of chlorophyll-*a* (open circles, dashed line) and ash-free dry mass (AFDM; black triangles, solid line) with trendlines with respect to total phosphorus collected in the Rideau River, Ontario, Canada in 2019. Each point provides the mean chlorophyll-*a* or ash-free dry mass for eight rocks at each site on one sampling date (June 4 or August 2, 2019), and the mean total phosphorus concentration of three water samples collected at each site on the corresponding sampling date (June 4 or August 2, 2019). Chlorophyll-*a*: $r^2 = 0.14$, p=0.19; AFDM: $r^2 = 0.15$, p=0.17.

Tab. 2. Summary of the results of the analyses of covariance (ANCOVA) to determine temporal variation (1995 *versus* 2019) in total phosphorus, conductivity, and periphyton biomass (chlorophyll-*a*, ash-free dry mass) in the Rideau River with respect to distance downstream.

Variable	Analysis of:	Type II sum of squares	df	F	p-value
LogTotal phosphorus	Intercept	0.0021	1	0.13	0.72
	Slope	0.238	1	1.51	0.22
Conductivity	Intercept	1587	1	4.68	0.056
	Slope	6716	1	14.83	0.027*
LogChlorophyll-*a*	Intercept	0.105	1	1.29	0.26
	Slope	0.395	1	4.81	0.030*
LogAsh-free dry mass	Intercept	0.278	1	4.61	0.033*
	Slope	0.048	1	0.78	0.38

**Represents p-value <0.05.*

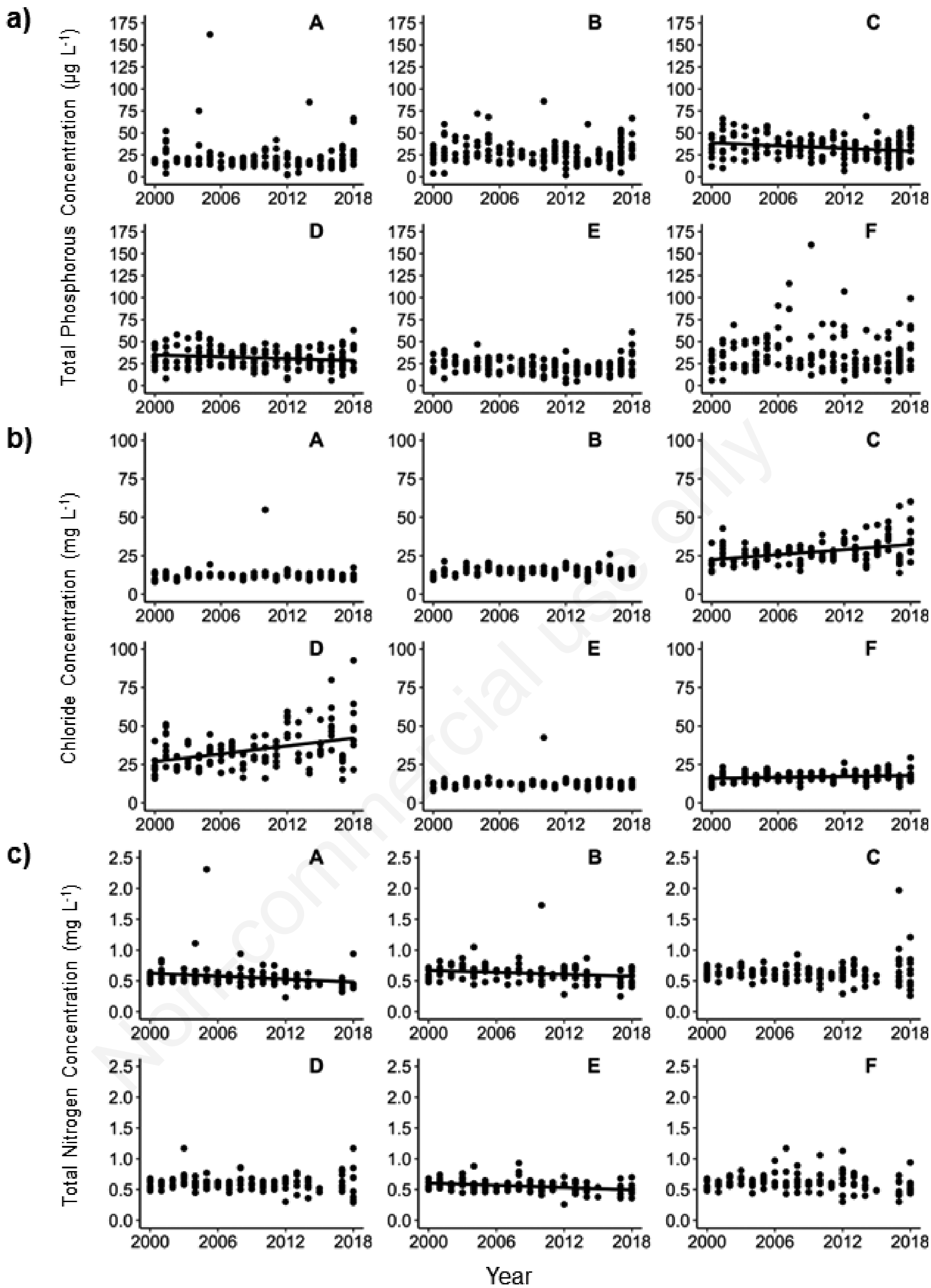

Fig. 4. The concentration of total phosphorus (a), chloride (b) and total nitrogen (c) at the six provincial water quality monitoring stations (A-F) in the Rideau River in Ontario, Canada between 2000 and 2018 with trendlines showing significant relationships. Stations are in order from upstream to downstream (top-right to bottom-left). Data acquired from the Ontario Provincial (Stream) Water Quality Monitoring Network (OMECP, 2018). a) Station A: $r^2 = 0.0061$, p=0.34; Station B: $r^2 = 0.0070$, p=0.31; Station C: $r^2 = 0.060$, p=0.0017; Station D: $r^2 = 0.031$, p=0.029; Station E: $r^2 = 0.023$, p=0.058; Station F: $r^2 = 0.00015$, p=0.88. b) Station A: $r^2 = 0.00017$, p=0.88; Station B: $r^2 = 0.13$, p=0.19; Station C: $r^2 = 0.16$, p=9.40×10^{-7}; Station D: $r^2 = 0.15$, p=3.31×10^{-6}; Station E: $r^2 = 0.00020$, p=0.87; Station F: $r^2 = 0.030$, p=0.041. c) Station A: $r^2 = 0.049$, p=0.013; Station B: $r^2 = 0.036$, p=0.036; Station C: $r^2 = 0.011$, p=0.24; Station D: $r^2 = 6.27 \times 10^{-5}$, p=0.93; Station E: $r^2 = 0.12$, p=6.73×10^{-5}; Station F: $r^2 = 0.0077$, p=0.34.

significantly related to water TP concentration in 1995 (Chételat *et al.*, 2000).

Declines in TP and increases in chloride at midstream sites may be a result of land-use change, specifically the recent urbanization of the suburbs south of Ottawa. Land use in southern Ontario has been progressing towards less agriculture in recent years (Hofmann *et al.*, 2005; Smith, 2015; DeBues *et al.*, 2019). A shift towards less agricultural and more urban land may be partially responsible for decreases in non-point sources of TP and TN, as applications of fertilizers or manure are reduced (Roberts *et al.*, 2009; Carpenter *et al.*, 1998). Evidence of declines in river TP concentrations have been noted in large scale studies across the United States and Sweden (Alexander and Smith, 2006; Huser *et al.*, 2018).

Together, declines in TP concentration and increases in chloride concentration have also been attributed to land-use changes trending towards urbanization with more impervious surfaces (Nagy *et al.*, 2012). Increasing conductivity and chloride concentrations have long been associated with urbanization, particularly in regions that experience cold winters with heavy snow and spring precipitation, such as our study region, because NaCl is applied to roads as a de-icing agent (Paul and Meyer, 2001; Godwin *et al.*, 2003), and can result in increased chloride concentrations throughout the year (Corsi *et al.*, 2015). Furthermore, as agricultural land is lost and we make way for greater impervious surfaces through urbanization, the movement of road salts and metals into waterways is also heightened (Kaushal *et al.*, 2005; Bazinet *et al.*, 2010).

The co-occurrence of declining TP concentrations and increasing chloride concentrations has also been found in recent analyses of Ontario streams. Among 113 stream sites in southern Ontario, 69% of the sites showed significant declines in TP concentration while 95% of sites showed significant increases in chloride concentration between 1975 and 2010 (Raney and Eimers, 2014). The urbanization of agricultural land was considered the leading cause for the co-occurrence of decreasing TP and increasing chloride (Raney and Eimers, 2014). Similar results were also described in an analysis of PWQMN data across 56 streams in Ontario by Stammler *et al.* (2017), where median TP concentration during the growing season (April to November) declined at 57% of streams between 1979 and 2011, and chloride concentrations increased at 88% of sites (Stammler *et al.*, 2017).

Although the present analysis of chloride concentrations from 2000 to 2018 showed an increasing trend over time at midstream sites, conductivity was reduced at upstream sites in 2019 compared to 1995. This reduction is especially evident at sites 1-3, where conductivity measurements were consistently lower in 2019 than in 1995. However, the underlying process responsible for this reduction in conductivity remains unclear, since conductivity can be impacted by numerous factors such as urban and agricultural run-off, wastewater inputs, and precipitation (Chapman *et al.,* 1996). In 1995, both AFDM and chlorophyll-*a* showed significant increasing trends with distance downstream (Chételat *et al.*, 2000), however, in 2019, there were no longitudinal patterns in AFDM nor chlorophyll-*a* with distance downstream, suggesting that periphyton production in the Rideau River is more spatially uniform than it was in 1995. This finding highlights the importance of long-term monitoring across larger river reaches because a lack of interannual variation at a single site may not reflect ecosystem-scale responses.

While there are many studies examining long-term trends in nutrient and chloride concentrations, long-term changes in periphyton biomass are not well documented. A 16-year study (1980-1996) on the Bow River in Alberta, Canada found that periphyton biomass declined in response to improved nutrient (phosphorus, nitrogen) removal at two wastewater treatment plants, but only at sites with water TP concentrations of 10 μg L^{-1} or less (Sosiak, 2002). However, when water TP concentrations were between 10-33 μg L^{-1}, periphyton biomass did not change in response to declines in TP (Sosiak, 2002). In the Rideau River, TP concentrations between 2000 and 2018 generally ranged between 27.4 and 29.4 μg L^{-1} (95% Confidence Interval; mean TP = 28.4 μg L^{-1}), suggesting that TP concentrations are not a limiting factor for the growth of periphyton. A decline in periphyton biomass in the Rideau River could occur if the phosphorus load to the river is reduced resulting in continually low TP concentrations below 10 μg L^{-1}. It should also be noted that one-third of water samples collected from 2000 to 2018 had a TP concentration ≥30.0 μg L^{-1}, thus exceeding the Ontario Provincial Water Quality Objective for the protection of aquatic life in streams (OMEE, 1994).

Elevated concentrations of chloride (sustained levels >600 mg L^{-1} for >8 weeks) have been correlated with declining zooplankton populations and increasing periphyton biomass (Van Meter *et al.*, 2011). However, chloride concentrations between 2000 and 2018 never exceeded 95 mg L^{-1} in the present study, which is below the concentration considered to be harmful to aquatic life over long-term exposure periods (120mg L^{-1}; CCME, 2011). Therefore, it is unlikely that temporal variations in chloride concentrations could have negatively impacted periphyton biomass in this study.

In 2019, neither chlorophyll-*a* nor AFDM were significantly related to water TP concentrations. This differs from the significant relationships observed in 1995 (Chételat *et al.*, 2000), and in many other studies (Biggs and Close, 1989; Chételat *et al.*, 1999; Dodds *et al.*, 2002). However, as reported in 2019, relationships between periphyton biomass and water TP concentration may become uncoupled by increased water flow rates (Lohman *et al.*, 1992; Biggs, 1995). Since water discharge rates in 2019 were substantially higher than those in 1995 due to high total precipitation, it is possible that increased water discharge rates played a role in uncoupling the nutrient-periphyton biomass relationship observed in this study.

In addition to the impacts of chemical and physical factors, periphyton biomass can also be altered by other biological factors, such as the introduction of invasive species (Strayer *et al.*, 1999). Zebra mussels (top-down effect) can impact periphyton directly through competition for space (Stević *et al.*, 2013) and indirectly by increasing water clarity (MacIsaac, 1996). While zebra mussel density was not analysed quantitatively in this study, many rocks sampled for periphyton in 2019 were covered with zebra mussels at sites 2-7; in some cases, upwards of 100 zebra mussel shells could be counted on a single fist-sized rock (specifically on rocks at sites 2 and 5 in August; personal observation,). This was not the case in 1995 when Chételat *et al.* (2000) collected periphyton samples, as zebra mussels were not observed upstream of Kars, Ontario (Martel and Madill, 2018). Surprisingly, increasing zebra mussel density appears to have had little impact on periphyton biomass at our study sites, indicated by very little change in periphyton biomass in 2019 compared to 1995 at site 2, despite a high density of zebra mussels on the rocks. A further analysis of zebra mussel density and periphyton biomass in this study area could verify the accuracy of the anecdotal observations described here, and quantify any relationships between the two.

CONCLUSIONS

Periphyton plays an important role in freshwater ecosystems; acting as the basis of benthic aquatic food webs and providing essential habitat for small organisms. Therefore, it is important that we continue to enhance our understanding of how periphyton biomass responds to long-term environmental changes to better predict the long-term response of river ecosystems to human disturbance. The results of this study suggest that periphyton biomass has become increasingly uniform in the Rideau River compared to 24 years ago, with periphyton biomass increasing in the upstream sections of the river and declining in midstream sections. As they often are, changes in biomass may be tied to modest shifts in local TP and chloride concentrations observed near these sites. However, it is also essential that future studies consider the impacts of anthropogenic change on the physical and biological aspects of freshwater ecosystems globally. Since river flow regimes are expected to change from their natural states and invasive species are expected to expand their distribution, the impacts of these factors must also be predicted and examined as they occur. To better understand how riverine ecosystems respond to cumulative environmental stressors, it is important that periphyton biomass in freshwater ecosystems is monitored to identify long-term, ecosystem-scale shifts.

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Conflict of interest

The authors declare there are no competing interests.

CONTRIBUTIONS

LLT, conceptualization, formal analysis, investigation, data curation, visualization, manuscript original draft; JC, FRP, conceptualization, methodology, manuscript review and editing; CV, conceptualization, manuscript review and editing, funding acquisition; PBH, conceptualization, manuscript review and editing; JCV, conceptualization, formal analysis, funding acquisition, supervision, manuscript review and editing.

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