



# **Salinisation des écosystèmes lacustres par les sels de voirie: perturbations chimiques et réponses des communautés microbiennes**

**Thèse**

**Isabelle Fournier**

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# **Salinisation des écosystèmes lacustres par les sels de voirie : perturbations chimiques et réponses des communautés microbiennes**

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**Isabelle Fournier**

Sous la direction de :

Warwick F. Vincent, directeur de recherche  
Rosa Galvez-Cloutier, codirectrice de recherche

## Résumé

La salinisation des eaux douces est un problème global qui, dans les régions tempérées nordiques, est lié à l'urbanisation. Les principaux ions responsables de cette salinisation sont les chlorures, le sodium et le calcium et leurs sources majeures sont les sels de déglaçage et l'usure de la chaussée. Durant l'hiver, ces ions s'accumulent dans la neige en bordure des routes et peuvent être transportés dans les eaux souterraines et de surface via l'eau de fonte et de ruissellement. Dans les dernières décennies, la salinité des lacs, et plus particulièrement leur concentration en chlorures, a augmenté de façon conséquente par rapport aux valeurs de référence. Par contre, pour la plupart des lacs, les niveaux atteints se situent en-deçà de la valeur seuil définie par le conseil canadien des ministres de l'environnement pour la protection de la vie aquatique qui est de  $120 \text{ mg Cl L}^{-1}$  pour une exposition chronique. Les impacts de la salinisation des écosystèmes d'eau douce sur les organismes aquatiques sont peu connus et le manque d'information est particulièrement marqué pour les communautés microbiennes. Les objectifs principaux de cette thèse étaient 1) d'identifier les voies qu'empruntent les ions pour se rendre dans les lacs, ainsi que les moments auxquels ces mouvements ont lieu et 2) de caractériser les changements que peuvent causer ces ions, particulièrement les chlorures, dans la composition taxonomique des communautés microbiennes.

Un suivi saisonnier dans le bassin versant du lac Saint-Charles (Québec, Canada) a permis de comparer la concentration de différents ions dans la neige en bordure des routes, dans les rivières et dans le lac. Ce suivi s'est échelonné de l'hiver (saison d'accumulation des ions dans la neige) au printemps (principale période de fonte) et combinait des mesures ioniques aux deux semaines dans la neige et les rivières et des mesures de conductivité prises aux 10 minutes par un mouillage dans le lac. Les résultats de ce suivi indiquent que les ions chlorures et sodium se déplacent de la neige vers le lac à tous les épisodes de fonte et que ces derniers surviennent aussi durant l'hiver. Dans les rivières, on observait une relation positive entre la concentration des ions et l'urbanisation du bassin versant, et ce dès qu'elle dépassait  $\approx 1\%$ .

Un suivi annuel de lacs présentant des variables limnologiques différentes a permis de mettre ces dernières en relation avec la composition des communautés microbiennes planctoniques. Les lacs Clair, Saint-Charles, Clément et Saint-Augustin, situés aux alentours

de la Ville de Québec, diffèrent entre autres en fonction de leur salinité, de leur morphométrie, de leur état trophique, et du niveau d'urbanisation de leur bassin versant. Les résultats ont souligné l'importance de la saison pour la composition taxonomique des communautés microbiennes, plus particulièrement celle de l'hiver, ou du couvert de glace. Ils ont aussi suggéré l'importance de la salinité comme facteur structurant, et ce à une conductivité d'environ  $1000 \mu\text{S cm}^{-1}$  et une concentration en chlorures de l'ordre de  $100 \text{ mg L}^{-1}$ . La salinité était, entre autres, positivement corrélée avec l'abondance de cryptophytes et d'haptophytes.

Une expérience de microcosme en laboratoire où une communauté microbienne du lac Saint-Charles a été exposée à des concentrations de chlorures de  $50 \text{ mg L}^{-1}$  (correspondant environ à une augmentation de la salinité par un facteur 2) a aussi été mise en place pour évaluer l'importance de ce facteur. Après deux semaines d'exposition aux chlorures, l'abondance de plusieurs taxons a augmenté, dont les cyanobactéries *Synechococcus* et *Pseudanabaena* et un cryptophyte du clade SA1-3C06. L'exposition aux chlorures, toujours à  $50 \text{ mg Cl L}^{-1}$ , mais combinée à la neige urbaine n'a pas causé de changements aussi marqués dans la composition taxonomique. Ces résultats suggèrent qu'une composante de la neige urbaine a atténué les effets des chlorures et que l'exposition aux chlorures seuls ne représente pas correctement les effets attendus dans le milieu naturel.

Cette thèse a permis de montrer que la salinité des lacs pouvait changer rapidement en réponse à des épisodes de fonte de neige, même en période hivernale et que l'augmentation de la salinité, tant en milieu naturel qu'en contexte expérimental, était corrélée avec des changements dans la composition taxonomique des communautés microbiennes. Ces résultats impliquent que l'augmentation de la salinité, même faible par rapport aux valeurs de référence, influence les écosystèmes d'eau douce, et que dans les régions froides, il devrait y avoir une gestion de l'eau de ruissellement des autoroutes afin de limiter la contamination des eaux par les sels de déglaçage.

## Abstract

Freshwater salinization is an ongoing global concern that in north temperate regions is linked to urbanization. Chloride, sodium, and calcium are the primary ions responsible for this salinization, and their major sources are road salts and pavement weathering. In winter, these solutes accumulate in roadside snow, and may then be transported to ground and surface waters via melting and runoff. In the last decades, the salinity of lakes and their chloride concentrations have significantly increased compared to background values. However, in most lakes, the levels reached are still below the guidelines of the Canadian Council of Ministers of the Environment for the protection of aquatic life, which is  $120 \text{ mg Cl L}^{-1}$  for chronic exposure. The impacts of salinization on freshwater biota are largely unknown, particularly for microbial communities. The main objectives of this thesis study were to 1) identify the flow pathways and timing of ion transport from roads to receiving waters; and 2) characterize the changes that major ions, particularly chloride, may have on the taxonomic composition of freshwater microbial communities.

Seasonal monitoring of the Lake Saint-Charles (Quebec, Canada) watershed allowed comparison of major ion concentrations in roadside snow, rivers within the lake basin, and the lake itself. These observations took place from winter (the season of ion accumulation in snow) to spring (the main melting period), and combined ion measurements in rivers and roadside snow at two-week intervals with conductivity measurements in the lake at 10-minute intervals. The results indicated that chloride and sodium moved from roadside snow to the lake during all melting events, which were also recorded during winter. There was a positive relationship between the major ion concentrations in the river and the watershed urbanization level, beyond a threshold of 1% pavement coverage of the catchment.

To assess the effects of chemical as well as other limnological variables on microbial community structure, plankton and associated samples were taken throughout an entire year from four lakes in the Quebec City region: lakes Clair, Saint-Charles, Clément, and Saint-Augustin. These lakes varied in terms of salinity, morphometry, trophic state, and watershed urbanization level. The results showed a strong seasonal effect on prokaryotic as well as eukaryotic taxonomic composition, and underscored the importance of winter ice cover. Salinity was also identified as a structuring factor, even at conductivities around  $1000 \mu\text{S cm}^{-1}$

$\text{L}^{-1}$  and chloride concentrations around 100 mg  $\text{L}^{-1}$ . Among other taxa, cryptophyte and haptophyte abundance were positively correlated with salinity.

In a laboratory microcosm experiment, the Lake Saint-Charles microbial community was exposed for two weeks to chloride at 50 mg  $\text{L}^{-1}$ , corresponding to a two-fold increase of salinity, to test hypotheses concerning the importance of this factor. After two weeks of exposure, the abundance of many taxa increased, notably the cyanobacteria *Synechococcus* and *Pseudanabaena* and a cryptophyte of the SA1-3C06 clade. The exposure to chlorides, still at 50 mg Cl  $\text{L}^{-1}$  but combined with urban snow, did not cause such marked changes in the taxonomic composition. These results suggest that a component of the snow mitigated the impacts of chloride, and that the exposure to chloride alone may not accurately represent the effects in natural ecosystems.

This thesis study has shown that lake salinity can change rapidly in response to roadside snowmelt events, even in winter. Increases in salinity, both in natural ecosystems and in the laboratory, were correlated with changes in taxonomic composition of the microbial communities. These results imply that increased salinities, even over a low range of values, can influence freshwater ecosystems, and that the environmental management of roads in cold regions should take measures to limit the contamination of waterways by road salts.

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## Avant-propos

Cette thèse de doctorat s'inscrit dans le programme de biologie et est présentée en 5 sections. La première section est une introduction conceptuelle sur les sels de voirie et leurs impacts sur les écosystèmes aquatiques, ainsi que plus particulièrement sur les bactéries et les eucaryotes microbiens. Les trois sections suivantes sont des articles scientifiques rédigés en anglais et publiés, ou destinés à être publiés, dans des revues scientifiques disposant d'un comité de révision par les pairs. La dernière section est une conclusion générale et relate l'importance des résultats de cette thèse dans un contexte plus vaste. J'ai participé, avec l'aide de ma co-directrice Rosa Galvez-Cloutier et de mon directeur Warwick Vincent à l'élaboration du projet de recherche. Les échantillons de cette thèse proviennent de lacs, de rivières et de neige en bordure de route situés aux alentours de la Ville de Québec. J'ai effectué l'échantillonnage en collaboration avec Agiro, un organisme de bassin versant anciennement connu sous le nom de l'Association pour la protection de l'environnement du Lac Saint-Charles et des Marais du Nord ou APEL, des techniciens forestiers du Ministère des Forêts, de la Faune et des Parcs, une stagiaire, des auxiliaires de recherche et des collègues. J'ai effectué les analyses de laboratoire avec la collaboration de la stagiaire Patricia Polquin et des professionnelles de recherche Marie-Josée Martineau et Marianne Potvin et certaines analyses chimiques ont été réalisées par les laboratoires de la division de la qualité de l'eau du Service de l'eau et de l'environnement de la Ville de Québec et l'Institut national de la recherche scientifique – Centre eau terre et environnement. J'ai aussi effectué l'analyse bio-informatique des échantillons avec l'aide de collègues, de Marianne Potvin et de la professeure Connie Lovejoy. J'ai finalement rédigé les articles scientifiques inclus dans cette thèse en prenant en compte l'expertise ainsi que les suggestions, commentaires et corrections des coauteurs.

Voici les détails des articles scientifiques inclus dans cette thèse:

Chapitre 1: Roadside snowmelt: a management target to reduce lake and river contamination.

Auteurs : Isabelle B. Fournier, Rosa Glavez-Cloutier et Warwick F. Vincent

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Chapitre 2: Salinization of urban lakes: is there an influence on under-ice and open-water microbiomes?

Auteurs: Isabelle B. Fournier, Connie Lovejoy et Warwick F. Vincent

Une version révisée de cet article a été acceptée pour publication dans la revue *Frontiers in Microbiology* avec le titre « Changes in the community structure of under-ice and open-water microbiomes in urban lakes exposed to road salts » (doi : [10.3389/fmicb.2021.660719](https://doi.org/10.3389/fmicb.2021.660719)).

Chapitre 3: Microbial plankton responses to salt versus urban snow in a spring snowmelt experiment.

Auteurs: Isabelle B. Fournier, Connie Lovejoy et Warwick F. Vincent

En préparation. Ma lettre d'intention (avec un résumé étendu) a été acceptée pour soumission du manuscrit en 2021 dans l'édition spéciale *Increasing salinity in freshwater and coastal ecosystems* de la revue *Limnology & Oceanography Letters*.

Certains des résultats de cette thèse ont aussi été présentés sous forme d'affiches et de présentations orales à plusieurs conférences nationales et internationales :

Fournier, I.B., Galvez-Cloutier, R. & Vincent, W.F. (2019). *Winter management of snowmelt runoff to prevent freshwater salinization*, Présentation orale présentée à la rencontre annuelle de la *North American Lake Management Society (NALMS)*, Burlington, Vermont, USA, 11-15 novembre.

Fournier, I.B. & Vincent, W.F. (2019). *Seasonally distinct below-ice microbial communities in four boreal lakes: Implications of climate warming*, Affiche présentée à la conférence Chapman: *Winter limnology in a changing world*, Polson, Montana, USA, 14-18 octobre.

Fournier, I.B., Lovejoy, C. & Vincent, W.F. (2018). *Response of the overwintering microbiome of a drinking water reservoir to road salt exposure*, Rencontre estivale de *Association for the Sciences of Limnology and Oceanography (ASLO)*, Victoria, British-Columbia, Canada, 10-15 juin.

Fournier, I.B., Galvez-Cloutier, R. & Vincent, W.F. (2017). *Physicochemical and biological impacts of road salts on urban lakes*. Présentation orale présentée à la rencontre annuelle de la *North American Lake Management Society (NALMS)*, Westminster, Colorado, USA, 6-9 novembre.

Fournier, I.B., Galvez-Cloutier, R. & Vincent, W.F. (2017). *Impacts de l'application de sels de voirie sur la physicochimie et la biologie des lacs urbains*. Rencontre annuelle de Chapitre Saint-Laurent et TOXEN, Montréal, Québec, Canada, 15-16 juin.

En 2018, à la rencontre estivale d'ASLO à Victoria en Colombie-Britannique, j'ai été invité à analyser des échantillons pour un projet de Shelley Arnott, professeure à *Queens University* en Ontario. Le projet était une expérience en mésocosmes pour étudier l'impact d'un gradient de chlorures (de 0,41 à 1500 mg Cl L<sup>-1</sup>) sur le zooplancton, le phytoplancton et les protistes (eucaryotes unicellulaires sans pigmentation). J'ai identifié en microscopie la composition taxonomique des communautés de phytoplancton et de protistes. Le résumé étendu de l'article en découlant, duquel je suis troisième auteure, a été accepté pour soumission dans l'édition spéciale *Increasing salinity in freshwater and coastal ecosystems* de la revue *Limnology & Oceanography Letters*.

En 2019, à la conférence Chapman *Winter limnology in a changing world* à Polson aux États-Unis, j'ai collaboré avec d'autres chercheurs à l'élaboration d'un cadre conceptuel pour l'étude des lacs durant l'hiver. Plus précisément, ce travail voulait exposer des liens entre les processus physiques, chimiques et biologiques ayant lieu durant l'hiver et leurs réponses à des conditions hivernales changeantes, particulièrement aux changements dans la couverture de glace. Ce travail, duquel je suis co-première auteure, a été soumis sous la forme d'un article scientifique dans l'édition spéciale *Limnology in a changing world* de la revue *Journal of Geophysical Research: Biogeosciences* en janvier 2021.

# Introduction

Dans les pays nordiques et dans plusieurs régions alpines à travers le monde, des sels de voirie sont épandus pour assurer la sécurité des usagers du réseau routier durant l'hiver (Table S0-1). Le chlorure de sodium ( $\text{NaCl}$ ) est le principal sel utilisé, mais d'autres sels de chlore tels que le chlorure de magnésium ( $\text{MgCl}_2$ ), le chlorure de potassium ( $\text{KCl}$ ) et le chlorure de calcium ( $\text{CaCl}_2$ ) peuvent aussi l'être, bien qu'en faible proportion (<1% au Canada, Evans et Frick 2001). Aux États-Unis, la charge annuelle de  $\text{NaCl}$ , à des fins de déglaçage, est estimée à 22 millions de tonnes (Bolen 2020). Au Canada, il s'agit d'environ 5 millions de tonnes et au Québec de 1,6 millions de tonnes (Evans et Frick 2001).

Bien que les sels de voirie réduisent le dérapage (Crinson et Martin 2008; Evans et al. 2008), et donc le nombre d'accidents routiers durant l'hiver (Kuemmel et Hanbali 1992; Usman et al. 2012), ils ont de nombreux impacts sur la société et l'environnement qui perdurent toute l'année. La présence de ces sels en grande concentration sur le bord des routes attire les grands mammifères en quête de sodium (Na), ce qui modifie leur comportement naturel d'évitement des routes et augmente les risques de collision avec les voitures (Grosman et al. 2011). Le sel en bordure des routes abîme aussi la végétation (Duk Lee et al. 2017) et favorise la prolifération d'espèces envahissantes telles que la phragmite d'Eurasie (Brisson et al. 2010) et l'ambroisie à feuille d'armoise (DiTommaso 2004). De plus, l'ion chlorure ( $\text{Cl}^-$ ) corrode les véhicules (Ratkovičius et al. 2014; Schoukens et al. 2017) et les infrastructures routières, particulièrement les routes (Ratkovičius et al. 2014; Duk Lee et al. 2017), les ponts (Kreislova et Geiplova 2012; Gode et Paeglitis 2014) et les viaducs (Commission d'enquête sur le viaduc de la Concorde 2007). Les sels sont, par définition, solubles dans l'eau. Ainsi, ils ne se retrouvent pas seulement là où on les épand, mais sont aussi transportés sur de grandes distances par l'eau de ruissellement, après des épisodes de pluie ou de fonte de neige. Il est estimé qu'environ la moitié du sel épandu finirait sa course dans l'eau de surface, que ce soit dans les lacs ou dans les rivières, et que l'autre moitié serait emmagasinée dans l'eau souterraine (Howard et Haynes 1993, cités par Environnement et santé Canada 2001; Müller et Gächter 2012). Dans le cadre de cette thèse, ce sont les impacts de l'arrivée des sels de voirie dans les eaux douces de surface qui étaient étudiés, particulièrement du point de vue de la qualité de l'eau et des communautés microbiennes.

## **Salinité des eaux naturelles**

Pour mieux comprendre les impacts de l'arrivée des ions Cl et Na dans les eaux douces, il faut d'abord bien caractériser ces dernières. De façon générale, une eau est considérée comme douce, si sa salinité est inférieure à  $500 \text{ mg L}^{-1}$  (Evans et Frick 2001). La salinité est la masse totale de tous les ions présents (Evans et Frick 2001) et elle peut être approximée par une mesure de la conductivité électrique (souvent en  $\mu\text{S cm}^{-1}$ ), puisque plus il y a d'ions dans une solution, plus cette dernière conduira le courant électrique. Cette relation est toutefois dépendante de la température de l'eau, alors, pour faciliter la comparaison entre différentes mesures, la conductivité est généralement corrigée à  $25^\circ\text{C}$ , ce qu'on appelle la conductivité électrique spécifique. Pour alléger la lecture du présent document, le terme conductivité sera utilisé pour désigner la conductivité électrique spécifique. L'eau douce a généralement une conductivité inférieure à  $925 \mu\text{S cm}^{-1}$  (valeur obtenue suite à l'utilisation d'un facteur de conversion de 0,54 sur la limite de  $500 \text{ mg L}^{-1}$ ; Singh et Kalra 1975). En limnologie, la conductivité est plus couramment utilisée que la salinité, laquelle l'est plus fréquemment en océanographie. Dans cette thèse, comme dans la littérature, il sera parfois question de salinité en masse/volume, parfois de conductivité en  $\mu\text{S cm}^{-1}$  et parfois de la concentration spécifique de certains ions en masse/volume, selon le contexte et les données existantes.

En eau douce, la salinité moyenne est de l'ordre d'une centaine de  $\text{mg L}^{-1}$  et les ions les plus abondants, appelés ions majeurs, sont le bicarbonate ( $\text{HCO}_3^-$ ), le sulfate ( $\text{SO}_4^{2-}$ ) et le calcium ( $\text{Ca}^{2+}$ ). Les ions Cl et Na, pour leur part, ne représentent ensemble qu'environ 15% de la salinité (Table 0-1). Cela constitue une différence majeure avec les océans où la salinité est de l'ordre de plusieurs dizaines de  $\text{g L}^{-1}$  et où les ions Cl et Na sont les ions majeurs (86%, Table 0-1). L'unicité de l'eau douce, comparativement à l'eau de mer, ne vient donc pas seulement d'une différence dans la quantité totale de sels, mais aussi dans la proportion des différents ions. Ces différences influencent fortement la façon dont les organismes aquatiques échangent du matériel avec le milieu environnant et régulent leur composition ionique interne. Le processus qui diffère est appelé « osmorégulation » et la perturbation de ce processus est à l'origine des effets néfastes directs observés après une augmentation de la salinité de l'eau douce. Ce processus, ainsi que les effets qu'entraînent sa perturbation par

une augmentation de la salinité de l'eau douce seront détaillés dans la section « Impacts sur les organismes aquatiques d'eau douce ».

**Table 0-1.** Composition ionique de lacs, de rivières et d'océans. %: pourcentage de la masse d'un ion donné sur la masse totale des ions présentés.

Ecosystèmes	Années	n	Unités	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	Data source
Lacs au Québec	1980-1990s	1377	mg L <sup>-1</sup>	2	0.5	0.7	0.4	4.1	4.4	0.4	Jeffries 1997
Lacs RLE <sup>1</sup>	1968-1969	40	mg L <sup>-1</sup>	1.6	0.9	0.9	0.4	4.1	3	1.4	Amstrong et Schindler 1971
Grands lacs	1958	5	mg L <sup>-1</sup>	25.3	6.7		5.1 <sup>2</sup>	51.7	8.2	4.6	Evans et Frick 2001
Rivières <sup>3</sup>	1983	ND	mg L <sup>-1</sup>	15.0	4.1	6.3	2.3	58.0	11.2	7.8	Evans et Frick 2001
Océans	1967	ND	mg L <sup>-1</sup>	409	1 300	10 770	388	140	2710	19 370	Evans et Frick 2001
			%	1.2	3.7	30.7	1.1	0.4	7.7	55.2	

<sup>1</sup>Région des lacs expérimentaux, en anglais *Experimental Lakes Area*

<sup>2</sup>Na + K

<sup>3</sup>Moyenne mondiale

Les sources naturelles de sels dans les eaux douces incluent l'infiltration d'eau de mer, l'apport de vapeur d'eau salée (seulement près des écosystèmes côtiers), les précipitations et l'érosion de la roche et des sols (Evans et Frick 2001). Les changements de volume d'eau liés à l'évaporation et à l'étiage sont aussi des facteurs naturels influençant la salinité de l'eau douce (Evans et Frick 2001; Rivett et al. 2016).

## Salinisation des eaux douces

Au fil des décennies, l'apport de sels par l'eau de ruissellement des zones urbaines a résulté en une augmentation de la concentration de Cl et de Na dans les rivières (Godwin et al. 2003; Kelly et al. 2012, 2019; Dailey et al. 2014; Corsi et al. 2015; Stets et al. 2018; Shoda et al. 2019) et les lacs (Chapra et al. 2012; Rogora et al. 2015; Dugan et al. 2017a). Par exemple, dans le bassin versant de la Mohawk River, entre les années 50 et 90, les concentrations de Na et Cl ont respectivement augmentées de 130 et 243%, atteignant 18 et 28 mg L<sup>-1</sup> (Godwin et al. 2003; Table 0-2); Entre les années 1900 et 2011, la concentration de Cl du Lac Michigan est passée de 4 à 12 mg L<sup>-1</sup> (Kelly et al. 2012; Table 0-2). Dans une étude sur les lacs de la région Nord-Américaine des lacs (en anglais *North American Lakes Region*, NALR), la concentration de Cl était stable dans 55% des lacs, mais a augmenté dans

34% des lacs dans les dernières décennies (Dugan et al. 2017a). En 2010, la dernière année considérée dans cette étude, la concentration de Cl variait entre 0,18 et 240,8 mg L<sup>-1</sup> et la médiane était de 6 mg L<sup>-1</sup> (Dugan et al. 2017a). D'autres exemples de l'augmentation des ions Cl et Na dans les lacs et les rivières (Table 0-2) montrent des concentrations en Cl qui doublent, ou plus, depuis les dernières décennies. Il est possible d'y remarquer que les concentrations «augmentées» sont beaucoup plus élevées pour les rivières que pour les lacs, pour lesquels on dispose de moins d'information, mais dont les concentrations sont largement sous 50 mg L<sup>-1</sup>. Une modélisation récente de la concentration de Cl dans des lacs des États-Unis (49432 lacs, basé sur une modélisation de 2773 lacs) suggère que la majorité des lacs (82%) ont une concentration inférieure à 20 mg L<sup>-1</sup>, et que seulement 4% ont une concentration plus grande que 50 mg L<sup>-1</sup> et 0.23% plus grande que 100 mg L<sup>-1</sup> (Dugan et al. 2020).

**Table 0-2.** Données sur les suivis à long-terme des concentrations de Cl et de Na dans les rivières et les lacs. La colonne concentration représente la concentration (en mg L<sup>-1</sup>) à la fin du suivi. n est le nombre de lacs ou des rivières suivis dans l'étude, dans les cas où n est plus grand que 1, la moyenne ou la médiane est présentée selon les données disponibles.

Début suivi	Fin suivi	n	Augmentation %		Concentration		Pays	Référence
			Na	Cl	Na	Cl		
<i>Rivières</i>								
1952	1998	1	130	243	18	28	États-Unis	Godwin et al. 2003
1985	2017	1	250	175	28	44	États-Unis	Kelly et al. 2019
1981	2005	59	-	75*	-	157*	États-Unis	Kelly et al. 2012
1967	2013	4	-	71*	-	65*	États-Unis	Dailey et al. 2014
1964	1994	1	100 <sup>†</sup>	100 <sup>†</sup>	24 <sup>†</sup>	56 <sup>†</sup>	États-Unis	Dailey et al. 2014
1990	2012	19	-	73* <sup>†</sup>	-	129* <sup>†</sup>	États-Unis	Corsi et al. 2015
1992	2012	141	-	65*	-	144	États-Unis	Stets et al. 2018
<i>Lacs</i>								
1900s	2011	1	-	300	-	12	États-Unis	Kelly et al. 2012
1940	2016	1	-	>1500*	-	50	États-Unis	Dugan et al. 2017c
1990	2012	5	17*	28*	3.3*	3.5*	Italie	Rogora et al. 2015

<sup>†</sup> valeurs estimées à partir d'un graphique de l'article

\* valeurs calculées à partir des données de l'article

En plus de cette évolution temporelle, l'impact des sels de voirie est particulièrement visible lorsque l'on compare la salinité des cours d'eau qui se trouvent dans des zones rurales ou forestières à celle des cours d'eau se trouvant dans des milieux urbains (Novotny et al. 2008; Kaushal et al. 2017). Par exemple, dans des rivières du Maryland, la concentration des ions,

entre autres de Cl, de Ca, de Na et de magnésium (Mg), était en moyenne 27 fois plus élevée en milieu urbain que dans les zones forestières (Moore et al. 2017). De façon générale, les concentrations de Cl et de Na sont corrélées avec la densité et la proximité des routes (Kelting et al. 2012; Dugan et al. 2017a) ainsi qu’avec l’urbanisation du territoire (Dugan et al. 2017a; Bird et al. 2018). Il semblerait qu’il existe un seuil d’environ 1% du territoire urbanisé (aire relative du bassin versant occupé par des surfaces pavées, des bâtiments ou des terres agricoles) pour voir une augmentation des concentrations relativement aux valeurs de référence (Valtanen et al. 2014; Dugan et al. 2017a; Kaushal et al. 2017; Bird et al. 2018).

Bien que l’application de sels de voirie soit la principale raison liant les routes et l’urbanisation à l’augmentation de Cl dans les eaux douces, les milieux urbains comptent plusieurs autres sources de Cl telles que les rejets des stations d’épuration, les fertilisants, les élevages (Corsi et al. 2015), les adoucisseurs d’eau, ainsi que les spas et les piscines au sel. Certaines des études citées précédemment présentent aussi l’augmentation de d’autres ions dans les zones urbaines, notamment du Ca et du Mg (Kaushal et al. 2017; Moore et al. 2017). Le terme émergent pour référer à ce problème global est la salinisation de l’eau douce (en anglais *freshwater salinization*). Il est à noter que si la salinisation est un problème global, les causes sont différentes selon les régions. Sous les climats nordiques où les sels de voirie sont utilisés, ceux-ci, et l’urbanisation à laquelle ils sont liés, sont les principales causes. C’est de ces régions dont il sera question dans cette thèse. Par contre, dans certaines régions arides, notamment en Australie et en Israël, on observe aussi une augmentation de la salinité des eaux douces et des sols, mais celle-ci résulte plutôt d’une désertification causée par le retrait de la végétation pour l’agriculture sèche et le détournement de cours d’eau pour l’irrigation (Banin et Fish 1995; Blinn et Bailey 2001; Blinn et al. 2004).

## **Impacts de la salinisation des eaux douces**

### Considérations de santé publique

La salinisation de l’eau douce a des répercussions sur l’environnement, mais il s’agit aussi d’un problème de santé publique, car la présence de Cl dans l’eau à des concentrations au-delà de 250 mg L<sup>-1</sup> compromet son utilisation comme source d’eau potable (Guidelines for Canadian Drinking Water Quality - Summary Table). Bien qu’à cette dose le Cl ne soit pas toxique pour l’humain (Guidelines for Canadian Drinking Water Quality - Summary Table),

il donne à l'eau un goût salé qui la rend impropre à la consommation et corrode les tuyaux, entraînant la libération de métaux comme le cuivre (Cu) et le plomb (Pb), qui eux, peuvent être toxiques (Pieper et al. 2018; Stets et al. 2018). Il y a peu d'exemples documentés de dépassements des normes dans des prises municipales d'eau potable de surface, mais des dépassements sont parfois observés dans des puits privés. Dans une étude récente présentée à la Communauté Métropolitaine de Québec, ≈1% des puits échantillonés présentaient des concentrations de chlorures au-delà de  $90 \text{ mg L}^{-1}$  (Proulx 2017). Les citoyens ont reçu des conseils afin de remédier au problème. Dans le même ordre d'idée, un communiqué destiné aux Néo-Brunswickois leur explique comment protéger leurs puits d'une contamination par les sels de voirie (Fiche d'information – Présence de sel dans les puits privés d'eau potable).

Toujours selon un critère de goût, le seuil recommandé pour le Na est de  $200 \text{ mg L}^{-1}$  (Guidelines for Canadian Drinking Water Quality - Summary Table). La concentration de Na dans l'eau potable n'est pas non plus une considération de santé, sauf pour des personnes à qui l'on a prescrit un régime réduit en sel et qui doivent idéalement consommer une eau dont la concentration de Na est inférieure à  $20 \text{ mg L}^{-1}$  (Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Sodium). Il n'y a pas de recommandations pour la conductivité totale, mais il en existe une pour la quantité totale de solides dissous (TDS, une mesure similaire à la salinité) qui est de  $500 \text{ mg L}^{-1}$  (Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Total Dissolved Solids). Ces valeurs seuils sont celles suggérées par le Gouvernement du Canada pour l'eau potable. Aux États-Unis, les valeurs seuils pour le Cl et les TDS recommandées par l'Agence de protection de l'environnement pour l'eau potable sont les mêmes qu'au Canada et aucune valeur n'a été définie pour le Na (US EPA 2020). Dans ces deux pays, il s'agit de recommandations et non de législation.

### Impacts sur les écosystèmes aquatiques

Lorsque les ions Cl et Na arrivent dans les lacs, ils y perturbent la composition ionique de l'eau, et ce faisant, ils peuvent aussi perturber des processus physiques et affecter les organismes. Cette section considère ces deux types d'influence sur les écosystèmes lacustres.

## Impacts sur les processus physiques

La densité de l'eau est influencée par la température (la densité de l'eau augmente entre 0 et 3.984 °C (maximum) et diminue plus l'eau se réchauffe parce que les molécules s'éloignent les unes des autres), la pression (une eau plus profonde est plus compressée et donc plus dense qu'une eau de surface) et la salinité (une eau plus salée contient plus de sels et est plus lourde, donc plus dense, Figure S0-1). La densité de l'eau, ou plus particulièrement la différence de densité entre des masses d'eau, est importante pour les processus de stratification et de mélange des lacs. En eau douce, on considère généralement que la densité de l'eau ne varie pas en fonction de la salinité, puisque les variations rencontrées en milieu naturel n'entraînent que des changements de densité négligeables. Par contre, dans les milieux influencés par les sels de déglaçage, les changements de densité peuvent être importants et entraîner des impacts qui le sont tout autant.

En Amérique du Nord, la plupart des lacs sont dimictiques, c'est-à-dire qu'ils sont mélangés deux fois par année : au printemps et à l'automne. Le mélange de printemps est le plus influencé par l'arrivée d'un affluent plus salé. En hiver, le gradient de densité entre la surface et le fond d'un lac est très faible et lorsque la glace fond au printemps, le vent est généralement capable de mélanger l'entièreté de la colonne d'eau, renouvelant l'oxygène utilisé durant l'hiver et redistribuant les nutriments. Un affluent plus salé arrivant à la fonte coulera vers le fond, celui-ci étant plus dense que l'eau du lac. Plus l'affluent est salé, plus la différence de densité sera grande entre cette couche d'eau profonde et le reste de la colonne d'eau. Si cette différence est suffisamment grande, il est possible que le vent ne puisse pas fournir suffisamment d'énergie pour mélanger l'entièreté de la colonne d'eau et l'eau profonde pourrait donc rester en déficit d'oxygène jusqu'au mélange d'automne (Judd 1970; Wiltse et al. 2020).

Un changement dans le régime d'oxygène en eau profonde a des répercussions diverses. Premièrement, il peut causer du stress ou de la mortalité chez les organismes vivant à ces profondeurs et nécessitant de l'oxygène pour leur respiration ou leur métabolisme. Deuxièmement, une faible quantité d'oxygène, voire de l'anoxie, près des sédiments peut causer le relargage de phosphore, lequel peut entretenir le processus d'eutrophisation causant lui aussi une anoxie en eau profonde. Ces effets extrêmes sur les processus de mélange et les

conséquences sur l’oxygène et le phosphore, avec les impacts potentiels qu’ils peuvent avoir sur l’utilisation de l’eau douce comme source d’eau potable, font partie des raisons pour lesquelles il est impératif de ne pas ignorer les impacts à long terme de l’augmentation de la salinité de l’eau douce.

### Impacts sur les organismes aquatiques d’eau douce

La concentration de Cl retrouvée dans les lacs et les rivières impactés par les sels de voirie est plus élevée que les valeurs de référence de leurs régions respectives. Toutefois, comme c’était le cas avec les recommandations de santé publique, les concentrations sont majoritairement aussi plus faibles que celles recommandées par les lignes directrices des différents organismes environnementaux pour la protection de la qualité de l’eau et de la vie aquatique. En effet, au Canada, les recommandations du conseil canadien des ministres de l’environnement (CCME) sont de  $120 \text{ mg Cl L}^{-1}$  pour une exposition chronique (plusieurs jours ou semaines d’exposition) et de  $630 \text{ mg Cl L}^{-1}$  pour une exposition aigüe (quelques heures à quelques jours d’exposition; CCME 2011). Au Québec et aux États-Unis, ces recommandations, respectivement définies par le Ministère de l’environnement et de la lutte contre les changements climatiques (MELCC) et l’*United States Environmental Protection Agency* (USEPA), sont de  $230 \text{ mg Cl L}^{-1}$  pour une exposition chronique et de  $860 \text{ mg Cl L}^{-1}$  pour une exposition aigüe (MELCC 2020; US EPA 1988). La différence de concentration entre les cours d’eau impactés et les recommandations suggère que les écosystèmes d’eau douce sont protégés. Or, pour le moment, on ne peut affirmer une telle chose puisque les études qui se sont penchées sur les effets de ces concentrations plus élevées, mais somme toute relativement faibles, sont rares. De plus, la concentration de Cl nécessaire pour induire des effets néfastes sur les communautés aquatiques d’eau douce varie énormément d’une étude à l’autre; certaines ne trouvant aucun effet aigüe à des concentrations aussi importantes que  $3500 \text{ mg Cl L}^{-1}$  (Blasius et Merritt 2002 et les références de cet article) et d’autres en trouvant à des concentrations de  $645 \text{ mg Cl L}^{-1}$  (Van Meter et al. 2011), voire même entre 50 et 90  $\text{mg Cl L}^{-1}$  (Wallace et Biastoch 2016).

La disparité entre les résultats de ces études peut en partie être expliquée par la différence de tolérance des différents organismes étudiés aux variations de salinité, mais aussi par le degré de complexité des écosystèmes ayant été intégré à l’étude. En effet, plusieurs de ces

études, ainsi que celles utilisées pour déterminer les recommandations des organismes gouvernementaux (critères de protection de la vie aquatique), ont été réalisées en laboratoire, souvent sur une seule espèce à la fois. Toutefois, dans les écosystèmes naturels, les organismes interagissent entre eux et avec leur environnement, ce qui peut moduler leur tolérance aux variations de salinité. Par exemple, la sensibilité des organismes au Cl varie selon l'état trophique (Denis et al. 2010), l'alcalinité de l'eau (Elphick et al. 2011; Soucek et al. 2011; Simmons 2012), la température (Silver et al. 2009), la disponibilité de la nourriture (Brown et Yan 2015), la présence de prédateur (Hintz et Relyea 2017a; Liu et Steiner 2017) et la source de Cl (Denis et al. 2010; Hintz et Relyea 2017b). Par ailleurs, les organismes en milieu naturel ne sont pas seulement exposés au Cl, mais au cocktail entier de substances et de contaminants provenant du bassin versant. En milieu urbain, cela inclut, mais ne se limite pas aux nutriments, aux métaux, aux hydrocarbures aromatiques polycycliques (HAPs) (Reinosdotter et Viklander 2007; Bartlett et al. 2012a, 2012b) et aux contaminants organiques tels que les particules libérées par l'usure des pneus (Tian et al. 2020) et les produits pharmaceutiques (Ondarza et al. 2019). Ils proviennent, entre autres, de sources aussi diverses que les sels de voirie eux-mêmes, l'essence, les voitures, le revêtement routier, les eaux usées et les fosses septiques.

L'augmentation de la concentration de Cl peut résulter en une modification de la structure des communautés aquatiques par ses effets directs sur la survie ou sur la reproduction (Blinn et al. 2004; Flöder et Burns 2004; Wu et al. 2009; Flöder et al. 2010; Chakraborty et al. 2011; Herlemann et al. 2011; Tang et al. 2012; Szöcs et al. 2014; Kirchman et al. 2017; Gutierrez et al. 2018). Par exemple, une espèce qui ne survit pas à l'augmentation de la salinité, ou qui ne peut plus se reproduire, verra son abondance diminuée par rapport à celle des espèces qui sont moins ou pas affectées. Le principal mécanisme par lequel une concentration élevée de Cl affecte les organismes aquatiques d'eau douce est que cela augmente les coûts d'osmorégulation, réduisant l'énergie disponible pour d'autres activités (Elphick et al. 2011). Lorsque deux liquides sont séparés par une membrane semi-perméable, le solvant, l'eau dans le cas d'une cellule, se déplace du milieu le moins concentré vers le milieu le plus concentré afin d'équilibrer la concentration de molécules osmotiquement actives de part et d'autre. Cela est appelé l'osmose et, de façon similaire à la salinité, la somme des molécules osmotiquement actives se nomme l'osmolarité. Les organismes vivants régulent de façon

constante ce mouvement d'eau afin de maintenir l'intégrité des cellules et une concentration en ions optimale pour leur métabolisme par un ensemble de processus appelé l'osmorégulation (Mayfield et Gates 2007). Le sens et l'intensité de l'osmorégulation varient selon le degré de différence entre l'osmolarité du milieu et celle de l'organisme. Les invertébrés marins sont généralement osmoconformes, c'est-à-dire qu'ils maintiennent leur osmolarité interne près de celle de leur environnement et sont donc presque isotoniques avec celui-ci. Leur métabolisme est adapté pour fonctionner à cette osmolarité. Les organismes d'eau douce, quant à eux, sont généralement plus concentrés en ions que leur environnement, créant un appel d'eau dont ils doivent constamment se débarrasser afin d'éviter l'éclatement de la cellule causé par une trop grande pression osmotique (Yancey 2005; Mayfield et Gates 2007; Lee et al. 2011). Les milieux d'eau douce et marins ne présentent donc pas les mêmes défis pour les espèces, ce qui rend difficile, et rare, la transition d'un milieu à l'autre. L'osmorégulation est un processus dynamique et énergivore qui demande la mise en place et la régulation de plusieurs mécanismes afin de compenser efficacement les différences d'osmolarité entre l'environnement et le milieu intracellulaire. Toutes les espèces n'ont pas développé le même mécanisme, ni la même tolérance aux variations d'osmolarité, et donc de salinité, et cela leur donne un potentiel d'adaptation différent face à un environnement changeant.

Des changements dans la structure des communautés en réponse à l'augmentation de la salinité peuvent aussi découler d'effets indirects, lesquels résultent de l'interaction entre diverses espèces n'y ayant pas la même sensibilité (Cleave et al. 1981; Berezina 2003; Bartolomé et al. 2009). Lorsque de tels changements sont observés, ils résultent majoritairement d'une modification de comportement ou de l'abondance des proies ou des prédateurs (Greenwald et Hurlbert 1993; Petranka et Doyle 2010; Van Meter et al. 2011; Strom et al. 2013; Hintz et Relyea 2017a; Hintz et al. 2017; Jones et al. 2017; Schuler et al. 2017; Gutierrez et al. 2018). Par exemple, on observe fréquemment une augmentation du phytoplancton (approximé par la chlorophylle *a*) suite à une augmentation des chlorures, dû à la mortalité du zooplancton qui y est plus sensible (e.g. Van Meter et al. 2011). Un gradient spatial de salinité peut aussi offrir un refuge contre la prédation, lorsqu'une proie et ses prédateurs n'ont pas la même tolérance (Strom et al. 2013). Il est aussi possible que de tels

changements puissent découler de relations de parasitisme (Milotic et al. 2017) et de compétition (Venâncio et al. 2017).

La plupart des études présentées jusqu'à maintenant se sont penchés sur les effets d'une augmentation de Cl sur le zooplancton, les macro-invertébrés, les poissons ou les amphibiens, mais très peu ont regardé les effets sur les bactéries et les eucaryotes unicellulaires. Ceux-ci sont toutefois à la base des réseaux trophiques, en étant des proies ou en faisant de la photosynthèse, et ont un rôle clé dans le recyclage des nutriments et de la matière organique, via la boucle microbienne (Figure S0-2). Les observations disponibles sur les effets de la salinité sur les bactéries ou les eucaryotes unicellulaires sont souvent limitées aux autotrophes et les données taxonomiques sont soit absentes (basées seulement sur la concentration du principal pigment photosynthétique, soit la chlorophylle *a* (chl *a*); Van Meter et al. 2011; Jones et al. 2017; Schuler et al. 2017), soit peu détaillées (identification aux grands groupes seulement à l'aide de trois pigments photosynthétiques; Chakraborty et al. 2011). Or, lorsque l'on étudie un phénomène qui varie de façon aussi importante d'une espèce à l'autre, la résolution taxonomique est importante.

Bien que moins étudié dans un contexte de salinisation des eaux douces par les sels de voirie, les changements dans la structure des communautés bactériennes en réponse à l'augmentation des concentrations en chlorures ont été étudiés intensivement dans les zones de transition entre les écosystèmes d'eau douce et les écosystèmes marins (Benlloch et al. 2002; Bouvier et del Giorgio 2002; Casamayor et al. 2002; Redden et Rukminasari 2008; Herlemann et al. 2011; Kirchman et al. 2017) ainsi que dans des lacs salés où l'on retrouve des salinités très élevées, et donc des espèces particulièrement résistantes (Blinn et al. 2004; Gutierrez et al. 2018). Ces études ont souligné l'importance de la salinité en tant que pression de sélection et ont permis d'identifier des taxons bactériens et des fonctions qui différaient systématiquement entre les deux habitats. Toutefois, l'information taxonomique est difficile à extraire de ces études, car les sites présentant des salinités inférieures à  $1\text{ g L}^{-1}$  ont été regroupés sous la bannière « eau douce » afin de permettre leur comparaison avec ceux dits « salés ». Le portrait est moins clair pour les eucaryotes unicellulaires, puisqu'ils ont, dans ce contexte comme dans bien d'autres, été moins étudiés que les procaryotes. Dans cette thèse, les effets de l'augmentation de la salinité sur la structure des communautés de bactéries

et d'eucaryotes unicellulaires seront étudiés, et ce, dans l'intervalle « eau douce » se situant en-deçà de 500 mg L<sup>-1</sup>.

## **Identification des communautés procaryotes et eucaryotes**

Les analyses standards de toxicité requièrent l'exposition d'espèces uniques en laboratoire afin de déterminer leur sensibilité aux contaminants. Cette approche permet de calculer des paramètres utiles, notamment la concentration efficace à X% (ECX) qui est la concentration qu'il faut atteindre pour observer un effet donné dans X% de la population étudiée (ex., mortalité, absence de reproduction ou de la croissance), ainsi que la LOEC ou la NOEC qui sont respectivement la concentration la plus faible et la concentration la plus élevée à laquelle un effet donné n'est pas observé. Le paramètre le plus couramment utilisé pour déterminer les critères de protection de la vie aquatique, dont ceux pour les chlorures, est la concentration efficace à 50% pour la mortalité, qui est aussi appelée concentration létale à 50%, concentration létale médiane ou LC50.

Si ces essais toxicologiques standards nous informent sur la sensibilité absolue des organismes, plusieurs raisons mentionnées dans la section précédente font en sorte qu'il est difficile d'extrapoler ces valeurs à l'extérieur du laboratoire. C'est notamment le cas de l'interaction des espèces entre elles et avec leur environnement, ainsi que de la présence de d'autres stresseurs. Ainsi, pour intégrer à la fois ces effets indirects et les effets directs liés à une augmentation de la salinité, le paramètre suivi dans cette thèse était la composition taxonomique, c'est-à-dire l'abondance relative des différentes espèces. Les communautés procaryotes ont été identifiées à l'aide de l'ARN ribosomique 16S (16S rRNA) et les communautés eucaryotes par les pigments photosynthétiques par chromatographie liquide à haute performance (HPLC), la microscopie optique, l'ARN ribosomique 16S des chloroplastes (chloroplasts 16S rRNA) et l'ARN ribosomique 18S (18S rRNA).

Pour les bactéries, seules les méthodes moléculaires permettent une identification à l'espèce. L'ARN 16S est le marqueur le plus fréquemment utilisé dans la littérature et c'est la raison pour laquelle il a été choisi (ex., Beall et al. 2016; Tran et al. 2018). Pour les eucaryotes, il existe plusieurs méthodes couramment utilisées, chacune ciblant un sous-ensemble de la diversité présente et présentant des forces et des faiblesses. L'analyse des

pigments par HPLC détecte les eucaryotes phototrophes et mixotrophes, mais aussi les bactéries phototrophes, dont les cyanobactéries (ex., Steinman 1998). Cette méthode permet seulement l'identification aux grands groupes taxonomiques (ex., diatomées, dinoflagellés ou algues vertes). La microscopie optique permet d'identifier les espèces dont la taille est plus grande que 5-10 µm, incluant les phototrophes et les mixotrophes, dont les cyanobactéries, mais aussi les hétérotrophes (Intergovernmental Oceanographic Commission of UNESCO 2010; Kalinowska and Grabowska 2016). Cette méthode demande une plus grande expertise que l'analyse des pigments, mais permet de calculer l'abondance ( $\text{individus L}^{-1}$ ) et les biovolumes (volume d'une cellule). L'ARN 16S des chloroplastes permet d'identifier les cyanobactéries, ainsi que les eucaryotes phototrophes et mixotrophes (ex., Beall et al. 2016). C'est une méthode qui dispose d'une base de données peu fournie, mais qui a été inclut puisqu'elle est utilisée dans la littérature pour identifier les eucaryotes et que les données sont générées par la même analyse que les procaryotes. Finalement, l'ARN 18S est un marqueur de plus en plus utilisé pour l'analyse moléculaire des eucaryotes microbiens. Il permet l'identification des phototrophes, des mixotrophes et des hétérotrophes, avec une résolution variant selon la richesse de la base de données.

Il est important de mentionner que dans la littérature, l'analyse moléculaire des marqueurs 16S et 18S est plus fréquemment basée sur le gène, donc sur l'ADN codant pour l'ARN ribosomique, que sur l'ARN en lui-même. Dans cette thèse, les analyses moléculaires ont été basées sur l'ARN. Ce choix découle du fait que l'échantillonnage incluait la période hivernale, dans laquelle on retrouve généralement plus de cellules mortes, en dégradation ou en dormance que durant le reste de l'année. Ces cellules pas ou peu actives dans l'écosystème sont considérées lorsque l'ADN est ciblé, mais pas, ou moins, lorsque la cible est l'ARN (Li et al. 2017). L'ARN permet de dresser un portrait des communautés plus près de l'activité que de la simple présence; L'ADN ribosomique est traduit en ARN lorsque la cellule a besoin d'assembler des protéines et l'ARN a une demi-vie très courte (de l'ordre de quelques minutes chez les bactéries; Li et al. 2017). Les communautés identifiées par l'ADN et l'ARN sont généralement semblables (Mohit et al. 2017), mais peuvent différer dans des régions ou à des moments où la succession des espèces est plus rapide (Kalenitchenko et al. 2019).

## **Contexte légal et administratif**

Déjà, durant les années 90, Environnement Canada et Santé Canada avaient mené une étude conjointe sur les sels de voirie à base de chlore et avaient conclu que ces produits représentaient une menace pour la faune et la flore et qu'ils devraient être ajoutés à la liste des substances toxiques selon la *Loi canadienne sur la protection de l'environnement* (Environnement Canada et Santé Canada 2001). Le développement d'une règlementation des sels de voirie s'est avéré complexe et contesté et l'idée a finalement été abandonnée. Les sels de voirie, n'ont donc pas été inscrits sur la liste des substances toxiques, mais seulement sur la liste des substances d'intérêt prioritaire, soient celles sur lesquelles on doit garder un œil. Pour ce faire, le gouvernement du Canada a mis en place de meilleures pratiques concernant l'approvisionnement, l'entreposage et l'épandage de ces sels, ainsi que sur l'élimination de la neige usée. Le *Code de pratique pour la gestion environnementale des sels de voirie* rapportant ces pratiques a été publié par le gouvernement du Canada en 2004. Ce code a pour but d'aider les municipalités à mieux gérer l'entreposage et l'utilisation des sels de voirie afin d'allier la sécurité routière à la protection de l'environnement. On trouve dans ce code des recommandations pour que l'entretien du réseau routier soit plus efficace, plus écoresponsable et moins coûteux. En somme, le code recommande d'utiliser la bonne quantité de sels, au bon moment et au bon endroit.

En 2010, le palier provincial a suivi l'initiative fédérale en publiant la *Stratégie québécoise pour une gestion environnementale des sels de voirie*. Ce document, résultant du travail conjoint de différents ministères et de partenaires municipaux, se veut un incitatif aux municipalités à participer, sur une base volontaire, à la démarche d'amélioration de la gestion des sels de voirie. Il est important de souligner le caractère volontaire des initiatives concernant les sels de voirie, car aucune loi, ni aucun règlement, n'encadrent leur utilisation sur les routes au Québec et au Canada (ni ailleurs dans le monde à ma connaissance). Dans le processus d'entretien hivernal du réseau routier, seule l'élimination de la neige usée est réglementée, et ce depuis 1997, par le *Règlement sur les lieux d'élimination de neige* (chapitre Q2-r.31) qui prévoit que la neige usée ne peut être déposée que dans des lieux accrédités à cet effet. Ce règlement découle de l'article 22 de la *Loi sur la qualité de l'environnement* du Québec.

Ce projet de doctorat s'inscrit dans ce contexte d'une volonté d'amélioration de la gestion des sels de voirie au Québec et permettra, par son identification de mécanismes de perturbations des écosystèmes par les sels et les contaminants qui y sont associés, de cibler des changements à privilégier pour un entretien hivernal du réseau routier plus écoresponsable. De plus, en identifiant les micro-organismes les plus tolérants à l'augmentation de la salinité, cette thèse permettra de mieux approximer ce à quoi pourrait ressembler un écosystème perturbé par les sels de voirie et d'ainsi évaluer si son fonctionnement pouvait en être affecté, dans l'absolu, et dans le cadre de son utilisation comme source d'eau potable.

## **Organisation de la thèse**

Dans les régions tempérées, l'urbanisation du territoire et l'utilisation des sels de voirie qui y est liée causent une augmentation de la salinité des eaux douces, phénomène que l'on appelle la salinisation. Cette salinisation peut compromettre l'utilisation de l'eau comme source d'eau potable, notamment à cause d'une augmentation des concentrations de Cl, et a le potentiel d'affecter les organismes aquatiques. Les objectifs principaux de cette thèse sont d'identifier des cibles de gestion des sels de voirie dans l'environnement et de déterminer les impacts de la salinisation de l'eau douce sur les communautés microbiennes planctoniques des lacs urbains. Il s'agit d'abord d'identifier les ions impliqués dans la salinisation, de déterminer les voies qu'empruntent ces ions pour atteindre les lacs et la proportion dans laquelle ils s'y retrouvent et d'ensuite vérifier s'ils peuvent être responsables d'un changement dans la composition taxonomique des communautés microbiennes. L'hypothèse principale de cette thèse est que l'environnement chimique des lacs à l'étude est perturbé par les ions Cl et Na provenant des sels de voirie et par d'autres ions y étant associés suivant un patron annuel et que cela se répercute sur la succession saisonnière des micro-organismes planctoniques. Les trois chapitres suivants correspondent à des articles scientifiques portant sur la réalisation de ces objectifs.

### Chapitre 1: “Roadside snowmelt: a management target to reduce lake and river contamination”.

Au cours de la saison hivernale, divers contaminants s'accumulent dans la neige au bord de la route. C'est notamment le cas des sels de voirie, mais aussi de métaux, d'hydrocarbures

aromatiques polycycliques (HAP) et de nutriments. Lors de la fonte printanière, l'eau de ruissellement transporte ces contaminants jusque dans les milieux récepteurs, ceux-ci pouvant être les rivières, les lacs et l'eau souterraine. Les objectifs principaux de ce chapitre sont de déterminer les voies qu'empruntent ces contaminants pour se rendre dans les lacs, le moment auquel ces déplacements ont lieu et la nature des perturbations chimiques pouvant être observées dans les lacs par leur arrivée. L'hypothèse que nous avons évaluée est que la neige usée contient des hauts niveaux de Cl et de Na, ainsi que des valeurs significatives de nutriments, de métaux et d'HAP et que l'arrivée de l'eau de fonte, au printemps, augmenterait la concentration de ces différentes substances dans les milieux récepteurs.

#### Chapitre 2: “Salinization of urban lakes: is there an influence on under-ice and open-water microbiomes”?

L'entrée principale des ions dans les lacs se faisant principalement au moment de la fonte de la neige, cela crée un patron annuel de variation de la salinité. Cette variation annuelle a le potentiel de modifier la succession des micro-organismes en fonction de leur tolérance. La salinité de base ainsi que l'intensité de cette variation diffèrent aussi d'un lac à l'autre, principalement en fonction de l'urbanisation du territoire. L'objectif principal du chapitre 2 était éclaircir la relation entre la composition taxonomique des communautés microbiennes planctoniques et la salinité des lacs en milieu naturel. Pour cela, deux approches ont été utilisées. La première a consisté à comparer la structure des communautés de lacs présentant différents niveaux de salinité et la deuxième à comparer la structure des communautés suivant la variation temporelle de salinité au sein d'un même lac. L'hypothèse évaluée était que la composition taxonomique des communautés microbiennes varie avec la salinité, autant d'un lac à l'autre que dans le temps au sein d'un même lac.

#### Chapitre 3: “Microbial plankton responses to salt versus urban snow in a spring snowmelt experiment”.

En milieu naturel, plusieurs facteurs autres que la salinité influencent la composition taxonomique des communautés microbiennes. Il est donc difficile d'isoler les effets de la salinité et de mettre en évidence des causalités. De plus, les principaux ions responsables de l'augmentation de la salinité, Cl et Na, n'arrivent pas seuls dans l'eau de fonte et leurs effets ne peuvent pas être isolés non plus. Le dernier chapitre de cette thèse visait donc à distinguer l'influence des sels de voirie de celle des autres contaminants contenus dans l'eau de fonte

de la neige urbaine. L'hypothèse qui a été testée est que la composition taxonomique des communautés microbiennes planctoniques est modifiée par les sels de voirie, indépendamment des autres substances contenues dans l'eau de fonte de la neige urbaine.

## Description des sites d'étude

La composition taxonomique des communautés microbiennes planctoniques étant influencée par de nombreux autres facteurs, notamment le niveau d'eutrophisation du lac, quatre lacs présentant différentes caractéristiques limnologiques ont été comparés: le lac Saint-Charles, réservoir d'eau potable de la Ville de Québec qui est mésotrophe et dont la salinité a modestement augmenté depuis les dernières décennies; le lac Clément qui présente un niveau de contamination en sel élevé jumelé avec un état trophique oligotrophe; le lac Saint-Augustin, un lac hypereutrophe présentant une salinité élevée, agissant à titre d'extrême tant au niveau de la salinité que de l'état trophique; ce qui, en comparaison avec le lac Clément, pourrait permettre une séparation des effets de la salinité de ceux de l'état trophique; et le lac Clair, qui agit à titre de contrôle puisqu'il est oligotrophe et qu'il n'a aucune route pavée dans son bassin versant. La section qui suit décrit chacun de ces lacs avec plus de détails.

### Lac Saint-Charles

Le lac Saint-Charles ( $46.94^{\circ}\text{N}$ ,  $71.39^{\circ}\text{W}$ ) est le réservoir pour la prise d'eau potable de la rivière Saint-Charles de la Ville de Québec et a aussi une vocation de plaisance pour des embarcations sans moteurs à essence (APEL 2014). C'est un lac de  $3,6 \text{ km}^2$  qui comprend deux bassins autour desquels sont distribuées six baies et dont la pointe nord se termine en marais (les Marais du Nord, Warren 2011). Le temps de résidence de l'eau varie d'une semaine à un mois selon le bassin et le moment de l'année (APEL 2014). Le niveau du lac est régulé à l'extrémité sud via un barrage construit en 1934 (Warren 2011). Le bassin versant de  $169 \text{ km}^2$  s'étend sur le territoire des villes de Québec et de Lac-Delage ainsi que sur les municipalités des Cantons-Unis de Stoneham-et-Tewkesbury, de Lac-Beauport et de Saint-Gabriel-de-Valcartier (APEL 2014). Ce territoire est couvert à plus de 76% de forêt, mais il comprend aussi un terrain de golf, un mont de ski, une carrière/sablière, des sites d'enfouissement, des milieux agricoles, des coupes forestières ou brûlis, deux usines de traitement d'eaux usées et de nombreuses résidences et commerces (APEL 2014). La

majorité de ce bassin versant (80 %) est drainé par la rivière des Hurons, le principal tributaire du lac Saint-Charles, qui s'y jette via les Marais du Nord (APEL 2014). L'effluent du lac Delage et 38 autres petits tributaires l'alimentent aussi (APEL 2014). La rivière Saint-Charles et le seul effluent du Lac Saint-Charles.

Le rôle important du lac Saint-Charles en tant que réservoir d'eau potable a fait de lui un lac particulièrement bien surveillé. Depuis les premiers signes d'une dégradation de sa qualité de l'eau, Agiro, anciennement connu sous le nom de l'Association pour la protection de l'environnement du lac Saint-Charles et des Marais du Nord ou APEL, y fait un suivi régulier, mesurant notamment les teneurs en nutriments, les coliformes fécaux et les niveaux de cyanobactéries. Les travaux de plusieurs chercheurs ont aussi contribués au fil des ans à l'importante base de données dont on dispose sur ce plan d'eau. Ainsi, il a pu être constaté une augmentation de l'azote entre 1996 ( $300 \mu\text{g L}^{-1}$ ) et 2013 ( $500 \mu\text{g L}^{-1}$ ), du phosphore ( $10 \mu\text{g L}^{-1}$  en 1996 vs  $14 \mu\text{g L}^{-1}$  en 2013) et de la conductivité ( $65 \mu\text{S cm}^{-1}$  en 1996 vs  $96 \mu\text{S cm}^{-1}$  en 2013) (Légaré 1998; APEL 2015). Sur une échelle de temps plus courte, on observe aussi une augmentation de la conductivité moyenne de la rivière des Hurons entre 2011 ( $70 \mu\text{S cm}^{-1}$ ) et 2013 ( $106 \mu\text{S cm}^{-1}$ ) (APEL 2015). La concentration de Na était d'environ  $10 \text{ mg L}^{-1}$  en 2014 et en 2015 (APEL 2014 et 2015). L'ion Cl avait à l'été 2012 une concentration moyenne de  $10 \text{ mg L}^{-1}$  (APEL 2014), alors que celle-ci a augmentée à  $16,2 \text{ mg L}^{-1}$  à l'été 2013 (APEL 2015). La contamination de ce lac par les sels de voirie est probablement causée par la proximité de l'autoroute 73 et du boulevard Talbot.

### Lac Clément

Le Lac Clément ( $46.94^\circ\text{N}$ ,  $71.35^\circ\text{W}$ ) est un petit lac urbain de  $0,086 \text{ km}^2$  ayant une profondeur maximale de 6 m (APEL 2011). Son bassin versant s'étend sur les territoires de la Ville de Québec et de Stoneham-et-Tewkesbury et est à 31% anthropisé, avec la plupart des constructions (routes pavées, maisons, etc.) situées aux abords immédiats du lac (APEL 2011). Le lac Clément est alimenté par l'eau souterraine et par trois petits tributaires, dont un qui passe sous trois axes routiers parallèles au lac, soient l'avenue de la rivière Jaune, le Boulevard Talbot et l'autoroute 73 (APEL 2011). Il s'agit donc d'un lac qui est fortement influencé par les sels de voirie, avec une conductivité de  $1000 \mu\text{S cm}^{-1}$  et une concentration

en chlorures, en surface, de  $150 \text{ mg L}^{-1}$ . Le lac Clément est toutefois oligotrophe (APEL 2011).

#### Lac Saint-Augustin

Le lac Saint-Augustin ( $46.75^\circ\text{N}$ ,  $71.39^\circ\text{W}$ ) est un plan d'eau récréatif situé principalement dans la municipalité de Saint-Augustin-de-Desmaures (Québec, Canada). C'est un lac de  $0,6 \text{ km}^2$  qui a une longueur de  $2,1 \text{ km}$ , une largeur moyenne de  $300 \text{ m}$  et une profondeur moyenne de  $3,6 \text{ m}$  (Bergeron et al. 2002). Le temps de résidence de l'eau est d'environ 6 mois. Son bassin versant naturel de  $7,46 \text{ km}^2$  recoupe la municipalité de Saint-Augustin-de-Desmaures et la ville de Québec (Québec, Canada) et compte de nombreuses résidences (Brin 2007), des milieux agricoles et plusieurs kilomètres de routes dont une portion de l'autoroute Félix-Leclerc (A40) (Galvez-Cloutier et al. 2006; OBV de la Capitale 2016). Il est principalement alimenté par les eaux souterraines, mais aussi via un réseau de drainage intermittent, le ruissellement de surface et un tributaire au nord-est (Galvez-Cloutier et al. 2006; Brin 2007). Son exutoire situé à l'extrémité sud alimente le fleuve Saint-Laurent (Galvez-Cloutier et al. 2006; Brin 2007).

La proximité de l'autoroute 40 et le fait que les principaux tributaires la traversent avant d'atteindre le lac le rendent particulièrement vulnérable à une dégradation de la qualité de l'eau. Il a donc, depuis la construction en 1974 du tronçon d'autoroute qui traverse son bassin versant (Galvez-Cloutier et al. 2006), fait l'objet de plusieurs études de caractérisation permettant un suivi à long terme. En 1977-1979, les concentrations de phosphore étaient de l'ordre de  $20 \text{ } \mu\text{g L}^{-1}$ , alors qu'en 2000-2001, il s'agissait plutôt de  $65 \text{ } \mu\text{g L}^{-1}$ . L'azote, pour sa part, est passé d'environ  $45 \text{ } \mu\text{g L}^{-1}$  en 1977-1979 à  $65 \text{ } \mu\text{g L}^{-1}$  en 2000-2001. Il en va de même pour le Na ( $35 \text{ } \mu\text{g L}^{-1}$  en 1977-1979 vs  $85 \text{ } \mu\text{g L}^{-1}$  en 2000-2001), le Cl ( $45 \text{ } \mu\text{g L}^{-1}$  en 1977-1979 vs  $135 \text{ } \mu\text{g L}^{-1}$  en 2000-2001) (Bergeron et al. 2002) et la conductivité ( $350 \text{ } \mu\text{S cm}^{-1}$  en 1979 vs  $900 \text{ } \mu\text{S cm}^{-1}$  en 2004; Galvez-Cloutier et al. 2006). Suite à un suivi de la qualité de l'eau de puits souterrains installés sur le bassin versant, Galvez-Cloutier et ses collègues (2006) soutiennent que les sels proviennent principalement de l'autoroute et rejoignent le lac via le ruissellement de surface.

Les sédiments, quant à eux, sont fortement contaminés par des métaux dont le cadmium, le chrome, le cuivre, le plomb, le nickel et le zinc (Brin 2007). À l'heure actuelle, le lac Saint-Augustin est hypereutrophe et présente des problèmes de floraisons de cyanobactéries qui ont menées, depuis 2009, à l'interdiction de la baignade.

### Lac Clair

Le lac Clair ( $46.96^{\circ}\text{N}$ ,  $71.69^{\circ}\text{W}$ ) est un plan d'eau de  $0,35 \text{ km}^2$  situé dans une zone de forêt expérimentale à Sainte-Catherine-de-la-Jacques-Cartier, laquelle est suivie par le Ministère des Forêts, de la Faune et des Parcs depuis les années '70. Le lac Clair n'a pas de tributaires, il n'est alimenté que par l'eau souterraine et l'eau de ruissellement, mais il a un effluent au nord (APEL 2016). Son bassin versant est exclusivement forestier (APEL 2016). Il s'agit d'un lac d'une profondeur moyenne de 11 m avec une fosse de 29 m près du centre du lac (APEL 2016). Le lac Clair est oligotrophe et présente une faible conductivité ( $\approx 20 \mu\text{S cm}^{-1}$ ) et une concentration faible de chlorures ( $\approx 0,3 \text{ mg L}^{-1}$ ), lesquels sont stables depuis les 50 dernières années (communication personnelle de Louis Duchesne, chercheur au Ministère des Forêts, de la Faune et des Parcs).

# **Chapitre 1 Roadside snowmelt: a management target to reduce lake and river contamination**

## **Résumé**

Dans plusieurs lacs et rivières des régions tempérées Nordiques, la concentration des ions majeurs a augmenté de façon importante dans les dernières décennies, principalement en réponse à l’application de sels de déglaçage sur les routes qui les entourent durant l’hiver. L’utilisation de sels de déglaçage est une pratique pour laquelle il n’y a présentement pas d’alternatives et qui va probablement augmenter dans le futur en raison des changements climatiques. La mise en place de stratégies de gestion préventive des impacts des sels de voirie sur les eaux de surface requiert une meilleure compréhension des voies qu’empruntent ces sels, et les contaminants qui y sont associés, pour se déplacer de la route aux milieux aquatiques récepteurs. Dans la présente étude, nous nous sommes penchés sur le cas du bassin versant de la rivière Saint-Charles et du réservoir en amont qui fournit de l’eau portable à la Ville de Québec, au Canada. La concentration des ions majeurs a été mesurée dans les rivières et dans la neige en bordure des routes, dans des sous-bassins versants présentant différents degrés d’urbanisation. Durant la même période, s’étendant du début de l’hiver à la fin du printemps, un système de mouillage était installé dans le réservoir afin d’enregistrer la conductivité en continu. Des différences significatives ont été observées entre les rivières en milieu urbanisé et celles en milieu forestier, et ce, tant au niveau de la concentration des ions que de leur patron de variation temporelle. La neige échantillonnée à 1 m de la route présentait des concentrations élevées de sels et de d’autres contaminants, et les concentrations les plus élevées mesurées dans le réservoir et les rivières étaient lors des événements de fonte. Les résultats indiquent que la gestion de l’eau de fonte provenant de la neige accumulée en bordure des routes pourrait prévenir une bonne partie de la contamination de l’eau de surface associée avec les sels de déglaçage. Les événements de fonte, particulièrement durant la période hivernale, vont demander une attention grandissante au fur et à mesure que le climat va se réchauffer.

## **Abstract**

Major ion concentrations have greatly increased in many northern temperate lakes and rivers over the last three decades as the result of de-icing materials applied to their surrounding roads in winter. Salt-based deicing will likely continue or increase in the future, and preventative management strategies require an improved understanding of the flow pathways and timing of road salt and associated contaminant fluxes to downstream receiving waters. In the present study, we focused on the catchment of the Saint-Charles River and its reservoir that provide drinking water for Quebec City, Canada. Major ion concentrations were measured in river waters and snowbanks along roads in sub-catchments that differed in their degree of urbanization, and during the same winter-spring period, a mooring system was installed in the reservoir to continuously record conductivity. There were large significant differences in the concentration and temporal behavior of ions between urbanized and forested watersheds. Snow sampled at 1-m distance from the roads had elevated concentrations of salts and other contaminants, and the highest solute concentrations in the reservoir and river waters occurred during snowmelt events. The results indicate that management of roadside snowmelt runoff during thawing events may largely prevent salt-associated contamination, and that winter snowmelt will require increasing attention as the climate continues to warm.

## 1.1 Introduction

Road runoff is a non-point source of many chemicals known to impair lake and river water quality. These runoff waters may contain aromatic polycyclic hydrocarbons (PAHs), nutrients, heavy metals, calcium (Ca) and magnesium (Mg) from concrete weathering and, in north temperate regions, sodium (Na) and chloride (Cl) from road salts. In Canada, sodium chloride (NaCl) is the most commonly used road salt for winter deicing, and the commercial product may also contain impurities such as phosphorus, nitrogen, iron (Fe), aluminum (Al), copper (Cu) and zinc (Zn, Howard and Beck 1993; Evans and Frick 2001). Freshwater salinization, evidenced by an increase of the total concentration of ions and the alteration of their ratios, has been widely observed in north temperate lakes and rivers, and in many cases attributed to road salt application (Kelting et al. 2012; Kaushal et al. 2017; Moore et al. 2017). This trend is of growing concern throughout the world given the potential negative impacts of road salts and associated contaminants on lake ecosystem functioning and services, including the provision of high quality drinking water (Bird et al. 2018; Kaushal et al. 2018).

The concentrations of chemicals in road runoff and in receiving waters are likely to vary with seasons, and with the degree of urbanization. Metals, nutrients and other ions have been observed to be in higher concentration in snowmelt than stormwater (Valtanen et al. 2014; Galfi et al. 2017), implying the importance of snow runoff as a management target (Zhu et al. 2012). The metal and ion concentrations in runoff and receiving waters appear to increase with the imperviousness of the catchment (Valtanen et al. 2014; Kaushal et al. 2017; Bird et al. 2018), with an observed threshold of around 1%. In Baltimore streams, impervious surface cover in the watershed needed to be greater than 1% to observe this relationship (Bird et al. 2018). The same 1% threshold was identified in North American lakes, for the association between impervious surface cover and long-term increase in Cl concentrations (Dugan et al. 2017a).

Lake and river contamination, like many other ecological perturbations, occurs gradually over time. It is generally difficult to determine which, if any, early warning indicators would allow the perturbation to be managed before it reaches a critical state (Contamin and Ellison 2009; Spears et al. 2017). Furthermore, there may be a considerable time lag, from months to decades, between the application of management strategies and the positive response in

water quality, due to factors such as water residence time, the properties of the contaminants and their pathways to the water (Phillips et al. 2005; Meals et al. 2010; Ficker et al. 2019). These problems of identifying the onset of severe perturbation and the delay in response to treatment are arguments to focus on preventative management strategies rather than retrospective approaches.

Good practices in road runoff management require the establishment of sustainable urban drainage systems and green infrastructure (Moore et al. 2018). While these have proven to be efficient in decreasing the concentrations of metals, total organic carbon, suspended solids and nutrients, even during winter in cold climates (Hilliges et al. 2013; Søberg et al. 2017), their ability to treat salted inflows may be limited (Rivett et al. 2016; Scarlett et al. 2018). Furthermore, such technologies rely on the infiltration of the runoff into the soil, which may displace solute peaks to other seasons (Cooper et al. 2014) and allow Na to mobilize major ions and metals from soils via cation exchange (CEC, Marsalek 2003; Cooper et al. 2014; Moore et al. 2017; Kaushal et al. 2018). There is a need to identify more effective ways to manage road runoff, and this requires an improved understanding of the timing of chemical fluxes, and their distribution and flow pathways through the landscape to downstream receiving waters.

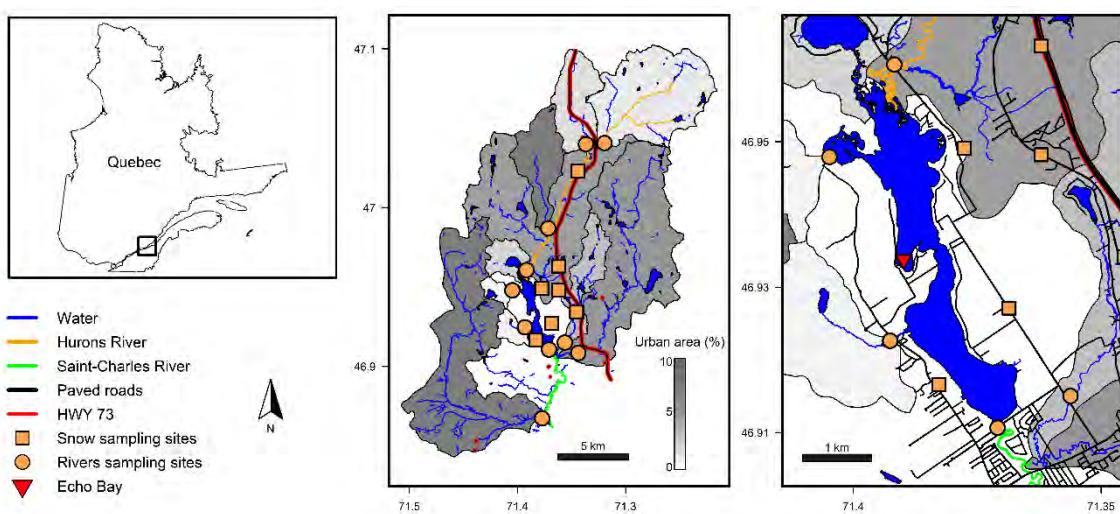
In the present study, we measured the temporal variations in road salt ions and other potential contaminants in a northern temperate drinking water reservoir and its associated rivers and roadside snow, during winter and spring. We compared these measurements with an undeveloped lake in the same region to evaluate the hypotheses that paved roads are associated with freshwater salinization, and that contaminant transfer from roadside snow to downstream waters occurred during spring snowmelt. In light of these results, we then identify preventative strategies to address this lake management issue, and consider the implications of ongoing climate change.

## 1.2 Methods

### 1.2.1 Study area

Lake Saint-Charles (lat. 47°20'N, long. 71°30'W) is a dimictic north temperate lake (Fig. 11 in Vincent 2018) and the drinking water reservoir for ca. 300,000 residents in Quebec

City, Canada. The drinking water intake is located on the Saint-Charles River, 11 km downstream of the reservoir (Fig. 1-1), and the water at that site is derived from a 348 km<sup>2</sup> catchment area (Fig. 1-1; Roche 2010). The region experiences 3 m of annual snowfall, with the snowpack persisting 140 days on average each year, from November to April. During that period, the mean air temperature is -5.6 °C (Government of Canada 2019a) and the temperature of the rivers is in the range 0 to +1 °C (MELCC 2019a). The rivers are partially to totally ice-covered, and lakes in this region are ice-covered throughout winter, from December to May.



**Figure 1-1.** Hydrological features of the study area and location of sampling sites. The shaded regions delimit the sub-catchments, with symbols in orange indicating the sampling sites. Latitude is in decimal degrees North and longitude is in decimal degrees West.

Lake Clair was selected as a reference lake for the study region. Like Lake Saint-Charles, it is dimictic and ice-covered for about six months of the year. It is located 20 km west of Lake Saint-Charles, and experiences a similar climate. Both lakes lie on the Canadian Shield, characterized by a bedrock of gneiss and granite, and a surface deposit of rock, gravel, sand, silt and clay (APEL 2011). Unlike Lake Saint-Charles, Lake Clair lies in a completely forested catchment with no paved roads, and there are therefore no maintenance activities during winter and no known anthropogenic perturbations (Houle et al. 2002). Lake Clair is smaller and deeper than Lake Saint-Charles, and also has a longer water residence time (Table 1-1), which means that it would take longer to respond to changes in its source water

quality. Water quality at the outflow of lake Clair has been monitored by the Québec Ministère des Forêts, de la Faune et des Parcs once to twice a month since 1989 (methods as in (Houle et al. 2002; Duchesne and Houle 2008)), providing a database to analyze long-term natural trends in the study area, and allowing the measurements of the present study to be placed in a longer term context.

**Table 1-1.** Physical and chemical characteristics of Lake Clair and Lake Saint-Charles. The chemical data are means for surface waters (0-50 cm) for the period January-May 2017. Numbers in parentheses are the coefficients of variation (SD as % mean; n=5). Physical data are from APEL (2014; 2016) and chemical data are from the present study.

Characteristics	Units	Lake Clair	Lake Saint-Charles
Physical			
Area	km <sup>2</sup>	0.4	3.6
Mean depth	m	11.7	4.1
Maximum depth	m	26.9	17.5
Water residence time	years	3	0.04
Mixing type	-	Dimictic	Dimictic
Chemical			
Conductivity	µS cm <sup>-1</sup>	18.5 (5)	97.3 (32)
Calcium	mg L <sup>-1</sup>	1.9 (12)	6.1 (23)
Sodium	mg L <sup>-1</sup>	1.0 (10)	9.5 (40)
Chloride	mg L <sup>-1</sup>	0.4 (39)	16.9 (50)

In the present study, rivers and roadside snow in the Saint-Charles River catchment were sampled for chemical analysis over winter-spring, every two weeks from 21 December 2016 to 08 May 2017. Lake Clair and Lake Saint-Charles were sampled once a month (Jan, Feb, Mar and May) during the study period and an automated in situ system was installed in Lake Saint-Charles to track changes in conductivity.

### 1.2.2 River sampling

We collected water at nine sites (Fig. 1-1) located on eight rivers. There were two sampling sites on the Hurons River, one upstream of Highway 73 and urbanized areas, and one downstream (Fig. 1-1). Other sampling sites included the main tributaries of the

reservoir, and two additional rivers upstream of the Quebec City water intake, the Nelson River and the Jaune River. The degree of urbanization of these sites varied, allowing the test of relationships between urbanization and ion concentrations, as well as analysis of temporal patterns.

Conductivity was measured in situ with a YSI EXO2 profiler (YSI Incorporated, Yellow Springs, OH) or in the laboratory with a Hydrolab DS5X profiler (OTT HydroMet, Loveland, CO) in water samples that had been collected in 1L plastic bottles that had been rinsed with Milli-Q and sample water. Water for chemical analysis was sampled in 500 mL acid-washed plastic bottles.

### 1.2.3 Snow sampling

Snow was sampled at seven sites along four major roads of the Saint-Charles River drinking water catchment (Fig. 1-1). These roads differed in their intensity of vehicle traffic and their maintenance activities (data from local authorities; Mochizuki 2011, MTQ 2019). Two sites were along the highway 73N (S6 and S7; 18 300 vehicles day<sup>-1</sup>) which is heavily treated with road salts in winter (around 46 tonnes km<sup>-1</sup> year<sup>-1</sup>). An additional four sites were along secondary roads: Avenue de la Grande-Ligne (S1 and S2; 3372 vehicles per day) and Boulevard Talbot (S3 and S4; 3067 vehicles day<sup>-1</sup>), which are likely maintained with a mix of road salts (around 0.12 tonnes km<sup>-1</sup> year<sup>-1</sup>) and salted abrasives (unknown quantities, 5% road salt in volume); and one site along Avenue du Lac Saint-Charles (S5; 733 vehicles day<sup>-1</sup>), which was maintained only with salted abrasives (application rate unknown). The road salt used in the study area was also sampled from the MTQ salt depot and analyzed to determine its chemical composition. The salt was diluted in Milli-Q water (500 mg L<sup>-1</sup>) before analysis, and results were then expressed in terms of elemental mass per total mass of the salt mixture.

Snow was sampled at 1, 5 and 20 m from the road at all sites; we combined snow from all depths as deeply as possible, but excluding any basal ice layer, to encompass the full snowbank profile. The snow was collected in 1 L acid-washed bottles for chemical analysis and in 2 L Milli-Q water washed plastic containers for conductivity measurement and was

allowed to melt at 4 °C before analysis. Conductivity was measured in the laboratory using a Hydrolab DS5X profiler.

#### 1.2.4 Lake sampling

Lake Clair and Lake Saint-Charles were sampled once a month during the study period, that is in January, February, March and May (April was excluded because of the dangerous ice-break-up conditions). Lake Clair was sampled at the deepest point, while Lake Saint-Charles was sampled in Echo Bay (Fig. 1-1). This particular location was chosen instead of the deepest point, because it is known for water quality problems, notably the highest occurrence in the lake of cyanobacterial blooms during summer (APEL 2014).

During the winter months, water was sampled just underneath the ice, through drilled holes, and open surface water (0-50 cm depth) was sampled during spring. Approximately 10 L of water were collected, in triplicates, with a 1 L Nalgene bottle and transfer into acid washed (HCl, 0.1 N) Aqua-Paks (Reliance, Manitoba, Canada) for chemical analysis. Conductivity profiles were obtained with a Hydrolab DS5X profiler (Loveland, Colorado, USA).

#### 1.2.5 Automated in situ measurements

An automated in situ system was installed in the Lake Saint-Charles reservoir to record specific conductivity at hourly intervals from 4 November 2016 to 2 June 2017, which encompassed the ice-cover period from 26 November to 29 April. The mooring system was deployed in Echo Bay (Fig.1-1) at a location with a maximum depth of 12 m, and consisted of a metal chain, a surface buoy to maintain the system upright and a bottom weight to maintain it in place. Conductivity loggers (Hobo U24-001, Onset Computer Corporation, Massachusetts, USA; conductivity resolution:  $1.0 \mu\text{S cm}^{-1}$ ) were attached to the metal chain at 1.5, 3, 7 and 8.5 m depth at the time of deployment. The system was designed to ensure that the loggers remained below the ice (maximum thickness 0.7 m) during winter. The loggers were calibrated with conductivity standards prior to their deployment in the mooring. Our Lake Saint-Charles profiling with the Hydrolab in January, February, March and May provided a cross-check of the loggers to verify that their calibration remained constant throughout the study period.

### 1.2.6 Chemical analysis

For the lake and the road salt samples, samples were filtered through Milli-Q water pre-rinsed cellulose acetate filters (0.2 µm pore size, Advantec Micro Filtration Systems), then acidified (for cations only, HNO<sub>3</sub> Trace Metal Grade 0.2% final) and kept at 4°C until analysis. Anion concentrations were measured by ion chromatography (ICS-2000, Dionex), major cations by atomic emission spectroscopy (ICP-AES, Varian Vista AX, Varian Medical Systems, Palo Alto, CA) and trace cations by mass spectroscopy (ICP-MS, Thermo X Series, Thermo Fisher Scientific, Bedford, MA).

For the rivers and the melted snow samples, the following elements were measured on unfiltered samples: Al, Ba, Br, Cd, Cl, Co, Cr, Cu, Fe, Fl, K, Mg, Mn, Mo, Na, Ni, Pb and Zn, as well as nitrate-N (NO<sub>3</sub>; not corrected for nitrite), total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC). The analyses were performed by the Laboratoires de la division de la qualité de l'eau du Service de l'eau et de l'environnement of Quebec City using standard methods (Baird et al. 2017).

### 1.2.7 Catchment variables

The flow gauge record of the Quebec Ministry of Environnement et de la Lutte contre les Changements Climatiques (MELCC) at the Saint-Charles River station 050904 (46°48'N, 71°19'W) provided an estimate of total discharge for the catchment (MELCC 2019b). This monitoring station is located 5 km downstream of all the sampled rivers of this study, and there are no large additional inflows in between. Decreases in snow height at the Jean Lesage Airport climate station (Government of Canada 2019b), 16 km south of Lake Saint-Charles, was used as a proxy for snowmelt during the sampling period, and also provided air temperature data that are likely similar across this region.

Urbanization level was calculated as the percentage of the entire watershed area that was covered by paved surfaces as well as buildings, golf clubs, pools, ski stations, landfill and industrial sites. The sampling sites were located at the mouth of the sampled rivers, as integrators of their entire watershed. The watershed delimitation data were taken from MELCC (2019c), while the land occupancy data were provided by the local conservation authority (APEL, unpublished data). The area of each land occupancy type was calculated

from those two data sets using the *sp* (v1.3-1) and the *rgeos* (v0.4-2) packages in R. The upstream and up-urbanization site on the Hurons River has a watershed with different soil occupancy than the rest of the Hurons River watershed, but no delimitation data were available. Watershed delimitations were therefore hand-drawn based on lines of flow perpendicular to isoheights, in the direction of highest to lowest altitude. The watershed delimitations were drawn using Open Street Map, and the resulting polygon was exported to R, where it was used to calculate areas of soil occupancy.

#### 1.2.8 Statistical analysis

The river and snow sampling sites were statistically compared and clustered according to their chemical composition. Measurements at different sampling times were used as replicates for sampling sites. The groups were separated using a hierarchical clustering analysis, with the *hclust* function, and the resulting dendrogram was separated into groups using the *cutree* function of the *dendextend* package (v.1.9.0). The environmental characteristics of these groups were then examined with a non-metric multidimensional scaling (NMDS) using the *metaMDS* function of the *vegan* package (v.2.5-3).

Mean chemical concentrations in the lakes, snow and rivers over the sampling period were compared between groups using Student's t-test (after log transformation) with the p-value corrected for multiple comparisons (Bonferroni corrected p-value of 0.0021). This was performed in R using the *t.test* function on the mean of the groups, which was calculated from all sampling dates and sites. For the snow, there were three sample groups (1, 5 and 20 m), and their means were compared for each pair of distances.

Relationships between the measured parameters and time were examined using scatterplots smoothed with a local regression using the *loess* function. The smoothing degree was 0.35, and degree 2 polynomial curves were fitted. To assess temporal variations, the means for each sampling dates (with sites as replicates) were compared using an ANOVA and a Tukey's test with, respectively, the *aov* and the *TukeyHSD* functions in R. The correlation between the measured ions and conductivity was calculated using the Pearson coefficient with the *cor.test* function. The slopes of the relationships between ion concentration and conductivity were compared with a Tukey test on the least-square means

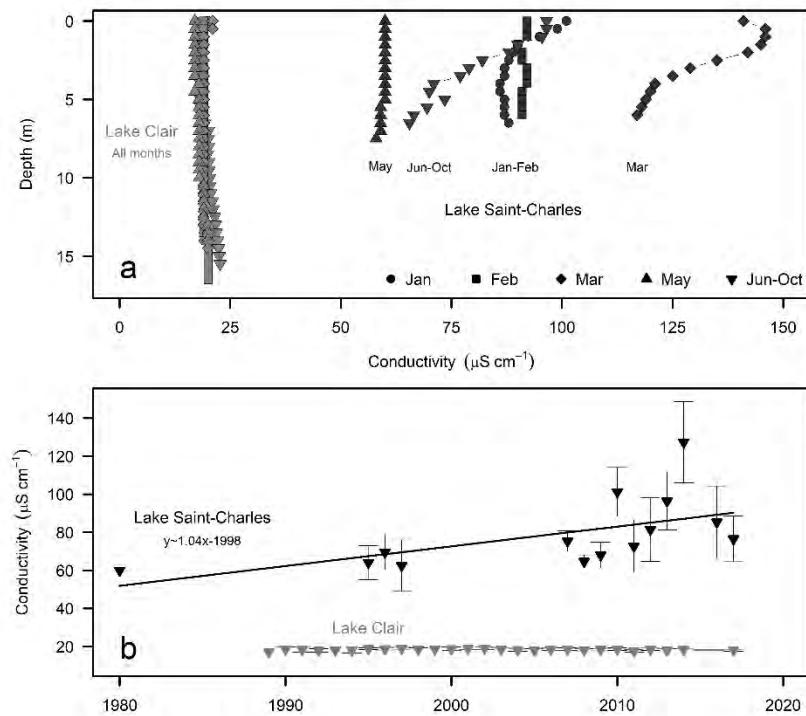
from a linear model. The functions *lstrends* and *pairs* from the *lsmeans* package as well as the function *lm* were used to do that.

The ion concentrations in rivers were box-plotted as a function of the urbanization level of their catchment. To evaluate the relationship between these two variables, linear and logarithmic models were fitted using the *lm* function on pooled samples (for all sites and dates, n=95). The long-term trends of conductivity in Lake Clair and Lake Saint-Charles were also investigated using the *lm* function on summer means for each year where data were available (n=27 and n=14, respectively). All statistical analyses were performed with R version 3.4.3 (R core team 2017).

## 1.3 Results

### 1.3.1 Lake temporal trends and water composition

During the present study period, Lake Saint-Charles conductivity increased throughout winter from around  $90 \mu\text{S cm}^{-1}$  in January to  $140 \mu\text{S cm}^{-1}$  in March, before sharply dropping to  $55 \mu\text{S cm}^{-1}$  in May (Fig. 1-2a). This temporal variability was also reflected in the vertical profiles, where up to a 40% difference in conductivity was observed between surface and bottom values, except in May due to spring mixing (Fig. 1-2a). In contrast, there was neither seasonal nor vertical variability in Lake Clair, where conductivity remained constant at around  $20 \mu\text{S cm}^{-1}$  throughout all months and depths (Fig. 1-2a).



**Figure 1-2.** Seasonal and long-term comparison of conductivity in Lake Clair and Lake Saint-Charles. a) Conductivity profiles in 2017, including the winter-spring study period and the subsequent June-October period. b) Conductivity in the surface waters between 1980 and 2017, averaged from June to October.

The difference in solute variability between the two lakes is also apparent in their long-term records. Conductivity in Lake Saint-Charles has steadily increased over the last four decades (Fig. 1-2b; linear model positive slope,  $p$ -value  $<0.05$ ), doubling from  $50 \mu\text{S cm}^{-1}$  in the 1980s to around  $100 \mu\text{S cm}^{-1}$  in recent years. Over the same period, the conductivity of reference Lake Clair has remained constant at around  $20 \mu\text{S cm}^{-1}$  (Fig. 1-2b; null linear model,  $p$ -value  $>0.05$ ).

The higher ion concentrations in Lake Saint-Charles relative to Lake Clair were especially pronounced for Cl. During the winter-spring study period, Cl concentrations were on average higher by a factor of 42 ( $16.9 \text{ vs. } 0.4 \text{ mg L}^{-1}$ , Table 1-1). The conductivity of the surface waters of Lake Saint-Charles was also more variable, with a coefficient of variation (CV%; standard deviation (SD) as a percentage of the mean) for winter-spring values that was 6.6-times higher than for Lake Clair ( $32.3 \text{ vs. } 4.9\%$ , Table 1-1). Sodium was the

dominant cation in Lake Saint-Charles, and its CV% was four times that in Lake Clair (40.3 vs. 10.1%), while Ca dominated in Lake Clair, with concentrations around twice those for Na. Consistent with these analyses, conductivity was significantly correlated with Na and Cl in Lake Saint-Charles but not Lake Clair, where it was correlated with Ca, Mg and K (Table S1-1).

### 1.3.2 River water composition

The chemical composition of river waters varied principally with the degree of urbanization of their catchments (NMDS, Fig. S1-1) and we therefore chose urbanization level (defined above) as the structuring variable. This separated the rivers into two distinct categories: rivers with a catchment of high (0.9-7.7%) or low (0.5-0.6%) urbanization levels. We hereafter referred to these two categories as urban rivers ( $n=6$ ) and forest rivers ( $n=3$ ), respectively. The two sampling sites on the main tributary of the Saint-Charles reservoir differed in their sub-watershed urbanization levels (0.62 vs. 4.1%), which also corresponded to differences in their chemical composition and their separation in the forest and urban river categories. Plots of river cation concentrations as a function of urbanization indicated a non-linear relationship, with a plateau above 2% (Fig. S1-2).

There were significant differences ( $p<0.05$ ) in the mean concentrations of measured ions (Ca, K, Na, Cl, Ba, Mn), as well as in TN, between the urban and forest rivers. The largest significant observed difference was for Na, with 10-times greater concentrations in the urban rivers (15.6 vs. 1.6 mg L<sup>-1</sup>, Table 1-2). In forest rivers, Cl was always at or below the limit of detection of 2 mg L<sup>-1</sup>. In the urban rivers, Na increased as a proportion of total cations with increasing conductivity (Fig. S1-3), indicative of the use of road salts. Overall, there was a close relationship between conductivity and the ion concentration. However, there were large differences in slopes. The slopes were non-significant for Mg and K, and the slopes of Na and Cl were four-times higher (respectively 96 and 88) than that of Ca (22, data not shown). As a result, at conductivities higher than 200  $\mu$ S cm<sup>-1</sup>, Na was the dominant cation in urban rivers, while in the low conductivity forest rivers, Ca was always the dominant cation. Consistent with this trend, the conductivity of urban rivers was most highly correlated with Cl and Na, but with Ca and Mg for forest rivers (correlation coefficients all >0.95; Table S1-1).

**Table 1-2.** Mean chemical composition of the sampled urban and forest rivers over the study period, and Student's t-test for significance of difference (\*\*\*: p <0.001; NS: non-significant at the Bonferroni corrected p-value of 0.0027). Numbers in parentheses are the coefficients of variation (SD as % mean; n=62 and 33 for the urban and forested rivers, respectively). All major ions, as well as TOC, TN and nitrate-N (NO<sub>3</sub>) are in mg L<sup>-1</sup>; all other values are in µg L<sup>-1</sup>.

Chemical variable	Urban	Forest	Significance
Cl	23.9 (36)	2.0 <sup>1</sup> (28)	***
Na	15.6 (39)	1.6 (65)	***
Ca	9.2 (33)	3.5 (37)	***
Mg	1.4 (44)	0.7 (51)	NS
K	0.7 (26)	0.3 (30)	***
TOC	3.5 (45)	2.2 (44)	NS
TN	0.6 (24)	0.3 (43)	***
NO <sub>3</sub>	0.3 (55)	0.2 (45)	NS
TP	11.1 (64)	7.2 (79)	NS
Fe	263.3 (56)	98.8 (70)	NS
Al	90.7 (60)	60.0 (101)	NS
Fl	59.1 (32)	50.3 (24)	NS
Mn	27.9 (32)	8.8 (67)	***
Ba	10.8 (22)	5.1 (8)	***
Br	6.4 (64)	2.9 (55)	NS
Zn	4.8 (31)	3.6 (44)	NS
Cu	0.8 (100)	0.3 (33)	NS
Ni	0.8 (113)	0.6 (83)	NS
Cr	0.4 (125)	0.4 (75)	NS
Mo	0.2 (50)	0.2 (150)	NS
Pb	0.2 (50)	0.1 (100)	NS
Co	0.1 (40)	0.04 (100)	NS
Cd	0.03 (100)	0.03 (200)	NS

### 1.3.3 Snow composition

The variables that influenced the chemical composition of snow across all sampling sites, distance, and dates were investigated with hierarchical clustering and NMDS analysis. The hierarchical clustering identified three groups that, from all measured variables, were best explained by distance from the road (Fig. S1-4). Points from all sampling sites (S1 to S7), and therefore from all vehicle traffic levels (low S5, intermediate S1 to S4 and high S6 and S7), were similar in the three groups, indicating that road and traffic site had little impact on the chemical composition of the snow. We therefore selected distance from the road as the grouping variable for further analysis.

Road salts (Na, Cl), other ions (Ca, Mg, K) and many trace metals concentrations in the snow decreased with distance from the road. The most significant decrease was observed for road salts and Ca with a two order of magnitude decline in concentrations between 1 and 20 m and a 20 to 40 times decrease between 1 and 5 m (Table 1-3). Throughout winter and spring, the concentrations of measured ions as a function of sample distance always followed the trend 1 m >> 5 m > 20 m (Fig. S1-5, Table 1-3). To simplify the figures, only the 1 and 20 m results are shown, but data for all three sampled distances are given in Table 1-3.

**Table 1-3.** Mean chemical composition of melted snow sampled at 1, 5 and 20 m from the road over the study period, and Student's t-test of the significance of differences (\*\*\*: p <0.001; NS: non-significant at the Bonferroni corrected p-value of 0.0027; NA: not available). Student's t-tests were done pairwise between sample distances; e.g., 1-5m: comparison of 1 m and 5 m means. Numbers in parentheses are the coefficients of variation (SD as % mean; n=56). All major ions, as well as TOC, TN, nitrate-N (NO<sub>3</sub>) and TP are in mg L<sup>-1</sup>; all other values are in µg L<sup>-1</sup>.

Chemical variable	Snow			Significance		
	1 m	5 m	20 m	1-5m	5-20m	1-20m
Cl	528.3 (135)	179.3 (198)	4.7 (85)	***	***	***
Na	355.3 (121)	121.8 (192)	2.8 (105)	***	***	***
Ca	150.8 (111)	38.9 (144)	1.7 (135)	***	***	***
Mg	2.2 (131)	0.6 (135)	0.04 (81)	***	***	***
K	2.6 (114)	0.9 (163)	0.07 (183)	***	***	***
TOC	9.2 (87)	NA	1.4 (110)	NA	NA	***
TN	0.7 (97)	0.4 (94)	0.3 (83)	***	NS	***
NO <sub>3</sub>	0.2 (187)	0.1 (61)	0.1 (164)	NS	NS	NS
TP	3.1 (87)	NA	0.07 (135)	NA	NA	***
Fe	980.5 (180)	318.0 (148)	13.5 (218)	NS	***	***
Al	729.6 (169)	220.5 (156)	12.7 (182)	NS	***	***
Fl	34.3 (51)	72.5 (462)	28.8 (77)	NS	NS	NS
Mn	181.7 (109)	43.5 (114)	4.8 (91)	***	***	***
Ba	62.3 (82)	18.9 (109)	1.6 (73)	***	***	***
Br	15.6 (125)	7.3 (156)	2.0 (0)	NS	***	***
Zn	104.1 (98)	53.7 (218)	7.6 (92)	NS	NS	***
Cu	10.5 (98)	4.3 (98)	0.6 (92)	***	***	***
Ni	1.9 (95)	0.6 (91)	0.1 (139)	***	***	***
Cr	1.5 (107)	0.7 (166)	0.2 (249)	NS	NS	***
Mo	0.5 (60)	0.2 (93)	0.1 (26)	***	NS	***
Pb	2.7 (115)	1.2 (99)	0.3 (111)	***	***	***
Co	5.3 (149)	1.1 (114)	0.0 (92)	***	***	***
Cd	0.1 (100)	0.05 (80)	0.0 (53)	***	***	***

For the snow immediately next to the road, the concentrations of major ions were high, with Cl values averaging  $500 \text{ mg L}^{-1}$  (Table 1-3). Total phosphorus values were in the  $\text{mg L}^{-1}$  range (between 0.2 and 12), and TN averaged  $0.7 \text{ mg L}^{-1}$ . Several trace metals were also in high concentration ( $>100 \text{ }\mu\text{g L}^{-1}$ ), notably Fe, Al, Mn and Zn. The analysis of road salts used for de-icing in the study area confirmed that they were mainly composed of Na and Cl (for a combined total of  $928 \text{ mg g}^{-1}$ ), but that they also contained Ca, TN, TP and metals (Table 1-4).

**Table 1-4.** Mean chemical composition in the commercial road salt used in the Quebec City region, and ratios for comparison with snow at 1 m from the road. The numbers in parentheses are the coefficients of variation (SD as % mean; n=3). All major ions, as well as TN, are in mg per g of road salt (dry weight); all other values are in  $\mu\text{g}$  per g of road salt (dry weight). The expected concentration (EC) of the different elements is in  $\text{mg L}^{-1}$  and was calculated as  $\text{EC} = [(\text{Cl concentration in roadside snow}) / (\text{Cl concentration in road salt})] \times \text{element concentration in road salt}$ . The concentration of the element in the snow at 1m (SN) presented in Table 3, was used to calculate the SN:EC ratio. An SN:EC ratio  $>1$  implies that there is more of the given element in the snow than expected if road salt was its only source.

Chemical variable	Concentration		Ratio SN:EC
	Road salt	EC	
Cl	559.5 (4)	528.3	1
Na	368.7 (4)	348.1	1
Ca	2.6 (61)	2.5	61
Mg	0.1 (22)	0.09	23
K	1.4 (22)	1.3	2
TN	0.1 (44)	0.09	7
TP	7.6 (48)	7.2	432
Fe	10 (7)	9.4	104
Al	10 (41)	9.4	77
Mn	2 (38)	1.9	96
Zn	9.1 (61)	8.6	12
Cu	4.6 (116)	4.3	2

Among the principal sources of Cl in urban areas (i.e., road salts, runoff from agricultural lands, livestock and waste water; Corsi et al. 2015), only road salts is likely to be a source of Cl to roadside snow. Therefore, by dividing the Cl concentration in the snow at 1 m from the

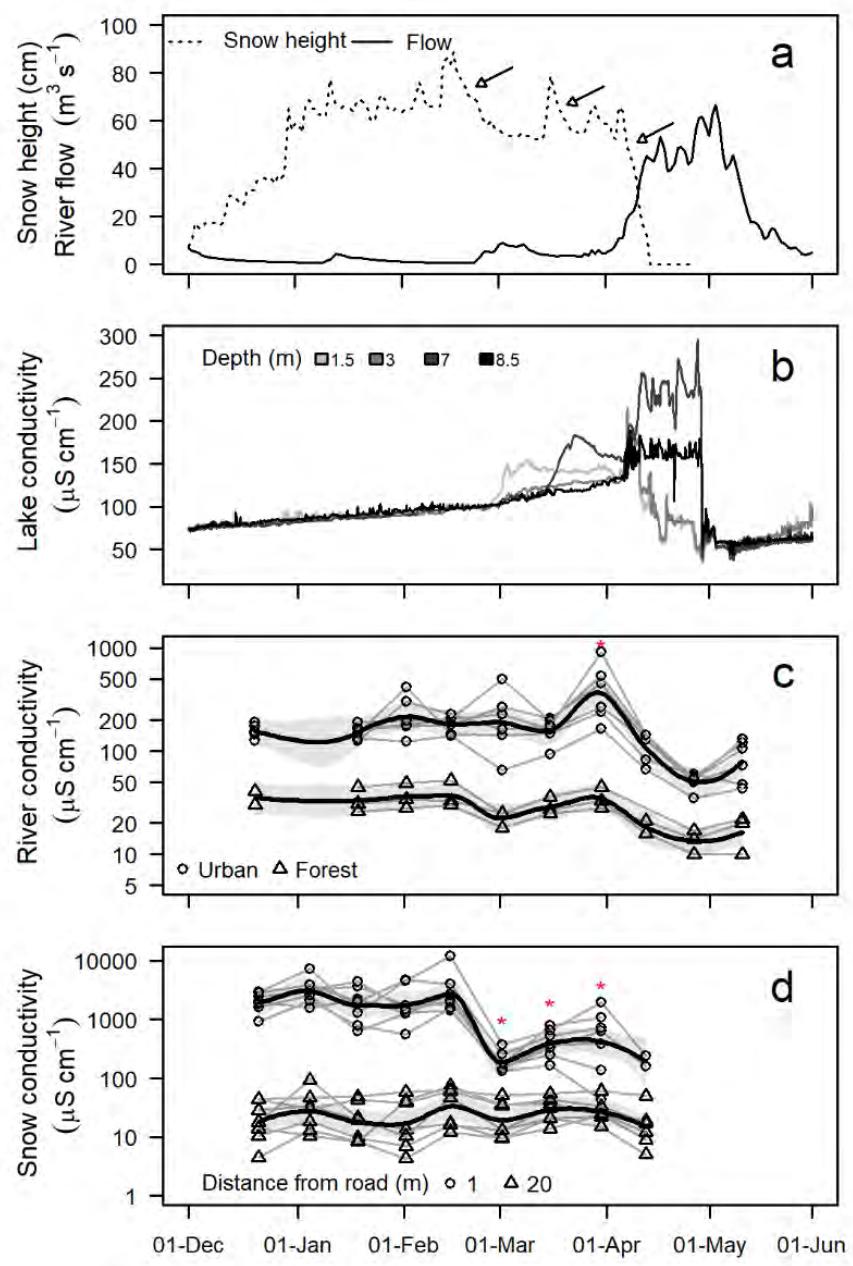
road (Table 1-3) by the Cl concentration in the road salt samples (Table 1-4), we estimated that there was 0.94 g of salt per L of melted snow. To evaluate if road salt was the main source of the other measured elements or if there were other sources, their expected concentration in the snow (EC) was calculated as:

$$EC = [Cl_{snow}/Cl_{salt}] \times E_{salt}$$

Where  $Cl_{snow}$  is the concentration of Cl in the snow,  $Cl_{salt}$  is the concentration of Cl in the road salt and  $E_{salt}$  is the element concentration in road salt (Table 1-4). The expected concentration of an element (EC) was compared to its measured concentration in the snow at 1m from the road (SN, Table 1-4). If SN:EC=1, the element likely came from the same source as Cl (i.e., road salt), whereas if SN:EC>1 there would be other sources of this element. Of all the measured elements, only Na showed a SN:EC ratio of 1. The highest SN:EC ratios were observed for TP, Fe, Mn, Al and Ca (respectively 437, 104, 96, 77 and 61).

#### 1.3.4 Melting events

The fluctuations in snow height indicated that there were three melting events during the study period (Fig. 1-3a). The first event (hereafter called first winter event) lasted from February 17 to March 1 and caused an increased flow in the Saint-Charles River from February 22 to March 16, rising to a maximum discharge of  $9 \text{ m}^3 \text{ s}^{-1}$  (Fig. 1-3a). A second event (hereafter called second winter event) lasted from March 17 to 24; less snow melted at this time and there was no pronounced increase in Saint-Charles River flow. The last melting event (hereafter called spring event) lasted from April 7 to 14 and was associated with the full melting and disappearance of the snowpack. This resulted in an increase in Saint-Charles River flow from April 4 to May 22, which reached a maximum of  $67 \text{ m}^3 \text{ s}^{-1}$  (Fig. 1-3a). The spring event coincided with conductivity increases in the urban rivers and lake water (Fig. 1-3b and 1-3c), as well as conductivity decreases in the snow (Fig. 1-3d). In all cases, conductivity was most highly correlated, among all the measured ions, with Na and Cl concentrations (Pearson correlation coefficients 0.93-0.98; p-values <0.0001).

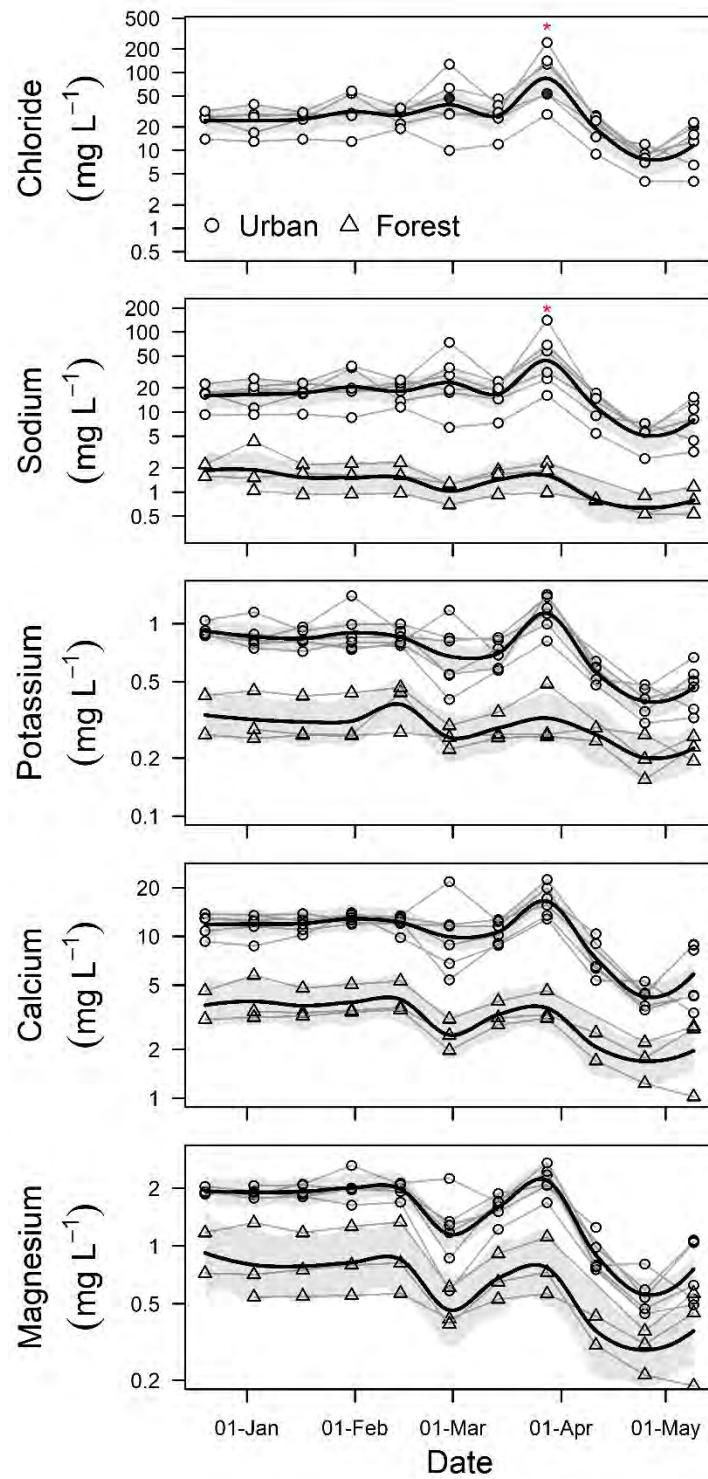


**Figure 1-3.** Snow pack, river flow and conductivity changes during winter-spring. a) Snow height (dash line) at Quebec City airport and flow of the Saint-Charles River downstream of the water intake. b) Conductivity at four depths in Lake Saint-Charles. c) Conductivity in the two classes of sampled rivers. d) Conductivity of the snow at 1m and 20m from all sampled roads. Note the log scales in panels c) and d). Arrows point to melting events and red asterisks denote significant differences (Tukey HSD,  $p<0.05$ ).

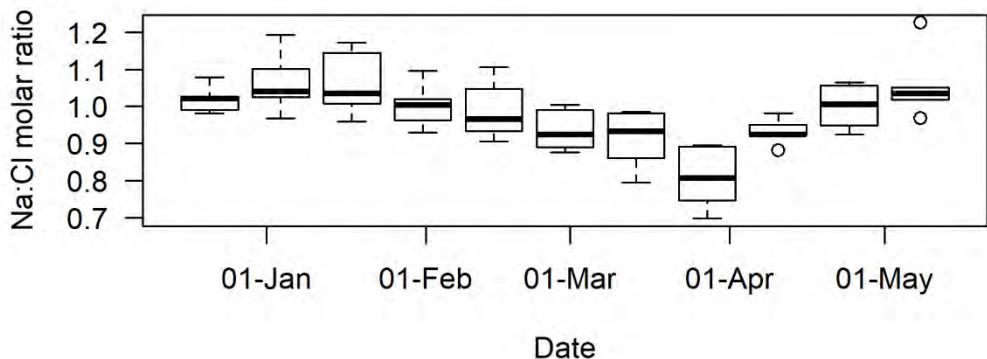
The temporal pattern of conductivity changes in Lake Saint-Charles varied with depth. During the first winter event, conductivity increased sharply in the surface waters and slightly increased at 3 m, indicating some mixing from the upper layer. Conductivity at these depths remained high until the spring event, when it sharply increased and then dropped some four days later. During the second winter event, conductivity increased only at 7 m. During the spring event, conductivity increased at 7 and 8.5 m, as in surface layers, but decreased about three weeks later, at the time of lake ice break-up and melting (Fig. 1-3b).

### 1.3.5 Seasonal variability in rivers and snow

The seasonal variations in ion concentrations and conductivity differed between the two categories of rivers. In urban rivers, the concentration of Cl, Na, Ca, Mg and K as well as the conductivity increased during the spring melting event (Tukey HSD  $p<0.05$ ), while it stayed constant in forest rivers (Fig. 1-3 and 1-4). In urban rivers ( $n=6$ ), the Na:Cl molar ratio decreased during the first winter and the spring melting events and more markedly during the spring event (Fig. 1-5).



**Figure 1-4.** Chloride (Cl), sodium (Na), calcium (Ca), magnesium (Mg), and potassium (K) concentrations through time for rivers in forested and urban watersheds. Note the log scales in all panels. Red asterisks denote significant differences (Tukey HSD,  $p < 0.05$ ).



**Figure 1-5.** Temporal changes in the Na/Cl molar ratio in urban rivers. Open circles are outliers; i.e., these values were more than 1.5 times the interquartile range above the upper quartile and below the lower quartile.

After the first winter event, the conductivity as well as the concentration of Cl and Na decreased in the snow at 1m from the road, while the other measured ions did not (Tukey HSD  $p<0.05$ , Fig. S1-5). At 20 m from the road, the concentration of measured ions and conductivity remained constant throughout the study period.

## 1.4 Discussion

Several features of Lake Saint-Charles indicate that it is strongly affected by roadside contamination during the winter-spring period of snowfall and snowmelt. The most conspicuous evidence of this contamination is that the conductivity of this reservoir has doubled since 1980, while that of Lake Clair, the reference lake in an undeveloped watershed without paved roads, has shown no long-term change. In addition, not only are the mean conductivity and major ion concentrations of the surface waters of Lake Saint-Charles much higher than in Lake Clair, but their variability is also higher. This was especially apparent for the road salt ions, which dominated in Lake Saint-Charles and showed large fluctuations throughout this period of the year. This salinization effect is consistent with many lakes in the north temperate region in which deicing salts are routinely used during winter. For example, road salt applications have resulted in large increases as well as high seasonal

variability in Cl concentrations in Minnesota lakes (Novotny et al. 2008). In the Halifax region of Canada, road salts have resulted in an increase of lake Cl levels over the last 35 years by a factor of 8 or greater (Scott et al. 2019).

Our analyses of river waters indicated a strong effect of urbanization, consistent with the hypothesis that paved roads and their winter maintenance have a major impact on water quality. The concentrations of major ions differed greatly between urban and forest rivers, with order of magnitude higher Na and Cl levels. Our results suggest that urban areas influence river chemistry at a low threshold of urbanization level (<2%; Fig. S1-5). This is in agreement with previous studies indicating that the concentration of many ions in lakes and streams is a function of impervious surface cover, with onset of effects at urbanization levels as low as 1% (Dugan et al. 2017a; Bird et al. 2018). Although such data are not available for our study area, in the North American lakes region (NALR), this 1% threshold represents around 7,770 lakes at risk of long-term salinization (Dugan et al. 2017a). The Cl concentration guideline for long-term protection of aquatic life is 120 mg L<sup>-1</sup> (from the Canadian Council of Ministers of the Environment, CCME 2011), and pavement coverage of around 25% would likely result in chloride concentrations beyond this limit in streams and lakes (Lax et al. 2017; Scott et al. 2019). Based on the measurements on some 500 North American and European lakes, between 2 and 10% of the lakes have impervious surface cover >25% in a 100-1500m buffer (Dugan et al. 2017b).

The urbanization effect on rivers sampled in the present study indicated effects on ionic ratios as well as concentrations. Among the cations, Na increased in relative proportion, and the urban rivers had about 30-times higher concentrations of Na than would be expected from their concentrations of Ca, Mg, and K based on global average ratios (as in Evans and Frick 2001). Such changes in the relative composition of major ions can affect acid-base properties, and the reactivity, solubility and mobility of nutrients, gases and contaminants (Kaushal et al. 2018a).

Our analyses of roadside snow showed the expected dominance of Na and Cl, but also high concentrations of Ca, Mg, K, and trace metals including potentially toxic Zn. Total nitrogen, nitrate and TP (of unknown bioavailability) were also high, indicating the potential for nutrient enrichment of receiving waters. Among the principal sources of Cl in urban areas

(i.e., road salts, runoff from agricultural lands, livestock, and wastewater; Corsi et al. 2015), only road salt is likely to contribute to the Cl in roadside snow. This makes Cl a good reference to estimate the quantity of road salt in the snow, and therefore the expected concentration of all other measured elements derived from road salt (Table 1-4). Based on this calculation, only Na has no other source. Among the chemicals with concentrations in the snow higher than expected, metals such as Zn are frequently associated with roadway pollution (Earon et al. 2012), while Ca and Mg may have originated from road weathering (Kaushal et al. 2017) and from sand abrasives applied to the roads. These abrasives, as well as vehicle-derived materials, including exhaust emissions, may also have been the sources of TN and TP (Hautala et al. 1995; Viklander 1999).

Almost all of the measured chemicals in the snow decreased sharply with distance from the road, with the most significant decrease observed for Na, Cl and other major ions, clearly pointing to the roads as the primary source. This is consistent with observations from elsewhere indicating that although contaminants may be dispersed by tens of meters, they are mostly localized to the immediate vicinity of the road (Blomqvist and Johansson 1999; Engelhard et al. 2007). Our analysis indicated that snow concentrations for Cl and many metals at 20 m from the roads were in the range reported in freshly fallen snow in urban areas (Bulskaya and Volchek 2014), while Ca, K and Mg were in the range of concentrations observed in snow from a forested part of the Rocky Mountains (Clow et al. 2002).

Observations in the present study indicate a preferential elution of Na and Cl from roadside snow, as they were the only constituents observed to decrease (by >95%) in the snow after the melting events. Although these ions only increased in the urban rivers during the spring melting event (a smaller sampling interval may have allowed us to observe more variation and track the winter events), the conductivity of Lake Saint-Charles increased in winter as well as in spring, suggesting that Cl and Na made their way into the reservoir and that contrary to our hypothesis, the mobilization of roadside snow contaminants is not limited to final spring thaw. Chloride peaks are often observed in lakes at the beginning of spring and sometimes represent the highest concentrations of the year (Thunqvist 2003; Scott et al. 2019), but although data for the winter period are sparse, Cl peaks at this time of year have also been reported (Novotny et al. 2008). During winter, the snowmelt runoff likely moves

as surface runoff to the lake, above the frozen soils. During the spring melting event, there is evidence that the runoff percolated through the soils. The Na:Cl molar ratio decreased during this event and was stable subsequently. A decrease of this ratio in the 0.5-0.7 range is often observed when water passes through soils, as Na adsorbs to clay particles while Cl does not (Moore et al. 2017; Bird et al. 2018).

The changes in conductivity in the reservoir were substantial (about a factor of two), and occurred rapidly, over days or even hours during the spring event. The high chemical loading associated with early rainfall or snowmelt is well known. This phenomenon has been called the “first flush” (Mayer et al. 2011; Westerlund and Viklander 2011), with evidence that the first 30% of snowmelt volume may carry 80-90% of the ions in the snowpack (Reinosdotter and Viklander 2007; Westerlund and Viklander 2011). During the winter events observed in the present study, air temperatures increased above the freezing point and allowed part of the snow to melt (the first flush), leading to a conductivity increase in the reservoir. When air temperatures returned below the freezing point after a few days, the melting stopped, preventing further discharge of meltwater to dilute the reservoir and allowing the conductivity to stay high. In spring, air temperatures did not decrease after the first flush, and subsequent meltwaters then diluted the reservoir. The increased air temperature, however, led to thermal stratification in the lake, which prevented the mixing of surface and deeper water. The conductivity of deeper water stayed high and decreased only after ice-melt, during spring turnover. All of these observations, along with the evidence of long-term chemical perturbation of Lake Charles in the absence of any changes in reference Lake Clair; Fig. 1-2b), indicate the need for preventative management attention to roadside snowmelt in both winter and spring.

Best practices in road runoff management currently rely on green infrastructure and retention basins. These have proven efficient in decreasing the concentrations of particulate pollutants, but they have much more limited capacity to retain dissolved pollutants such as road salts. Studies on biofiltration swales, vegetation strips and other storm-water control measures have shown that these only slightly decrease (or even enrich) dissolved solutes such as Ca and Cl (Stagge et al. 2012; Scarlett et al. 2018; Flanagan et al. 2018). Salt tolerant plants in filtration basins may decrease the concentrations of dissolved pollutants by

adsorption and absorption. However, in winter or early spring, when the salt in runoff may be maximal (as shown in the present study), they have almost negligible effect as the plants are biologically inactive (Zhu et al. 2012; Roy 2015). These roadside technologies can reduce solute peaks by retaining salted runoff for a longer period and allowing dilution by less salted inflows such as rainfall (Trenouth et al. 2018; Gu et al. 2019), but this may not decrease the total discharge load of contaminants, and the cumulative transfer of these chemicals to downstream ecosystems.

A more effective preventative strategy to protect rivers and lakes is to use green infrastructure systems with an impermeable liner and underdrain that can prevent the infiltration of runoff into natural soils, but that instead collects and diverts this runoff to a wastewater treatment plant or a local facility where a desalinization treatment is available. A detailed example of such an installation for enhanced roadside drainage and the protection of sensitive areas is provided by Trenouth et al. (2018), with a novel design that can be modified for specific pollutant loads and regulatory guidelines. These enhanced road drainage systems are costly to construct and maintain, and would need to be strategically deployed (Betts et al. 2014; Trenouth et al. 2018), for example in specific zones of meltwater runoff around drinking water supplies. Based on the decrease in concentrations from 1 to 20 m observed in the present study, a 1-m wide roadside swale combined with such a collection system would catch up to 85% of the road salts (calculated from an exponential model fitted to our data), as well as the other possible contaminants.

The ultimate method to control roadside snowmelt contamination is to stop or substantially reduce the use of road salts. Cities in Finland and Sweden have partially or even totally reduced the use of road salts, and their winter maintenance activities is limited to gritting and spreading of sand and rock (Salminen et al. 2011; Westerlund and Viklander 2011). Improved practices for the application of road salts, such as pre-wetting, automated-calibrated spreading and adjustments based on meteorological data are also gaining greater attention (Salminen et al. 2011; Rivett et al. 2016).

Salt deicing significantly reduces skidding (Crinson and Martin 2008) and the incidence of winter road accidents (Kuemmel and Hanbali 1992; Usman et al. 2012), and changing weather conditions may maintain the pressure to continue or even increase the use of road

salts. The Intergovernmental Panel on Climate Change (IPCC 2019) projections indicate that warming over the course of this century will result in increased variability in weather conditions, and greater likelihood of extreme events. Of relevance to road salt management for northern lakes, this may translate into more common melting days during winter, as well as more frequent rain-on-snow events (Palko and Lemmen 2017), which may mobilize roadside salts during the winter-spring period. For example, projections for the period 2030–50 in the Quebec City region (Bush et al. 2019) indicate that mean annual air temperatures may increase by 1.5 °C (RCP2.6) by that time. Simply adding this value to current winter temperatures gives an increase in winter melting days (days with mean temperatures above 0 °C for the period 1 December to 31 March) from 13 at present to 21; this estimate is likely to be conservative given that the warming trends are greater in winter than other seasons (IPCC 2019). Additionally, more frequent thaw-then-freeze cycles may increase the need for more regular application of road salts during winter, as indicated in transport projections (Palko and Lemmen 2017). As road transport infrastructure continues to proliferate northwards into the rapidly warming, lake-rich subarctic and Arctic, the issue of salted roadside snowmelt contamination of freshwater ecosystems will be an expanding problem that requires special management attention over an increasingly vast area of the northern hemisphere.

## 1.5 Conclusions

As demonstrated here, roadside snow in areas of deicing management contain high concentrations of salts as well as nutrients and certain trace elements. In the Lake Saint-Charles catchment, this contamination effect was highly localized, and especially apparent in snow from within 1 m of the road. Our results show that episodes of snowmelt were followed by increased salinities in the river inflows and reservoir, indicating that road salts likely contribute to the elevated, highly variable and increasing chloride levels in Lake Saint-Charles relative to reference Lake Clair. There was a clear separation in conductivity and chemical composition between rivers in urbanized catchments and those in forested catchments. Contrary to our hypothesis that this salt mobilization would be limited to the spring snowmelt period, we also observed winter melting of roadside snow and subsequent effects on the river and lake waters. These winter episodes of roadside snowmelt are likely to become more common as the global climate continues to warm. Preventative management

strategies against freshwater contamination will need to increasingly target winter as well as spring roadside meltwaters for localized collection and treatment.

## **1.6 Acknowledgements**

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# **Chapitre 2 Salinization of urban lakes: is there an influence on under-ice and open-water microbiomes?**

## **Résumé**

On observe une salinisation des lacs et des rivières dans les régions où l'on utilise des sels de voirie pour entretenir les routes durant l'hiver, mais on en connaît très peu sur ses effets sur les communautés microbiennes qui sous-tendent le fonctionnement des écosystèmes aquatiques. Dans cette étude, nous avons analysé le microbiome planctonique de quatre lacs différent selon leurs degrés d'urbanisation, d'eutrophisation et de salinisation, d'un lac oligotrophe n'ayant aucune route pavée dans son bassin versant à un lac eutrophe et lourdement impacté par les sels de voirie via sa proximité avec une autoroute. Nous avons évalué l'hypothèse que l'influence de la salinité allait s'ajouter à celle des saisons et de l'état trophique. Nous avons suivi les changements dans la composition taxonomique des communautés microbiennes via le séquençage de l'ARN ribosomique 16S (16S rRNA) pour les bactéries, et via quatre méthodes pour les eucaryotes microbiens : l'analyse du 16S rRNA des chloroplastes, le séquençage de l'ARN ribosomique 18S (18S rRNA), l'analyse de pigments photosynthétiques et la microscopie inversée. En accord avec notre hypothèse, la composition taxonomique variait avec le degré de salinisation, et ce, autant durant la période de couverture de glace (*ice-cover*) que durant la période d'eau libre (*open-water*). La concentration de Cl et d'azote total étaient parmi les facteurs les plus importants expliquant les différences dans la composition du microbiome, indépendamment de la période ou de la méthode d'identification. Ces facteurs étaient notamment positivement corrélés avec l'abondance de cryptophytes, d'haptophytes et de cyanobactéries. La couverture de glace était un facteur majeur expliquant la composition du microbiome, avec des différences marquées avec celle de la période d'eau libre; des cellules plus petites contenant plus de chlorophylle ont été sélectionnées, mais leur abondance totale était plus faible. Des bactéries capables de faire de la nitrification ou d'oxyder le méthane étaient plus abondantes durant l'hiver, suggérant l'importance des processus anaérobiques prenant place dans les sédiments et libérant des composés réduits dans la colonne d'eau couverte de glace. Les quatre méthodes d'analyse des communautés microbiennes eucaryotes ont fourni de l'information complémentaire. Le séquençage de l'ARNr 18S est fortement influencé par la présence de

ciliés, puisque ces derniers contiennent beaucoup de ribosomes. Toutefois, c'est aussi la méthode qui a révélé la plus grande richesse taxonomique, qui a permis la plus grande séparation entre les échantillons et qui a le plus mis en évidence les effets de la saison et ceux, potentiels, de la salinité.

## **Abstract**

Salinization of lakes and rivers is increasingly observed in regions where de-icing salts are applied to the roads in winter, but little is known about the effects on microbial communities that underpin aquatic ecosystem functioning. In this study, we analyzed the aquatic microbiomes of four lakes that differed in degree of urbanization, eutrophication and road salt influence, from a pristine, oligotrophic reference lake with no surrounding roads, to a eutrophic, salinized lake receiving runoff from a highway that is heavily treated with de-icing salts. We tested the hypothesis that a salinity influence would be superimposed on the effects of season and trophic status. We evaluated the successional changes in microbial community structure by 16S rRNA transcripts sequencing for bacteria, and by four methods for microbial eukaryotes: 16S rRNA transcripts analysis of chloroplasts; 18S rRNA transcripts sequencing, photosynthetic pigment analysis and inverted microscopy. Consistent with our hypothesis, taxonomic composition varied with degree of salinization in both the ice-cover and the open-water periods. Cl concentration was among the most important statistical factors explaining the differences in microbiome structure, along with total nitrogen (TN), independently of periods and identification methods. These factors were notably positively correlated with the abundance of cryptophytes, haptophytes and cyanobacteria. Ice-cover was also a major structuring factor, with clear differences between the winter communities and those of the open-water period. There was a selection for smaller cells with a higher chlorophyll content than in the open-water period, and the overall cell abundance was lower. Nitrifying and methane oxidizing bacteria were more abundant in all lake waters in winter, suggesting the importance of anaerobic sediment processes and release of reduced compounds into ice-covered water column. The four methods for eukaryotic analysis provided complementary information, with 18S rRNA amplicon sequencing strongly influenced by the presence of ribosome-rich ciliates, but revealing a much higher degree of taxonomic richness and greater separation of lakes, seasonal changes and potential salinity effects than the other methods.

## 2.1 Introduction

In many cold temperate lakes and rivers, the use of road de-icing salts in their catchments during winter has resulted in a pronounced increase of ion concentrations, especially of the main road salt constituents: sodium and chloride (Dugan et al. 2017a; Kaushal et al. 2018b). This salinization effect has been shown to increase with increasing intensity of urbanization of the watershed (Dugan et al. 2017a; Bird et al. 2018) and is especially apparent in winter and early spring due to the runoff from melting roadside snow (Novotny et al. 2008; Scott et al. 2019). Road-contaminated snow has a high salt content, and the subsequent meltwater inflow into lakes can cause a sudden rise in major ion concentrations; for example, salinity rose by a factor of two within a few hours of a snowmelt event in Lake Saint-Charles, Quebec (Fournier et al. 2020). These acute seasonal peaks, as well as chronic long-term salinization, have the potential to affect aquatic communities.

Most studies to assess salinization effects on freshwater biota have been done via short term (days) laboratory experiments, often at salt concentrations that are well above the usual levels of perturbation (e.g., Van Meter et al. 2011), or have been conducted by sampling over freshwater to marine salinity gradients (e.g., Herlemann et al. 2011). Smaller salinity gradients within the freshwater range have been less studied (Wallace and Biastoch 2016), and consideration has not been given to salt impacts on freshwater microbiomes, here defined as the consortia of Bacteria, Archaea and microbial eukaryotes that underpin biological productivity, food webs and biogeochemical cycling processes in lakes and rivers. Such consideration requires a focus on natural microbial communities, with their diversity of taxa and their potential responses to the presence of multiple stressors and other variables that may lessen or exacerbate the influence of salt inputs in the environment.

Given the seasonal timing of salt contamination in cold temperate lakes, analysis of salinization effects requires special attention to the overwintering microbial community. There is increasing research interest in under-ice microbiomes in lakes, and observations to date show that lakes maintain active microbial assemblages throughout winter with prokaryotic and eukaryotic communities that differ from those in summer (e.g., Tran et al. 2018; Kalinowska et al. 2019). The seasonal differences in metabolism and community composition of Bacteria may be due to changes in substrate availability caused by a shift in

the microbial eukaryote community (Bertilsson et al. 2013), and to large winter-summer differences in physical and chemical conditions (Vigneron et al. 2019). Winter assemblages of microbial eukaryotes in different aquatic systems vary greatly and include ice-associated filamentous diatom assemblages in large lakes with clear ice (Lake Baikal, Bashenkhaeva et al. 2015; Lake Erie, Beall et al. 2016). In lakes with ice and snow cover, there appears to be a functional convergence toward dominance by phytoflagellates (chrysophytes and cryptophytes), but with large differences in species composition among different waters (Butts and Carrick 2017). Less is known, however, about whether there is a similar taxonomic diversity with functional convergence among non-pigmented heterotrophic microbial eukaryotes (colourless protists) in winter.

Our aim in the present study was to identify the impacts of freshwater salinization on lake microbiomes, including during the less studied ice-cover period. We hypothesized that road salt exposure causes taxonomic changes in microbiome composition, and acts in concert with other known structuring factors, notably trophic state and seasonal forcing. To assess this hypothesis, we investigated microbial community structure along with physical and chemical variables throughout all seasons, in four boreal lakes that were chosen to encompass contrasting salinity perturbations and trophic regimes.

We used four methods to analyze the community structure of the microbial eukaryotes: classic microscopy, pigment analysis by High Pressure Liquid Chromatography (HPLC), and high throughput amplicon sequencing of the V4 region of two types of ribosomal transcripts in order to monitor the taxonomic distribution of functional activity: 18S rRNA in cellular ribosomes and additionally the 16S rRNA in chloroplasts. Sequencing of 16S rRNA amplicons was also applied to analyze the prokaryotic communities. These methods each have their own biases, strengths and weaknesses, but potentially offered complementary information to evaluate taxonomic structure in the four lakes.

## 2.2 Methods

### 2.2.1 Study sites

The four lakes selected for this study (Lake Saint-Charles, Lake Clément, Lake Saint-Augustin, and Lake Clair) are located within a 40 km-radius of Quebec City, Canada, and span a range of morphometries, trophic states, mixing regimes and other environmental conditions, including a gradient of urbanization impacts (Table 2-1). During the study, the mean air temperature for the ice-cover period (Jan-Feb-Mar) was -11°C, with 254 cm of snowfall. The mean air temperature for the open-water period (rest of the year) was +12°C, with rainfall totaling 751 cm (Government of Canada 2019b).

Lake Saint-Charles (LSC) is a drinking water supply for Quebec City, with the drinking water intake located 11 km downstream on its outflow, the Saint-Charles River. The lake is located in an urban area and is close to a major road (Highway 73), but most of its watershed is forested (Figure 2-1, Table 2-1), and is considered here as a low impacted urban lake.

Lake Clément (LCL) is a recreational lake that can only be accessed by residents. It is located near LSC, but closer to Highway 73, under which its main tributary flows (APEL 2011). Its salinity is high (specific conductivity around  $1000 \mu\text{S cm}^{-1}$ ), mainly due to the input of NaCl road salt from the highway, with chloride concentrations often exceeding the chronic exposure criteria for the protection of aquatic life of  $120 \text{ mg L}^{-1}$  (CCME 2011). This lake is considered here as a moderately impacted urban lake.

Lake Saint-Augustin (LSA) is a recreational urban lake with public access, where motor boating and seaplane landing are permitted. It is close to a major four-lane road (Highway 40), and like LCL, it has an elevated salinity as a result of road salt application. Its specific conductivity increased rapidly with the completion and full operation of the highway, from  $255 \mu\text{S cm}^{-1}$  in 1970 to  $700 \mu\text{S cm}^{-1}$  in 2000, with a stabilisation since that time in the range 650 to 800 (Figure S2-1). The lake has other water quality issues, including eutrophication and summer-long cyanobacterial blooms of *Aphanizomenon flos-aquae*, *Microcystis aeruginosa* and *Dolichospermum* spp. It was included here as a heavily impacted urban lake. This lake lies in the St-Lawrence Lowlands physiographic region, while the other lakes are

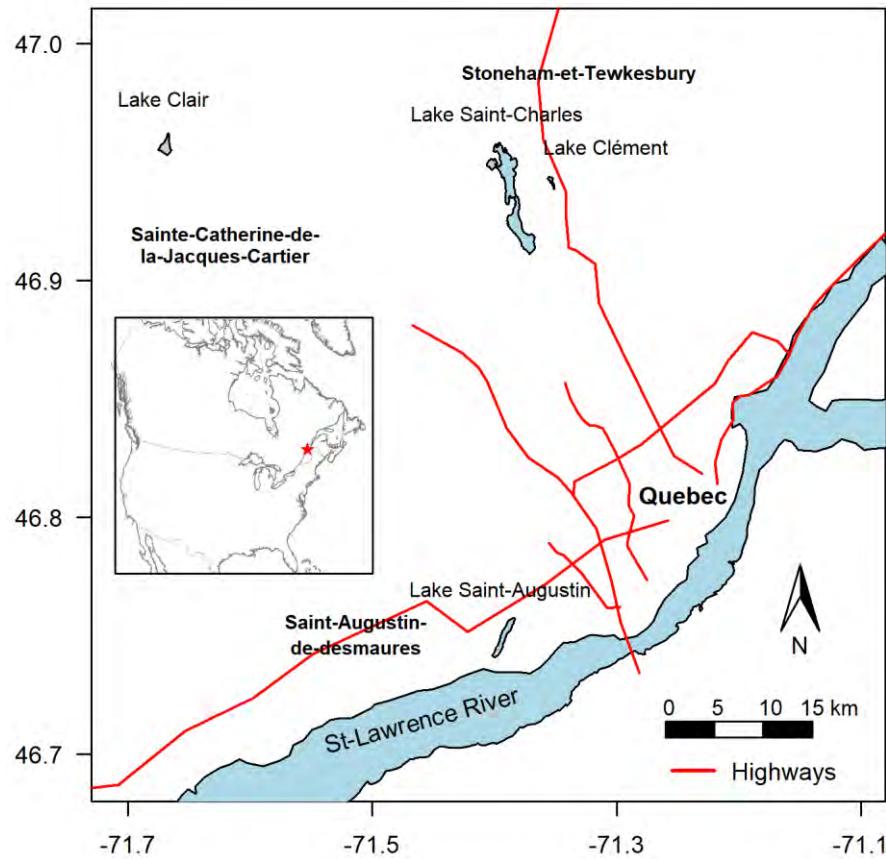
on the Canadian Shield; this difference in bedrock result in higher natural background alkalinity and conductivity.

Finally, Lake Clair (LCR) is located in the Duchesnay nature reserve, and although it is in close proximity to urban areas and the other studied lakes (Figure 2-1), its watershed is entirely forested, is without paved roads, and has not experienced anthropogenic perturbations (Houle et al. 2002). It was included in the present study as a non-urban reference lake. All the lakes experience a similar climate and are covered by continuous ice and snow from December to May.

**Table 2-1.** Physico-chemical characteristics of Lake Clair, Lake Saint-Charles, Lake Clément, and Lake Saint-Augustin, and the percentage of urbanization of their watersheds. Sources : APEL 2011, 2014, 2016 ; OBV de la Capitale 2018

	Mean depth	Max depth	Max length	Max width	Area	Trophic status	Mixing type	Urbanization
	(m)				(km <sup>2</sup> )			%
Lake Clair	11.7	9.62	242	982	464	Oligotrophic	Dimictic	0
Lake Saint-Charles	4.1	5961	1184	5948	66.	Mesotrophic	Dimictic	8
Lake Clément	2.3	.65	.9	666	4642	Oligotrophic	Dimictic	31
Lake Saint-Augustin	3.6	46.	9544	644	46.	Eutrophic	Polymictic	70*

\* estimated from Roberge 2004 (Fig. 4) and OBV de la Capitale 2018



**Figure 2-1.** Localization of the sampled lakes.

### 2.2.2 Lake sampling

The four lakes were sampled throughout the year between January and October 2017, except in April, when conditions were too precarious due to ice break-up, with 3 sampling dates in the under-ice period and 3-4 sampling dates in the open-water period (Table S2-1). Samples were taken in triplicate in a 5 m radius. During the open-water period, water was sampled at the surface (0-30 cm) directly into Aqua-Pak containers (Reliance, Manitoba, Canada) that had been acid-washed (HCl, 0.1 N), then rinsed 3 times with sample water. During the ice-cover periods, water was sampled just below the lake ice through 20 cm holes with a Kemmerer bottle, then transferred to cleaned and rinsed Aqua-Pak containers. Water from the containers was used for chemical analyses, photosynthetic pigment analyses, and characterization of the microbial communities by light microscopy. For both periods, water

was also collected in sterile Nalgene bottles for RNA-based characterization of the microbial communities. Conductivity, dissolved oxygen, and temperature profiles were measured at all sampling sites with a Hydrolab DS5X profiler (Loveland, Colorado, USA).

### 2.2.3 Chemical analyses

Total nitrogen (TN) and total phosphorus (TP) samples were acidified ( $\text{H}_2\text{SO}_4$ , 0.1% final concentration), then kept at 4°C until concentrations were determined by a colorimetric method after persulfate digestion. Samples for major ions, dissolved organic carbon (DOC) and colored dissolved organic carbon (CDOM) were filtered through Milli-Q water pre-rinsed cellulose acetate filters (0.2  $\mu\text{m}$  pore size, Advantec Micro Filtration Systems), then acidified (for cations only, Trace Metal Grade  $\text{HNO}_3$  at 0.2% final concentration) and kept at 4°C until analysis. Anion concentrations were measured by ion chromatography (ICS-2000, Dionex), major cations by atomic emission spectroscopy (ICP-AES, Varian Vista AX), and trace cations by mass spectroscopy (ICP-MS, Thermo X Series). DOC was measured by combustion catalytic oxidation (Shimadzu TOC-5000A carbon analyzer calibrated with potassium biphenyl), and CDOM was quantified by absorption at 440 nm measured in a Varian Cary 100 dual beam spectrophotometer (Varian Inc., Canada; Watanabe et al. 2011). Alkalinity (in calcium carbonate equivalents) was determined with titration by 0.01 M sulfuric acid (Wetzel and Likens, 2000), and DIC was calculated from alkalinity and the initial sample pH.

### 2.2.4 Photosynthetic pigments

Samples for photosynthetic pigment analyses were filtered under low light through Whatman GF/F 25 mm filters. These were then folded, wrapped in aluminum foil and stored at -80 °C until extraction. The pigments were extracted for one hour on ice in 2.7 mL of 95% methanol after sonication (3 times for 20 s at 17 W intensity). The solution was then filtered into an HPLC vial using a PTFE 0.2  $\mu\text{m}$  filter. During extraction and in the HPLC vial, headspaces were filled with argon to avoid oxidation.

HPLC analyses were performed by injecting the filtrates (100  $\mu\text{L}$ ) into a Thermo Scientific system (Thermo Scientific, West Palm Beach, FL, USA) fitted with a Hypersil Gold C8 HPLC column (3.0- $\mu\text{m}$  pore size, 4.6 x 150 mm, Thermo Scientific) at 25°C, with a

C8 guard column. The pigments were eluted by a succession of polar and non-polar solvents as described in Zapata et al. (2000) and were detected by Photodiode Array (PDA) and fluorescence spectroscopy as they exited the columns; absorbance chromatograms for carotenoids were obtained at 450 nm, and fluorescence chromatograms for chlorophylls by excitation at 440 nm and emission at 650 nm. The HPLC was calibrated for 30 pigments using standards from DHI International Agency (Holshørn, Denmark) or Sigma Aldrich (Missouri, United States): 9-cis-neoxanthin, 19-but-fucoxanthin, 19-hex-fucoxanthin, alloxanthin, antheraxanthin, aphanizophyll, astaxanthin,  $\beta,\beta$ -carotene,  $\beta,\varepsilon$ -carotene, canthaxanthin, chlorophyll *a*, chlorophyll *b*, chlorophyll *c2*, chlorophyll *c3*, chlorophyllide *a*, crocoxanthin, diadinoxanthin, diatoxanthin, echinenone, fucoxanthin, lutein, lycopene, MgDVP, myxoxanthophyll, peridinin, pheophorbide *a*, pheophytin *a*, prasinoxanthin, violaxanthin and zeaxanthin. This calibration allowed the conversion from absorbance to concentration. For unidentified pigments with a PDA spectrum, the conversion factor of the known pigment with both the closest spectrum and retention time was used, while for those with concentrations that were too small to resolve the spectrum,  $\beta,\beta$ -carotene and chlorophyll *a* conversion factors were used for carotenoids and chlorophylls respectively.

The abundance of different pigments was compared in terms of molar ratios to chlorophyll *a*, with molecular weights obtained from Roy et al. (2011). For unknown pigments, the molecular weight of  $\beta,\beta$ -carotene, and chlorophyll *a* were used for carotenoids and chlorophylls, respectively. For some analyses, pigments were pooled as total carotenoids (Tot Caro: sum of known and unknown carotenoids), total chlorophylls (Tot Chl: sum of known and unknown chlorophylls, excluding degradation products of chlorophyll *a*, namely pheophytins and pheophorbides) and total chlorophyll *a* (Tot Chla: sum of chlorophyll *a* and chlorophyll *a* allomers).

A combination of 17 pigments was used to infer the proportion of the photosynthetic community associated with chlorophytes, chrysophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, euglenophytes, and haptophytes. A Bayesian method based on CHEMTAX, and available in R (*lse1* function of the *limSolve* package), was used for these calculations (Van den Meersche et al. 2008). The input table (Table S2-2) was constructed

from data for freshwater species (Schluter et al. 2006), except for haptophytes for which the ratio from the CHEMTAX marine paper was used (Mackey et al. 1996).

#### 2.2.5 Microscopic analyses

Samples for microscopy were preserved with acid Lugol's solution (glacial acetic acid 10% v/v, potassium-iodide, and iodine; 3 µL of Lugol's solution per mL<sup>-1</sup> of the sample) and stored in the dark at 4°C until analysis. Microscopy samples were analyzed using the Utermöhl protocol as described in Intergovernmental Oceanographic Commission of UNESCO (2010). Briefly, 50 to 100 mL of the preserved samples were placed in a settling chamber and allowed to settle for 40 to 80 h, depending on the volume. The sedimented samples were examined with a Zeiss Axiovert 100 inverted microscope. The organisms were counted under visible light in phase contrast at 400X or 1000X, depending on the cell size, until 400 cells/colonies per sample were reached at each magnification.

The microbial eukaryotes were identified at the lowest taxonomic resolution possible and separated into subgroups by morphology. Biovolumes were estimated from simple geometric forms (Hillebrand et al. 1999) from photomicrographs taken through an ocular with a calibrated micrometer and the MB-Ruler software (MB-Softwaresolutions, Germany). Biovolumes were calculated from the averaged measurements of 20 specimens per cells or colony.

#### 2.2.6 Sequencing and rRNA analysis

Samples for molecular analysis were filtered through 0.2 µm Sterivex units (Millipore), which were immediately filled with RNAlater (Life Technologies) and frozen at -80°C until extraction. The RNA was extracted using the AllPrep DNA/RNA Mini Kit (Qiagen) and the Qiagen protocol modified as follows: RLT<sup>+</sup>+β-ME was injected into the Sterivex units, which were then incubated at 37°C for 45 min. Lysozyme and proteinase k were then added in the units, and they were incubated at 65°C for 15 min. In the RNA purification phase, a DNA digestion step was added between Qiagen kit steps 8 and 9: 10 µL of DNase (Qiagen RNase free DNase set) diluted in 55 µL RDD Buffer was added in the RNeasy spin columns and left for 15 min at room temperature. RNeasy spin columns were then rinsed with Buffer RW1 as in step 8. Extracted RNA was tested for DNA contamination by a PCR and then

converted to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems-Ambion). The transcription was carried out in 20 µL (10 µL RNA template, 2 µL RT Buffer 5X, 0.8 µL dntps, 2 µL RT primers, 1 µL transcriptase and 4.2 µL water) with the following thermal cycle: 25°C for 10 min, 37°C for 120 min and 85°C for 5 min. The cDNA was stored at -80°C until further analysis.

The cDNA was amplified using two sets of primers modified with Illumina adaptors: (1) 515F/806R as modified by Apprill et al. (2015), which targets the V4 region of the 16S rRNA gene in bacteria, archaea and chloroplasts; and (2) 572F/1009R from Comeau et al. (2011), which targets the V4 region of the eukaryote 18S rRNA gene. PCR was carried out in a total volume of 25 µL (1 µL cDNA template, 5 µL PCR buffer (New England Biolabs), 1.25 µL reverse and forward primers, 0.5 µL mix dNTP, 0.25 µL Q5 High-Fidelity DNA Polymerase (New England Biolabs) and 15.75 µL water). Conditions of the PCR thermal cycling for each set of primers are given in Table S2-3. PCR products were purified with ethanol and magnetic beads (Agencourt AMPure XP), and a second PCR was run to introduce sample tags. This reaction had an initial denaturation temperature of 98°C for 30 s, then 13 cycles of 10 s denaturation at 98°C, 30 s annealing at 55°C and 30 s elongation at 72°C followed by a final extension of 4.5 min at 72°C. The second amplicons were purified with beads as previously described, quantified with the Nanodrop 1000 (Thermo Fisher Scientific), pooled in an equimolar ratio, and then sequenced with Illumina MiSeq at the IBIS/Laval University Plate-forme d'analyses génomiques (Quebec City, QC).

Forward and reverse read pairs were merged using BBMerge v37.36 (Bushnell et al., 2017), and the obtained merged reads were filtered with maximum expected error of 1 (Rognes et al., 2016). Reads with the same sequences were identified as well as abundance- and size-filtered to discard potential chimera (>300bp) and singletons using VSEARCH (Rognes et al., 2016). UPARSE was then used to cluster reads for Operational Taxonomic Units (OTUs) at 98% similarity level for 18S rRNA and at 97% similarity level for 16S rRNA (Edgar, 2013). We used mothur v1.39 (Schloss et al., 2009) to assign the taxonomy of the most abundant sequence of each OTU based on the Protist Ribosomal Reference database (PR<sup>2</sup>; Guillou et al., 2013) for 18S rRNA and SILVA 132 (Quast et al., 2013) for 16S rRNA. OTU tables were constructed with the number of reads per OTU in each sample.

The 18S rRNA sequences identified as embryophytes, arthropods, rotifers and mitochondria were removed from further analysis. A separate analysis was made of chloroplast reads from the 16S rRNA data. When pertinent, unidentified OTUs were submitted to a BLAST search to the nr database of NCBI GenBank and identified to the closest match. The nucleotide sequence data reported are available in the NCBI database under the project number PRJNA681583.

#### 2.2.7 Statistical analyses

The dissimilarities between the samples were visualized using non-metric multidimensional scaling (NMDS) analysis, with the *metaMDS* function of the *vegan* package (v2.5-6, Oksanen et al. 2019). The role of different explanatory variables in the 16S rRNA, chloroplast 16S rRNA, 18S rRNA (reads pooled at the OTU level) and microscopy analyses, was evaluated with distance-based redundancy analysis (dbRDA) and partial dbRDA. The dbRDA computes the overall variation among the communities that could be attributed to the provided explanatory variables, while the partial dbRDA calculates the contribution of those explanatory variables individually. These analyses are similar to redundancy analysis but allow for non-Euclidean distances (Legendre and Anderson 1999). In the present study, they were carried out using the *capscale* function of the *vegan* package, using Bray-Curtis distance on abundances after a Hellinger transformation. Among all measured physico-chemical parameters, those included in the dbRDA model as explanatory variables were selected by the stepwise model building function *ordistep*, from the *vegan* package. The selected explanatory variables were: Al, DIC, Fe, lake, Cl, period, Si, TN, and TP. The final analysis was conducted by period, so this variable was removed, and we kept only variables that had a correlation of less than 0.6 with one another; DIC and Fe were therefore removed because they were respectively correlated with Cl and Al.

The partial dbRDA and the NMDS permitted us to identify variables that explained the greatest variation between the samples. The organisms that responded the most to those variables were discriminated using *DESeq*, a test to detect differential abundances, using the *DESeq2* package (v1.26.0, Love et al. 2014), which normalized the samples to correct for the difference in sequencing depth. The difference in abundance between the levels of the variables was then calculated as the log base 2 of the fold change. The OTUs that contributed

significantly to the difference between groups were plotted on a heatmap, using the *ComplexHeatmap* package (v2.3.4, Gu 2016), where each OTU (column) and each combination of seasonal period and lake (rows) were sorted using hierarchical clustering with the *dendsort* function of the *dendsort* package (v0.3.3, Sakai et al. 2015). The same method was also applied to microscopy data, using the lowest taxonomic ranks.

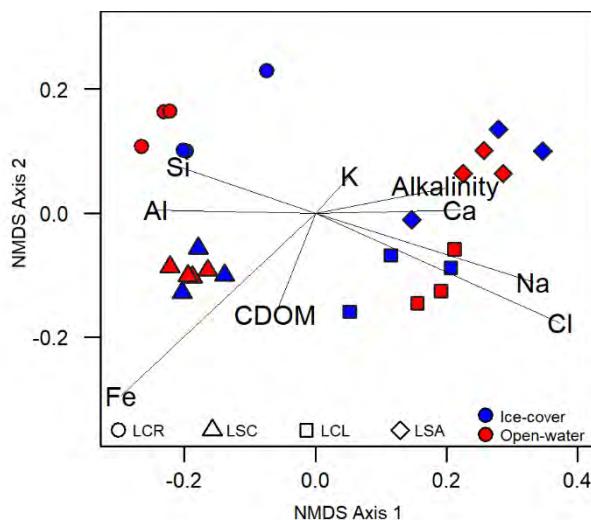
Diversity was evaluated by the Shannon index using the *diversity* function of the *vegan* package, and evenness was evaluated by the Pielou index, which was calculated as the Shannon index divided by the natural logarithm of the number of OTUs/species in a given sample. For diversity and evenness analyses, the 16S rRNA, chloroplast 16S rRNA and 18S rRNA reads data sets were rarefied, to respectively 7000, 550, and 2000 reads per sample, using the *rarefy* function of the *vegan* package to account for differences in sequencing depth between samples.

The effect of the groups on various variables was determined by a Student t test followed by a post hoc Dunn test with Bonferroni correction on the p-value using, respectively, the *t.test* function of the *stats* base package and the *dunn.test* function of the *dunn.test* package (v1.3.5, Dinno 2017). To evaluate the effect of groups on community composition, we used non-parametric multivariate analysis of variance (PERMANOVA) followed by a pairwise post hoc test using respectively the *adonis* function of the *vegan* package and the *pairwise.adonis* function from the *pairwiseAdonis* package (v0.0.1, Martinez Arbizu 2020). To evaluate the relationship between various parameters, and statistical significance, linear models were constructed using the *lm* function of the *stats* base package. Differences in distribution were evaluated with Pearson chi-square test using the *chisq.test* function of the base *stats* package. All statistical analyses were executed in the R free software (v3.6.3, R core team 2013). Environmental data and analyses are available on GitHub at: [https://github.com/isaza233/Road\\_salts\\_lakes\\_microbiome](https://github.com/isaza233/Road_salts_lakes_microbiome).

## 2.3 Results

### 2.3.1 Lake chemistry

The four lakes differed greatly in their chemical properties. The 25 samples clustered into four water chemistry groups that separated according to lake (ANOVA  $p<0.05$ ; NMDS, Figure 2-2), and there was no distinction between the ice-cover and the open-water periods (ANOVA  $p>0.05$ ). The first axis was correlated with alkalinity, conductivity, Ca, K, Na, and Cl (linear models  $R^2>0.75$ ;  $p\text{-value}<0.05$ ), while the second axis was only weakly correlated with Fe and cDOM ( $R^2$  from linear models  $<0.5$ ;  $p\text{-value}<0.05$ ). In this ordination, the lakes remained distinct and separated throughout the year, and the alkalinity/salinity gradient on the first axis also persisted throughout the year.



**Figure 2-2.** Non-metric multidimensional scaling analysis of samples based on chemical variables. Axis 1 is associated with alkalinity, conductivity, Ca, Cl, Alkalinity, K, and Na (linear models  $R^2>0.75$ ;  $p<0.05$ ), while Axis 2 is weakly associated with Fe and CDOM ( $R^2$  from linear models are  $<0.4$ ;  $p<0.05$ ).

The chemical variation between the sampled lakes was mainly due to the concentration and relative proportion of Ca, Cl and Na (Table 2-2). The latter ions were 3-50 times lower in Lake Clair compared to Lake Saint-Charles and 20-400 times lower than in the other two lakes. In Lake Clair, Ca was the dominant cation, and Cl concentration was  $<1.5 \text{ mg L}^{-1}$ , while in the other lakes, Na was the dominant cation, and Cl concentration varied between

10 and 183 mg L<sup>-1</sup>, reflecting their contamination by road salts (Mochizuki, 2011; Guesdon et al., 2016; Fournier et al., 2020). As expected, the highest concentration of TP was found in Lake Saint-Augustin (14-134 µg L<sup>-1</sup>), and the lowest concentration of TN and TP were found in Lake Clair (respectively 0.1-0.2 mg L<sup>-1</sup> and 4-10 µg L<sup>-1</sup>).

**Table 2-2.** Mean (coefficient of variation, SD as % mean) of chemical variables in surface water (0-30 cm) for Lake Clair, Lake Saint-Charles, Lake Clément, Lake Saint-Augustin during ice-cover (Jan-Feb-Mar) and open-water (rest of the year) periods.

Variable	Units	Lake Clair		Lake Saint-Charles		Lake Clément		Lake Saint-Augustin	
		Ice-cover	Open-water	Ice-cover	Open-water	Ice-cover	Open-water	Ice-cover	Open-water
Conductivity	µS cm <sup>-1</sup>	19 (0)	17 (3)	110 (24)	77 (14)	694 (45)	707 (9)	624 (5)	644 (3)
Alkalinity	mg CaCO <sub>3</sub> L <sup>-1</sup>	8 (9)	7 (5)	17 (26)	15 (8)	66 (18)	71 (30)	114 (9)	117 (28)
CDOM	mg C L <sup>-1</sup>	1 (89)	1 (86)	13 (23)	17 (14)	16 (19)	10 (9)	18 (36)	10 (91)
Ca	mg L <sup>-1</sup>	2 (9)	2 (1)	7 (12)	6 (18)	21 (22)	29 (12)	40 (26)	43 (13)
Cl	mg L <sup>-1</sup>	0.4 (32)	0.6 (92)	19 (45)	13 (16)	77 (15)	149 (17)	86 (22)	112 (2)
DIC	mg L <sup>-1</sup>	3 (5)	3 (18)	5 (4)	4 (18)	18 (27)	18 (13)	31 (27)	29 (6)
Na	mg L <sup>-1</sup>	1 (7)	1 (27)	11 (34)	7 (16)	50 (22)	87 (16)	57 (21)	70 (2)
Si	mg L <sup>-1</sup>	1.3 (3)	1.3 (3)	3.4 (4)	2.4 (10)	2.2 (23)	1.4 (18)	2.0 (27)	2.4 (33)
TN	mg L <sup>-1</sup>	0.2 (18)	0.1 (8)	0.6 (11)	0.4 (15)	0.7 (7)	0.4 (6)	0.7 (23)	0.7 (35)
Al	µg L <sup>-1</sup>	11 (9)	17 (39)	48 (13)	43 (29)	18 (18)	32 (32)	14 (81)	31 (25)
Fe	µg L <sup>-1</sup>	13 (4)	2 (102)	178 (9)	156 (35)	86 (19)	58 (95)	14 (72)	7 (51)
TP	µg L <sup>-1</sup>	6 (42)	8 (25)	9 (18)	16 (29)	11 (171)	8 (46)	20 (28)	79 (56)

DIC: Dissolved inorganic carbon. TN: total nitrogen. TP: total phosphorus. CDOM: colored dissolved organic carbon.

### 2.3.2 Water column profiles

All lakes exhibited similar year-round patterns of temperature with warmer water during the open-water period and inverted profiles (cold overlying warmer water) during the ice-cover period (Figure S2-2). A thermocline was observed during most of the open-water period, except during spring and autumn overturn.

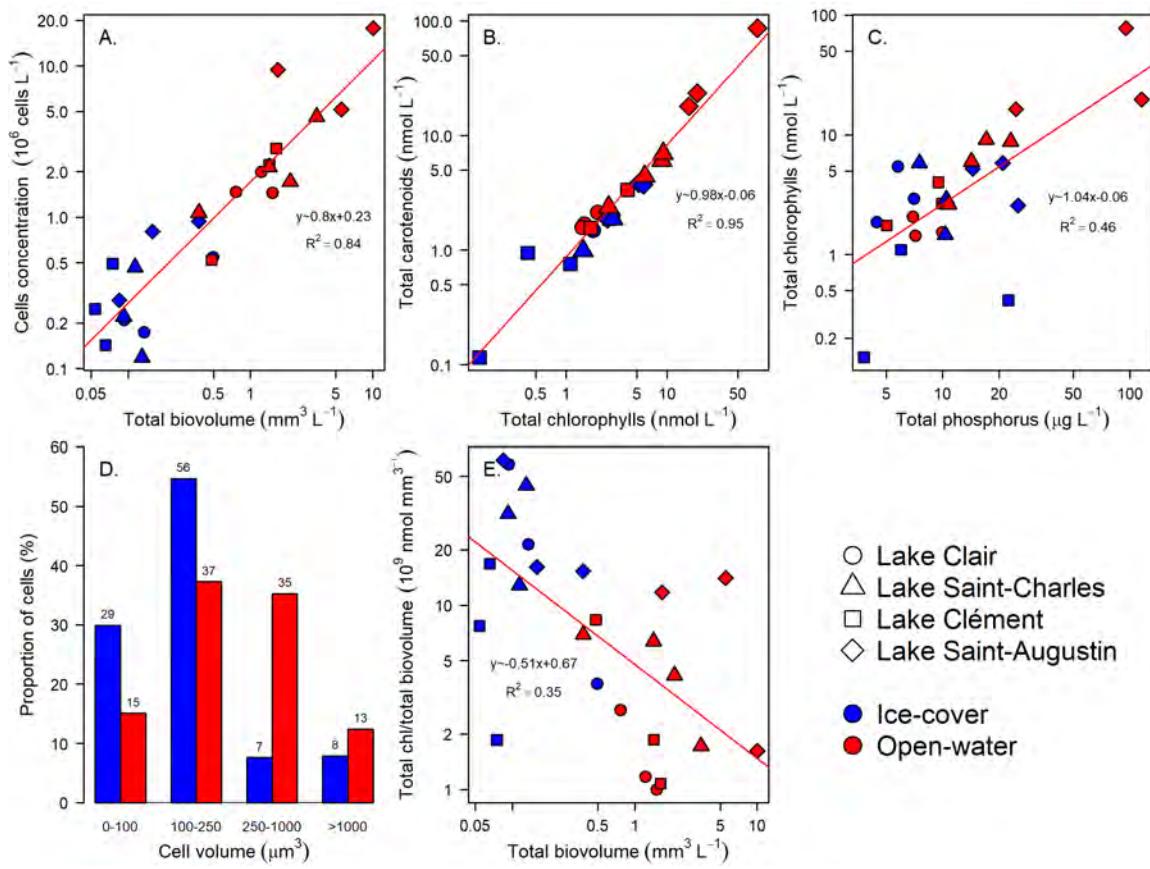
Oxygen in the surface water was near saturation with the atmosphere throughout the year in all lakes, while in the bottom water, oxygen levels were often well below saturation ( $\approx 50\%$ ), including periods of anoxia during summer/autumn in Lake Saint-Augustin and Lake Clément (Figure S2-2).

Salinity, as measured by specific conductivity, was the most variable parameter, whether by depth or among lakes (Figure S2-2). Lake Clair exhibited minor fluctuations in conductivity down the water column and over the year (in the range 17-22  $\mu\text{S cm}^{-1}$ ), with small increases in bottom water during the open-water period. Lake Saint-Charles conductivity was about 10-times greater than Lake Clair, and always around 50% greater in surface compared to bottom waters. Conversely, in Lake Clément and Lake Saint-Augustin, conductivity was always 2 to 4 times greater in the bottom water, consistent with these lakes being fed by denser, saltier runoffs from the roads. Also, the conductivities of the two lakes were 3 to 13 times higher than in Lake Saint-Charles. In these three lakes, maximum conductivity was observed during the late summer period of lowest inflows and the winter period of road salt use.

For the chemical and the biological analyses, all water was sampled from the surface, and the sampled communities from the four lakes were therefore from similar temperature and oxygenation levels for any period of sample collection, but with large differences in conductivity among sites (<25 to  $\approx 1000 \mu\text{S cm}^{-1}$ ).

### 2.3.3 Description of the biological data sets

The five identification methods provided complementary information on the taxonomic composition of the microbial community. During the ice-cover period, there was a selection for smaller cells with a higher chlorophyll content than in the open-water period, and the overall cell abundance was lower (Figure 2-3A, D, E). In both periods, the total carotenoids were correlated with total chlorophylls (Figure 2-3B,  $R^2=0.95$ ). The chlorophyll concentration was correlated with total phosphorus when all samples were considered, but when samples from different periods were analyzed separately, this relationship was only significant during the open-water period (Figure 2-3C,  $R^2=0.82$ ).



**Figure 2-3.** General relationships for the microbial eukaryote community from Lake Clair, Lake Saint-Charles, Lake Clément and Lake Saint-Augustin during the ice-cover and the open-water periods based on microscopy and photosynthetic pigment analyses.

The 16S rRNA primers yielded 1 079 370 non-chloroplasts reads, with a mean of 25 101 per sample. They were grouped in 711 OTUs, of which 97% were identified at the phylum level, 38% at the genus level, and only 5% at the species level. The communities were dominated by Proteobacteria (44-69%), represented mainly by the Betaproteobacteria (24-47%) and Alphaproteobacteria (9-27%) classes (Figure S3). At the genus level there was more variation between lakes and periods, but some common genera emerged including *Polynucleobacter*, *Sediminibacterium*, *Polaromonas*, *Rhodoferax*, and *Caulobacter* (Tables S2-4 to S2-11).

We obtained 337 290 chloroplast reads from the 16S rRNA amplicons, that were grouped in 137 OTUs, with a mean of 7 844 reads per sample. The majority of the OTUs were

identified at the phylum level (91%), 77% were identified at the genus level, and 72% at the species level. Depending on the lake, communities were either dominated by chrysophytes (2-79%) or cryptophytes (2-44%, Figure S2-4). The most abundant genera were *Synura*, *Cryptomonas*, and *Florensiella* (Tables S2-4 to S2-11).

The 18S rRNA primers yielded 251 390 reads, with a mean of 5 982 reads per sample. They were placed into 812 OTUs, of which 97% were identified at the Phylum level, 29% at the genus level and 10% at the species level. The dominant phylum differed between lakes and periods. The three most abundant phyla were Ochrophyta (2-40%, mostly chrysophytes except for 1 OTU affiliated to the diatom *Stephanodiscus*), cryptophytes (2-38%) and ciliates (2-55%, Figure S2-3). In these groups, activity of *Dinobryon*, *Synura*, *Cryptomonas*, *Mesodinium*, and ciliates from the Class Spirotrichae was common and abundant (Tables S2-4 to S2-11).

Among all lakes and the two periods, a total of 79 different microorganisms were observed by inverted microscopy, 78% of which were identified at the genus level and 22% at the species level. The communities were dominated by chlorophytes (2-38% by biovolume) and chrysophytes (0-64% by biovolume, Figure S2-5). *Strobilidium*, *Dinobryon*, *Cryptomonas*, and *Chlamydomonas* were the most common genera (Tables S2-4 to S2-11).

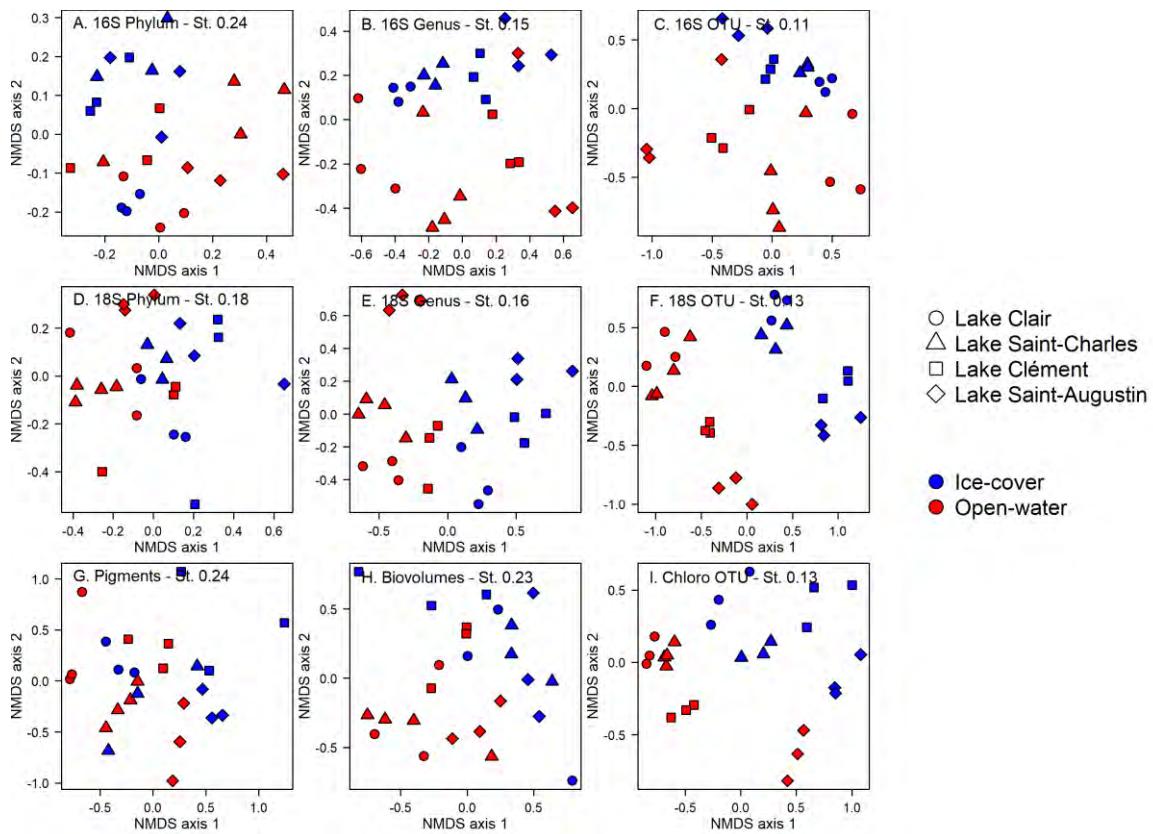
The HPLC analysis detected 159 photosynthetic pigments, with a mean of 35 pigments per sample. The number of chlorophylls and carotenoids were similar (respectively 43% and 57%). Some pigments were identified by comparison to the standards (19%) or related to them (similar spectrum but different retention time, 21%), while the rest had different spectra and retention times, and could not be identified. The most abundant pigments were chlorophyll *a*, *c1* and *c2*, fucoxanthin, alloxanthin, and diadinoxanthin (data not shown). The communities, based on the composition inferred by the ratio of 17 selected pigments, were dominated by chrysophytes (13-79%), dinoflagellates (0-62%), and cryptophytes (6-46%, Figure S2-4).

Although the four methods used to identify the microbial eukaryotes were consistent at the group and even genus levels, there were also major differences. Pigments associated with diatoms were detected, and diatoms were observed by microscopy (e.g., *Asterionella*,

*Tabellaria*, and *Synedra*) and chloroplast 16S rRNA (e.g., *Acanthoceras* and *Skeletonema*) in half or more of the samples, while only 1 OTU affiliated with this group in one sample was identified by 18S rRNA (*Stephanodiscus*, Figure S2-4). The analysis of photosynthetic pigments revealed the presence of euglenophytes. These were also observed in microscopic analysis of live samples from 10 µm plankton net hauls (unpublished observations), but they were rare in the microscopic counts of preserved samples and not detected at all by the molecular analysis, neither chloroplast 16S rRNA nor 18S rRNA (Figure S2-4). More chlorophytes were identified with microscopy than with any other method (Figure S2-4). The chloroplast 16S rRNA analysis was the method that identified the least number of different phototrophs with no detection of dinoflagellates, euglenophytes, or chlorophytes (Figure S2-4). The 18S rRNA analyses detected many more ciliates than microscopy (Figure S2-3 vs. Figure S2-5). For example, in Lake Clair during the ice-cover period, *Mesodinium* made up 40% of the reads, while in microscopy, it represented 0.4% of the biovolume (Table S2-5).

#### 2.3.4 Identification of driving environmental drivers

At the phylum level for the prokaryotes, there was no differentiation between lakes and periods (PERMANOVA  $p>0.05$ , Figure 2-4A). However, at the genus and the OTU levels, the lakes and periods were clearly separated (PERMANOVA  $p<0.01$ , Figure 2-4B, C). A pairwise post-hoc test further identified Lake Clair and Lake Saint-Charles as being similar at the OTU level, the two lakes with the lowest salinization, while Lake Clément and Lake Saint-Augustin were different from them and from each other (post hoc test  $p<0.01$ ). Despite these distinctions, most of the OTUs were shared between the ice-cover and the open-water periods (77%); of the OTUs found only during a given period, 5% were from the ice-cover period and 95% from the open-water period. Almost half of the OTUs (40%) were shared among all the lakes, and only a few found only in either low or high conductivity lakes (respectively 8 and 3%). The Shannon diversity indices were around 4, and neither this metric nor evenness (around 0.75) of the bacterial communities showed any influence by period (ice-cover versus open-water) or salinity (t test  $p>0.05$ ).



**Figure 2-4.** Non-metric multidimensional scaling analysis of microbial community structure in the surface waters of Lake Clair, Lake Saint-Charles, Lake Clément and Lake Saint-Augustin for the ice-cover and the open-water periods. A-F) 16S rRNA and 18S rRNA sequences pooled at the phylum (A-D), genus (B-E) and OTU (C-F) levels G) pigment concentrations, H) microscopy biovolumes, and I) chloroplast 16S rRNA reads pooled at OTU level from St NMDS stress.

The photosynthetic pigments indicated an effect of season (PERMANOVA,  $p=0.037$ ) and lakes (PERMANOVA,  $p<0.01$ , Figure 2-4G), but for the latter, it was only between Lake Clair and Lake Saint-Augustin, the two extremes of the salinization/urbanization gradient. Only a small proportion of the pigments were detected in all the lakes (23%), and a smaller proportion, respectively 5 and <1%, were uniquely found in the highest and the lowest salinity lakes (data not shown). About half of the pigments were detected year-round, and among those detected only in one period, the majority (89%) were detected in the open-water period.

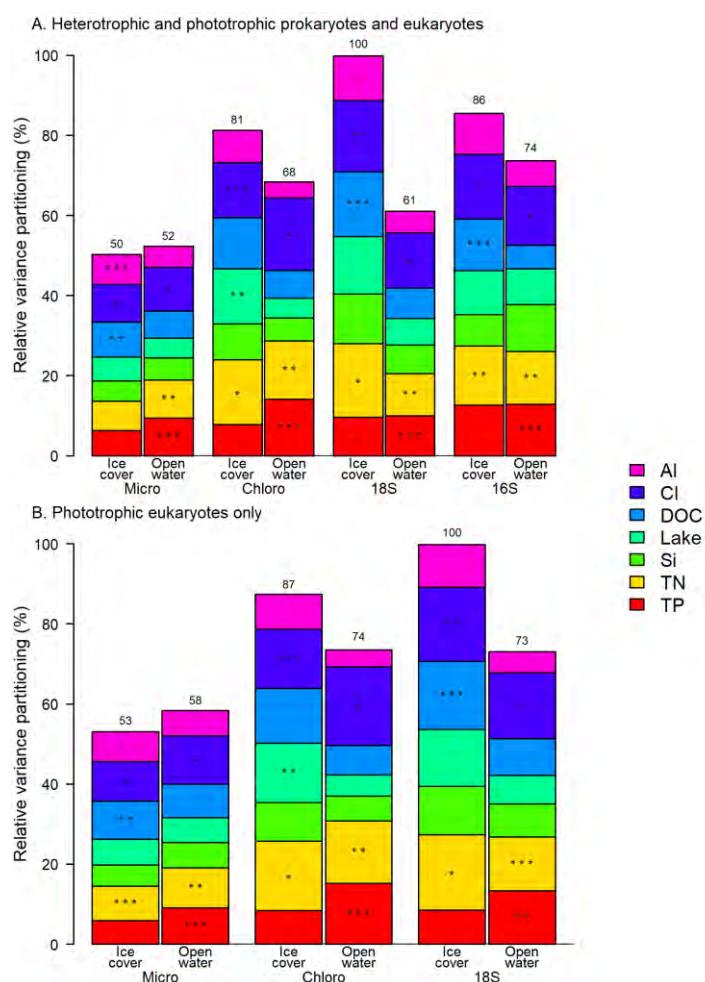
The microscopy-derived biovolume data showed significant differences between the two periods (PERMANOVA,  $p<0.01$ ), but not among lakes (Figure 2-4H). The diversity of the community was higher during the open-water period (t-test  $p<0.01$ , 1.81 vs. 1.14), but there was no difference in evenness. The diversity index was about two times lower when calculated from microscopy than from the chloroplast 16S rRNA or 18S rRNA. A proportion of species were shared between lakes (22%) and periods (40%). No species was unique to the lakes with the highest salinization, while 13% of the species were only found in the lowest salinization lakes. There were unique species observed in the open-water period (53%), while few were unique to the ice-cover period (7%).

The photosynthetic eukaryotic community inferred from the chloroplast 16S rRNA showed significant differences between periods and among the lakes (PERMANOVA,  $p<0.01$ , Figure 2-4I). The lakes with the lowest salinization, Lake Clair and Lake Saint-Charles, were different from Lake Saint-Augustin, the lake with the highest salinization (post hoc test  $p<0.01$ ). The diversity was higher during the open-water period (t-test  $p<0.01$ , 3.09 vs. 1.85), while the evenness was higher during the ice-cover period (t-test  $p<0.01$ , 0.97 vs. 0.78). The majority of OTUs were shared between the two periods (79%, data not shown), while only a small proportion of the OTUs was shared among all lakes (35%, data not shown). Concerning the OTUs that were only found in one period, the majority were unique to the open-water (93%, data not shown). No OTU was unique to the lakes with the highest salinization, while 15% of the OTUs were only found in the lakes with the lower salinization.

The 18S rRNA analyses showed differences between the ice-cover and the open-water at the phylum, the genus, and the OTU levels (PERMANOVA,  $p<0.01$ , Figure 2-4D, E, F). The effect of the lake was only apparent at the genus and the OTU levels (PERMANOVA,  $p<0.01$ , Figure 2-4E, F); a pairwise post-hoc test revealed that, at the OTU level, Lake Clair and Lake Saint-Charles were similar, and Lake Clément and Lake Saint-Augustin were similar, but the two types of lakes differed ( $p<0.01$ ), with only 17% of OTUs shared by all lakes. A small proportion of the OTUs were unique to the lakes with the higher salinization (3%), while 19% of the OTUs were found only in the lakes with the lower salinization. About half of the OTUs were shared between the two periods, while 13% were unique to the ice-cover period, and 40% were exclusive to the open-water. The 18S rRNA communities were

more diverse (t-test  $p<0.01$ , 4.11 vs. 3.14) and more even (t-test  $p<0.01$ , 0.79 vs. 0.67) during the open-water period.

The variation of the microbial community was correlated in similar proportion to the selected factors for both prokaryotes and eukaryotes, with all applied methods (Figure 2-5A). The relative importance of each selected factor remained the same when only the phototrophic eukaryotes were considered (Figure 2-5B). For both periods, the two most consistently important factors were chloride (Cl) and total nitrogen (TN) concentrations.



**Figure 2-5.** Relative contribution to the variance of selected physico-chemical factors for A) the entire prokaryote and eukaryote communities, and B) phototrophic eukaryotes. Asterisks represent the order of the three most important factors and the number over the column is the total variance explained by the model relative to the highest explained variance, which was for 18S ice-cover in both panels. Micro microscopy biovolumes; chloro chloroplast 16S rRNA reads; 18S 18S rRNA reads; 16S 16S rRNA reads.

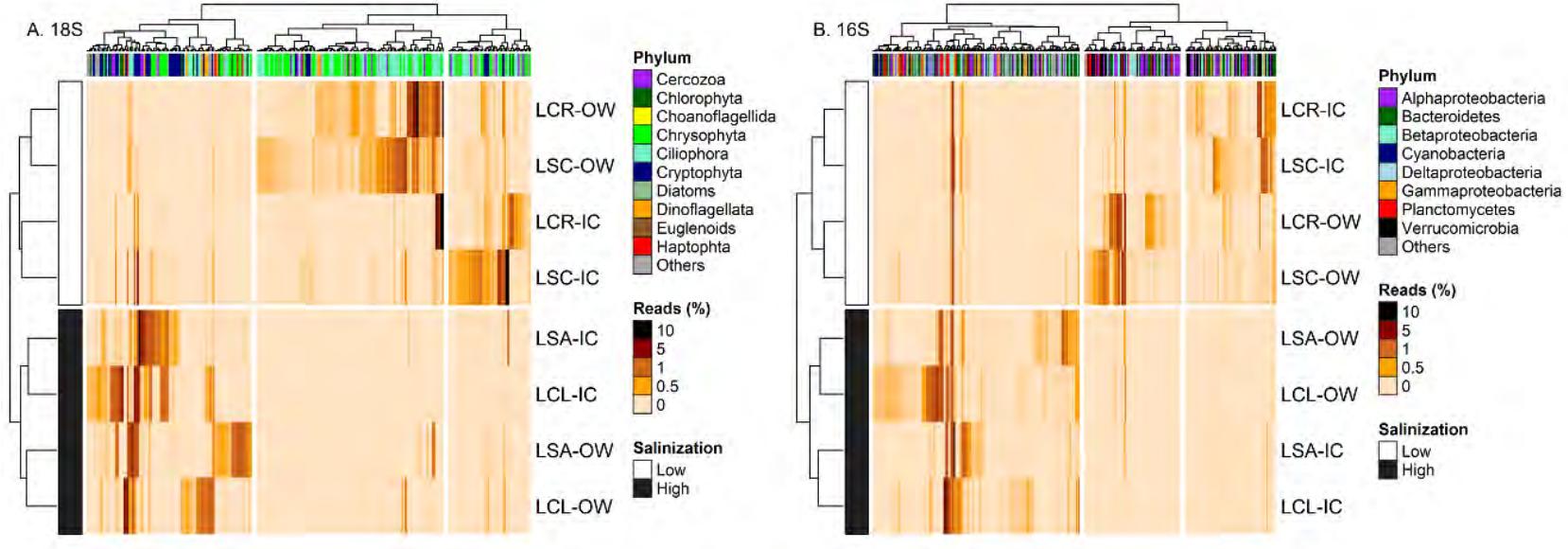
### 2.3.5 Taxonomic responses to environmental drivers

Data from the two methods with the highest resolution in the separation of the samples (16S rRNA and 18S rRNA) were further used to identify changes in the abundance of taxonomic groups in response to the salinization gradient and ice-cover versus open-water periods; for the microbial eukaryotes, the results from the microscopy and chloroplast 16S rRNA were similar to those from 18S rRNA and are presented in supplementary material (Tables S2-12 and S2-13).

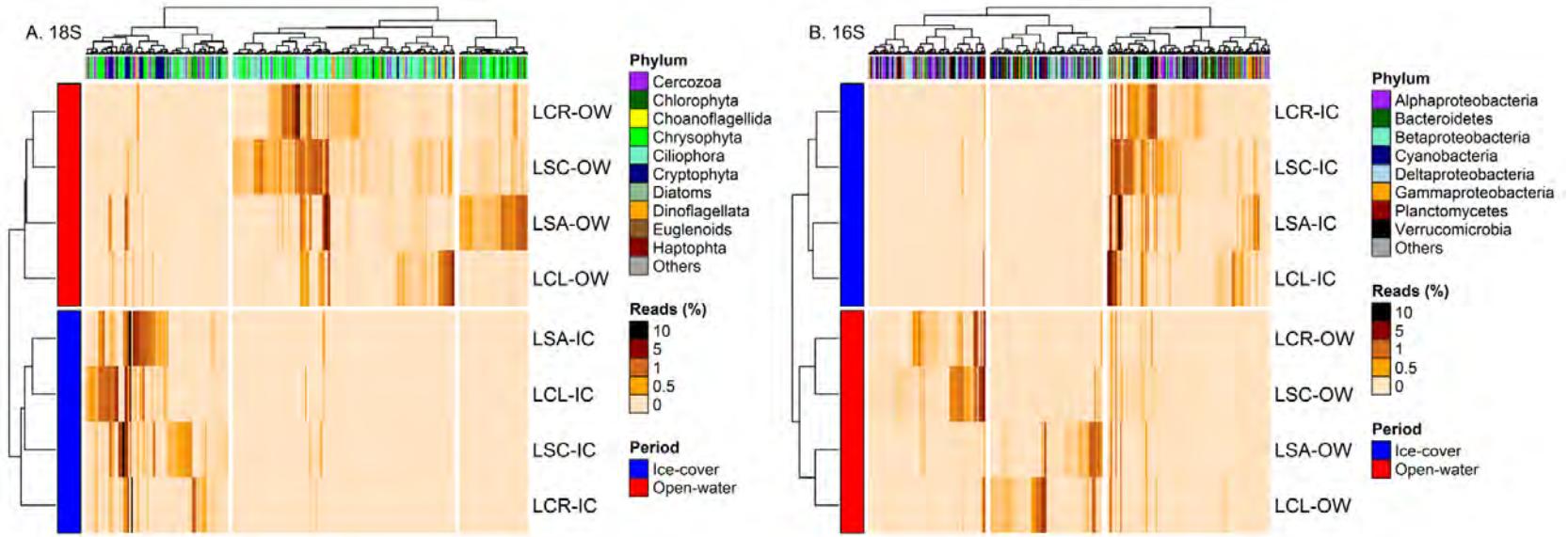
For the 18S rRNA, 368 OTUs were identified as differentially abundant between the lakes with the lowest (Lake Clair and Lake Saint-Charles) and the highest (Lake Clément and Lake Saint-Augustin) urbanization levels (Figure 2-6A). The lakes with the lowest salinization and urbanization levels were characterized by chrysophytes and ciliates, mainly *Dinobryon sociale* and *Mesodinium* sp. The other group of lakes was characterized by an increase of dinoflagellates, the cryptophytes *Plagioselmis* and *Cryptomonas*, and the haptophyte *Diacronema* (Figure 2-6A, Table S2-12). A second DESeq analysis was conducted to identify OTUs (n=394) that discriminated between the ice-cover and open-water periods (Figure 2-7A). The ice-cover period was characterized by chrysophytes, ciliates, and cryptophytes, with the more influential being *Synura* sp., and spirotrich ciliates (Table S2-13). During the open-water period, there were also more chrysophytes and ciliates, mainly *Dinobryon* sp., and Litostomatea ciliates (Figure 2-7A, Table S2-13).

For the 16S rRNA analyses, certain OTUs differed between the two sets of lakes (Lake Clair and Lake Saint-Charles vs. Lake Clément and Lake Saint-Augustin, Figure 2-6B). The most prominent OTUs in the least salinized lakes were identified as Verrucomicrobia *Chthoniobacter* sp. and soil-related Bacteroidetes (Table S2-12). The lakes with the highest salinization were characterized by an increase in Planctomycetes, Bacteroidetes, and cyanobacteria such as *Algoriphagus hongiella* and *Candidatus Cuspidothrix LMECYA-163* (Table S2-12). A further DESeq analysis identified OTUs that were differentially abundant between the ice-cover and the open-water periods (Figure 2-7B). For both periods, these OTUs were mainly represented by Proteobacteria and Bacteroidetes, with *Methylobacter* sp., *Nitrosospira* sp. and *Flavobacterium* sp. showing the largest increase in abundance during the ice-cover period, and among the OTUs that increase the most in abundance during the

open-water period most were unclassified at the genus level, but some were identified as *Dinghuibacter* sp. and *Rhizorhapis* sp. (Table S2-13). An increase in the abundance of reads Deltaproteobacteria and Cyanobacteria were almost exclusively seen during the open-water period (Figure 2-7B). Hierarchical clustering analysis reinforced the similarity of Lake Clair and Lake Saint-Charles, and of Lake Clément and Lake Saint-Augustin (Figure 2-7B).



**Figure 2-6.** Heatmap of the abundance of OTUs (columns) identified by DESeq to discriminate between the lakes with lowest (LCR and LSC) and highest (LCL and LSA) **salinization levels** for A) 18S rRNA ( $n=368$ ,  $p<0.05$ ) and B) 16S rRNA ( $n=382$ ,  $p<0.05$ ). Phylum to which these OTUs are affiliated is displayed. The analysis was conducted on the two periods (ice-cover and open-water) combined. Rows and columns are ordered by hierarchical clustering. LCR Lake Clair; LSC Lake Saint-Charles; LCL Lake Clément; LSA Lake Saint-Augustin; IC ice-cover period; OW open-water period.



**Figure 2-7.** Heatmap of the abundance of OTUs (columns) identified by DESeq to discriminate between the **ice-cover** (Jan-Feb-Mar) and the **open-water periods** for A) 18S rRNA ( $n=394$ ,  $p<0.05$ ) and B) 16S rRNA ( $n=393$ ,  $p<0.05$ ). Phylum to which these OTUs are affiliated is displayed. The analysis was conducted on the four lakes combined. Rows and columns are ordered by hierarchical clustering. LCR Lake Clair; LSC Lake Saint-Charles; LCL Lake Clément; LSA Lake Saint-Augustin; IC Ice-cover period; OW Open-water period.

## 2.4 Discussion

In this study, we analyzed the seasonal microbiomes of four lakes across a gradient of road salt exposure. The results revealed that the taxonomic composition varied with degree of salinization (from unimpacted to strongly contaminated by road salts) in both the ice-cover and the open-water periods. The variables related to salinization, such as Cl concentration, were among the most explanatory statistical factors, along with total nitrogen (TN), independent of the period and the identification method. For the eukaryotes, all methods resulted in the identification of the same factors with similar proportions of the total variation, but the depiction of community composition and its associated changes differed by method.

### 2.4.1 Identification of environmental drivers

During both periods, the concentration of chloride (Cl), and the concentration of total nitrogen (TN) were the most important drivers of the taxonomic composition of the microbial communities, both prokaryotes and eukaryotes, with all methods. In the studied lakes, Cl concentration is likely a proxy for total salinity, which is mostly influenced by bedrock type and urbanization level, as well as by the related road salt use. It was also correlated with alkalinity, which has previously been observed in road salt contaminated freshwater and attributed to pavement weathering and cation exchange (O'Brien and Majewski 1975; Moore et al. 2017). Sodium ions from road salts are exchanged with other major ions in soils, including calcium, which would contribute to the increased alkalinity (Shanley 1994). This phenomenon likely occurred in the urbanized lakes as the sodium to chloride ratio was <1 (in the 0.5-0.7 range), while it would have otherwise been at least 1 due to the main source of sodium and chloride from NaCl road salt. Total nitrogen was not correlated with chloride, but its concentration was still higher in the three more urbanized lakes, compared to reference Lake Clair. The results suggest that the two most important drivers of taxonomic composition were related to the urbanization level of the watersheds.

While salinity is known to be a structuring factor for microbial communities across freshwater, brackish and marine environments, the results of this study are among the few providing evidence that there might also be an effect of salinity (or an unknown correlate of salinity) between the lowest and the highest ends of the freshwater range (Butts and Carrick 2017; Toporowska et al. 2018). For bacteria, the increase in salinity that occurs in the

transition from a freshwater to a marine ecosystem, generally leads to a decrease in abundance of Actinobacteria, Verrucomicrobia and Betaproteobacteria and an increase in abundance of Alpha-, and Gammaproteobacteria (Bouvier and del Giorgio 2002; Herlemann et al. 2011; Eiler et al. 2014). This switch of phylum dominance was not observed in the present study, most likely because the salinity range was too small, but two representatives of the Gammaproteobacteria were identified as having a higher abundance in the lakes with the higher salinity. For cyanobacteria, the most well-known genus with halotolerant species is *Synechococcus*, but this was not detected in the present study (Celepli et al. 2017). However, the filamentous cyanobacterium *Dolichospermum* was detected, and is known to include halotolerant strains, for example in the Baltic Sea (Celepli et al. 2017). The response of eukaryotes was more pronounced, consistent with previous observations that bacteria are generally less responsive to changes in salinity than eukaryotes (Casamayor et al. 2002). Chrysophytes and ciliates were more abundant in the lakes with the lower salinity, while cryptophytes and haptophytes were more characteristic of lakes of higher salinity. Taxa with the highest increase in abundance between these two sets of lakes include halotolerant genera. The haptophyte *Diacronema* is frequently observed in brackish and marine environments (Balzano et al. 2015). The haptophyte *Chrysochromulina* was found to increase along a transect from a river mouth to the sea (Bazin et al. 2014). The cryptophyte *Plagioselmis*, along with other cryptophytes, were observed to be the dominant components of a phytoplankton bloom in an estuary with a salinity of  $\approx 20$  ppt (Bazin et al. 2014). Cryptophyte abundance was also found to increase with salinity in a microcosm experiment from freshwater to 51 ppt (Greenwald and Hurlbert 1993), and to be positively correlated with conductivity in freshwater lakes (Toporowska et al. 2018).

Urbanization is often associated with eutrophication, and species characteristic of urban lakes are therefore often bloom-forming cyanobacteria (Napiórkowska-Krzelbietke and Dunalska 2015; Lévesque et al. 2020). Other species known in urban lakes include dinoflagellates, and diatoms such as *Cyclotella* and *Stephanodiscus*, which were identified as constituents in the present study (Napiórkowska-Krzelbietke and Dunalska 2015). The bloom-forming cyanobacterium *Dolichospermum* was found in the most urbanized lakes in the present study, but this response was mainly driven by its high abundance in the hyper-eutrophic Lake Saint-Augustin. However, the urbanized Lake Clément, has no eutrophication

problem, nor a higher abundance of cyanobacteria, yet the composition of its microbial community was more similar to the highly urbanized and hyper-eutrophic Lake Saint-Augustin, than to the less eutrophic, but also less urbanized Lake Clair and Lake Saint-Charles. This indicates the importance of other factors related to urbanization, such as the road salts, or of other common contaminants of urban watersheds including pharmaceuticals, hydrocarbons and metals from wastewater, sewage and roads (Hong et al. 2018; Ondarza et al. 2019; dos Santos et al. 2020). While the exact factor, or combination of factors, to which they respond remain uncertain, the results of the present study suggest that an increase in abundance of bloom-forming cyanobacteria, cryptophytes or haptophytes is exacerbated by urbanization and the associated road salt effects.

#### 2.4.2 The structuring effect of ice-cover

The presence of an ice-cover leads, directly or indirectly, to many physical (light, temperature, exchange with atmosphere, wind-driven mixing), chemical (redox gradients, dissolved gas concentrations) and ecological (predator populations) changes that result in a markedly different environment than in the open-water period. The distinct communities observed in the present study between the ice-cover and the open-water periods, are likely a complex response to this combination of differences. In the Saint-Charles River, ice-cover was identified as a major driving factor of community composition for both prokaryotes (Craaud et al. 2019a) and eukaryotes (Craaud et al. 2019b). As in the present study, phytoplankton and ciliate communities sampled year-round in three lakes in Poland did not cluster according to the four seasons, but rather by winter (ice-cover) and the rest of the year (Kalinowska et al. 2019). In Lake Erie, the expanse of ice cover during winter was also a main segregating factor for both bacteria and microbial eukaryote communities (Beall et al. 2016).

The bacterial communities during the ice-cover period appeared to have distinctive biogeochemical functions. In the present study, *Nitrospira* and *Nitrotoga* were identified as more abundant during the ice-cover than the open-water period. These nitrifying bacteria are often found together in wastewater treatment plants, and *Nitrotoga* has been identified as a low-temperature adapted genus (Wegen et al. 2019). Their greater abundance during winter is consistent with reports drawing attention to the importance of nitrification in lakes under

winter ice cover (Cavaliere and Baulch 2019; Massé et al. 2019). Another genus that was conspicuously more abundant in the ice-cover period was *Methylobacter*, a methane oxidizer typically found in proximity to anoxic habitats containing methanogens. It was observed in the four lakes, but with a higher abundance in Lake Clément and Lake Saint-Charles (these lakes had respectively 65 and 32% of the reads associated with this genus). Although the surface waters of the studied lakes were almost fully oxygenated during the ice-cover period, the bottom waters had oxygen saturation around 50%, with likely much lower oxygen tensions and possible anoxia in the lake sediments that would liberate reduced substrates into the water column. In contrast, the open-water period was characterized by the presence of cyanobacteria such as the bloom-forming *Microcystis* and *Dolichospermum*, as well as *Rabdotiderma* and many unclassified picocyanobacteria.

Phytoplankton communities during the ice-cover period are generally dominated by either cyanobacteria or diatoms or by small heterotrophic or mixotrophic flagellates such as chrysophytes and cryptophytes (Öterler 2017). The shift in dominance between these groups is mainly related to the trophic status of the lake, and the thickness of the snow and ice cover, with chrysophytes and cryptophytes negatively correlated with the former and positively with the latter (Beall et al. 2016; Kalinowska and Grabowska 2016). During winter, the studied lakes were covered with  $\approx$ 70 cm of ice and  $\approx$ 40 cm of snow that would greatly attenuate the light, and lead to the expected dominance of chrysophytes and cryptophytes. However, while cryptophytes were almost exclusively found during the ice-cover period, chrysophytes also dominated during the open-water period, but not the same genera. There was also an increase in abundance of various chlorophytes during the ice-cover period, mainly small ovoid flagellates such as *Chlamydomonas* and *Oophila*. *Chlamydomonas* is frequently encountered during the ice-cover period, including in perenially ice covered systems (e.g., Bégin et al. 2020).

In the present study, the alpha-diversity (within sample) of prokaryotes was not influenced by the period, but the alpha-diversity of eukaryotes was higher during open-water period with all methods. Along the same lines, there were more species/OTUs unique to the open-water period than to the ice-cover period, both for prokaryotes and microbial eukaryotes, as has been previously observed for prokaryotes (Wilhelm et al. 2014). While

the ice-cover period offers niches that are not found during the open-water period, it is more stable in time and therefore limits species succession. The stability however leads to the establishment of physico-chemical gradients that may offer a variety of niches. In comparison, the open-water period goes through a low light mixing period with high nutrient levels (spring), a higher light stratified period with lower nutrient level and bottom water hypoxia/anoxia (summer), a lower light mixing period with lower nutrient (autumn), and multiple transition stages. Furthermore, the greater connexion with the surrounding watershed during the open-water period, with stream inputs of allochthonous materials, would contribute to a greater diversity of available substrates.

#### 2.4.3 Comparison of methods

One of our aims in this study was to compare a variety of methods to evaluate their complementarity and to provide a broad depiction of the taxonomic community structure. We were concerned that DNA analyses in winter could be biased towards dormant and degrading cells that were not active in the under-ice microbiome, and our molecular analyses therefore focused on RNA rather than DNA. Specifically, we targeted 16S or 18S rRNA (via conversion to cDNA) as a measure of ribosomal potential for protein synthesis and a more accurate guide to potential *in situ* activity than 16S or 18S rRNA genes (Blazewicz et al. 2013). While some studies have shown a correspondence between the two approaches (e.g., Mohit et al. 2017), there can be differences in regions or times of rapid successional change (e.g., Kalenitchenko et al. 2019).

The biggest differences in community composition observed between the methods was the over-representation of ciliates and the under-representation of diatoms with 18S rRNA, and the absence of dinoflagellates with chloroplast 16S rRNA. The disparity in the abundance of ciliates between 18S rRNA and microscopy, has mainly been observed as a result of their high number of gene copies (Medinger et al. 2010). However, when the 18S rRNA (cDNA) is analyzed instead of 18S rDNA, there is a better correlation in the abundance of different groups between sequencing and microscopy (Giner et al. 2016; Pingping et al. 2020). In those studies, the size distribution of the compared groups was more uniform, because they were limited to picoeukaryotes or ciliates, respectively. In the present study, the bias toward ciliates might have arisen from size effects as the cellular quantity of rRNA increases with

size (Fu and Gong 2017), and ciliates are generally bigger than most of the other identified groups. It is also likely that delicate ciliates are differentially lost during the preservation of microscopy samples (Stoecker et al. 1994; Medinger et al. 2010). Lower diatom abundances in 18S rRNA reads have been attributed to the cell-lysis step of RNA/DNA extraction, which may be less efficient for this group due to their frustules (Medinger et al. 2010). The unusual attributes of dinoflagellate chloroplasts might explain why they were not amplified by the 16S rRNA primer when they were detected, sometimes in high relative abundance, with the three other methods; the genome of dinoflagellate chloroplasts is fragmented with certain gene functions transferred into the nucleus (Bennke et al. 2018). Furthermore, some members of this group are kleptoplastidic, with the ability to steal chloroplasts from other photosynthetic algae (Bennke et al. 2018). Some ciliates, such as the genus *Mesodinium* identified in the present study by 18S rRNA and microscopy, are also capable of kleptoplastidy (Bennke et al. 2018), which means that some of the chloroplast 16S rRNA reads might not represent the actual community, but the identity of the original bearers of the chloroplasts. Some of the differences might also arise from differences in transcription regulation between chloroplast and nuclear rRNA (Marín-Navarro et al. 2007). In interpreting these differences, the differential affinity and resolution of the primers for sequences of different groups and species at a given location in their genome (e.g., V4) must also be considered (Rimet et al. 2018; Choi and Park 2020). Compounds present in the initial water sample, such as humic materials in high concentration, can also interfere with nucleic acid extraction or inhibit the PCR steps (Crevecoeur et al. 2015).

Among all methods, 18S rRNA detected the highest  $\alpha$  diversity (number of OTUs per sample), followed by microscopy and 16S rRNA in chloroplasts. The higher diversity using 18S rRNA as compared to microscopy has been attributed to its better detection of rare species due to higher total counts (in the present study there was around 6000 reads per sample with 18S rRNA, versus 800 cells per sample by microscopy), its detection of cryptic or rare species, and to factors linked to sequencing errors and OTU groupings (Medinger et al., 2010; Rimet et al., 2018). The detection of species by microscopy is also limited by the skills of the analyst and the size of the cells (Intergovernmental Oceanographic Commission of UNESCO 2010). Furthermore, some species are lost during the preservation protocol with acid Lugol's (Gieskes and Kraay 1983; Stoecker et al. 1994) or are altered by it, for example

by break-up of colonies (Mukherjee et al. 2014), loss of flagella and discoloration (Intergovernmental Oceanographic Commission of UNESCO 2010). Phase contrast and staining agents (e.g., silver staining of ciliates) can help with the identification of colourless or discolored species (Kim and Jung 2017). The lowest diversity detected with chloroplast 16S rRNA was mainly due to the fact that it only included phototrophs. However, the database for chloroplast 16S rRNA marker is still limited (Bennke et al. 2018), therefore many species are lumped together. The nature of certain chloroplasts, as mentioned for dinoflagellates, might also contribute to this lower resolution of species.

Microscopy, HPLC pigments and molecular analysis with either chloroplast 16S rRNA or 18S rRNA each provide useful ways to identify the factors driving differences in the taxonomic composition of microbial communities among sites, sampling times or experimental treatments. It is unusual to apply all five methods in a single study, but previous studies have used pairs of methods to differentiate communities: fluorescence (pigments) and microscopy (Lévesque et al. 2020); 18S rRNA/18S rRNA genes and microscopy (Gao et al. 2018; Rimet et al. 2018; Minerovic et al. 2020); and chloroplast 16S rRNA and microscopy (Eiler et al. 2013; Bennke et al. 2018). In the present study, 18S rRNA and chloroplast 16S rRNA offered better resolution for the separation of the samples among lakes and sample period than microscopy, as observed elsewhere (Yan et al. 2007). However, the combination of methods provides the best estimate of taxonomic composition of the microbial community, including by way of biovolume proportions that can be obtained by microscopy (Auinger et al. 2008; McManus and Katz 2009; Xiao et al. 2014), albeit with the caveats noted above. These methods can additionally be augmented to consider functional activities and diversity by the use of primers for genes and transcripts of enzyme participating in specific biochemical pathways (e.g., methanotrophy; Crevecoeur et al., 2017) and omics methods to functionally as well as taxonomically analyze the entire community (e.g., winter versus summer microbiomes; Vigneron et al., 2019).

## **2.5 Conclusions**

Consistent with our hypothesis, there was a statistical relationship between community structure and chloride concentrations, notably on the relative abundance of cryptophytes and haptophytes, suggesting an influence of salinization across our sampled gradient of lakewater conditions. Large differences in trophic status and other limnological properties were insufficient to blanket out this effect, implying that the salt-rich runoff from roadside snowmelt may cause a shift in lake microbiomes during ice-cover as well as open-water periods. The application of diverse methods in the present study underscored the large differences between under-ice and open-water microbial communities. The seasonal differences included the tendency of the under-ice communities to have smaller algal cells, greater pigmentation, and higher relative abundances of methanotrophs, nitrifiers, and cryptophytes than the open-water communities. These results attest to the importance of extending limnological studies to all seasons, including winter, to capture the full range of microbiome compositions, functions and responses to contaminant exposure.

## **2.6 Acknowledgements**

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# **Chapitre 3 Microbial plankton responses to salt versus urban snow in a spring snowmelt experiment.**

## **Résumé**

La salinisation de l'eau douce est un problème grandissant dans les lacs des régions tempérées nordiques, mais on en connaît pourtant très peu sur ses effets sur les microbes. Nous avons évalué l'hypothèse que les sels de voirie causent des changements dans la structure taxonomique des communautés microbiennes et que ces changements sont amplifiés par les autres contaminants que l'on retrouve dans l'eau de fonte de la neige urbaine. Le plancton du Lac Saint-Charles, prélevé en mars, a graduellement été acclimaté, dans un incubateur, à des conditions similaires à celles que l'on retrouve au printemps. Il a ensuite été exposé pour une durée de 2 semaines à du NaCl ou à de l'eau de fonte de neige urbaine provenant d'un dépôt à neige. La structure taxonomique des communautés microbiennes, déterminée par l'analyse de l'ARNr 16S/18S, a changé en réponse à l'ajout de chlorures, avec certains taxons rares qui sont devenus plus abondants. Contrairement à notre hypothèse, l'ajout de chlorures a causé un changement plus prononcé que l'eau de fonte de la neige urbaine. Ces résultats suggèrent que les sels de voirie ont le potentiel d'influencer les microbes des lacs, mais que les essais écotoxicologiques basés sur l'exposition aux sels seuls ne reproduit pas correctement les effets combinés de tous les contaminants que l'on retrouve dans la neige urbaine.

## **Abstract**

Freshwater salinization is an ongoing concern for north temperate lakes. However, little is known about its impacts on microbes. We tested the hypotheses that road salt induces changes in microbial community structure, and that these effects are amplified by additional chemicals in urban snowmelt. Overwintering plankton in lake water were gradually acclimated in a laboratory incubator to spring-like conditions, and then exposed for 2 weeks to either NaCl or melted snow from a road snow disposal site. Microbial community structure as determined by 16S and 18S rRNA analysis showed changes in response to chloride enrichment, with certain rare taxa becoming more prominent. Contrary to our hypothesis, the salt treatment caused a more pronounced response than the snow. These results indicate that road salt has the potential to impact lake microbes, but that ecotoxicology assays based on a single salt addition do not adequately reproduce the combined chemical effects of urban snowmelt mixtures.

### **3.1 Introduction**

Chloride concentrations in many northern freshwater lakes are increasing due to road salt applications for driving safety in winter. However, few of the salt-affected lakes contain chloride concentrations beyond the guideline limits of 230 mg L<sup>-1</sup> (USA) or 120 mg L<sup>-1</sup> (Canada) for chronic exposure of freshwater aquatic life, and most lake values are well below this limit. A recent analysis of estimated chloride concentrations in more than 49 000 lakes in the United States indicated that less than 5% have chloride levels in excess of 50 mg L<sup>-1</sup> (Dugan et al. 2020). Most studies on the ecotoxicology of chloride are carried out at much higher concentrations, and the effects of moderate increases above natural background levels are poorly known.

Research on chloride effects at environmentally relevant concentrations has mainly focused on zooplankton, insects, amphibians and fish, while little attention has been accorded to microbial communities (Hintz and Relyea 2019), despite their fundamental role in underpinning aquatic ecosystem function. Studies on the effects of chloride on microbes have been restricted to a single microbial species (Cleave et al. 1981) or have analysed changes in community properties such as photosynthetic pigments, without information on taxonomic composition (e.g., Chakraborty et al. 2011). Changes are known to occur in bacterial community structure across salinity gradients, with the transition from freshwater to marine ecosystems accompanied by shifts in bacterial taxa and functions (Herlemann et al. 2011). However, the estuarine salinity range is much greater than typically found in salt-affected lakes, and little is known about the sensitivity of microbial communities at the low end of this range.

In addition to the question of how salt and especially chloride affects microbial communities, the combined effects of multiple stressors must also be considered. Chloride can be delivered to lakes via roadside snowmelt, which is a cocktail of numerous constituents including inorganic nutrients, metals and organic contaminants such as polycyclic aromatic hydrocarbons (e.g., Bartlett et al. 2012a,b; Fournier et al. 2020). In many temperate lakes and rivers, the snowmelt period at the beginning of spring corresponds to peak chloride loads and concentrations (Ruth 2003), and information is therefore needed to understand the sensitivity

of the overwintering communities to this seasonal rise in chloride and associated road-derived contaminants.

Our aim in the present study was to evaluate the response of planktonic microbial prokaryote and eukaryote communities to chloride at environmentally relevant concentrations, either as pure sodium chloride or as a component of urban snowmelt that contains many other chemicals. Specifically, our objective was to test the hypotheses that chloride induces a shift in the taxonomic structure of overwintering microbial communities in lakes, and that these changes are exacerbated by the presence of other chemical stressors in urban snowmelt. To address these hypotheses, we sampled the overwintering plankton of a freshwater reservoir (Lake Saint-Charles, Quebec, Canada) and exposed subsamples to different chemical treatments in a 2-week laboratory controlled experiment. Microbial community structure (bacteria and eukaryotes) was assessed at the beginning and end of the experiment by 16S and 18S rRNA transcripts analysis.

## 3.2 Methods

### 3.2.1 Lake and snow sampling

Lake Saint-Charles (lat. 46.94°N, long. 71.39°W), a reservoir that supplies drinking water for around 285 000 people in Quebec City, Canada was sampled in late winter (11 March 2016). The conductivity of this lake increases during spring (April-May) each year as a result of inputs from melting roadside snow that contains de-icing salts (Fournier et al. 2020). Surface water (0-50 cm) was sampled through holes cut in the ice in Echo Bay (Fig. S3-1). In addition to water for the experiment, subsamples were taken for chemical analysis, and characterization of the initial microbial plankton community. Physicochemical parameters (pH, conductivity, dissolved oxygen and temperature) at the time of sampling were measured *in situ* using a Hydrolab DS5X profiler (Loveland, Colorado, USA).

Road-influenced snow was obtained from the “de la Colline” snow depot (lat. 46.87°N, long. 71.35°W; 17<sup>th</sup> March 2016). This site is where the snow collected from roads, sidewalks, parking lots and other municipal spaces in the southern part of Lake Saint-Charles watershed is disposed of. The snow sample was allowed to melt in the dark at 4°C and then used for chemical analysis and the experiment. Water and snow samples were analyzed for total nitrogen (TN), total phosphorus (TP), dissolved ions, dissolved organic carbon (DOC), alkalinity, and polycyclic aromatic hydrocarbons (PAHs). Additional sampling and analysis details are given in the Supporting Information (Annexe Chapitre 3: Supporting Information).

### 3.2.2 Experimental design

The lake water for the experiment was filtered through a 250 µm mesh to remove zooplankton, and then dispensed into twelve 4-L LDPE Cubitainers (Thermo Fisher Scientific). The Cubitainers were placed in a Sanyo environmental incubator at the Laboratoire aquatique de recherche en sciences environnementales et médicales (LARSEM) at Laval University (Quebec, QC) under initial conditions of 4°C, a photoperiod of 12:12, and a daytime irradiance cycle of 40-60-40 µmol photons m<sup>-2</sup> s<sup>-1</sup>. This light and temperature regime was chosen to mimic lake conditions under the ice. Prior to initiating the experimental treatments, the incubator temperature was gradually increased to 15°C (around 2°C per day over 7 days), while irradiance was gradually increased to 80-150-80 µmol photons m<sup>-2</sup> s<sup>-1</sup>.

(around 10-20-10  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  per day; same photoperiod as before) to simulate the onset of spring conditions.

For the experiment, the water was incubated at two Cl concentrations: without salt amendment, the original lake water Cl concentration was 20 mg Cl L<sup>-1</sup>, and with a doubling of conductivity to give a final concentration of around 50 mg Cl L<sup>-1</sup>. The sodium chloride was either supplied as NaCl (reagent grade) dissolved in Milli-Q water (hereafter referred to as the “Salt treatment”) or as melted urban snow containing Na<sup>+</sup> and Cl<sup>-</sup> as major ions (hereafter referred to as the “Snow treatment”). The snow was not sterilized prior to the experiment, because heat or filtration would have denatured or filtered out some of the contaminants and it may have resulted in different toxicity than the entire mixture (Boxall and Maltby 1997). Furthermore, in the environment, microbial community from the snow will likely join the lake with the snowmelt runoff (Comte et al. 2018). Phosphorus was added at a low concentration to the Salt and Snow treatments (+4  $\mu\text{g P L}^{-1}$  final concentration, as KH<sub>3</sub>PO<sub>4</sub>) to prevent phosphorus limitation of microbial growth and was added on its own as a test for any effects of this nutrient addition (“P-only treatment”). Three Cubitainers were selected randomly for each treatment (n=9), and three others were incubated with only Milli-Q addition (“Control”). Additions in all treatments represented 5% of the total volume. A subsample (300 mL) for RNA analysis was taken from each Cubitainer after two weeks of incubation under the different treatments.

### 3.2.3 RNA analysis

All samples for rRNA amplicon analysis were filtered through a 0.2  $\mu\text{m}$  Sterivex units (Millipore), then filled with RNAlater (Life Technologies) and frozen at -80°C until analysis. Nucleic acids were extracted from the Sterivex units using an AllPrep DNA/RNA Mini Kit (Qiagen), and following testing for contaminating DNA, the RNA was converted to cDNA with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems-Ambion). The cDNA was amplified using two sets of primers modified with Illumina adaptors: (1) 515F/806R, which targets the V4 region of the 16S rRNA (prokaryotes); and (2) 572F/1009R, which targets the V4 region of the 18S rRNA (eukaryotes). The amplicons were sequenced on an Illumina MiSeq at the IBIS/Laval University Plate-forme d’analyses génomiques (Quebec City, QC). Further details on laboratory protocols, bioinformatics, and

statistical analyses are given in the Supporting Information (Annexe Chapitre 3: Supporting Information).

### 3.3 Results

#### 3.3.1 Properties of the lake water and urban snow

On the date of lake sampling, the 3 m water column was covered by 50 cm of ice and 10 cm of snow. The air temperature was 0.5°C, and the surface water that rose up into the hole (0-50 cm) had a temperature of 0.17°C and conductivity of 125  $\mu\text{S cm}^{-1}$ , with 11 mg dissolved oxygen  $\text{L}^{-1}$  (76% of saturation) and pH 7.18. The concentration of chloride was 20 mg  $\text{L}^{-1}$  (Table 3-1), and additional information is given in Supporting Information (Annexe Chapitre 3: Supporting Information). On the date of urban snow sampling, the air temperature was -1.4°C. All measured chemical constituents in the snow were in higher concentrations than the lake, with the exception of total nitrogen and potassium that were around half of lake water concentrations (Table 3-1). Three PAHs (benzo(a)pyrene, fluoranthene and pyrene) were measured in the snow in concentrations higher than the CCME guidelines, which are respectively 0.015, 0.04, and 0.025  $\mu\text{g L}^{-1}$ , but were below detection in the lake water.

**Table 3-1.** Chemical composition of Lake Saint-Charles surface water (0-50 cm) in March 2016, urban snow and experimental treatments. The values are means for triplicate samples (SD as percent mean in parentheses). Alkalinity is in mg  $\text{CaCO}_3 \text{ L}^{-1}$ , and all other values are in mg  $\text{L}^{-1}$ .

Variable	Natural medium		Treatments			
	Lake ( $t_0$ )	Snow	Control <sup>a</sup>	P-Only <sup>a</sup>	Snow <sup>a</sup>	Salt <sup>a</sup>
Alkalinity	15.6 (2)	40.9 (13)	14.7 (2)	14.7 (2)	18.0 (1)	14.7 (2)
Calcium	6.4 (1)	14.8 (23)	6.0 (1)	6.0 (1)	7.3 (1)	6.0 (1)
Chloride	20.0 (9)	17.8 (68)	18.9 (9)	18.9 (9)	49.6 (3)	48.9 (3)
Nitrogen <sup>b</sup>	0.8 (6)	0.4 (46)	0.7 (6)	0.7 (6)	0.7 (6)	0.7 (6)
Phosphorus <sup>b</sup>	11.4 (22)	84.3 (46)	10.8 (22)	14.8 (16)	16.5 (15)	14.8 (16)
Potassium	0.7 (2)	0.3 (27)	0.7 (2)	0.7 (2)	0.7 (2)	0.7 (2)
Sodium	11.1 (7)	10.6 (63)	10.4 (7)	10.4 (7)	30.4 (2)	30.1 (2)
Sulfate	4.6 (3)	5.3 (72)	4.3 (3)	4.3 (3)	4.9 (3)	4.3 (3)

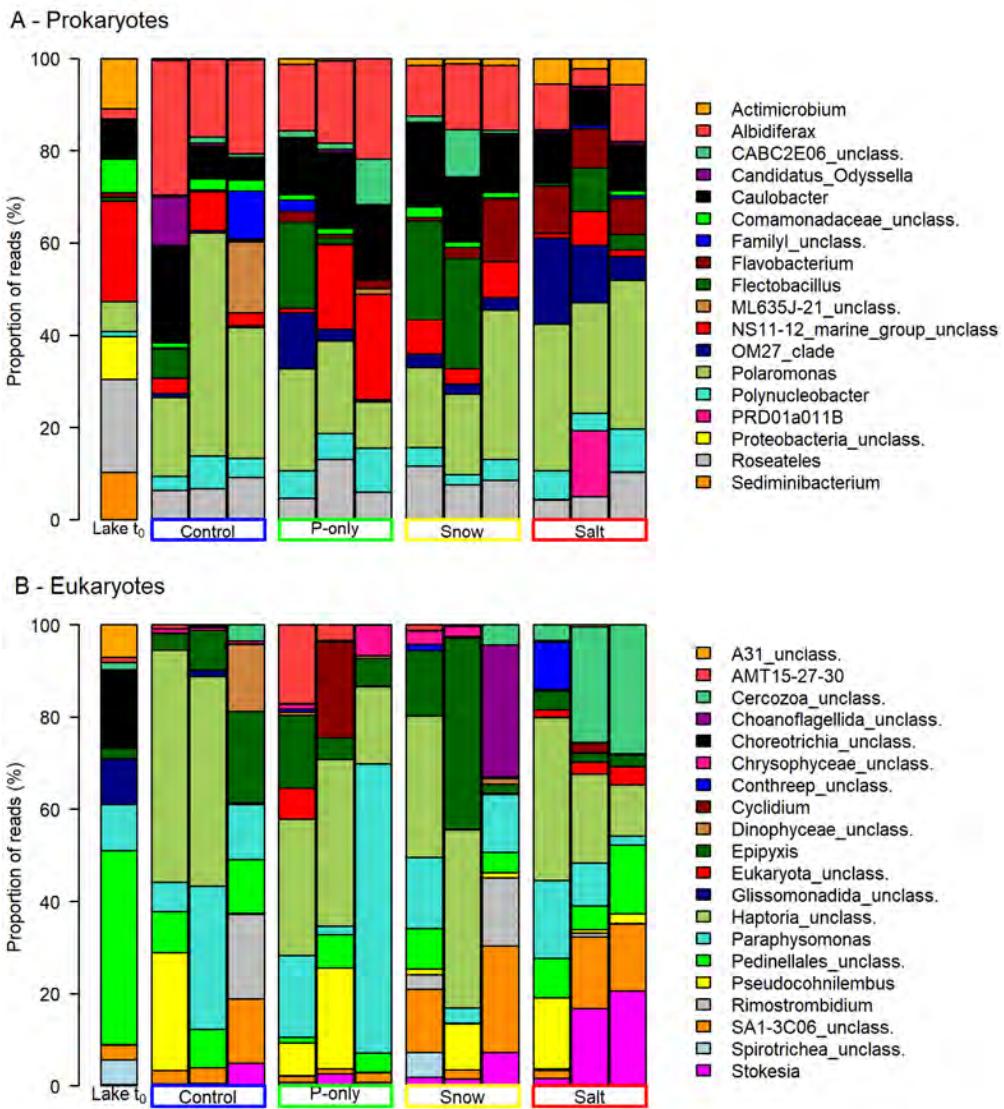
<sup>a</sup>Calculated from added chemicals

<sup>b</sup>Total (dissolved+particulate)

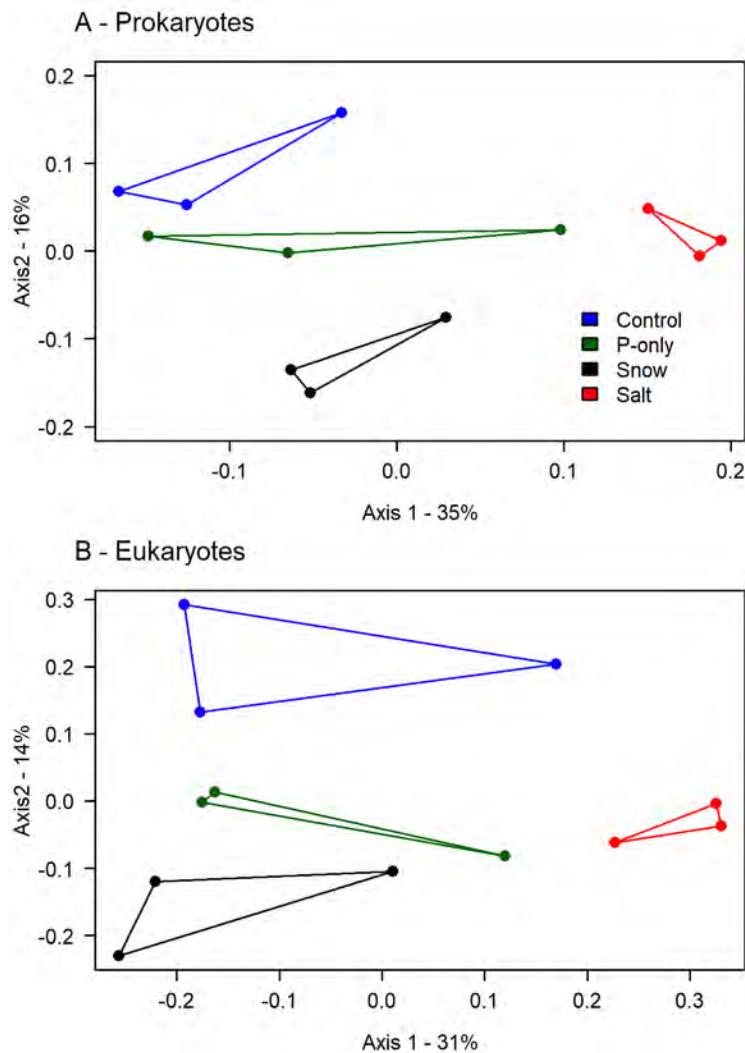
### 3.3.2 Microbial plankton responses

The 77,137 reads obtained for the lake sample of March 2016 using the 16S rRNA primer were distributed in 307 OTUs. The dominant phyla were *Proteobacteria* (65% of total reads), comprising *Betaproteobacteria* (48% of total reads), *Gammaproteobacteria* (10%) and *Alphaproteobacteria* (7%); and *Bacteroidetes* (29%). The 18S rRNA primer yielded 44,163 reads, distributed among 155 OTUs, and the dominant phyla were ochrophytes (45.2% of total reads), mostly chrysophytes (45.1%); ciliophora (32%); dinoflagellates (10%) and cercozoa (5%).

The experimental treatments resulted in changes in both the prokaryotic and eukaryotic communities (Fig. 3-1). The treatments explained 25% and 77% of the variation for these two groups respectively (PERMANOVA;  $F=2.50$  and  $F=2.14$ ;  $p=0.002$  and  $p=0.007$ ). A PCoA constructed from a Bray-Curtis dissimilarity matrix revealed one cluster for each treatment, with the Salt treatment separating well apart from the others on the first axis (Fig. 3-2). The first axis explained 35% and 31% of the variation, while the second axis explained 16% and 14% of the variation for the prokaryotes and the eukaryotes respectively.



**Figure 3-1.** Relative proportion of 16S or 18S rRNA reads (%) for lowest resolved taxonomic groups having at least 5% of reads in one sample. A. Prokaryotes. B. Eukaryotes. Each bar is a biological replicate.



**Figure 3-2.** Principal coordinates analysis (PcoA) on a Bray-Curtis dissimilarity matrix for microbial community structure in the experimental treatments. A. Prokaryotes. B. Eukaryotes communities. Each point is a biological replicate.

By the end of the experiment under the simulated spring warming conditions, the Control treatment had measurably shifted from the original lake community. For the bacteria, there was a shift in dominance from *Flavobacterium* (16 to 3%) to an unclassified *Comamonadaceae* (4 to 22%) and a 15-fold increase of *Roseatales* (1 to 15%, Table 3-2). Of the 16 most abundant genera in the lake, half remained in the control at a read abundance level of  $\geq 1\%$ . Most of the other common taxa were still detectable, but in lower extent, except for two Gammaproteobacteria methanotrophs; *Methylococcales*, *Crenothrix*, *Gracilibacteria*

and an unclassified cyanobacterium were initially detected in the lake water, but were undetected at the end of the experiment. For the eukaryotes, five of the most abundant genera in the original lake water sample were detected in the Control with a relative abundance of  $\geq 1\%$ . Of the other dominants, five were undetectable or had order of magnitude lower abundances at the end. These included *Pseudocohnilembus* (ciliate), *Stokesia* (ciliate), *Gymnodinium* (naked dinoflagellate), an unclassified hypotrich (ciliate) and an unclassified chrysophyte of the CCI40 order.

**Table 3-2.** The relative abundance (% of total reads) of the twelve most abundant lowest resolved groups (to genus when possible) within each treatment for prokaryotes. Results are presented as means of triplicate incubations (SD as percent mean in parentheses).

Taxa	Control	Taxa	P-Only	Taxa	Snow	Taxa	Salt
Comamonadaceae unclass.	22 (52)	<i>Roseateles</i> sp.	12 (31)	Comamonadaceae unclass.	14 (23)	Comamonadaceae unclass.	18 (10)
<i>Roseateles</i> sp.	15 (34)	Comamonadaceae unclass.	12 (19)	ML635J-21 unclass.	11 (87)	FamilyI unclass.	8 (57)
<i>Polynucleobacter</i> sp.	8 (85)	<i>Polynucleobacter</i> sp.	10 (21)	<i>Polynucleobacter</i> sp.	10 (30)	<i>Polynucleobacter</i> sp.	6 (17)
<i>Albidiferax</i> sp.	5 (13)	<i>Flavobacterium</i> sp.	10 (87)	<i>Roseateles</i> sp.	9 (16)	NS11-12 marine group unclass.	5 (17)
<i>Rhodobacter</i> sp.	4 (28)	<i>Albidiferax</i> sp.	5 (51)	<i>Albidiferax</i> sp.	6 (32)	<i>Roseateles</i> sp.	5 (46)
<i>Flavobacterium</i> sp.	3 (66)	<i>Caulobacter</i> sp.	5 (43)	<i>Flavobacterium</i> sp.	4 (38)	<i>Caulobacter</i> sp.	4 (36)
<i>Flectobacillus</i> sp.	3 (171)	ML635J-21 unclass.	4 (155)	NS11-12 marine group unclass.	3 (116)	<i>Albidiferax</i> sp.	4 (44)
<i>Caulobacter</i> sp.	3 (45)	FamilyI unclass.	3 (124)	Proteobacteria unclass.	3 (132)	<i>Candidatus Odyssella</i>	3 (169)
PRD01a011B	3 (159)	Proteobacteria unclass.	3 (122)	Sphingomonadaceae unclass.	3 (49)	Rhizobiales unclass.	3 (57)
<i>Pseudarcicella</i> sp.	3 (52)	Myxococcales unclass.	3 (45)	GKS98 freshwater group	2 (35)	<i>Sediminibacterium</i> sp.	3 (39)
<i>Ferruginibacter</i> sp.	2 (39)	<i>Rhodobacter</i> sp.	3 (76)	<i>Caulobacter</i> sp.	2 (28)	ML635J-21 unclass.	3 (118)
OM27 clade	2 (168)	<i>Pseudarcicella</i> sp.	2 (52)	Planctomycetaceae unclass.	2 (31)	Myxococcales unclass.	2 (81)

The Salt and Snow treatments formed two distinct clusters, indicating that the microbial community responded differently to the chloride supplied alone versus at the same concentration in the snowmelt cocktail (Fig. 3-2). Seven bacterial genera, representing a total of around 6% of the community reads increased in relative abundance in the Salt treatment (ANOVA and Tukey HSD, Table S3-1). The most abundant were *Synechococcus* sp. (0.63%), *Chtonibacter* sp. (0.07%), and *Pirellula* sp. (0.01%). The filamentous cyanobacterium *Pseudanabaena* sp. was detected solely in the Salt treatment (ANOVA;  $F=235.6$ ;  $p<0.01$ ), while other salt-stimulated genera were found in all the other treatments, but at 2-10 times lower abundance (Table S3-1). In the Snow treatment, some prokaryotic taxa showed an increase in relative abundance, notably the psychrophilic/psychrotolerant genus *Psychrobacter* (present only in low abundance in the lake, around 0.001% of total reads), *Hirschia* sp., and *Planctomyces* sp. These taxa collectively represented <2% of the total community reads. For the eukaryotes, the most conspicuous change was that of an unclassified cryptophyte in the SA1-3C06 clade (Table S3-2), which represented around 1% of the community reads in the other treatments, but became co-dominant in the Salt treatment with a mean relative abundance of 18% (Table 3-3). *Mallomonas* also responded to chloride addition (Table S3-2) by a 20-fold increase in abundance, but still represented less than 1% to the total reads. The chrysophyte *Oikomonas* (Table S3-2) was detected only in the Salt treatment. There were no significant differences among the Control, P-only and Snow treatments in any eukaryotic taxon.

**Table 3-3.** The relative abundance (% of total reads) of the twelve most abundant lowest resolved groups (to genus when possible) within each treatment for eukaryotes. Results are presented as means of triplicates (SD as percent mean).

Taxa	Control	Taxa	P-Only	Taxa	Snow	Taxa	Salt
<i>Cyclidium</i> sp.	30 (86)	<i>Cyclidium</i>	27 (38)	<i>Cyclidium</i> sp.	23 (87)	<i>Cyclidium</i> sp.	21 (55)
Conthreep unclass.	16 (77)	Conthreep unclass.	26 (113)	Cercozoa unclass.	13 (82)	SA1-3C06 unclass.	18 (72)
Chrysophyceae _unclass.	9 (18)	Choreotrichia unclass.	10 (116)	Conthreep unclass.	10 (60)	A31 unclass.	13 (77)
Choreotrichia unclass.	8 (170)	Haptoria unclass.	7 (173)	<i>Rimostrombidium</i> sp.	10 (173)	Cercozoa unclass.	10 (73)
Cercozoa unclass.	6 (92)	Spirotrichea unclass.	7 (132)	Choanoflagellida unclass.	6 (131)	Chrysophyceae unclass.	9 (54)
Choanoflagellida unclass.	6 (173)	Chrysophyceae unclass.	4 (71)	Chrysophyceae unclass.	4 (97)	Conthreep unclass.	9 (79)
Glissomonadida unclass.	5 (173)	Pedinellales unclass.	3 (127)	Choreotrichia unclass.	4 (123)	Choreotrichia unclass.	6 (133)
<i>Leucocryptos</i> sp.	2 (83)	<i>Epipyxis</i> sp.	2 (173)	A31 unclass.	4 (91)	<i>Paraphysomonas</i> sp.	3 (169)
A31 unclass.	2 (138)	<i>Leucocryptos</i> sp.	1 (158)	Pedinellales unclass.	2 (73)	<i>Epipyxis</i> sp.	3 (42)
SA1-3C06 unclass.	1 (157)	Cercozoa unclass.	1 (35)	AMT-15-27-30	2 (173)	<i>Synura</i> sp.	1 (72)
Pedinellales unclass.	1 (39)	A31 unclass.	1 (81)	SA1-3C06 unclass.	2 (170)	Haptoria unclass.	1 (171)
<i>Chrysamoeba</i> sp.	1 (119)	<i>Bolidomonas</i> sp.	1 (141)	Spirotrichea unclass.	1 (121)	<i>Bolidomonas</i> sp.	1 (60)

### 3.4 Discussion

The aim of this study was to evaluate the effects of environmentally relevant concentrations of chloride on microbial community structure when supplied alone (Salt treatment) or in combination with urban snowmelt (Snow treatment). The added chloride changed the Lake Saint-Charles microbial community structure during this incubation under simulated spring warming, which itself also modified the community. The chloride effect was particularly strong on the eukaryotic community but differed markedly between the Snow and the Salt treatments, with a much more pronounced effect in the latter.

The 2-week exposure to chloride at 50 mg L<sup>-1</sup> changed the relative abundance of many bacteria and eukaryotes. These results contrast with laboratory studies on single species in which Cl concentrations higher than 1000 mg L<sup>-1</sup> are needed to induce detrimental effects, for example in terms of reduced growth or photosynthesis (Simmons 2012). In communities of multiple species, trophic and other interactions may influence and even offset the direct toxicity of chloride (Bray et al. 2019). For example, exposure of *Daphnia pulex* to chloride induced the production of stress eggs (ephyppia), but not when coupled with the threat of predation by rainbow trout, suggesting that this crustacean did not have sufficient energy to deal with both stressors combined (Hintz and Relyea 2017a). In addition to predation, the exposure to chloride may also impact bacterivory (Denis et al. 2010), parasitism (Merrick and Searle 2019), and competition (Venâncio et al. 2017). For example, *Raphidocelis subcapitata* outcompeted *Chlorella vulgaris* (two green algae) when they were grown together in controlled conditions, but with increasing salinities, the more halotolerant *C. vulgaris* achieved similar or even higher growth rates (Venâncio et al. 2017). These studies suggest that even at sub-lethal concentrations, chloride may affect interactions with other species, with consequences for ecological success. The response of the Lake Saint-Charles microbial community to increased chloride is more likely the result of modified interactions due to differences in sub-lethal tolerance, rather than any niche opening due to mortality of salt-sensitive species.

Among the bacterial taxa that were found to increase in response to the Salt treatment, only the cyanobacterial genera *Synechococcus* and *Pseudanabaena* have been previously identified as halotolerant. *Synechococcus* is frequently encountered in habitats of diverse

salinity with the replacement of freshwater strains by estuarine then marine strains along the downstream-offshore gradient (Herlemann et al. 2011). Nonetheless, individual strains have broad salinity tolerances that may extend over more than an order of magnitude range in salt concentrations (Junier et al. 2013). The exceptional halotolerance of freshwater *Synechococcus* strains has been attributed to their ability to pump out cellular sodium and to produce osmolytes and exopolysaccharides, as well as a capacity to resist cell volume changes associated with the entry of ions and water into the cell (Ladas and Papageorgiou 2000). *Pseudanabaena* is a commonly identified genus in freshwater plankton (Öterler 2017) but has also been identified in many brackish and marine ecosystems (Caroppo et al. 2006). Positive correlations have been observed between *Pseudanabaena* sp. and salinity (Zhao et al. 2015), as well as chloride (Miranda and Krishnakumar 2015). Any salt-induced changes in community structure towards cyanobacteria may be detrimental to the freshwater food web, since although some taxa can be grazed by zooplankton, they have low nutritional quality and their ingestion can result in reduced growth rates (e.g., *Daphnia pulex* feeding on *Synechococcus*; Przytulska et al. 2015).

In the present study, the microbial communities were composed of a few dominants and a large number of rare taxa ('the rare biosphere'), as is typically found in natural assemblages (Coveley et al. 2015). All of the taxa that responded positively to the Salt treatment, for both eukaryotes and prokaryotes, were originally present at low relative abundance, highlighting the importance of the rare biosphere as a reservoir of diversity and potential resilience of the ecosystem. Similar effects have been noted in marine-influenced freshwater pools, where community shifts were attributed to the growth of rare taxa rather than the arrival of new species (Zhang et al. 2014). Compensatory growth by rare taxa can be observed when dominant taxa are negatively affected by the perturbation, leaving a niche open for the success of others (Coveley et al. 2015). However, the response to small perturbations is more likely to be a shift in species proportions than a complete replacement of the community (Brown et al. 2016), and ongoing functional resilience. For example, a mesocosm study of pesticide impacts on freshwater microbial plankton showed that while the community shifted at fine-scale phylogenetic levels, there was a replacement by taxa in the same genera, and an overall maintenance of metabolic capabilities (Barobosa da Costa et al. 2020).

In the Salt treatment of our experiment, the chemical stress was insufficient to induce a shift in dominance. For the eukaryote 18S rRNA sequences, the ciliate *Cyclidium* sp. continued to dominate, but the taxon ‘un-classified conthreep’ decreased while the cryptophyte SA1-3C06 increased. The chrysophyte *Mallomonas* sp. and all prokaryotes that responded to the Salt treatment were among the rare taxa and despite their increase, had a final abundance of <1%. These responses indicate rare taxa that may become more prominent if a salt perturbation persists or becomes more severe and underscore the need to identify changes at the lowest taxonomic resolution possible.

While the chloride added alone led to pronounced changes in community structure, the exposure to the same chloride concentrations within the urban snowmelt addition did not. The Snow treatment led only to the increased abundance of three prokaryotic taxa, one of which, *Psychrobacter*, is psychrophilic or psychrotolerant, and has also been associated with high salinities and anthropogenic pressure such as aquatic environments polluted with hydrocarbons (Azevedo et al. 2013). This population may have been derived from the snow itself and was not a response to chloride from the Snow treatment. These results imply that contaminants in the urban snow did not lead to adverse effects on the microbial community, and that one or more components of the snowmelt cocktail moderated the microbial response to increased chloride. The principal contaminants measured in the snow were road salts, heavy metals, and PAHs, which are known to be the major components of urban snow elsewhere (Reinosdotter and Viklander 2007; Bartlett et al. 2012b, 2012a). However, their concentrations in our snowmelt sample were low compared to polluted sites elsewhere, and closer to those values measured at reference or suburban sites (Moghadas et al. 2015). The snow used in this study was from an urban snow disposal site and was likely collected from roads before it had time to substantially accumulate contaminants, in contrast to snowbanks that persist along roads throughout winter that accumulate contaminants (Moghadas et al. 2015; Fournier et al. 2020).

Two factors that are known to decrease chloride toxicity are food or nutrients levels (Brown and Yan 2015; Park et al. 2020) and alkalinity/calcium (e.g., Simmons 2012). For example, the growth rate of the green alga *Scenedesmus quadricauda* was less negatively affected in response to increasing salinity when higher nitrogen and phosphorus levels were

also provided (Park et al. 2020). For the macrophyte *Lemna minor* and the green alga *Pseudokirchneriella subcapitata*, chloride was less toxic when added with calcium rather than sodium (Simmons 2012). In the present study, the concentration of dissolved organic carbon, total nitrogen and total phosphorus were not higher in the Snow than in the Salt treatment, but calcium concentrations and alkalinity were higher. Although the difference in alkalinity and calcium between the Snow and the Salt treatments was modest as compared with the Simmons (2012) study, it may have increased the tolerance of the lake microbiome to salt stress.

Microcosm experiments have a number of limitations that need to be considered when interpreting the data presented here. In the analysis of nutrient enrichment effects, there can be good consistency between small volume results and whole ecosystem responses (Spivak et al. 2011). However, the release from grazing pressure by removing large zooplankton, the effects of the container surface, the change in mixing regime and advection, and the removal from any influence by the lower water column and sediments are all factors that may influence the microbial community and its responses to chemical perturbation. In the case of chloride exposure, assays based on actual snowmelt mixtures as opposed to a single salt are one step closer to understanding the actual environment responses. However, our experimental design did not take into account the spatio-temporal separation of ions and contaminants that may occur as a result of the melting process and the movement of the resulting runoff from the roads to the receiving waters. When urban snow melts, the dissolved chemicals are mobilized in the runoff and a proportion of chemicals that are adsorbed to particles are left behind. There is also evidence for differential elution among the mobilized chemicals, with chloride leaving the snowpack sooner than other constituents (Reinosdotter and Viklander 2007; Westerlund and Viklander 2011). This could lead to successive waves of ion and contaminant inputs to road-influenced lakes, with different compositions in each wave. It is possible, for example, that chloride and calcium do not reach the receiving waters at the same time, as chloride leaves the snowpack earlier than calcium (Fournier et al. 2020), which would delay any offsetting effect.

Controlled laboratory experiments are useful tools to evaluate the toxicity of contaminants to organisms or community. However, in the case of a more complex exposure

to multiple stresses with landscape scaling questions, as with snowmelt, there is a need for whole lake or whole watershed monitoring and analysis. This would address the multiple factors that may influence both the chemical loading and the response of the microbial communities, such as higher trophic levels (zooplankton and fish), spring turnover and temporal separation of the ions and contaminants in lake water that result from the melting process and their interactions with soils. In the same vein, routinely including chloride along with conductivity measurements in monitoring programs would greatly increase the data available for meta-analysis across a full range of urban lakes, many of which are already monitored for nutrients, harmful algal blooms and drinking water quality.

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## **Conclusion générale**

De plus en plus de lacs d'eau douce des régions tempérées se salinisent en réponse à l'apport important d'ions des milieux urbanisés, particulièrement des ions chlorures et sodium associés aux sels de déglaçage. Bien que les concentrations de chlorures atteintes par la plupart de ces lacs salinisés se situent largement sous les critères chroniques de protection de la vie aquatique, elles sont généralement beaucoup plus élevées que les valeurs de référence. Cette thèse s'est penchée sur l'identification d'endroits et de moments critiques concernant l'apport d'ions aux lacs et sur les réponses des communautés planctoniques microbiennes à ces augmentations de la salinité, mais aussi plus précisément de la concentration en chlorures, en milieu naturel et dans un contexte expérimental.

### **Hétérogénéité spatio-temporelle des ions dans la neige, les rivières et les lacs durant l'hiver et le printemps**

La concentration de différents ions dans la neige et les rivières du bassin versant de la prise d'eau potable de la rivière Saint-Charles variait de façon importante, tant dans l'espace que dans le temps. Cette hétérogénéité spatio-temporelle était particulièrement marquée pour les ions chlorures et sodium.

Dans la neige en bordure des routes, la concentration de tous les éléments mesurés, à l'exception de l'azote, diminuait de façon exponentielle en fonction de la distance. À 1 m des routes, on retrouvait plusieurs centaines de  $\text{mg L}^{-1}$  de chlorures et de sodium, une centaine de  $\text{mg L}^{-1}$  de calcium, en plus de concentrations élevées de phosphore total (de l'ordre du  $\text{mg L}^{-1}$ ) et de plusieurs métaux. À 20 m des routes, on retrouvait plutôt des valeurs plus typiques d'une neige fraîchement tombée. Ces résultats suggèrent que les routes, ainsi que les activités qui y sont associées, sont une source importante d'ions dans l'environnement, particulièrement de chlorures, de sodium, de calcium et de phosphore.

Une différence importante dans la concentration de ces ions a aussi été observée dans les rivières, en fonction du niveau d'urbanisation de leur bassin versant. En effet, les rivières dont le bassin versant était plus urbanisé (avec un seuil à moins de 2% du territoire urbanisé) avaient des concentrations de chlorures et de sodium, ainsi que de calcium et de phosphore, respectivement 10 et 2 fois plus élevées que celles en milieu forestier. De plus, ces rivières

urbaines avaient, proportionnellement aux autres ions, plus de chlorures et de sodium que les rivières en milieu forestier.

La variation temporelle de la concentration des ions dans la neige et les rivières, ainsi que celle de la conductivité du Lac Saint-Charles, était principalement reliée aux épisodes de fonte de neige. Lors de ces épisodes de fonte, la concentration des ions de la neige diminuait, alors que leur concentration dans les rivières et la conductivité du lac augmentaient. Contrairement à notre hypothèse, les épisodes de fonte n'étaient pas limités au printemps, mais survenaient aussi durant l'hiver. Ces épisodes de fonte hivernaux sont particulièrement importants, puisque leur amplitude était similaire à celui de la fonte de printemps, c'est-à-dire qu'ils ont causé une augmentation relative de la conductivité aussi importante, mais que leur augmentation a duré plus longtemps. Les résultats suggèrent aussi une élution différentielle des ions présents dans la neige, notamment une élution préférentielle des ions chlorures et sodium.

Dans un contexte de gestion et de prévention de l'augmentation de la salinité de façon générale, et des chlorures de façon plus précise, les variations spatiales suggèrent que pour être plus efficaces, des endroits particuliers pourraient être ciblés, notamment le bord des routes importantes et les quartiers les plus urbanisés, alors que les variations temporelles impliquent qu'il est important de considérer à la fois les apports de l'hiver et du printemps. L'implantation d'unités de gestion de l'eau de ruissellement capables de traiter, ou du moins de retenir ou de dévier, l'eau salée à l'hiver et au printemps réduirait de façon considérable l'apport des ions aux lacs.

## **Importance de la salinité et de l'urbanisation comme variables structurantes des communautés microbiennes en milieu naturel**

Après les saisons, ou plus précisément la présence ou l'absence d'un couvert de glace, la salinité était la variable la plus corrélée aux variations de composition taxonomique des communautés microbiennes des lacs à l'étude. Ce résultat était le même que les communautés aient été déterminées suite à l'analyse des pigments photosynthétiques, au comptage par microscopie optique ou par le séquençage à haut débit.

La présence d'un couvert de glace influence de façon importante la plupart des variables limnologiques, résultant en un environnement qui est très différent de ce qu'on trouve lorsque l'eau est libre. Par exemple, elle diminue la pénétration de la lumière et l'intensité du brassage. Pour les bactéries, la période avec un couvert de glace était associée avec des genres ayant un métabolisme oxydatif, par exemple *Methylobacter* et l'oxydation du méthane, alors que la période d'eau libre était associée avec la photosynthèse, via diverses cyanobactéries. Pour les eucaryotes, la période avec un couvert de glace était associée à des mixotrophes flagellés tels que des cryptophytes et des chrysophytes, alors que la période d'eau libre était moins caractéristique.

Dans les lacs avec la salinité, et l'urbanisation, la plus élevée, on retrouvait davantage des chrysophytes et de cryptophytes, notamment des genres ayant déjà été identifiés comme halotolérants comme *Cryptomonas*. Au lac Saint-Augustin, qui avait à la fois l'urbanisation la plus élevée, une salinité élevée et était eutrophe, on retrouvait en plus une augmentation significative de l'abondance de cyanobactéries filamentueuses pouvant former des floraisons toxiques. En comparaison, les lacs avec la salinité, et l'urbanisation, la moins élevée étaient associés avec une plus grande abondance de chrysophytes et de ciliés.

## Réponses des communautés microbiennes aux chlorures dans un contexte expérimental

L'exposition des communautés microbiennes du Lac Saint-Charles aux chlorures à une faible concentration a résulté en des changements de composition taxonomique, particulièrement pour les eucaryotes. En effet, après deux semaines d'exposition à une concentration de 50 mg Cl L<sup>-1</sup>, l'abondance relative d'un cryptophyte du clade SA1-3C06 a augmenté d'un facteur 20. Ce dernier est même devenu co-dominant, alors qu'il représentait moins de 1% de la communauté de départ. Les autres groupes dont l'abondance relative a augmenté en réponse à l'exposition aux chlorures représentaient aussi moins de 1% de la communauté de départ (les membres de la biosphère dits rares), mais n'ont pas augmenté de façon aussi importante et sont restés rares. Parmi ceux-là, on retrouve le chrysophage *Mallomonas* sp. et les cyanobactéries *Synechococcus* sp. et *Pseudanabaena* sp.

L'exposition aux chlorures, toujours à 50 mg Cl L<sup>-1</sup>, combinée à la neige urbaine n'a pas causé de changements aussi marqués dans la composition taxonomique. Seuls quelques

groupes ont vu leur abondance relative augmenter, dont *Psychrobacter* un genre psychrotolérant, voire psychrophile, provenant potentiellement de la neige elle-même. Ces résultats suggèrent qu'une composante de la neige urbaine a atténué les effets des chlorures, soit en diminuant sa toxicité ou en causant des effets antagonistes qui balançait les changements. Suite à l'analyse de la composition chimique de la neige, notre hypothèse est que l'atténuation des effets a été causée par une plus grande alcalinité, ou une plus grande concentration de carbonates ou de calcium.

Les résultats de cette expérience de laboratoire suggèrent que les chlorures en faible concentration ont le potentiel de modifier la composition taxonomique des communautés microbiennes, mais que l'exposition aux chlorures seuls ne représente pas correctement, voir surestime, les effets attendus dans le milieu naturel.

## **Implication scientifique**

La salinisation de l'eau douce est un problème grandissant dans les régions tempérées et nordiques. Des corrélations ont fréquemment été observées entre ce phénomène et l'urbanisation du territoire. Par exemples, la proportion de surfaces pavées et la densité de routes dans un rayon de 500m autour d'un lac étaient les meilleurs prédicteurs d'une tendance à long-terme à la hausse de la concentration en chlorure (Amérique du Nord, excluant le Québec; Dugan et al. 2017a) et il y avait une relation positive entre la proportion de surfaces pavées dans le bassin versant de rivières et les concentrations de chlorures, de sodium, de calcium et de bicarbonate (États-Unis; Bird et al. 2018). Dans le même ordre d'idée, dans la neige au bord de la route, les concentrations de chlorures et de sodium, ainsi que de plusieurs métaux, étaient positivement corrélées avec le trafic (Norvège; Moghadas et al. 2015) et négativement corrélées avec la distance à la route (Finlande; Hautala et al. 1995). Cette thèse a permis d'observer que ces corrélations étaient aussi valides au Québec, qui est un des endroits en Amérique du Nord où l'on utilise le plus de sels de voirie, mais sur lequel on a le moins d'information et de suivis (Evans et Frick 2001; Dugan et al. 2017a). Cela implique que des gestionnaires peuvent explorer des travaux effectués dans d'autres régions lorsque des données locales plus précises ne sont pas disponibles.

Cette thèse s'est aussi penchée sur les voies qu'empruntent les ions responsables de cette salinisation pour se déplacer de leur source (les routes) vers les plans d'eau, ainsi que sur les moments auxquels ces déplacements ont lieu. Les données suggèrent qu'en hiver, le ruissellement de surface est l'écoulement préférentiel, alors qu'une percolation à-travers le sol semble avoir davantage lieu au printemps. Dans le cadre de ce suivi hivernal, la présence d'un mouillage dans le Lac Saint-Charles mesurant la conductivité à chaque heure a permis d'observer que la conductivité pouvait changer rapidement en réponse à des épisodes de fonte de neige (de l'ordre de quelques heures à quelques jours), même en période hivernale. Ces données sont particulièrement importantes pour la gestion des eaux de ruissellement, puisqu'elles impliquent que les méthodes utilisées doivent l'être de façon constante et ne peuvent pas seulement être mises en place en fin de saison ou suivant un événement précis comme l'augmentation de la température au-dessus de 0°C.

Les effets de la salinisation de l'eau douce sur les organismes aquatiques ont principalement été étudiés sur le zooplancton, les amphibiens et les poissons des rivières et des étangs, alors que les organismes vivant dans les lacs, particulièrement les micro-organismes, ont reçu moins d'attention. Le phytoplancton a parfois été intégré dans des études, mais leur intégration se limitait à une mesure de leur biomasse estimée par la concentration de chlorophylle a (e.g., Van Meter et al. 2011), ou à une identification à de grands groupes taxonomiques grâce à la combinaison de d'autres pigments photosynthétiques (e.g., Chakraborty et al. 2011). Cette thèse a évalué les effets d'une augmentation de la salinité sur les communautés microbiennes avec des méthodes permettant d'en étudier la composition taxonomique avec plus de précision, et ce, en milieu naturel et dans un contexte expérimental. En milieu naturel, la concentration de chlorures était une variable clé associée aux variations de composition taxonomique, particulièrement avec l'abondance de cryptophytes. Dans le contexte expérimental, l'ajout de chlorures a résulté en une variation de l'abondance relative de plusieurs groupes, notamment un cryptophyte du clade SA1-3C06. Cela suggère qu'une augmentation de l'abondance des cryptophytes pourrait être utilisée comme un marqueur potentiel de l'augmentation de la salinité. De façon générale, ces résultats impliquent que les chlorures influencent les écosystèmes aquatiques même à des niveaux se situant en-deçà des critères chroniques de protection de la vie aquatique (230 mg L<sup>-1</sup> pour le Québec et les États-Unis et de 120 mg L<sup>-1</sup> pour le Canada). Il

serait primordial que le Québec adopte, au minimum, les critères de protection de la vie aquatique canadiens. Ces résultats impliquent aussi que les chlorures, ou la salinité de façon générale, devraient être inclus dans les suivis environnementaux comme une variable confondante.

## Perspectives

Les résultats de cette thèse suggèrent que l'exposition combinée à de la neige urbaine et à une augmentation des chlorures pourraient réduire les effets de ces derniers sur les communautés microbiennes. Pour poursuivre ces travaux, d'autres expériences pourraient être mises en place, par exemple en augmentant l'alcalinité, les carbonates ou le calcium ou en utilisant de la neige prélevée en bordure de la route, laquelle serait potentiellement plus contaminée que la neige provenant d'un dépôt à neige.

La réponse des organismes à l'augmentation de la concentration en chlorures varient selon plusieurs autres facteurs chimiques ou biologiques. Il serait donc intéressant d'étudier les effets combinés de l'augmentation de la salinité avec la présence de niveaux trophiques supérieurs, comme le zooplancton ou les poissons, avec d'autres contaminants aussi retrouvés en zone urbaine comme les pesticides ou les médicaments, ou avec d'autres stresseurs naturels comme un changement de la température de l'eau, de la quantité d'oxygène ou de l'état trophique.

Des études récentes suggèrent que les organismes s'adaptent à l'augmentation de la concentration en chlorures, c'est-à-dire que les individus qui survivent à une exposition antérieure produisent une descendance qui est plus tolérante à une exposition future (e.g., Coldsnow et al. 2017; Hintz et al. 2018). Dans les milieux naturels, cette évolution de la tolérance pourrait survenir au cours d'une même saison dû aux variations de la concentration en chlorures suivant les multiples épisodes de fonte de neige ou au fil des ans dû à sa tendance à la hausse. Afin de mieux prendre en compte cette réalité, des expériences pourraient être mises en place pour comparer la réponse d'organismes provenant d'un lac dont la concentration en chlorures est stable, autant sur une année qu'entre les années, comme le Lac Clair utilisé comme lac témoin dans cette thèse, et celle d'organismes provenant d'un lac

avec de grandes variations annuelles et une tendance à long-terme à la hausse, comme c'était le cas des trois autres lacs à l'étude.

Dans le même ordre d'idée, il pourrait être intéressant de voir si les communautés microbiennes sont résilientes à l'augmentation de la salinité, c'est-à-dire de voir si 1) on retrouve les mêmes fonctions dans les communautés modifiées par l'augmentation des chlorures que dans les communautés de départ et si 2) les communautés peuvent retrouver leur composition taxonomique et leurs fonctions d'origine lorsque la salinité est remise à son niveau de départ. Ce concept de résilience a été observé sur des communautés bactériennes exposées au glyphosate (un herbicide, Barbosa da Costa et al. 2020).

Finalement, d'un point de vue de gestion et de prévention d'une augmentation de la salinité et de ses effets sur les écosystèmes aquatiques, il est impératif de continuer le développement de méthodes de contrôle d'ingénierie, vertes ou durables de préférence, comme celles mentionnées dans le chapitre 1. Combiner ce développement avec des meilleures pratiques d'utilisation des sels de voirie (e.g., pré-mouillage) ainsi que des suivis locaux pour cibler des endroits et des moments sensibles dans différents bassins versants pourrait faire une réelle différence sur notre capacité à continuer d'utiliser les sels de voirie lorsque nécessaire tout en réduisant leurs effets.

## Bibliographie

- Aghazadeh, N., Nojavan, M., and Mogaddam, A.A. 2012. Effects of road-deicing salt (NaCl) and saline water on water quality in the Urmia area, northwest of Iran. *Arabian Journal of Geosciences* **5**: 565–570. doi: [10.1007/s12517-010-0210-6](https://doi.org/10.1007/s12517-010-0210-6)
- [APEL] Association pour la protection de l'environnement du lac Saint-Charles et des Marais du Nord. 2011. Suivi du lac Clément Évaluation de la contamination par les sels de voirie.
- [APEL] Association pour la protection de l'environnement du lac Saint-Charles et des Marais du Nord. 2014. Diagnose du lac Saint-Charles, rapport final.
- [APEL] Association pour la protection de l'environnement du lac Saint-Charles et des Marais du Nord. 2015. Suivi du lac Saint-Charles – Bilan des campagnes 2011 à 2013.
- [APEL] Association pour la protection de l'environnement du lac Saint-Charles et des Marais du Nord. 2016. Lac Clair: Évaluation de l'état trophique, étude de la communauté cyanobactérienne, inventaire des herbiers aquatiques et évaluation de la conductivité spécifique - Faits saillants de la campagne d'échantillonnage 2015.
- Apprill, A., McNally, S., Parsons, R., and Weber, L. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology* **75**: 129–137. doi: [10.3354/ame01753](https://doi.org/10.3354/ame01753)
- Auinger, B.M., Pfandl, K., and Boenigk, J. 2008. Improved methodology for identification of protists and microalgae from plankton samples preserved in Lugol's iodine solution: Combining microscopic analysis with single-cell PCR. *Applied and Environmental Microbiology* **74**: 2505–2510. doi: [10.1128/AEM.01803-07](https://doi.org/10.1128/AEM.01803-07)
- Azevedo, J.S.N., Correia, A., and Henriques, I. 2013. Molecular analysis of the diversity of genus *Psychrobacter* present within a temperate estuary. *FEMS Microbiology Ecology* **84**: 451–460. doi: [10.1111/1574-6941.12075](https://doi.org/10.1111/1574-6941.12075)
- Baird, R.B., Rice, E.W., Eaton, A.D., editors. 2017. Standard methods for the examination of water and wastewater, 23rd ed, American Public Health Association.
- Balzano, S., Abs, E., and Leterme, S.C. 2015. Protist diversity along a salinity gradient in a coastal lagoon. *Aquatic Microbial Ecology* **74**: 263–277. doi: [10.3354/ame01740](https://doi.org/10.3354/ame01740)
- Banin, A., and Fish, A. 1995. Secondary desertification due to salinization of intensively irrigated lands: The Israeli experience. *Environmental Monitoring and Assessment* **37**: 17–37. doi: [10.1007/BF00546878](https://doi.org/10.1007/BF00546878)
- Barbosa da Costa, N., Fugère, V., Hébert, M.-P., Xu, C.C.Y., Barrett, R.D.H., Beisner, B., Bell, G., Yargeau, V., Fussmann, G.F., Gonzalez, A., and Shapiro, B.J. 2020. Resistance, resilience, and functional redundancy of freshwater microbial communities facing multiple agricultural stressors in a mesocosm experiment. *BioRxiv, Ecology*. doi: [10.1101/2020.04.12.038372](https://doi.org/10.1101/2020.04.12.038372)
- Bartlett, A.J., Rochfort, Q., Brown, L.R., and Marsalek, J. 2012a. Causes of toxicity to *Hyalella azteca* in a stormwater management facility receiving highway runoff and

- snowmelt. Part II: Salts, nutrients, and water quality. *Science of the Total Environment* **414**: 238–247. doi: [10.1016/j.scitotenv.2011.11.036](https://doi.org/10.1016/j.scitotenv.2011.11.036)
- Bartlett, A.J., Rochfort, Q., Brown, L.R., and Marsalek, J. 2012b. Causes of toxicity to *Hyalella azteca* in a stormwater management facility receiving highway runoff and snowmelt. Part I: Polycyclic aromatic hydrocarbons and metals. *Science of the Total Environment* **414**: 227–237. doi: [10.1016/j.scitotenv.2011.11.041](https://doi.org/10.1016/j.scitotenv.2011.11.041)
- Bartolomé, M.C., D'ors, A., and Sanchez-Fortun, S. 2009. Toxic effects induced by salt stress on selected freshwater prokaryotic and eukaryotic microalgal species. *Ecotoxicology* **18**: 174–179. doi: [10.1007/s10646-008-0269-y](https://doi.org/10.1007/s10646-008-0269-y)
- Bashenkhaeva, M.V., Zakharova, Y.R., Petrova, D.P., Khanaev, I.V., Yury, P., and Likhoshway, Y.V. 2015. Sub-ice microalgal and bacterial communities in freshwater Lake Baikal, Russia. *Microbial Ecology* **70**. doi: [10.1007/s00248-015-0619-2](https://doi.org/10.1007/s00248-015-0619-2)
- Bazin, P., Jouenne, F., Deton-Cabanillas, A.-F., Pérez-Ruzafa, A., and Véron, B. 2014. Complex patterns in phytoplankton and microeukaryote diversity along the estuarine continuum. *Hydrobiologia* **726**: 155–178. doi: [10.1007/s10750-013-1761-9](https://doi.org/10.1007/s10750-013-1761-9)
- Beall, B.F.N., Twiss, M.R., Smith, D.E., Oyserman, B.O., Rozmarynowycz, M.J., Binding, C.E., Bourbonniere, R.A., Bullerjahn, G.S., Palmer, M.E., Reavie, E.D., Waters, L.M.K., Woityra, L.W.C., and McKay, R.M.L. 2016. Ice cover extent drives phytoplankton and bacterial community structure in a large north-temperate lake: implications for a warming climate: Effect of ice cover on microbial community structure. *Environmental Microbiology* **18**: 1704–1719. doi: [10.1111/1462-2920.12819](https://doi.org/10.1111/1462-2920.12819)
- Bégin, P.N., Rautio, M., Tanabe, Y., Uchida, M., Culley, A.I. and Vincent W.F. 2020. The littoral zone of polar lakes: Inshore-offshore contrasts in an ice-covered High Arctic lake. *Arctic Science* **6**. doi: [10.1139/as-2020-0026](https://doi.org/10.1139/as-2020-0026)
- Benlloch, S., López-lópez, A., Casamayor, O., Øvreås, L., Goddard, V., Daae, F.L., Smerdon, G., Massana, R., Joint, I., Thingstad, F., Pedrós-alió, C., and Rodríguez-valera, F. 2002. Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. *Environmental Management* **4**: 349–360. doi: [10.1046/j.1462-2920.2002.00306.x](https://doi.org/10.1046/j.1462-2920.2002.00306.x)
- Bennke, C.M., Pollehne, F., Müller, A., Hansen, R., Kreikemeyer, B., and Labrenz, M. 2018. The distribution of phytoplankton in the Baltic Sea assessed by a prokaryotic 16S rRNA gene primer system. *Journal of Plankton Research* **40**: 244–254. doi: [10.1093/plankt/fby008](https://doi.org/10.1093/plankt/fby008)
- Berezina, N.A. 2003. Tolerance of freshwater invertebrates to changes in water salinity. *Russian Journal of Ecology* **34**: 296–301. doi: [10.1023/A:1024597832095](https://doi.org/10.1023/A:1024597832095)
- Bergeron, M., Corbeil, C., and Arsenault, S. 2002. Diagnose écologique du lac Saint-Augustin. Document préparé pour la municipalité de Saint-Augustin-de-Desmaures par EXXEP Environnement.
- Bertilsson, S., Burgin, A., Carey, C.C., Fey, S.B., Grossart, H.P., Grubisic, L.M., Jones, I.D., Kirillin, G., Lennon, J.T., Shade, A., and Smyth, R.L. 2013. The under-ice

- microbiome of seasonally frozen lakes. *Limnology and Oceanography* **58**: 1998–2012. doi: [10.4319/lo.2013.58.6.1998](https://doi.org/10.4319/lo.2013.58.6.1998)
- Betts, A.R., Gharabaghi, B., and McBean, E.A. 2014. Salt vulnerability assessment methodology for urban streams. *Journal of Hydrology* **517**: 877–888. doi: [10.1016/j.jhydrol.2014.06.005](https://doi.org/10.1016/j.jhydrol.2014.06.005)
- Bird, D.L., Gro, P.M., Salice, C.J., and Moore, J. 2018. Steady-state land cover but non-steady-state major ion chemistry in urban streams. *Environmental Science and Technology* **52**: 13015–13026. doi: [10.1021/acs.est.8b03587](https://doi.org/10.1021/acs.est.8b03587)
- Blasius, B.J., and Merritt, R.W. 2002. Field and laboratory investigations on the effects of road salt (NaCl) on stream macroinvertebrate communities. *Environmental Pollution* **120**: 219–231. doi: [10.1016/S0269-7491\(02\)00142-2](https://doi.org/10.1016/S0269-7491(02)00142-2)
- Blazewicz, S.J., Barnard, R.L., Daly, R.A., and Firestone, M.K. 2013. Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *The ISME Journal* **7**: 2061–2068. doi: [10.1038/ismej.2013.102](https://doi.org/10.1038/ismej.2013.102)
- Blinn, D.W., and Bailey, P.C.E. 2001. Land-use influence on stream water quality and diatom communities in Victoria, Australia: A response to secondary salinization. *Hydrobiologia* **466**: 231–244. doi: [10.1023/A:1014541029984](https://doi.org/10.1023/A:1014541029984)
- Blinn, D.W., Halse, S.A., Pinder, A.M., Shiel, R.J., and Mcrae, J.M. 2004. Diatom and micro-invertebrate communities and environmental determinants in the western Australian wheatbelt: A response to salinization. *Hydrobiologia* **528**: 229–248. doi: [10.1007/s10750-004-2350-8](https://doi.org/10.1007/s10750-004-2350-8)
- Blomqvist, G., and Johansson, E.-L. 1999. Airborne spreading and deposition of de-icing salt - a case study. *Science of the Total Environment* **235**: 161–168. doi: [10.1016/S0048-9697\(99\)00209-0](https://doi.org/10.1016/S0048-9697(99)00209-0)
- Bolen, W. 2020. Salt statistics and information. USGS. [<https://prd-wret.s3-us-west-2.amazonaws.com/assets/palladium/production/atoms/files/mcs-2019-salt.pdf>], consulté le 26 novembre 2020.
- Bouvier, T.C., and del Giorgio, P.A. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnology and Oceanography* **47**: 453–470. doi: [10.4319/lo.2002.47.2.0453](https://doi.org/10.4319/lo.2002.47.2.0453)
- Boxall, A.B.A., and Maltby, L. 1997. The Effects of Motorway Runoff on Freshwater Ecosystems: 3. Toxicant Confirmation. *Archives of Environmental Contamination and Toxicology* **33**: 9–16. doi: [10.1007/s002449900216](https://doi.org/10.1007/s002449900216)
- Bray, J.P., Reich, J., Nichols, S.J., Kon Kam King, G., Mac Nally, R., Thompson, R., O'Reilly-Nugent, A., and Kefford, B.J. 2019. Biological interactions mediate context and species-specific sensitivities to salinity. *Philosophical Transactions of the Royal Society B* **374**: 20180020. doi: [10.1098/rstb.2018.0020](https://doi.org/10.1098/rstb.2018.0020)
- Brin, M.-È. 2007. Étude de la biodisponibilité des contaminants (éléments traces métalliques et phosphore) contenus dans les sédiments du lac Saint-Augustin (Québec). Master's thesis. Université Laval.

- Brisson, J., Blois, S.D., and Lavoie, C. 2010. Roadside as invasion pathway for common reed (*Phragmites australis*). *Invasive Plant Science and Management* **3**: 506–514. doi: [10.1614/IPSM-09-050.1](https://doi.org/10.1614/IPSM-09-050.1)
- Brown, A.H., and Yan, N.D. 2015. Food quantity affects the sensitivity of *Daphnia* to road salt. *Environmental Science and Technology* **49**: 4673–4680. doi: [10.1021/es5061534](https://doi.org/10.1021/es5061534)
- Brown, B.L., Downing, A.L., and Leibold, M.A. 2016. Compensatory dynamics stabilize aggregate community properties in response to multiple types of perturbations. *Ecology* **97**: 2021–2033. doi: [10.1890/15-1951.1](https://doi.org/10.1890/15-1951.1)
- Bulskaya, I., and Volchek, A. 2014. Inorganic constituents in surface runoff from urbanised areas in winter: The case study of the city of Brest, Belarus. *Oceanologia* **56**: 373–383. doi: [10.5697/oc.56-2.373](https://doi.org/10.5697/oc.56-2.373)
- Bush, E., and Lemmen, D.S. editors. 2019. Canada's changing climate report. Ottawa (ON): Government of Canada.
- Bushnell, B., Rood, J., and Singer, E. 2017. BBMerge – Accurate paired shotgun read merging via overlap. *PLoS ONE* **12**: e0185056. doi: [10.1371/journal.pone.0185056](https://doi.org/10.1371/journal.pone.0185056)
- Butts, E., and Carrick, H.J. 2017. Phytoplankton seasonality along a trophic gradient of temperate lakes: Convergence in taxonomic composition during winter ice-cover. *Northeastern Naturalist* **24**: B167–B187. doi: [10.1656/045.024.s719](https://doi.org/10.1656/045.024.s719)
- Caroppo, C., Turicchia, S., and Margheri, M.C. 2006. Phytoplankton assemblages in coastal waters of the northern Ionian Sea (eastern Mediterranean), with special reference to cyanobacteria. *Journal of the Marine Biological Association of the United Kingdom* **86**: 927–937. doi: [10.1017/S0025315406013889](https://doi.org/10.1017/S0025315406013889)
- Casamayor, E.O., Massana, R., Benlloch, S., Øvreås, L., Díez, B., Goddard, V.J., Gasol, J.M., Joint, I., Rodríguez-valera, F., and Pedrós-alió, C. 2002. Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environmental Microbiology* **4**: 338–348. doi: [10.1046/j.1462-2920.2002.00297.x](https://doi.org/10.1046/j.1462-2920.2002.00297.x)
- Cavaliere, E., and Baulch, H.M. 2019. Winter nitrification in ice-covered lakes. *PLoS ONE* **14**: e0224864. doi: [10.1371/journal.pone.0224864](https://doi.org/10.1371/journal.pone.0224864)
- [CCME] Canadian Council of Ministers of the Environment. 2011. Scientific Criteria. Document for the development of the Canadian water quality guidelines for the protection of aquatic life: chloride ion. PN 1460. ISBN 978-1-896997-77-3 PDF. Winnipeg (ON).
- Celepli, N., Sundh, J., Ekman, M., Dupont, C.L., Yooseph, S., Bergman, B., and Ininbergs, K. 2017. Meta-omic analyses of Baltic Sea cyanobacteria: diversity, community structure and salt acclimation. *Environmental Microbiology* **19**: 673–686. doi: [10.1111/1462-2920.13592](https://doi.org/10.1111/1462-2920.13592)
- Chakraborty, P., Acharyya, T., Babu, P.V.R., and Bandhyopadhyay, D. 2011. Impact of salinity and pH on phytoplankton community in a tropical freshwater system : An investigation with pigment analysis by HPLC. *Journal of Environmental Monitoring* **13**: 614–620. doi: [10.1039/c0em00333f](https://doi.org/10.1039/c0em00333f)

- Chapra, S.C., Dove, A., and Warren, G.J. 2012. Long-term trends of Great Lakes major ion chemistry. *Journal of Great Lakes Research* **38**: 550–560. doi: [10.1016/j.jglr.2012.06.010](https://doi.org/10.1016/j.jglr.2012.06.010)
- Choi, J., and Park, J.S. 2020. Comparative analyses of the V4 and V9 regions of 18S rDNA for the extant eukaryotic community using the Illumina platform. *Scientific Reports* **10**: 6519. doi: [10.1038/s41598-020-63561-z](https://doi.org/10.1038/s41598-020-63561-z)
- Cleave, M.L., Porcella, D.B., and Adams, D.V. 1981. The application of batch bioassay techniques to the study of salinity toxicity to freshwater phytoplankton. *Water Research* **15**: 573–584. doi: [10.1016/0043-1354\(81\)90020-8](https://doi.org/10.1016/0043-1354(81)90020-8)
- Clow, D.W., Ingersoll, G.P., Mast, M.A., Turk, J.T., and Campbell, D.H. 2002. Comparison of snowpack and winter wet-deposition chemistry in the Rocky Mountains, USA: implications for winter dry deposition. *Atmospheric Environment* **36**: 2337–2348. doi: [10.1016/S1352-2310\(02\)00181-4](https://doi.org/10.1016/S1352-2310(02)00181-4)
- Coldsnow, K.D., Mattes, B.M., Hintz, W.D., and Relyea, R.A. 2017. Rapid evolution of tolerance to road salt in zooplankton. *Environmental Pollution* **222**: 367–373. doi: [10.1016/j.envpol.2016.12.024](https://doi.org/10.1016/j.envpol.2016.12.024)
- Comeau, A.M., Li, W.K.W., Tremblay, J.-É., Carmack, E.C., and Lovejoy, C. 2011. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PLoS ONE* **6**: e27492. doi: [10.1371/journal.pone.0027492](https://doi.org/10.1371/journal.pone.0027492)
- Commission d'enquête sur le viaduc de la Concorde. 2007. [<https://apigq.qc.ca/wp-content/uploads/2013/08/CEIC-R-2446.pdf>], consulté le 3 avril 2019.
- Comte, J., Culley, A.I., Lovejoy, C., and Vincent, W.F. 2018. Microbial connectivity and sorting in a High Arctic watershed. *The ISME Journal* **12**: 2988–3000. doi: [10.1038/s41396-018-0236-4](https://doi.org/10.1038/s41396-018-0236-4)
- Contamin, R., and Ellison, A.M. 2009. Indicators of regime shifts in ecological systems: What do we need to know and when do we need to know it. *Ecological Applications* **19**: 799–816. doi: [10.1890/08-0109.1](https://doi.org/10.1890/08-0109.1)
- Cooper, C.A., Mayer, P.M., and Faulkner, B.R. 2014. Effects of road salts on groundwater and surface water dynamics of sodium and chloride in an urban restored stream. *Biogeochemistry* **121**: 149–166. doi: [10.1007/s10533-014-9968-z](https://doi.org/10.1007/s10533-014-9968-z)
- Corsi, S.R., Cicco, L.A.D., Lutz, M.A., and Hirsch, R.M. 2015. River chloride trends in snow-affected urban watersheds: Increasing concentrations outpace urban growth rate and are common among all seasons. *Science of the Total Environment* **508**: 488–497. doi: [10.1016/j.scitotenv.2014.12.012](https://doi.org/10.1016/j.scitotenv.2014.12.012)
- Coveley, S., Elshahed, M.S., and Youssef, N.H. 2015. Response of the rare biosphere to environmental stressors in a highly diverse ecosystem (Zodletone spring, OK, USA). *PeerJ* **3**:e1182. doi: [10.7717/peerj.1182](https://doi.org/10.7717/peerj.1182)
- Crevecoeur, S., Vincent, W.F., Comte, J., and Lovejoy, C. 2015. Bacterial community structure across environmental gradients in permafrost thaw ponds: methanotroph-rich ecosystems. *Frontiers in Microbiology* **6**: 192. doi: [10.3389/fmicb.2015.00192](https://doi.org/10.3389/fmicb.2015.00192)

- Crevecoeur, S., Vincent, W.F., Comte, J., Matveev, A., and Lovejoy, C. 2017. Diversity and potential activity of methanotrophs in high methane-emitting permafrost thaw ponds. *PLoS ONE* **12**: e0188223. doi: [10.1371/journal.pone.0188223](https://doi.org/10.1371/journal.pone.0188223)
- Crinson, L., and Martin, J. 2008. An assessment of the effect of de-icers on skidding accidents. TRL Published Project Report PPR220, Transport Research Laboratory, Wokingham.
- Cruaud, P., Vigneron, A., Fradette, M., Dorea, C.C., Culley, A.I., Rodriguez, M.J., and Charette, S.J. 2019a. Annual bacterial community cycle in a seasonally ice-covered river reflects environmental and climatic conditions. *Limnology and Oceanography* **65**: S21-S37. doi: [10.1002/limo.11130](https://doi.org/10.1002/limo.11130)
- Cruaud, P., Vigneron, A., Fradette, M.-S., Dorea, C.C., Culley, A.I., Rodriguez, M.J., and Charette, S.J. 2019b. Annual protist community dynamics in a freshwater ecosystem undergoing contrasted climatic conditions: The Saint-Charles River (Canada). *Frontiers in Microbiology* **10**: 2359. doi: [10.3389/fmicb.2019.02359](https://doi.org/10.3389/fmicb.2019.02359)
- Dailey, K.R., Welch, K.A., and Lyons, W.B. 2014. Evaluating the influence of road salt on water quality of Ohio rivers over time. *Applied Geochemistry* **47**: 25–35. doi: [10.1016/j.apgeochem.2014.05.006](https://doi.org/10.1016/j.apgeochem.2014.05.006)
- Denis, C.H.S., Pinheiro, M.D.O., Power, M.E., and Bols, N.C. 2010. Effect of salt and urban water samples on bacterivory by the ciliate, *Tetrahymena thermophila*. *Environmental Pollution* **158**: 502–507. doi: [10.1016/j.envpol.2009.08.014](https://doi.org/10.1016/j.envpol.2009.08.014)
- Dinno, A. 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.5. [<https://CRAN.R-project.org/package=dunn.test>], consulté le 2 novembre 2020.
- DiTommaso, A. 2004. Germination behavior of common ragweed (*Ambrosia artemisiifolia*) populations across a range of salinities. *Weed Science* **52**: 1002–1009. doi: [10.1614/WS-04-030R1](https://doi.org/10.1614/WS-04-030R1)
- Dos Santos, V.M., de Andrade, L.C., Tiecher, T., and de Oliveira Camargo, F.A. 2020. The urban pressure over the sediment contamination in a southern Brazil metropolis: The case of Diluvio stream. *Water, Air, and Soil Pollution* **231**: 156. doi: [10.1007/s11270-020-04504-2](https://doi.org/10.1007/s11270-020-04504-2)
- Duchesne, L., and Houle, D. 2008. Impact of nutrient removal through harvesting on the sustainability of the boreal forest. *Ecological Applications* **18**: 1642–1651. doi: [10.1890/07-1035.1](https://doi.org/10.1890/07-1035.1)
- Dugan, H.A., Bartlett, S.L., Burke, S.M., Doubek, J.P., Krivak-tetley, F.E., Skaff, N.K., Summers, J.C., Farrell, K.J., McCullough, I.M., Morales-williams, A.M., Roberts, D.C., Ouyang, Z., Scordo, F., Hanson, P.C., and Weathers, K.C. 2017a. Salting our freshwater lakes. *Proceedings of the National Academy of Sciences USA* **114**: 4453–4458. doi: [10.1073/pnas.1620211114](https://doi.org/10.1073/pnas.1620211114)
- Dugan, H.A., Summers, J.C., Skaff, N.K., Krivak-tetley, F.E., Doubek, J.P., Burke, S.M., Bartlett, S.L., Arvola, L., Monteith, D., Moore, K., Rogora, M., and Hanson, P.C. 2017b. Data Descriptor : Long-term chloride concentrations in North American and European freshwater lakes. *Scientific Data* **4**: 170101. doi: [10.1038/sdata.2017.101](https://doi.org/10.1038/sdata.2017.101)

- Dugan, H.A., Helmueller, G., and Magnuson, J.J. 2017c. Ice formation and the risk of chloride toxicity in shallow wetlands and lakes. *Limnology and Oceanography: Letters* **2**: 150-158. doi: [10.1002/lo2.10045](https://doi.org/10.1002/lo2.10045)
- Dugan, H.A., Skaff, N.K., Doubek, J.P., Bartlett, S.L., Burke, S.M., Krivak-Tetley, F.E., Summers, J.C., Hanson, P.C., and Weathers, K.C. 2020. Lakes at risk of chloride contamination. *Environmental Science and Technology* **54**: 6639–6650. doi: [10.1021/acs.est.9b07718](https://doi.org/10.1021/acs.est.9b07718)
- Duk Lee, B., Suk Choi, Y., Geun Kim, Y., Sun Kim, I., and Ik Yang, E. 2017. A comparison study of performance and environmental impacts of chloride-based deicers and eco-label certified deicers in South Korea. *Cold Regions Science and Technology* **143**: 43–51. doi: [10.1016/j.coldregions.2017.08.010](https://doi.org/10.1016/j.coldregions.2017.08.010)
- Earon, R., Olofsson, B., and Renman, G. 2012. Initial effects of a new highway section on soil and groundwater. *Water, Air, and Soil Pollution* **223**: 5413–5432. doi: [10.1007/s11270-012-1290-6](https://doi.org/10.1007/s11270-012-1290-6)
- Edgar, E.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998. doi: [10.1038/nmeth.2604](https://doi.org/10.1038/nmeth.2604)
- Eiler, A., Drakare, S., Bertilsson, S., Pernthaler, J., Peura, S., Rofner, C., Simek, K., Yang, Y., Znachor, P., and Lindström, E.S. 2013. Unveiling distribution patterns of freshwater phytoplankton by a Next Generation Sequencing based approach. *PLoS ONE* **8**: e53516. doi: [10.1371/journal.pone.0053516](https://doi.org/10.1371/journal.pone.0053516)
- Eiler, A., Zaremba-niedzwiedzka, K., Martínez-garcía, M., McMahon, K.D., Stepanauskas, R., Andersson, S.G.E., and Bertilsson, S. 2014. Productivity and salinity structuring of the microplankton revealed by comparative freshwater metagenomics. *Environmental Microbiology* **16**: 2682–2698. doi: [10.1111/1462-2920.12301](https://doi.org/10.1111/1462-2920.12301)
- Elphick, J.R.F., Bergh, K., and Bailey, H.C. 2011. Chronic toxicity of chloride to freshwater species: effects of hardness and implications for water quality guidelines. *Environmental Toxicology and Chemistry* **30**: 239–246. doi: [10.1002/etc.365](https://doi.org/10.1002/etc.365)
- Engelhard, C., De Toffol, S., Lek, I., Rauch, W., and Dallinger, R. 2007. Environmental impacts of urban snow management - The alpine case study of Innsbruck. *Science of the Total Environment* **382**: 286–294. doi: [10.1016/j.scitotenv.2007.04.008](https://doi.org/10.1016/j.scitotenv.2007.04.008)
- Environnement et santé Canada. 2001. Liste des substances d'intérêt prioritaire - Rapport d'évaluation pour les sels de voirie [<https://www.canada.ca/fr/sante-canada/services/sante-environnement-milieu-travail/rapports-publications/contaminants-environnementaux/loi-canadienne-protection-environnement-1999-liste-substances-interet-prioritaire-rapport-evaluation-sels-voirie.html#a252>], consulté le 2 novembre 2020.
- Evans, M., and Frick, C. 2001. The effects of road salts on aquatic ecosystems. Direction general des sciences et de la technologie, eau – Environnement Canada. WSTD Contribution No. 02-308.
- Evans, M.G., Jordan, R.W., and Roe, P.G. 2008. Road trials to determine the effects of de-icers on skid resistance. TRL Published Project Report PPR219, Transport Research Laboratory, Wokingham.

Fiche d'information – Présence de sels dans les puits privés d'eau potable. [<https://www2.gnb.ca/content/dam/gnb/Departments/env/pdf/Water-Eau/PresenceSelPuitsPrivesDeauPotable.pdf>], consulté le 9 mars 2021.

Ficker, H., Luger, M., Pamminger-Lahnsteiner, B., Achleitner, D., Jagsch, A., and Gassner, H. 2019. Diluting a salty soup: Impact of long-lasting salt pollution on a deep Alpine lake (Traunsee, Austria) and the downside of recent recovery from salinization. *Aquatic Sciences* **81**: 7. doi: [10.1007/s00027-018-0602-3](https://doi.org/10.1007/s00027-018-0602-3)

Flanagan, K., Branchu, P., Boudahmane, L., Caupos, E., Demare, D., Deshayes, S., Dubois, P., Meffray, L., Partibane, C., Saad, M., and Gromaire, M.-C. 2018. Field performance of two biofiltration systems treating micropollutants from road runoff. *Water Research* **145**: 562–578. doi: [10.1016/j.watres.2018.08.064](https://doi.org/10.1016/j.watres.2018.08.064)

Flöder, S., and Burns, C.W. 2004. Phytoplankton diversity of shallow tidal lakes : Influence of periodic salinity changes on diversity and species number of natural assemblage. *Journal of Phycology* **40**: 54–61. doi: [10.1046/j.1529-8817.2004.03050.x](https://doi.org/10.1046/j.1529-8817.2004.03050.x)

Flöder, S., Jaschinski, S., Wells, G., and Burns, C.W. 2010. Dominance and compensatory growth in phytoplankton communities under salinity stress. *Journal of Experimental Marine Biology and Ecology* **395**: 223–231. doi: [10.1016/j.jembe.2010.09.006](https://doi.org/10.1016/j.jembe.2010.09.006)

Fournier, I.B., Galvez-Cloutier, R., and Vincent, W.F. 2020. Roadside snowmelt: a management target to reduce lake and river contamination. *Inland Waters*. doi: [10.1080/20442041.2020.1801312](https://doi.org/10.1080/20442041.2020.1801312)

Fu, R., and Gong, J. 2017. Single cell analysis linking ribosomal (r)DNA and rRNA copy numbers to cell size and growth rate provides insights into molecular protistan ecology. *Journal of Eukaryotic Microbiology* **64**: 885–896. doi: [10.1111/jeu.12425](https://doi.org/10.1111/jeu.12425)

Galfi, H., Österlund, H., Marsalek, J., and Viklander, M. 2017. Mineral and anthropogenic indicator inorganics in urban stormwater and snowmelt runoff: Sources and mobility patterns. *Water, Air, and Soil Pollution* **228**: 263. doi: [10.1007/s11270-017-3438-x](https://doi.org/10.1007/s11270-017-3438-x)

Galvez-Cloutier, R., Leroueil, S., and Pérez, J.C. 2006. Le lac Saint-Augustin, sa problématique d'eutrophisation et le lien avec les produits d'entretien de l'autoroute Félix-Leclerc. Rapport technique final 3605'3\_06 présenté au ministère des Transports du Québec.

Galvez-Cloutier, R., Saminathan, S.K.M., Boillot, C., Triffaut-Bouchet, G., Bourget, A., and Soumis-Dugas, G. 2012. An evaluation of several in-lake restoration techniques to improve the water quality problem (eutrophication) of Saint-Augustin Lake, Quebec, Canada. *Environmental Management* **49**: 1037–1053. doi: [10.1007/s00267-012-9840-7](https://doi.org/10.1007/s00267-012-9840-7)

Gao, W., Chen, Z., Li, Y., Pan, Y., Zhu, J., Guo, S., Hu, L., and Huang, J. 2018. Bioassessment of a drinking water reservoir using plankton: High Throughput Sequencing vs. traditional morphological method. *Water* **10**: 82. doi: [10.3390/w10010082](https://doi.org/10.3390/w10010082)

Gieskes, W.W.C., and Kraay, G.W. 1983. Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. *Marine Biology* **75**: 179–185. doi: [10.1007/BF00406000](https://doi.org/10.1007/BF00406000)

- Giner, C.R., Forn, I., Romac, S., Logares, R., de Vargas, C., and Massana, R. 2016. Environmental sequencing provides reasonable estimates of the relative abundance of specific picoeukaryotes. *Applied and Environmental Microbiology* **82**: 4757–4766. doi: [10.1128/AEM.00560-16](https://doi.org/10.1128/AEM.00560-16)
- Gode, K., and Paeglis, A. 2014. Concrete bridge deterioration caused by de-icing salts in high traffic volume road environment in Latvia. *The Baltic Journal of Road and Bridge Engineering* **9**: 200–207. doi: [10.3846/bjrbe.2014.25](https://doi.org/10.3846/bjrbe.2014.25)
- Godwin, K.S., Hafner, S.D., and Buff, M.F. 2003. Long-term trends in sodium and chloride in the Mohawk River, New York: The effect of fifty years of road-salt application. *Environmental Pollution* **124**: 273–281. doi: [10.1016/S0269-7491\(02\)00481-5](https://doi.org/10.1016/S0269-7491(02)00481-5)
- Government of Canada. 2019a. Past weather and climate Database. 2019 – Climate normals and averages: Government of Canada. [[http://climate.weather.gc.ca/climate\\_normals/results\\_1981\\_2010\\_e.html?searchType=stnProv&lstProvince=QC&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=5251&dispBack=0](http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?searchType=stnProv&lstProvince=QC&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=5251&dispBack=0)], consulté le 1er août 2019.
- Government of Canada. 2019b. Past weather and climate Database. 2019 – Historical data: Government of Canada. [[http://climat.meteo.gc.ca/climate\\_data/daily\\_data\\_e.html?hlyRange=2005-03-24%7C2019-05-26&dlyRange=1992-12-04%7C2019-05-26&mlyRange=1998-01-01%7C2016-03-01&StationID=26892&Prov=QC&urlExtension=.f.html&searchType=stnName&optionLimit=yearRange&StartYear=1840&EndYear=2019&selRowPerPage=25&Line=0&searchMethod=contains&txtStationName=lesage&timeframe=2&Day=27&Year=2017&Month=4](http://climat.meteo.gc.ca/climate_data/daily_data_e.html?hlyRange=2005-03-24%7C2019-05-26&dlyRange=1992-12-04%7C2019-05-26&mlyRange=1998-01-01%7C2016-03-01&StationID=26892&Prov=QC&urlExtension=.f.html&searchType=stnName&optionLimit=yearRange&StartYear=1840&EndYear=2019&selRowPerPage=25&Line=0&searchMethod=contains&txtStationName=lesage&timeframe=2&Day=27&Year=2017&Month=4)], consulté le 1er août 2019.
- Greenwald, G.M., and Hurlbert, S.H. 1993. Microcosm analysis of salinity effects on coastal lagoon plankton assemblages. *Hydrobiologia* **267**: 307–335. doi: [10.1007/BF00018810](https://doi.org/10.1007/BF00018810)
- Grosman, P.D., Jaeger, J.A.G., Biron, P.M., Dussault, C., and Ouellet, J. 2011. Trade-off between road avoidance and attraction by roadside salt pools in moose : An agent-based model to assess measures for reducing moose-vehicle collisions. *Ecological Modelling* **222**: 1423–1435. doi: [10.1016/j.ecolmodel.2011.01.022](https://doi.org/10.1016/j.ecolmodel.2011.01.022)
- Gu, Z. 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**: 2847-9. doi: [10.1093/bioinformatics/btw313](https://doi.org/10.1093/bioinformatics/btw313)
- Gu, C., Cockerill, K., Anderson, W.P., Shepherd, F., Groothuis, P.A., Mohr, T.M., Whitehead, J.C., Russo, A.A., and Zhang, C. 2019. Modeling effects of low impact development on road salt transport at watershed scale. *Journal of Hydrology* **574**: 1164–1175. doi: [10.1016/j.jhydrol.2019.04.079](https://doi.org/10.1016/j.jhydrol.2019.04.079)
- Guesdon, G., de Santiago-Martin, A., Raymond, S., Messaoud, H., Michaux, A., Roy, S., and Galvez, R. 2016. Impacts of salinity on Saint-Augustin Lake, Canada: Remediation measures at watershed scale. *Water* **8**: 285. doi: [10.3390/w8070285](https://doi.org/10.3390/w8070285)
- Guidelines for Canadian Drinking Water Quality - Summary Table. [<https://www.canada.ca/en/health-canada/services/environmental-workplace->

[health/reports-publications/water-quality/guidelines-canadian-drinking-water-quality-summary-table.html#12](https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-summary-table.html#12)], consulté le 2 novembre 2020.

Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Sodium. [<https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-sodium.html>], consulté le 2 novembre 2020.

Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Total Dissolved Solids (TDS). [<https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-total-dissolved-solids-tds.html>], consulté le 2 novembre 2020.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, JR., Dunthorn, M., Edvardsen, B., Holzman, M., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., and Christen, R. 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research* **41**: D597–D604. doi: [10.1093/nar/gks1160](https://doi.org/10.1093/nar/gks1160)

Gutierrez, M.F., Tavsanoglu, U.N., Vidal, N., Yu, J., Teixeira-de Mello, F., Çakiroglu, A.I., He, H., Liu, Z., and Jeppesen, E. 2018. Salinity shapes zooplankton communities and functional diversity and has complex effects on size structure in lakes. *Hydrobiologia* **813**: 237–255. doi: [10.1007/s10750-018-3529-8](https://doi.org/10.1007/s10750-018-3529-8)

Hautala, E.-L., Rekila, R., Tarhanen, J., and Ruuskanen, J. 1995. Deposition of motor vehicle emissions and winter maintenance along roadside assessed by snow analyses. *Environmental Pollution* **87**: 45–49. doi: [10.1016/S0269-7491\(99\)80006-2](https://doi.org/10.1016/S0269-7491(99)80006-2)

Herlemann, D.P.R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., and Andersson, A.F. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME Journal* **5**: 1571–1579. doi: [10.1038/ismej.2011.41](https://doi.org/10.1038/ismej.2011.41)

Hillebrand, H., Durselen, C.-D., Kirschtel, D., Pollingher, U., and Zohary, T. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* **35**: 403–424. doi: [10.1046/j.1529-8817.1999.3520403.x](https://doi.org/10.1046/j.1529-8817.1999.3520403.x)

Hilliges, R., Schriewer, A., and Helmreich, B. 2013. A three-stage treatment system for highly polluted urban road runoff. *Journal of Environmental Management* **128**: 306–312. doi: [10.1016/j.jenvman.2013.05.024](https://doi.org/10.1016/j.jenvman.2013.05.024)

Hintz, W.D., Jones, D.K., and Relyea, R.A. 2018. Evolved tolerance to freshwater salinization in zooplankton: Life-history trade-offs, cross-tolerance and reducing cascading effects. *Philosophical Transactions of the Royal Society B* **374**: 20180012. doi: [10.1098/rstb.2018.0012](https://doi.org/10.1098/rstb.2018.0012)

Hintz, W.D., Mattes, B.M., Schuler, M.S., Jones, D.K., Stoler, A.B., Lind, L.A., and Relyea, R.A. 2017. Salinization triggers a trophic cascade in experimental freshwater communities with varying food-chain length. *Ecological Applications* **27**: 833–844. doi: [10.1002/eap.1487](https://doi.org/10.1002/eap.1487)

- Hintz, W.D., and Relyea, R.A. 2017a. A salty landscape of fear : Responses of fish and zooplankton to freshwater salinization and predatory stress. *Oecologia* **185**: 147–156. doi: [10.1007/s00442-017-3925-1](https://doi.org/10.1007/s00442-017-3925-1)
- Hintz, W.D., and Relyea, R.A. 2017b. Impacts of road deicing salts on the early-life growth and development of a stream salmonid: Salt type matters. *Environmental Pollution* **223**: 409–415. doi: [10.1016/j.envpol.2017.01.040](https://doi.org/10.1016/j.envpol.2017.01.040)
- Hintz, W.D., and Relyea, R.A. 2019. A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. *Freshwater Biology* **64**: 1081–1097. doi: [10.1111/fwb.13286](https://doi.org/10.1111/fwb.13286)
- Hong, B., Lin, Q., Yu, S., Chen, Y., Chen, Y., and Chiang, P. 2018. Urbanization gradient of selected pharmaceuticals in surface water at a watershed scale. *Science of the Total Environment* **634**: 448–458. doi: [10.1016/j.scitotenv.2018.03.392](https://doi.org/10.1016/j.scitotenv.2018.03.392)
- Houle, D., Duchesne, L., Ouimet, R., Paquin, R., Meng, F.-R., and Arp, P.A. 2002. Evaluation of the FORHYM2 model for prediction of hydrologic fluxes and soil temperature at the Lake Clair Watershed (Duchesnay, Quebec). *Forest Ecology and Management* **159**: 249–260. doi: [10.1016/S0378-1127\(01\)00438-8](https://doi.org/10.1016/S0378-1127(01)00438-8)
- Howard, K.W.F., and Beck, P.J. 1993. Hydrogeochemical implications of groundwater contamination by road de-icing chemicals. *Journal of Contaminant Hydrology* **12**: 245–268. doi: [10.1016/0169-7722\(93\)90010-P](https://doi.org/10.1016/0169-7722(93)90010-P)
- Intergovernmental Oceanographic Commission of UNESCO. 2010. Karlson, B., Cusack, C., and Bresnan, E. editors. Microscopic and molecular methods for quantitative phytoplankton analysis. Paris, UNESCO. (IOC Manuals and Guides, no. 55.) (IOC/2010/MG/55).
- [IPCC] Intergovernmental Panel on Climate Change. 2019. Special report on the ocean and cryosphere in a changing climate [<https://www.ipcc.ch/srocc/>], consulté le 5 décembre 2019.
- Jones, D.K., Mattes, B.M., Hintz, W.D., Schuler, M.S., Stoler, A.B., Lind, L.A., Cooper, R.O., and Relyea, R.A. 2017. Investigation of road salts and biotic stressors on freshwater wetland. *Environmental Pollution* **221**: 159–167. doi: [10.1016/j.envpol.2016.11.060](https://doi.org/10.1016/j.envpol.2016.11.060)
- Judd, J.H. 1970. Lake stratification caused by runoff from street deicing. *Water Research* **4**: 521–532. doi: [10.1016/0043-1354\(70\)90002-3](https://doi.org/10.1016/0043-1354(70)90002-3)
- Junier, P., Kim, O., Imhoff, J.F., Witzel, K., and Hadas, O. 2013. Effect of salinity on cyanobacterial community composition along a transect from Fuliya spring into the water of Lake Kinneret, Israel. *Fundamental and Applied Limnology* **182**: 99–107. doi: [10.1127/1863-9135/2013/0407](https://doi.org/10.1127/1863-9135/2013/0407)
- Kalenitchenko, D., Joli, N., Potvin, M., Tremblay, J.-É., and Lovejoy, C. 2019. Biodiversity and species change in the Arctic Ocean: A view through the lens of Nares Strait. *Frontiers in Marine Science* **6**: 479. doi: [10.3389/fmars.2019.00479](https://doi.org/10.3389/fmars.2019.00479)

- Kalinowska, K., and Grabowska, M. 2016. Autotrophic and heterotrophic plankton under ice in a eutrophic temperate lake. *Hydrobiologia* **777**: 111–118. doi: [10.1007/s10750-016-2769-8](https://doi.org/10.1007/s10750-016-2769-8)
- Kalinowska, K., Napiórkowska-Krzelbietke, A., Bogacka-Kapusta, E., and Stawecki, K. 2019. Comparison of ice-on and ice-off abiotic and biotic parameters in three eutrophic lakes. *Ecological Research* **34**: 687–698. doi: [10.1111/1440-1703.12039](https://doi.org/10.1111/1440-1703.12039)
- Kaushal, S.S., Duan, S., Doody, T.R., Haq, S., Smith, R.M., Newcomer Johnson, T.A., Delaney Newcomb, K., Gorman, J., Bowman, N., Mayer, P.M., Wood, K.L., Belt, K.T., and Stack, W.P. 2017. Human-accelerated weathering increases salinization, major ions, and alkalinization in fresh water across land use. *Applied Geochemistry* **83**: 121–135. doi: [10.1016/j.apgeochem.2017.02.006](https://doi.org/10.1016/j.apgeochem.2017.02.006)
- Kaushal, S.S., Likens, G.E., Pace, M.L., Haq, S., Wood, L., Galella, J.G., Morel, C., Doody, T.R., Wessel, B., Skinner, V., Utz, R., Kortelainen, P., Ra, A., and Jaworski, N. 2018a. Novel ‘chemical cocktails’ in inland waters are a consequence of the freshwater salinization syndrome. *Philosophical Transactions of the Royal Society of London - Series B Biological Sciences* **374**: 201800. doi: [10.1098/rstb.2018.0017](https://doi.org/10.1098/rstb.2018.0017)
- Kaushal, S.S., Likens, G.E., Pace, M.L., Utz, R.M., Haq, S., Gorman, J., and Grese, M. 2018b. Freshwater salinization syndrome on a continental scale. *Proceedings of the National Academy of Sciences USA* **115**: E574–E583. doi: [10.1073/pnas.1711234115](https://doi.org/10.1073/pnas.1711234115)
- Kelly, V.R., Findlay, S.E., Hamilton, S.K., Lovett, G.M., and Weathers, K.C. 2019. Seasonal and long-term dynamics in stream water sodium chloride concentrations and the effectiveness of road salt best management practices. *Water, Air, and Soil Pollution* **230**: 13. doi: [10.1007/s11270-018-4060-2](https://doi.org/10.1007/s11270-018-4060-2)
- Kelly, W.R., Panno, S.V., and Hackley, K. 2012. The sources, distribution, and trends of chloride in the waters of Illinois. *Illinois State Water Survey Bulletin* **B-74**(March).
- Kelting, D.L., Laxson, C.L., and Yerger, E.C. 2012. Regional analysis of the effect of paved roads on sodium and chloride in lakes. *Water Research* **46**: 2749–2758. doi: [10.1016/j.watres.2012.02.032](https://doi.org/10.1016/j.watres.2012.02.032)
- Kim, J.H., and Jung, J.-H. 2017. Cytological staining of protozoa: a case study on the impregnation of hypotrichs (Ciliophora: spirotrichea) using laboratory-synthesized protargol. *Animal Cells and Systems* **21**: 412–418. doi: [10.1080/19768354.2017.1376707](https://doi.org/10.1080/19768354.2017.1376707)
- Kirchman, D.L., Cottrel, M.T., and Ditullio, G.R. 2017. Shaping of bacterial community composition and diversity by phytoplankton and salinity in the Delaware Estuary, USA. *Aquatic Microbial Ecology* **78**: 93–106. doi: [10.3354/ame01805](https://doi.org/10.3354/ame01805)
- Kreislova, K., and Geiplova, H. 2012. Evaluation of corrosion protection of steel bridges. *Procedia Engineering* **40**: 229–234. doi: [10.1016/j.proeng.2012.07.085](https://doi.org/10.1016/j.proeng.2012.07.085)
- Kuemmel, D., and Hanbali, R. 1992. Accident analysis of ice control operations. Transportation Research Center: Accident analysis of ice control operations. Marquette University, Department of Civil, Construction, and Environmental Engineering.

- Ladas, N.P., and Papageorgiou, G.C. 2000. The salinity tolerance of freshwater cyanobacterium *Synechococcus* sp. PCC 7942 is determined by its ability for osmotic adjustment and presence of osmolyte sucrose. *Photosynthetica* **38**: 343–348. doi: [10.1023/A:1010957117237](https://doi.org/10.1023/A:1010957117237)
- Lax, S.M., Peterson, E.W., and Van der Hoven, S.J. 2017. Stream chloride concentrations as a function of land use: A comparison of an agricultural watershed to an urban agricultural watershed. *Environmental Earth Sciences* **76**: 708. doi: [10.1007/s12665-017-7059-x](https://doi.org/10.1007/s12665-017-7059-x)
- Lecointre, G., and Le Guyader, H. 2018. The tree of life: a phylogenetic classification, Belknap press of Harvard University Press.
- Lee, C.E., Kiergaard, M., Gelembiuk, G.W., Eads, B.D., and Posavi, M. 2011. Pumping ions : rapid parallel evolution of ionic regulation following habitat invasions. *Evolution* **65**: 2229–2244. doi: [10.1111/j.1558-5646.2011.01308.x](https://doi.org/10.1111/j.1558-5646.2011.01308.x)
- Légaré 1998. Étude limnologique du Lac Saint-Charles 1996-1997. Université Laval et APEL.
- Legendre, P., and Anderson, M.J. 1999. Distance-based redundancy analysis : Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* **69**: 512. doi: [10.1890/0012-9615\(1999\)069\[0001:DBRATM\]2.0.CO;2](https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2)
- Lévesque, D., Pinel-Alloul, B., Giani, A., Kufner, D.C.L., and Mimouni, E.-A. 2020. Are fluorometric, taxonomic, and functional indicators of phytoplankton community structure linked to environmental typology of urban ponds and lakes? *Inland Waters* **10**: 71–88. doi: [10.1080/20442041.2019.1678970](https://doi.org/10.1080/20442041.2019.1678970)
- Li, R., Min Tun, H., Jahan, M., Zhang, Z., Kumar, A., Dilantha Fernando, W.G., Farenhorst, A., and Khafipour, E. 2017. Comparison of DNA-, PMA-, and RNA-based 16S rRNA Illumina sequencing for detection of live bacteria in water. *Scientific Reports* **7**: 5752. doi: [10.1038/s41598-017-02516-3](https://doi.org/10.1038/s41598-017-02516-3)
- Liu, X., and Steiner, C.F. 2017. Ecotoxicology of salinity tolerance in *Daphnia pulex* : Interactive effects of clonal variation, salinity stress and predation. *Journal of Plankton Research* **39**: 687–697. doi: [10.1093/plankt/fbx027](https://doi.org/10.1093/plankt/fbx027)
- Love, M.I., Huber, W., and Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550. doi: [10.1186/s13059-014-0550-8](https://doi.org/10.1186/s13059-014-0550-8)
- Mackey, M., Mackey, D., Higgins, H., and Wright, S. 1996. CHEMTAX - a program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. *Marine Ecology Progress Series* **144**: 265–283. doi: [10.3354/meps144265](https://doi.org/10.3354/meps144265)
- Marín-Navarro, J., Manuell, A.L., Wu, J., and Mayfield, S.P. 2007. Chloroplast translation regulation. *Photosynthesis Research* **94**: 359–374. doi: [10.1007/s11120-007-9183-z](https://doi.org/10.1007/s11120-007-9183-z)
- Marsalek, J. 2003. Road salts in urban stormwater: An emerging issue in stormwater management in cold climates. *Water Science and Technology* **48**: 61–70. doi: [10.2166/wst.2003.0493](https://doi.org/10.2166/wst.2003.0493)

Martinez Arbizu, P. 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.0.1.

Massé, S., Botrel, M., Walsh, D.A., and Maranger R. 2019. Annual nitrification dynamics in a seasonally ice-covered lake. *PLoS ONE* **14**: e0213748. doi: [10.1371/journal.pone.0213748](https://doi.org/10.1371/journal.pone.0213748)

Mayer, T., Rochfort, Q., Marsalek, J., Parrott, J., Servos, M., Baker, M., McInnis, R., Jurkovic, A., and Scott, I. 2011. Environmental characterization of surface runoff from three highway sites in Southern Ontario, Canada: 2. Toxicology. *Water Quality Research Journal of Canada* **46**: 121–136. doi: [10.2166/wqrjc.2011.036](https://doi.org/10.2166/wqrjc.2011.036)

Mayfield, A.B., and Gates, R.D. 2007. Osmoregulation in anthozoan – dinoflagellate symbiosis. *Comparative Biochemistry and Physiology, Part A* **147**: 1–10. doi: [10.1016/j.cbpa.2006.12.042](https://doi.org/10.1016/j.cbpa.2006.12.042)

McManus, G.B., and Katz, L.A. 2009. Molecular and morphological methods for identifying plankton: what makes a successful marriage? *Journal of Plankton Research* **31**: 1119–1129. doi: [10.1093/plankt/fbp061](https://doi.org/10.1093/plankt/fbp061)

Meals, D.W., Dressing, S.A., and Davenport, T.E. 2010. Lag time in water quality response to best management practices: A review. *Journal of Environmental Quality* **39**: 85–96. doi: [10.2134/jeq2009.0108](https://doi.org/10.2134/jeq2009.0108)

Medinger, R., Nolte, V., Pandey, R.V., Jost, S., Ottenwälder, B., Schlötterer, C., and Boenigk, J. 2010. Diversity in a hidden world: potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology* **19**: 32–40. doi: [10.1111/j.1365-294X.2009.04478.x](https://doi.org/10.1111/j.1365-294X.2009.04478.x)

[MELCC] Ministère de l'Environnement et de la Lutte contre les changements climatiques. 2019a. Atlas interactif de la qualité des eaux et des écosystèmes aquatiques. Station 05090017  
[[http://www.environnement.gouv.qc.ca/eau/Atlas\\_interactif/stations/stations\\_riviere\\_s.asp](http://www.environnement.gouv.qc.ca/eau/Atlas_interactif/stations/stations_riviere_s.asp)], consulté le 1er août 2019.

[MELCC] Ministère de l'Environnement et de la Lutte contre les changements climatiques. 2019b. Centre d'expertise hydrique du Québec. 2019. Historique des niveaux et des débits de différentes stations hydrométriques [[https://www.cehq.gouv.qc.ca/hydrometrie/historique\\_donnees/fiche\\_station.asp?NoStation=050904](https://www.cehq.gouv.qc.ca/hydrometrie/historique_donnees/fiche_station.asp?NoStation=050904)], consulté le 1er août 2019.

[MELCC] Ministère de l'Environnement et de la Lutte contre les changements climatiques. 2019c. Données Québec. Bassins hydrographiques multiéchelles du Québec [[https://www.donneesquebec.ca/recherche/fr/dataset/bassins-hydrographiques-multi-echelles-du-quebec?fbclid=IwAR3SzSBpXU71lpApGrekHJrz6W9pkIcE\\_5xsnGrz6iu3UN5TtI-gNwPyqpU](https://www.donneesquebec.ca/recherche/fr/dataset/bassins-hydrographiques-multi-echelles-du-quebec?fbclid=IwAR3SzSBpXU71lpApGrekHJrz6W9pkIcE_5xsnGrz6iu3UN5TtI-gNwPyqpU)], consulté le 1er août 2019.

[MELCC] Ministère de l'Environnement et de la Lutte contre les changements climatiques. 2020. Critères de qualité de l'eau de surface.

[[http://www.environnement.gouv.qc.ca/eau/criteres\\_eau/details.asp?code=S0118](http://www.environnement.gouv.qc.ca/eau/criteres_eau/details.asp?code=S0118)], consulté le 2 novembre 2020.

Merrick, A.M., and Searle, C.L. 2019. Combined effects of salinity and infectious disease on *Daphnia dentifera* at multiple scales. *Freshwater Biology* **64**: 601–607. doi: [10.1111/fwb.13245](https://doi.org/10.1111/fwb.13245)

Meunier, P., and Alain, J. 1979. Rapport de la diagnose écologique lac Saint-Augustin. Service de la qualité des eaux. Direction générale des eaux. Ministère des richesses naturelles du Québec.

Milotic, D., Milotic, M., and Koprivnikar, J. 2017. Effects of road salt on larval amphibian susceptibility to parasitism through behavior and immunocompetence. *Aquatic Toxicology* **189**: 42–49. doi: [10.1016/j.aquatox.2017.05.015](https://doi.org/10.1016/j.aquatox.2017.05.015)

Minerovic, A.D., Potapova, M.G., Sales, C.M., Price, J.R., and Enache, M.D. 2020. 18S-V9 DNA metabarcoding detects the effect of water-quality impairment on stream biofilm eukaryotic assemblages. *Ecological Indicators* **113**: 106225. doi: [10.1016/j.ecolind.2020.106225](https://doi.org/10.1016/j.ecolind.2020.106225)

Miranda, J., and Krishnakumar, G. 2015. Microalgal diversity in relation to the physicochemical parameters of some industrial sites in Mangalore, South India. *Environmental Monitoring and Assessment* **187**: 664. doi: [10.1007/s10661-015-4871-1](https://doi.org/10.1007/s10661-015-4871-1)

Mochizuki, J. 2011. Évaluation de la contamination du lac Clément, de son bassin versant et de la nappe phréatique par les sels de voirie – Charlesbourg, Québec. Master's essay. Université Laval.

Moghadas, S., Paus, K.H., Muthanna, T.M., Herrmann, I., Marsalek, J., and Viklander, M. 2015. Accumulation of traffic-related trace metals in urban winter-long roadside snowbanks. *Water, Air, and Soil Pollution* **226**: 1–15. doi: [10.1007/s11270-015-2660-7](https://doi.org/10.1007/s11270-015-2660-7)

Mohit, V., Culley, A., Lovejoy, C., Bouchard, F., and Vincent, W.F. 2017. Hidden biofilms in a far northern lake and implications for the changing Arctic. *npj Biofilms and Microbiomes* **3**: 17. doi: [10.1038/s41522-017-0024-3](https://doi.org/10.1038/s41522-017-0024-3)

Moore, J., Bird, D.L., Dobbs, S.K., and Woodward, G. 2017. Nonpoint source contributions drive elevated major ion and dissolved inorganic carbon concentrations in urban watersheds. *Environmental Science and Technology: Letters* **4**: 198–204. doi: [10.1021/acs.estlett.7b00096](https://doi.org/10.1021/acs.estlett.7b00096)

Moore, T.L., Rodak, C.M., Ahmed, F., and Vogel, J.R. 2018. Urban stormwater characterization, control and treatment. *Water Environment Research* **90**: 1821–1871. doi: [10.2175/106143018X15289915807452](https://doi.org/10.2175/106143018X15289915807452)

[MTQ] Ministère des Transports du Québec. 2019. Données Québec. Débit de circulation [<https://www.donneesquebec.ca/echerché/fr/dataset/debit-decirculation>], consulté le 1er août 2019

Mukherjee, A., Das, S., Bhattacharya, T., De, M., Maiti, T., and Kumar De, T. 2014. Optimization of phytoplankton preservative concentrations to reduce damage during

- long-term storage. *Biopreservation and Biobanking* **12**: 139–147. doi: [10.1089/bio.2013.0074](https://doi.org/10.1089/bio.2013.0074)
- Müller, B., and Gächter, R. 2012. Increasing chloride concentrations in Lake Constance: Characterization of sources and estimation of loads. *Aquatic Sciences* **74**: 101–112. doi: [10.1007/s00027-011-0200-0](https://doi.org/10.1007/s00027-011-0200-0)
- Napiórkowska-Krzelbietke, A., and Dunalska, J. 2015. Phytoplankton-based recovery requirement for urban lakes in the implementation of the Water Framework Directive's ecological targets. *Oceanological and Hydrobiological Studies* **44**: 109–119. doi: [10.1515/ohs-2015-0011](https://doi.org/10.1515/ohs-2015-0011)
- Novotny, E.V., Murphy, D., and Stefan, H.G. 2008. Increase of urban lake salinity by road deicing salt. *Science of the Total Environment* **406**: 131–144. doi: [10.1016/j.scitotenv.2008.07.037](https://doi.org/10.1016/j.scitotenv.2008.07.037)
- O'brien, J.E., and Majewaki, J.C. 1975. Effects of de-icing salt on ground water characteristics. *Environmental Letters* **8**: 303–313. doi: [10.1080/00139307509437440](https://doi.org/10.1080/00139307509437440)
- [OBV de la Capitale] Organisme des bassins versants de la Capitale. 2016. Bassin du lac Saint Augustin / Lacs. [<http://www.obvcapitale.org/bassin-du-lac-saint-augustin-lacs>], consulté le 2 novembre 2020.
- [OBV de la Capitale] Organisme des bassins versants de la Capitale. 2018. Diagnose du lac Saint-Augustin – Campagnes de terrain 2014-2015. Pour la Ville de Saint-Augustin-de-Desmaures. Version finale mise à jour en janvier 2018.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., and Wagner, H. 2019. vegan: Community Ecology Package. R package version 2.5-6. [<https://CRAN.R-project.org/package=vegan>], consulté le 2 novembre 2020.
- Ondarza, P.M., Haddad, S.P., Avigliano, E., Miglioranza, K.S.B., and Brooks, B.W. 2019. Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: Implications for protected areas influenced by urbanization. *Science of the Total Environment* **649**: 1029–1037. doi: [10.1016/j.scitotenv.2018.08.383](https://doi.org/10.1016/j.scitotenv.2018.08.383)
- Öterler, B. 2017. Winter phytoplankton composition occurring in a temporarily ice-covered lake : a case study. *Polish Journal of Environmental Studies* **26**: 2677–2688. doi: [10.15244/pjoes/74015](https://doi.org/10.15244/pjoes/74015)
- Palko, K.G., and Lemmen, D.S. 2017. Climate risks and adaptation practices: For the Canadian transportation sector 2016. [<https://apps.uqo.ca/LoginSigparb/LoginPourRessources.aspx?url=http://www.deslibris.ca/ID/10093815>], consulté le 13 février 2020.
- Paradis, E., and Schliep, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**: 526–528. doi: [10.1093/bioinformatics/bty633](https://doi.org/10.1093/bioinformatics/bty633)
- Park, M.-H., Park, C.-H., Sim, Y.B., and Hwang, S.-J. 2020. Response of *Scenedesmus quadricauda* (Chlorophyceae) to salt stress considering nutrient enrichment and

- intracellular proline accumulation. *International Journal of Environmental Research and Public Health* **17**: 3624. doi: [10.3390/ijerph17103624](https://doi.org/10.3390/ijerph17103624)
- Petraska, J.W., and Doyle, E.J. 2010. Effects of road salts on the composition of seasonal pond communities : Can the use of road salts enhance mosquito recruitment? *Aquatic Ecology* **44**: 155–166. doi: [10.1007/s10452-009-9286-z](https://doi.org/10.1007/s10452-009-9286-z)
- Phillips, G., Kelly, A., Pitt, J.-A., Sanderson, R., and Taylor, E. 2005. The recovery of a very shallow eutrophic lake, 20 years after the control of effluent derived phosphorus. *Freshwater Biology* **50**: 1628–1638. doi: [10.1111/j.1365-2427.2005.01434.x](https://doi.org/10.1111/j.1365-2427.2005.01434.x)
- Pienitz, R., Roberge, K., and Vincent, W.F. 2006. Three hundred years of human-induced change in an urban lake: paleolimnological analysis of Lac Saint-Augustin, Québec City, Canada. *Canadian Journal of Botany* **84**: 303-320. doi: [10.1139/b05-152](https://doi.org/10.1139/b05-152)
- Pieper, K.J., Tang, M., Jones, C.N., Weiss, S., Greene, A., Mohsin, H., and Edwards, M.A. 2018. Impact of road salt on drinking water quality and infrastructure corrosion in private wells. *Environmental Science and Technology* **52**: 14078–14087. doi: [10.1021/acs.est.8b04709](https://doi.org/10.1021/acs.est.8b04709)
- Pingping, H., Feng, Z., and Kuidong, X. 2020. Complementary DNA sequencing (cDNA): an effective approach for assessing the diversity and distribution of marine benthic ciliates along hydrographic gradients. *Journal of Limnology and Oceanography*. doi: [10.1007/s00343-020-9234-2](https://doi.org/10.1007/s00343-020-9234-2)
- Proulx, F. 2017. Rapport de caractérisation de l'eau des puits privés des bassins versants des prises d'eau situées dans la rivière Saint-Charles et la rivière Montmorency. Rapport présenté à la Communauté Métropolitaine de Québec.
- Przytulska, A., Bartosiewicz, M., Rautio, M., Dufresne, F., and Vincent, W.F. 2015. Climate effects on high latitude *Daphnia* via food quality and thresholds. *PLoS ONE* **10**: 1–16. doi: [10.1371/journal.pone.0126231](https://doi.org/10.1371/journal.pone.0126231)
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**: D590-D596. doi: [10.1093/nar/gks1219](https://doi.org/10.1093/nar/gks1219)
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [<http://www.R-project.org/>], consulté le 2 novembre 2020.
- Ratkevičius, T., Laurinavičius, A., Tuminienė, F., and Bradulienė, J. 2014. Reduction of negative impact of salts used for winter road maintenance on the environment. The 9th International Conference “Environmental Engineering”. eISSN 2029-7092 / eISBN 978-609-457-640-9.
- Redden, A.M., and Rukminasari, N. 2008. Effects of increases in salinity on phytoplankton in the Broadwater of the Myall Lakes, NSW, Australia. *Hydrobiologia* **608**: 87–97. doi: [10.1007/s10750-008-9376-2](https://doi.org/10.1007/s10750-008-9376-2)

- Reinosdotter, K., and Viklander, M. 2007. Road salt influence on pollutant releases from melting urban snow. *Water Quality Research Journal of Canada* **42**: 153–161. doi: [10.2166/wqrj.2007.019](https://doi.org/10.2166/wqrj.2007.019)
- Rimet, F., Vasselon, V., A.-Keszte, B., and Bouchez, A. 2018. Do we similarly assess diversity with microscopy and high-throughput sequencing? Case of microalgae in lakes. *Organisms Diversity and Evolution* **18**: 51–62. doi: [10.1007/s13127-018-0359-5](https://doi.org/10.1007/s13127-018-0359-5)
- Rivett, M.O., Cuthbert, M.O., Gamble, R., Connon, L.E., Pearson, A., Shepley, M.G., and Davis, J. 2016. Highway deicing salt dynamic runoff to surface water and subsequent infiltration to groundwater during severe UK winters. *Science of the Total Environment* **565**: 324–338. doi: [10.1016/j.scitotenv.2016.04.095](https://doi.org/10.1016/j.scitotenv.2016.04.095)
- Roberge, K. 2004. Paléolimnologie du Lac Saint-Augustin. Reconstitution de l'histoire trophique par l'étude des diatomées fossiles, des pigments d'algues et de la géochimie des sédiments. Master's thesis. Université Laval.
- Roche. 2010. État de la situation du bassin versant de la prise d'eau de la rivière St-Charles. N/Réf: 56692-100.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584. doi: [10.7717/peerj.2584](https://doi.org/10.7717/peerj.2584)
- Rogora, M., Mosello, R., Kamburska, L., Salmaso, N., Cerasino, L., Leoni, B., Garibaldi, L., Soler, V., Lepori, F., Colombo, L., and Buzzi, F. 2015. Recent trends in chloride and sodium concentrations in the deep subalpine lakes (Northern Italy). *Environmental Science and Pollution Research* **22**: 19013–19026. doi: [10.1007/s11356-015-5090-6](https://doi.org/10.1007/s11356-015-5090-6)
- Roy, S. 2015. Traitement du ruissellement routier chargé en sels de déglaçage par marais épuration au moyen de plantes halophytes. Master's thesis. Université Laval.
- Roy, S., Llewellyn, C.A., Skarstad, E., and Johnsen, G. 2011. Phytoplankton pigments. Characterization chemotaxonomy and applications in oceanography, Cambridge University Press.
- Ruth, O. 2003. The effects of de-icing in Helsinki urban streams, Southern Finland. *Water Science and Technology* **48**: 33–43. doi: [10.2166/wst.2003.0486](https://doi.org/10.2166/wst.2003.0486)
- Sakai, R., Winand, R., Verbeiren, T., Vande Moere, A., and Aerts, J. 2015. dendsort: modular leaf ordering methods for dendrogram representations in R [version 1; peer review: 2 approved]. *F1000Research* **3**: 177. doi: [10.12688/f1000research.4784.1](https://doi.org/10.12688/f1000research.4784.1)
- Salminen, J.M., Nystén, T.H., and Tuominen, S.M. 2011. Review of approaches to reducing adverse impacts of road deicing on groundwater in Finland. *Water Quality Research Journal of Canada* **46**: 166–173. doi: [10.2166/wqrjc.2011.002](https://doi.org/10.2166/wqrjc.2011.002)
- Scarlett, R.D., McMillan, S.K., Bell, C.D., Clinton, S.M., Jefferson, A.J., and Rao, P.S.C. 2018. Influence of stormwater control measures on water quality at nested sites in a small suburban watershed. *Urban Water Journal* **15**: 868–879. doi: [10.1080/1573062X.2019.1579347](https://doi.org/10.1080/1573062X.2019.1579347)
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,

- Thallinger, G.G., Van Horn, D.J., and Weber, C.F. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology* **75**: 7537–41. doi: [10.1128/AEM.01541-09](https://doi.org/10.1128/AEM.01541-09)
- Schluter, L., Lauridsen, T.L., Krogh, G., and Jorgensen, T. 2006. Identification and quantification of phytoplankton groups in lakes using new pigment ratios – a comparison between pigment analysis by HPLC and microscopy. *Freshwater Biology* **51**: 1474–1485. doi: [10.1111/j.1365-2427.2006.01582.x](https://doi.org/10.1111/j.1365-2427.2006.01582.x)
- Schoukens, I., Cavezza, F., Cerezo, J., Vandenberghe, V., C. Gudla, V., and Ambat, R. 2017. Influence of de-icing salt chemistry on the corrosion behavior of AA6016. *Materials and Corrosion* **69**: 881–887. doi: [10.1002/maco.201709907](https://doi.org/10.1002/maco.201709907)
- Schuler, M.S., Hintz, W.D., Jones, D.K., Lind, L.A., Mattes, B.M., Stoler, A.B., Sudol, K.A., and Relyea, R.A. 2017. How common road salts and organic additives alter freshwater food webs : In search of safer alternatives. *Journal of Applied Ecology* **54**: 1353–1361. doi: [10.1111/1365-2664.12877](https://doi.org/10.1111/1365-2664.12877)
- Scott, R., Goulden, T., Letman, M., Hayward, J., and Jamieson, R. 2019. Long-term evaluation of the impact of urbanization on chloride levels in lakes in a temperate region. *Journal of Environmental Management* **244**: 285–293. doi: [10.1016/j.jenvman.2019.05.029](https://doi.org/10.1016/j.jenvman.2019.05.029)
- Shanley, J.B. 1994. Effects of ion-exchange on stream solute fluxes in a basin receiving highway deicing salts. *Journal of Environmental Quality* **23**: 977–986. doi: [10.2134/jeq1994.00472425002300050019x](https://doi.org/10.2134/jeq1994.00472425002300050019x)
- Shoda, M.E., Sprague, L.A., Murphy, J.C., and Riskin, M.L. 2019. Water-quality trends in U.S. rivers, 2002 to 2012: Relations to levels of concern. *Science of the Total Environment* **650**: 2314–2324. doi: [10.1016/j.scitotenv.2018.09.377](https://doi.org/10.1016/j.scitotenv.2018.09.377)
- Silver, P., Rupprecht, S.M., and Stauffer, M.F. 2009. Temperature-dependent effects of road deicing salt on chironomid larvae. *Wetlands* **29**: 942–951. doi: [10.1672/08-212.1](https://doi.org/10.1672/08-212.1)
- Simmons, J.A. 2012. Toxicity of major cations and anions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$ ) to a macrophyte and an alga. *Environmental Toxicology and Chemistry* **31**: 1370–1374. doi: [10.1002/etc.1819](https://doi.org/10.1002/etc.1819)
- Singh, T., and Kalra, Y.P. 1975. Specific conductance method for in situ estimation of total dissolved solids. *American Water Works Association* **67**: 99–100. doi: [10.1002/j.1551-8833.1975.tb02168.x](https://doi.org/10.1002/j.1551-8833.1975.tb02168.x)
- Søberg, L.C., Viklander, M., and Blecken, G.T. 2017. Do salt and low temperature impair metal treatment in stormwater bioretention cells with or without a submerged zone? *Science of the Total Environment* **579**: 1588–1599. doi: [10.1016/j.scitotenv.2016.11.179](https://doi.org/10.1016/j.scitotenv.2016.11.179)
- Soucek, D.J., Linton, T.K., Tarr, C.D., Dickinson, A., Wickramanayake, N., Delos, C.G., Cruz, L.A., Elos, C.H.G.D., Ruz, L.U.I.S.A.C., Soucek, D.J., Linton, T.K., Tarr, C.D., Dickinson, A., Wickramanayake, N., Delos, C.G., and Cruz, L.A. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive

- freshwater invertebrates. *Environmental Toxicology and Chemistry* **30**: 930–938. doi: [10.1002/etc.454](https://doi.org/10.1002/etc.454)
- Spears, B.M., Futter, M.N., Jeppesen, E., Huser, B.J., Ives, S., Davidson, T.A., Adrian, R., Angeler, D.G., Burthe, S.J., Carvalho, L., Daunt, F., Gsell, A.S., Hessen, D.O., Janssen, A.B.G., Mackay, E.B., May, L., Moorhouse, H., Olsen, S., Søndergaard, M., Woods, H., and Thackeray, S.J. 2017. Ecological resilience in lakes and the conjunction fallacy. *Nature Ecology and Evolution* **1**: 1616–1624. doi: [10.1038/s41559-017-0333-1](https://doi.org/10.1038/s41559-017-0333-1)
- Spivak, A.C., Vanni, M.J., and Mette, E.M. 2011. Moving on up : can results from simple aquatic mesocosm experiments be applied across broad spatial scales ? *Freshwater Biology* **56**: 279–291. doi: [10.1111/j.1365-2427.2010.02495.x](https://doi.org/10.1111/j.1365-2427.2010.02495.x)
- Stagge, J.H., Davis, A.P., Jamil, E., and Kim, H. 2012. Performance of grass swales for improving water quality from highway runoff. *Water Research* **46**: 6731–6742. doi: [10.1016/j.watres.2012.02.037](https://doi.org/10.1016/j.watres.2012.02.037)
- Steinman, A.D., Havens, K.E., Louda, J.W., Winfree, N.M., and Baker, E.W. 1998. *Canadian Journal of Fisheries and Aquatic Sciences* **55**: 206–219. doi: [10.1139/f97-239](https://doi.org/10.1139/f97-239)
- Stets, E.G., Lee, C.J., Lytle, D.A., and Schock, M.R. 2018. Increasing chloride in rivers of the conterminous U.S. and linkages to potential corrosivity and lead action level exceedances in drinking water. *Science of the Total Environment* **613–614**: 1498–1509. doi: [10.1016/j.scitotenv.2017.07.119](https://doi.org/10.1016/j.scitotenv.2017.07.119)
- Stoecker, D., Gifford, D., and Putt, M. 1994. Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. *Marine Ecology Progress Series* **110**: 293–299. doi: [10.3354/meps110293](https://doi.org/10.3354/meps110293)
- Strom, S.L., Harvey, E.L., Fredrickson, K.A., and Menden-Deuer, S. 2013. Broad salinity tolerance as a refuge from predation in the harmful raphidophyte alga *Heterosigma akashimo* (raphidophyceae). *Journal of Phycology* **49**: 20–31. doi: [10.1111/jpy.12013](https://doi.org/10.1111/jpy.12013)
- Szöcs, E., Corring, E., Bäthe, J., and Schäfer, R.B. 2014. Effects of anthropogenic salinization on biological traits and community composition of stream macroinvertebrates. *Science of the Total Environment* **468–469**: 943–949. doi: [10.1016/j.scitotenv.2013.08.058](https://doi.org/10.1016/j.scitotenv.2013.08.058)
- Taka, M., Kokkonen, T., Kuoppamäki, K., Niemi, T., Sillanpää, N., Valtanen, M., Warsta, L., and Setälä, H. 2017. Spatio-temporal patterns of major ions in urban stormwater under cold climate. *Hydrological Processes* **31**: 1564–1577. doi: [10.1002/hyp.11126](https://doi.org/10.1002/hyp.11126)
- Tang, X., Xie, G., Shao, K., Chen, Y., and Gao, G. 2012. Influence of salinity on the bacterial community composition in Lake Bosten, a large oligosaline lake in arid northwestern China. *Applied and Environmental Microbiology* **78**: 4748–4751. doi: [10.1128/AEM.07806-11](https://doi.org/10.1128/AEM.07806-11)
- Thaler, M., Vincent, W.F., Lionard, M., Hamilton, A.K., and Lovejoy, C. 2017. Microbial community structure and interannual change in the last epishelf lake ecosystem in the

North Polar Region. *Frontiers in Marine Science* **3**: 275. doi: [10.3389/fmars.2016.00275](https://doi.org/10.3389/fmars.2016.00275)

Thunqvist, E.L. 2003. Increased chloride concentration in a lake due to deicing salt application. *Water Science and Technology* **48**: 51–59. doi: [10.2166/wst.2003.0491](https://doi.org/10.2166/wst.2003.0491)

Thunqvist, E.L. 2004. Regional increase of mean chloride concentration in water due to the application of deicing salt. *Science of the Total Environment* **325**: 29–37. doi: [10.1016/j.scitotenv.2003.11.020](https://doi.org/10.1016/j.scitotenv.2003.11.020)

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundein, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., and Kolodziej, E.P. 2020. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science*: eabd6951. doi: [10.1126/science.abd6951](https://doi.org/10.1126/science.abd6951)

Toporowska, M., Ferencz, B., and Dawidek, J. 2018. Impact of lake-catchment processes on phytoplankton community structure in temperate shallow lakes: Impact of lake-catchment processes on phytoplankton. *Ecohydrology* **11**: e2017. doi: [10.1002/eco.2017](https://doi.org/10.1002/eco.2017)

Tran, P., Ramachandran, A., Khawasek, O., Beisner, B.E., Rautio, M., Huot, Y., and Walsh, D.A. 2018. Microbial life under ice: Metagenome diversity and *in situ* activity of Verrucomicrobia in seasonally ice-covered lakes. *Environmental Microbiology* **20**: 2568–2584. doi: [10.1111/1462-2920.14283](https://doi.org/10.1111/1462-2920.14283)

Trenouth, W.R., Gharabaghi, B., and Farghaly, H. 2018. Enhanced roadside drainage system for environmentally sensitive areas. *Science of the Total Environment* **610–611**: 613–622. doi: [10.1016/j.scitotenv.2017.08.081](https://doi.org/10.1016/j.scitotenv.2017.08.081)

US EPA. 1988. Ambient Water Quality Criteria for Chloride – 1988. [<https://www.epa.gov/sites/production/files/2018-08/documents/chloride-aquatic-life-criteria-1988.pdf>], consulté le 2 novembre 2020.

US EPA. 2020. Secondary Drinking Water Standards: Guidance for Nuisance Chemicals. [<https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals>], consulté le 2 novembre 2020.

Usman, T., Fu, L., and Miranda-moreno, L.F. 2012. A disaggregate model for quantifying the safety effects of winter road maintenance activities at an operational level. *Accident Analysis and Prevention* **48**: 368–378. doi: [10.1016/j.aap.2012.02.005](https://doi.org/10.1016/j.aap.2012.02.005)

Valtanen, M., Sillanpää, N., and Setälä, H. 2014. The effects of urbanization on runoff pollutant concentrations, loadings and their seasonal patterns under cold climate. *Water, Air, and Soil Pollution* **225**: 1977. doi: [10.1007/s11270-014-1977-y](https://doi.org/10.1007/s11270-014-1977-y)

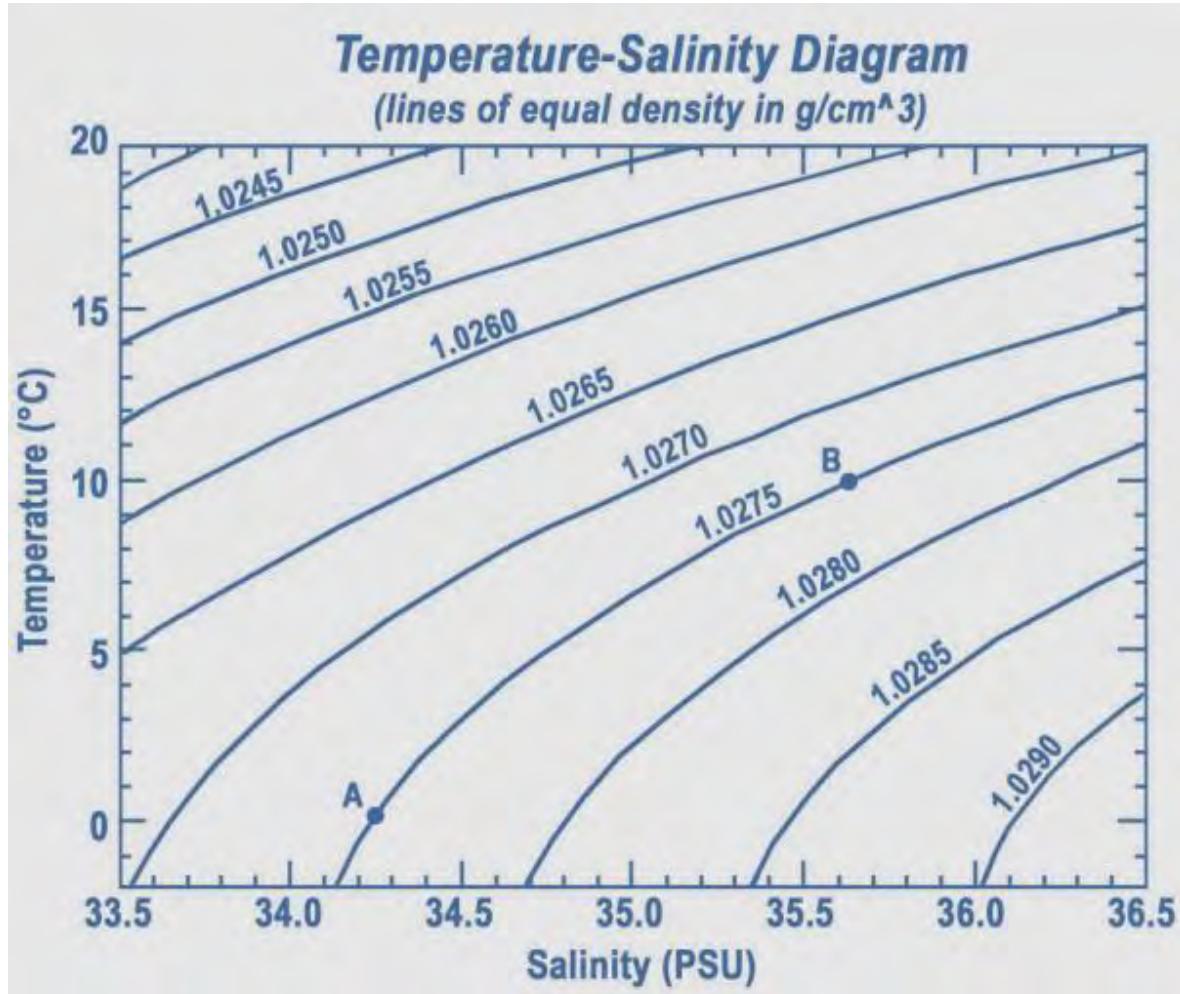
Van den Meersche, K., Soetaert, K., and Middelburg, J.J. 2008. A Bayesian compositional estimator for microbial taxonomy based on biomarkers: Bayesian compositional estimator. *Limnology and Oceanography: Methods* **6**: 190–199. doi: [10.4319/lom.2008.6.190](https://doi.org/10.4319/lom.2008.6.190)

- Van Meter, R.J., Swan, C.M., Leips, J., and Snodgrass, J.W. 2011. Road salt stress induces novel food web structure and interactions. *Wetlands* **31**: 843–851. doi: [10.1007/s13157-011-0199-y](https://doi.org/10.1007/s13157-011-0199-y)
- Venâncio, C., Anselmo, E., Soares, A., and Lopes, I. 2017. Does increased salinity influence the competitive outcome of two producer species? *Environmental Science and Pollution Research* **24**: 5888–5897. doi: [10.1007/s11356-016-8346-x](https://doi.org/10.1007/s11356-016-8346-x)
- Vigneron, A., Lovejoy, C., Cruaud, P., Kalenitchenko, D., Culley, A., and Vincent, W.F. 2019. Contrasting winter versus summer microbial communities and metabolic functions in a permafrost thaw lake. *Frontiers in Microbiology* **10**: 1656. doi: [10.3389/fmicb.2019.01656](https://doi.org/10.3389/fmicb.2019.01656)
- Viklander, M. 1999. Substances in urban snow. A comparison of the contamination of snow in different parts of the city of Luleå, Sweden. *Water, Air, and Soil Pollution* **114**: 377–394. doi: [10.1023/A:1005121116829](https://doi.org/10.1023/A:1005121116829)
- Vincent, W.F. 2018. Lakes: a very short introduction, Oxford University Press.
- Wallace, A.M., and Biastoch, R.G. 2016. Detecting changes in the benthic invertebrate community in response to increasing chloride in streams in Toronto, Canada. *Freshwater Science* **35**: 353–363. doi: [10.1086/685297](https://doi.org/10.1086/685297)
- Warren, A. 2011. Suivi des cyanobactéries en milieu lacustre par fluorimétrie in vivo. Master's thesis. Université Laval.
- Watanabe, S., Laurion, I., Chokmani, K., Pienitz, R., and Vincent, W.F. 2011. Optical diversity of thaw ponds in discontinuous permafrost: A model system for water color analysis. *Journal of Geophysical Research* **116**: G02203. doi: [10.1029/2010JG001380](https://doi.org/10.1029/2010JG001380)
- Wegen, S., Nowka, B., and Speck, E. 2019. Low temperature and neutral pH define “*Candidatus Nitrotoga* sp.” as a competitive nitrite oxidizer in coculture with *Nitrospira defluvii*. *Applied Environmental Microbiology* **85**: e02569-18. doi: [10.1128/AEM.02569-18](https://doi.org/10.1128/AEM.02569-18)
- Westerlund, C., and Viklander, M. 2011. Pollutant release from a disturbed urban snowpack in northern Sweden. *Water Quality Research Journal of Canada* **46**: 98–109. doi: [10.2166/wqrjc.2011.025](https://doi.org/10.2166/wqrjc.2011.025)
- Wetzel, R.B., and Likens, G. 2000. Limnological analyses, 3rd edition, Springer.
- Wilhelm, S.W., LeCleir, G.R., Bullerjahn, G.S., McKay, R.M., Saxton, M.A., Twiss, M.R., and Bourbonniere, R.A. 2014. Seasonal changes in microbial community structure and activity imply winter production is linked to summer hypoxia in a large lake. *FEMS Microbiology Ecology* **87**: 475–485. doi: [10.1111/1574-6941.12238](https://doi.org/10.1111/1574-6941.12238)
- Wiltse, B., Yerger, E.C., and Laxson, C.L. 2020. A reduction in spring mixing due to road salt runoff entering Mirror Lake (Lake Placid, NY). *Lake and Reservoir Management* **36**: 109–121. doi: [10.1080/10402381.2019.1675826](https://doi.org/10.1080/10402381.2019.1675826)
- Wu, Q.L., Chatzinotas, A., Wang, J., Boenigk, J., and Boenigk, J. 2009. Genetic diversity of eukaryotic plankton assemblages in eastern Tibetan lakes differing by their salinity and altitude. *Microbial Ecology* **58**: 569–581. doi: [10.1007/s00248-009-9526-8](https://doi.org/10.1007/s00248-009-9526-8)

- Xiao, X., Sogge, H., Lagesen, K., Tooming-klunderud, A., Jakobsen, K.S., and Rohrlack, T. 2014. Use of High Throughput Sequencing and light microscopy show contrasting results in a study of phytoplankton occurrence in a freshwater environment. *PLoS ONE* **9**: e106510. doi: [10.1371/journal.pone.0106510](https://doi.org/10.1371/journal.pone.0106510)
- Yan, Q.Y., Yu, Y.H., Feng, W.S., Deng, W.N., and Song, X.H. 2007. Genetic diversity of plankton community as depicted by PCR-DGGE fingerprinting and its relation to morphological composition and environmental factors in Lake Donghu. *Microbial Ecology* **54**: 290–297. doi: [10.1007/s00248-006-9200-3](https://doi.org/10.1007/s00248-006-9200-3)
- Yancey, P.H. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *The Journal of Experimental Biology* **208**: 2819–2830. doi: [10.1242/jeb.01730](https://doi.org/10.1242/jeb.01730)
- Zapata, M., Jeffrey, S.W., Wright, S.W., Rodríguez, F., Garrido, J.L., and Clementson, L. 2004. Photosynthetic pigments in 37 species (65 strains) of Haptophyta: implications for oceanography and chemotaxonomy. *Marine Ecology Progress Series* **270**: 83–102. doi: [10.3354/meps270083](https://doi.org/10.3354/meps270083)
- Zehetner, F., Rosenfellner, U., Mentler, A., and Gerzabek, M.H. 2009. Distribution of road salt residues, heavy metals and polycyclic aromatic hydrocarbons across a highway-forest interface. *Water, Air, and Soil Pollution* **198**: 125–132. doi: [10.1007/s11270-008-9831-8](https://doi.org/10.1007/s11270-008-9831-8)
- Zhang, L., Gao, G., Tang, X., and Shao, K. 2014. Can the freshwater bacterial communities shift to the “marine-like” taxa? *Journal of Basic Microbiology* **54**: 1264–1272. doi: [10.1002/jobm.201300818](https://doi.org/10.1002/jobm.201300818)
- Zhao, H., Wang, Y., Yang, L., Yuan, L., and Peng, D. 2015. Relationship between phytoplankton and environmental factors in landscape water supplemented with reclaimed water. *Ecological Indicators* **58**: 113–121. doi: [10.1016/j.ecolind.2015.03.033](https://doi.org/10.1016/j.ecolind.2015.03.033)
- Zhu, H., Xu, Y., Yan, B., and Guan, J. 2012. Snowmelt runoff: A new focus of urban nonpoint source pollution. *International Journal of Environmental Research and Public Health* **9**: 4333–4345. doi: [10.3390/ijerph9124333](https://doi.org/10.3390/ijerph9124333)

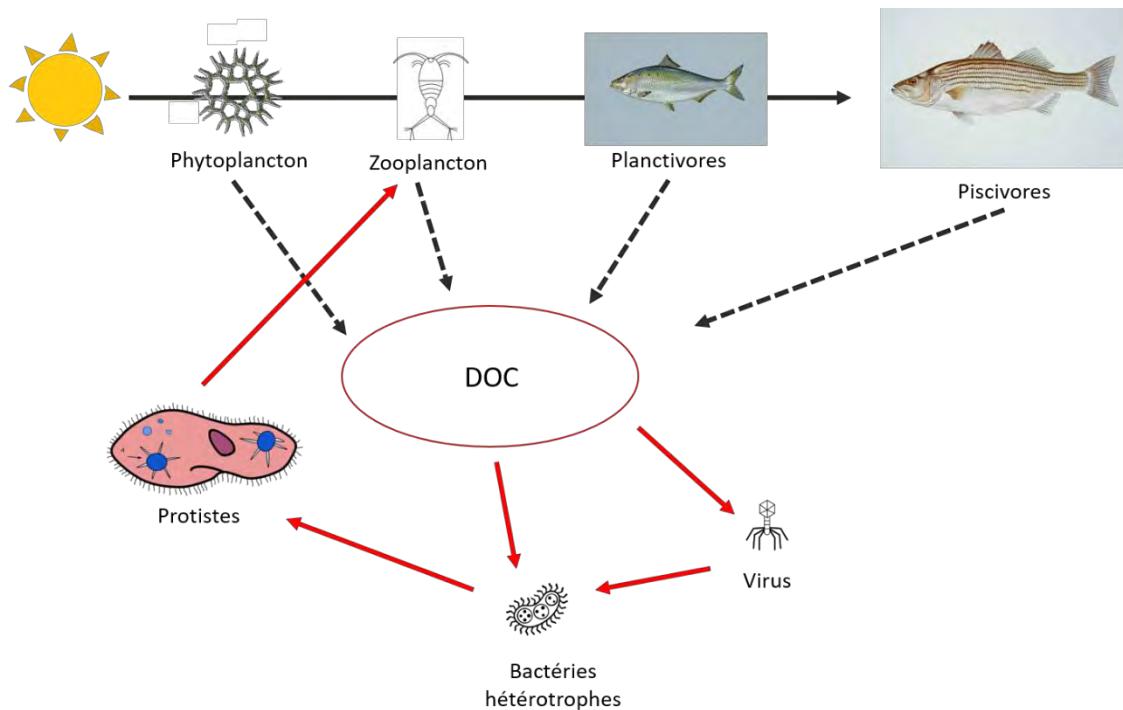
## Annexes

### Introduction



**Figure S0-1.** Masse volumique (densité) de l'eau en fonction de la température et de la salinité. À une température donnée, plus on augmente la salinité, plus la masse volumique augmente.

Source : <https://commons.wikimedia.org/wiki/File:WaterDensitySalinity.png>



**Figure S0-2.** Insertion de la boucle microbienne (en rouge) dans le réseau alimentaire.  
DOC *Dissolved Organic Carbon*.

Sources des images:

Paramécie:

[https://commons.wikimedia.org/wiki/File:Contractile\\_Vacuole\\_In\\_Paramecium.gif](https://commons.wikimedia.org/wiki/File:Contractile_Vacuole_In_Paramecium.gif)

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*Pediastrum*: Modifiée de [https://commons.wikimedia.org/wiki/File:Pediastrum\\_duplex.jpg](https://commons.wikimedia.org/wiki/File:Pediastrum_duplex.jpg)

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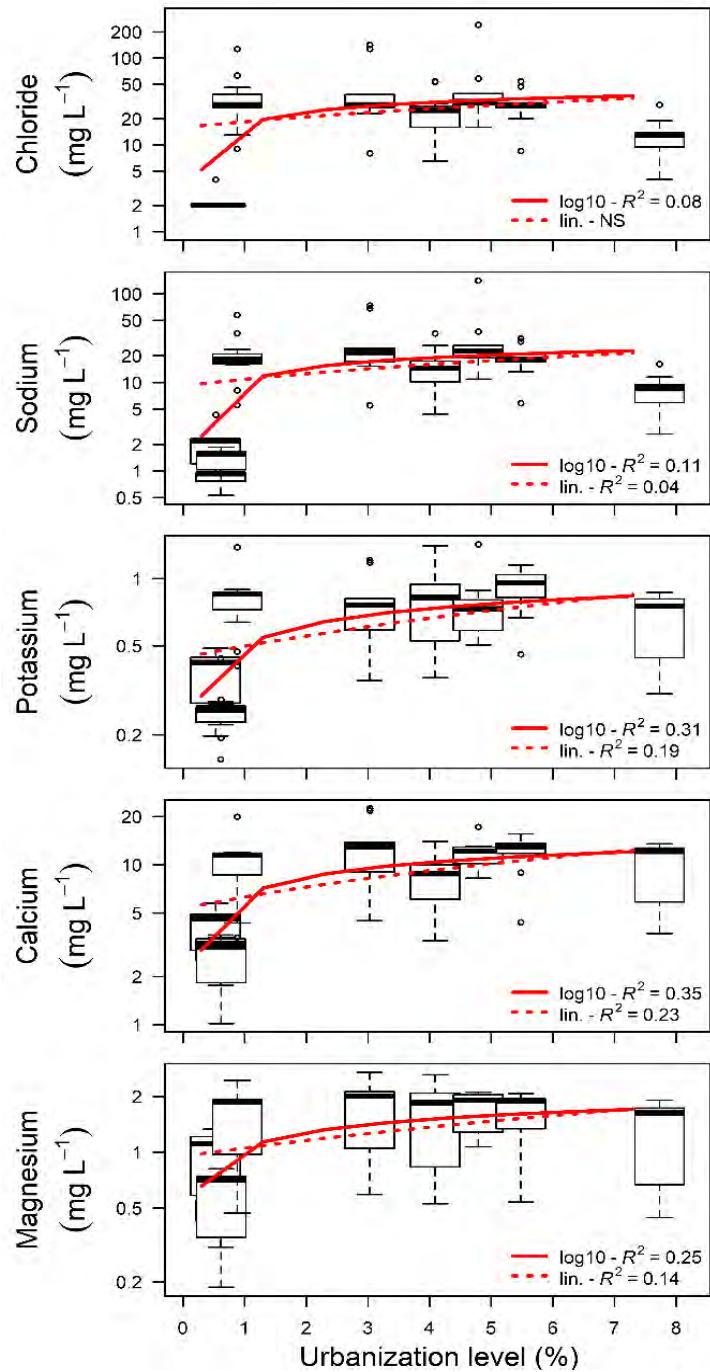
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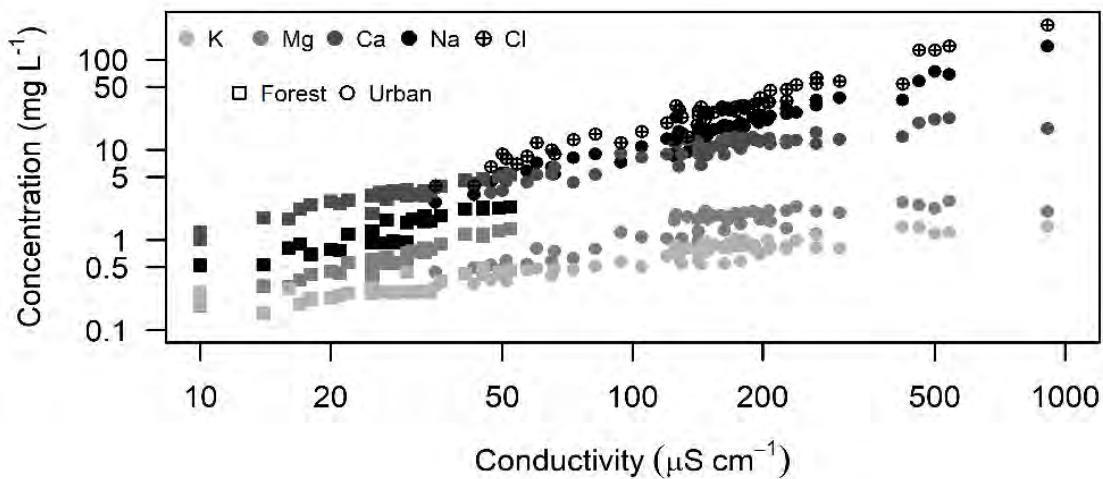
**Table S0- 1.** Liste non-exhaustive des pays qui utilisent des sels de déglaçage (NaCl).

Pays	Référence
Allemagne	Müller et Gächter 2012
Autriche	Müller et Gächter 2012
Biélorussie	Bulskaya et Volchek 2014
Canada	Evans et Frick 2001
Corée du sud	Duk Lee et al. 2017
États-Unis	Corsi et al. 2015
Finlande	Taka et al. 2017
Italie	Rogora et al. 2015
Iran	Aghazadeh et al. 2012
Lituanie	Ratkevičius et al. 2014
République de Lettonie	Gode et Paeglis 2014
République tchèque	Zehetner et al. 2009
Royaume-Unis	Rivett et al. 2016
Russie	Hilliges et al. 2013
Suède	Thunqvist 2004
Suisse	Müller et Gächter 2012

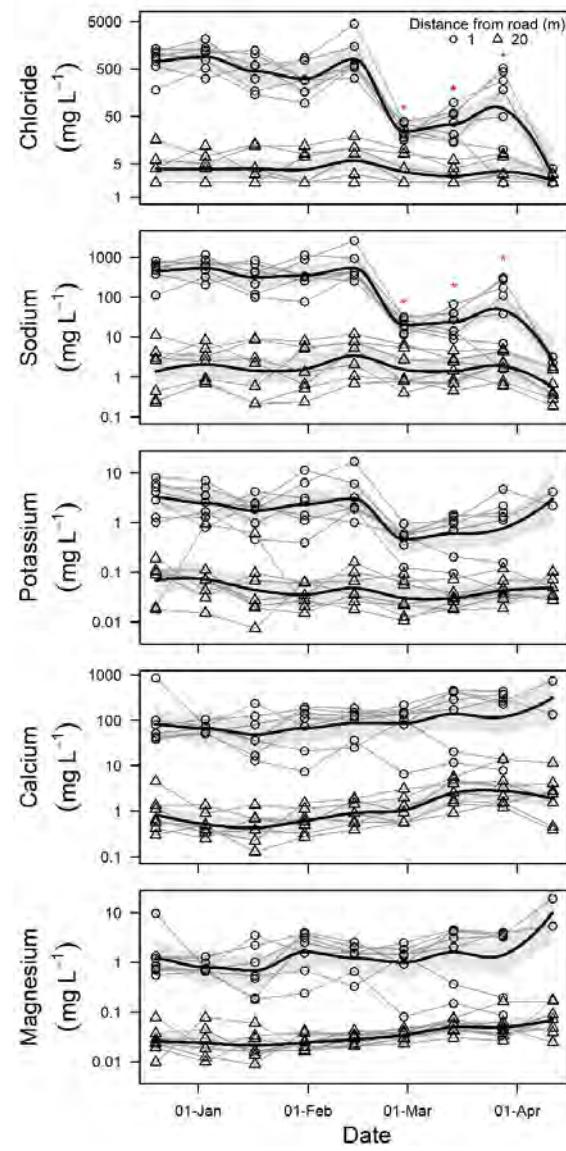
## Chapitre 1



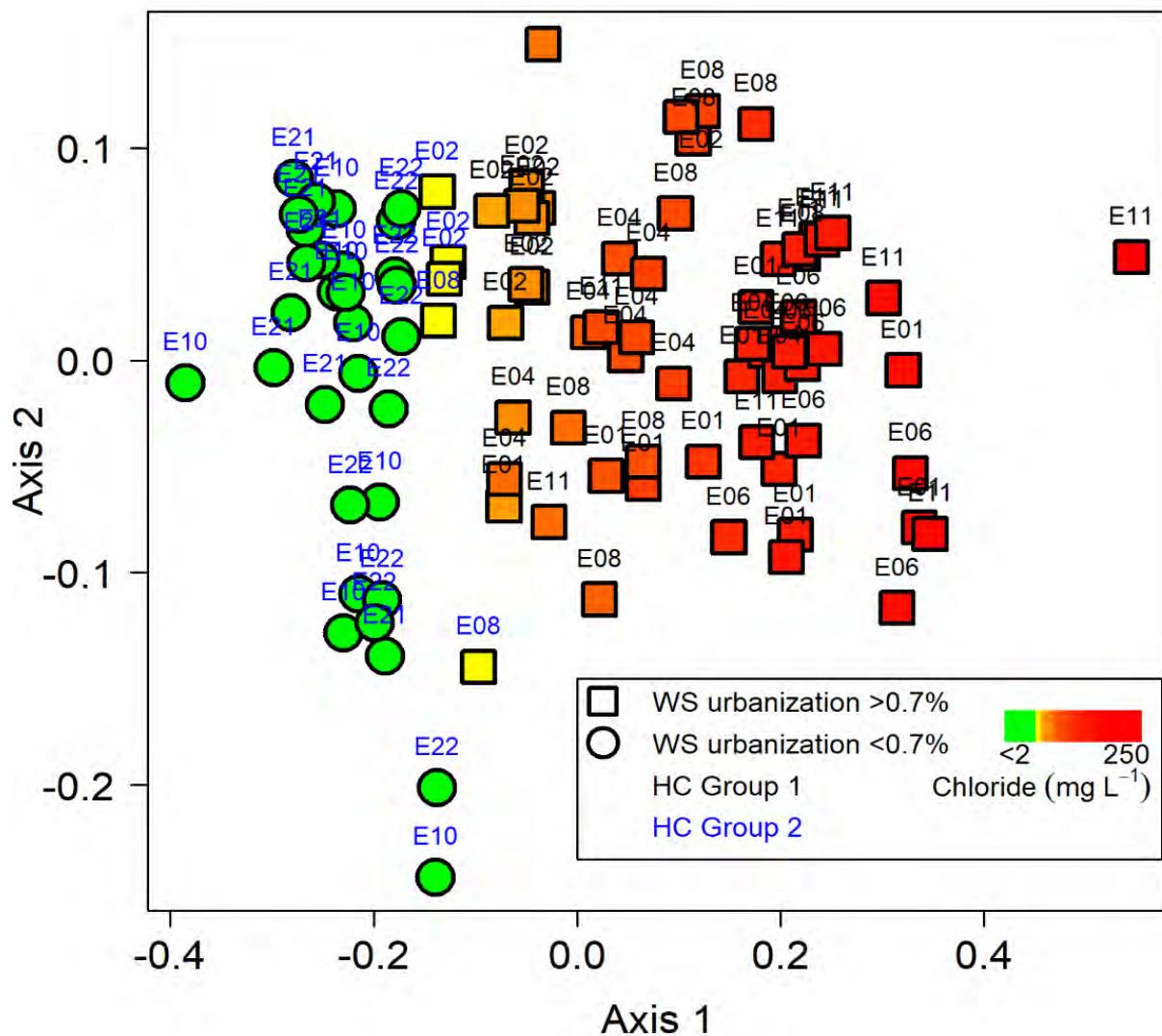
**Figure S1-1.** Chloride (Cl), sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) concentration in rivers as a function of impervious surface cover (%). Added lines are regression fits for all data ( $n = 95$ ) and are all significant at  $p < 0.001$ . Log10: logarithmic model  $y = a * \log_{10}(x) + b$ . Lin: linear model  $y = a * x + b$ . Open circles are outliers, i.e. values outside 1.5 times the interquartile range above the upper quartile and below the lower quartile.



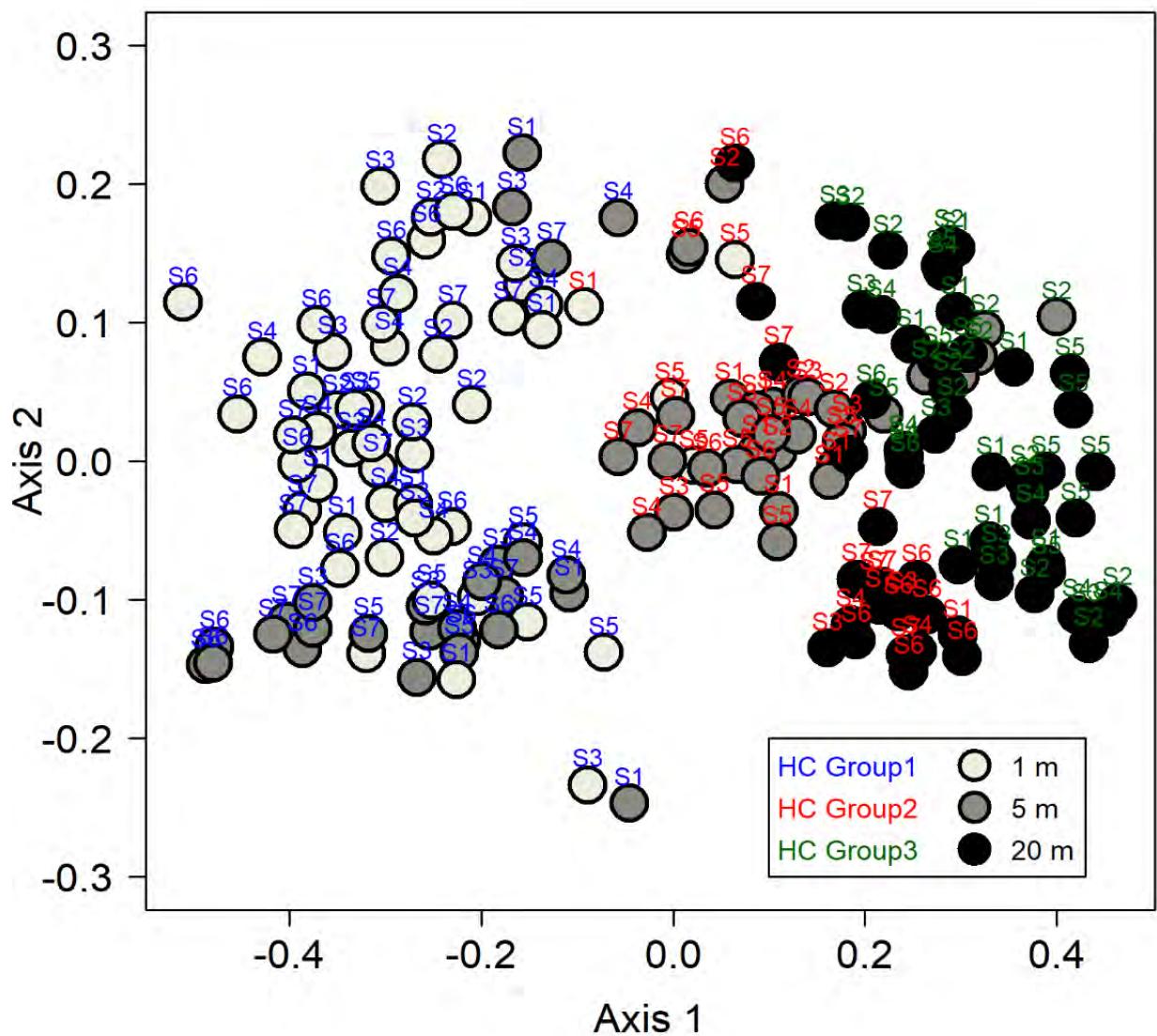
**Figure S1-2.** Chloride (Cl), sodium (Na), calcium (Ca), magnesium (Mg), and potassium (K) concentration in rivers of urban (circles) and forest (squares) watersheds as a function of conductivity. There is a close log-log relationship between the concentration of each ion and conductivity (for all relationships  $R^2 > 73$  and  $p < 0.001$ ; for all data ( $n=95$ )).



**Figure S1-3.** Chloride (Cl), sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) concentration in snow as a function of time. Note the log scales in all panels.

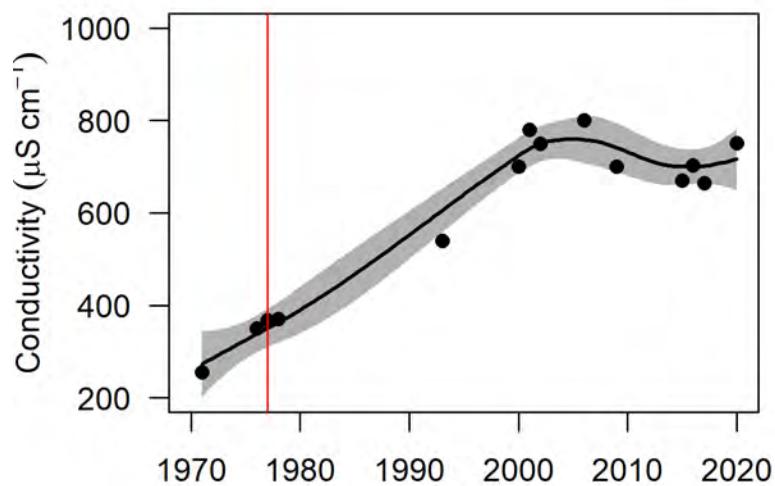


**Figure S1-4.** Non-metric multidimensional scaling (NMDS) of the rivers based on their chemical composition. WS: Watershed. HC: hierarchical clustering. EXX: sampling site ID. The stress value was 0.09. Each point represent a unique combination of sampling site and date.



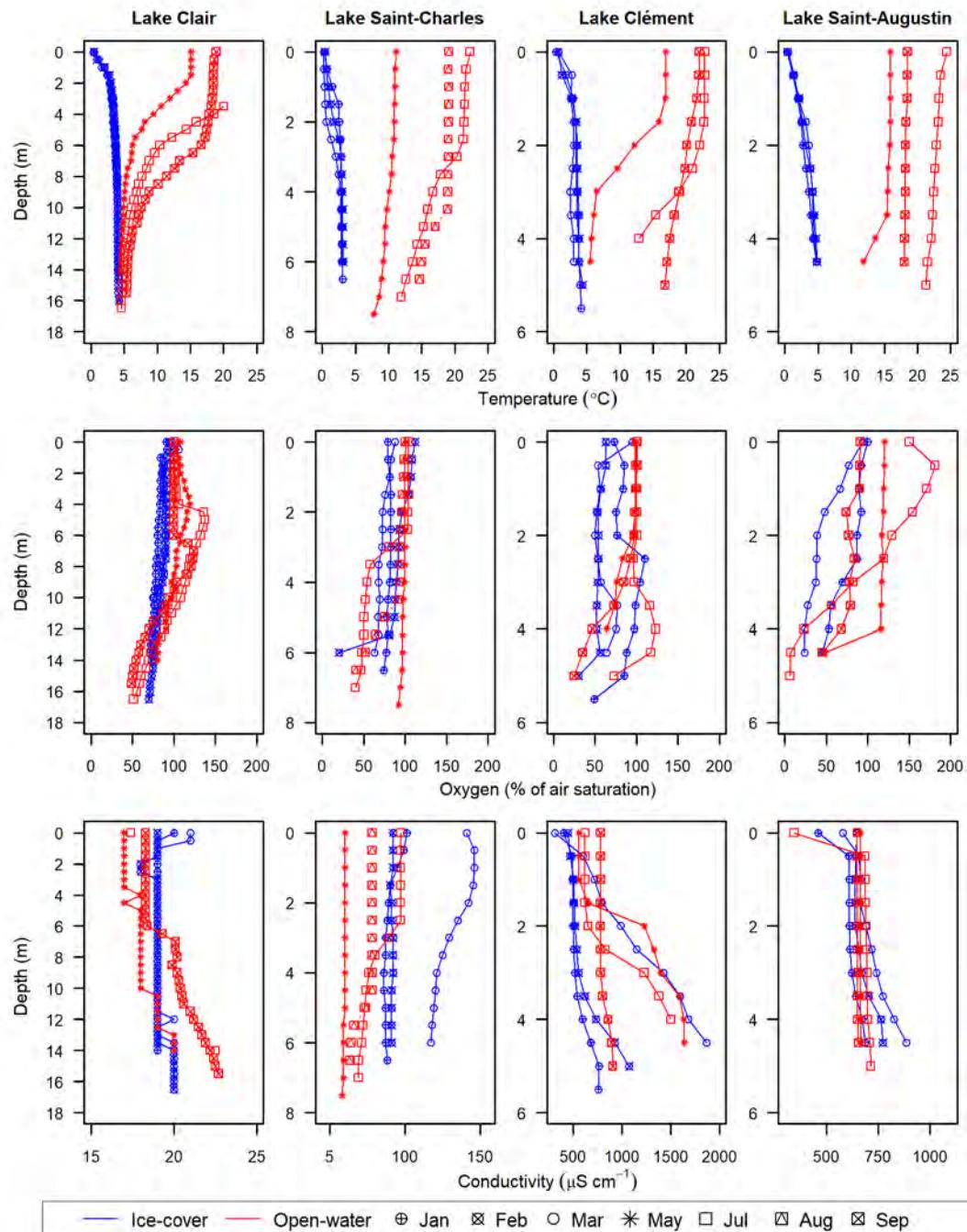
**Figure S1-5.** Non-metric multidimensional scaling (NMDS) of the snow based on its chemical composition. HC: hierarchical clustering. SX: sampling site ID. The stress value was 0.128. Each point represent a unique combination of sampling site, distance, and date. Sampling sites vary according to their traffic level, with low (S5), intermediate (S1-S4) and high (S6-S7).

## Chapitre 2

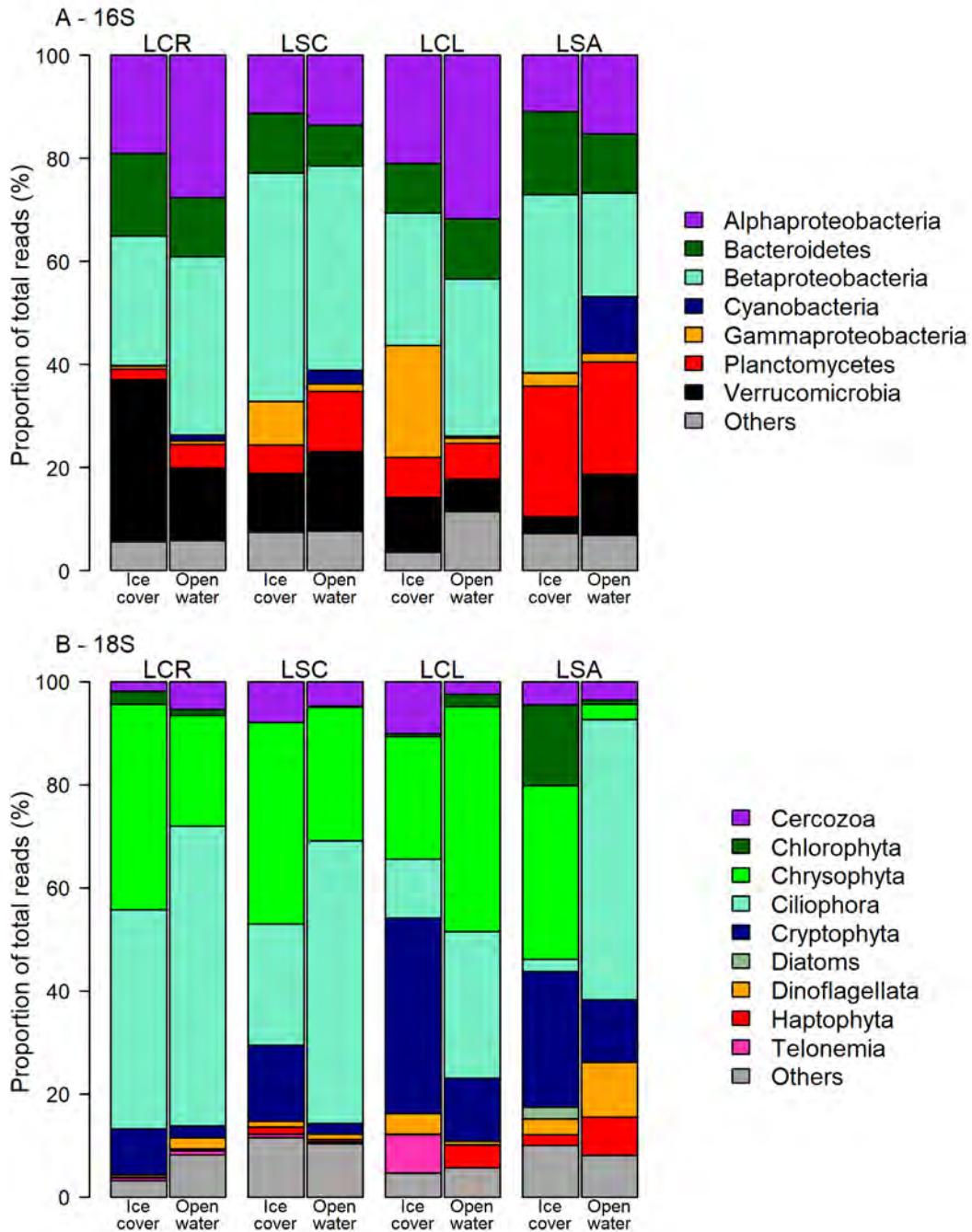


**Figure S2-1.** Changes in the specific conductivity of Lake Saint-Augustin surface water from 1971 to present. Red line marks the Highway 40 construction in the lake watershed (1977, Pienitz et al. 2006). Black line is a degree 2 polynomial fit of span 0.75.

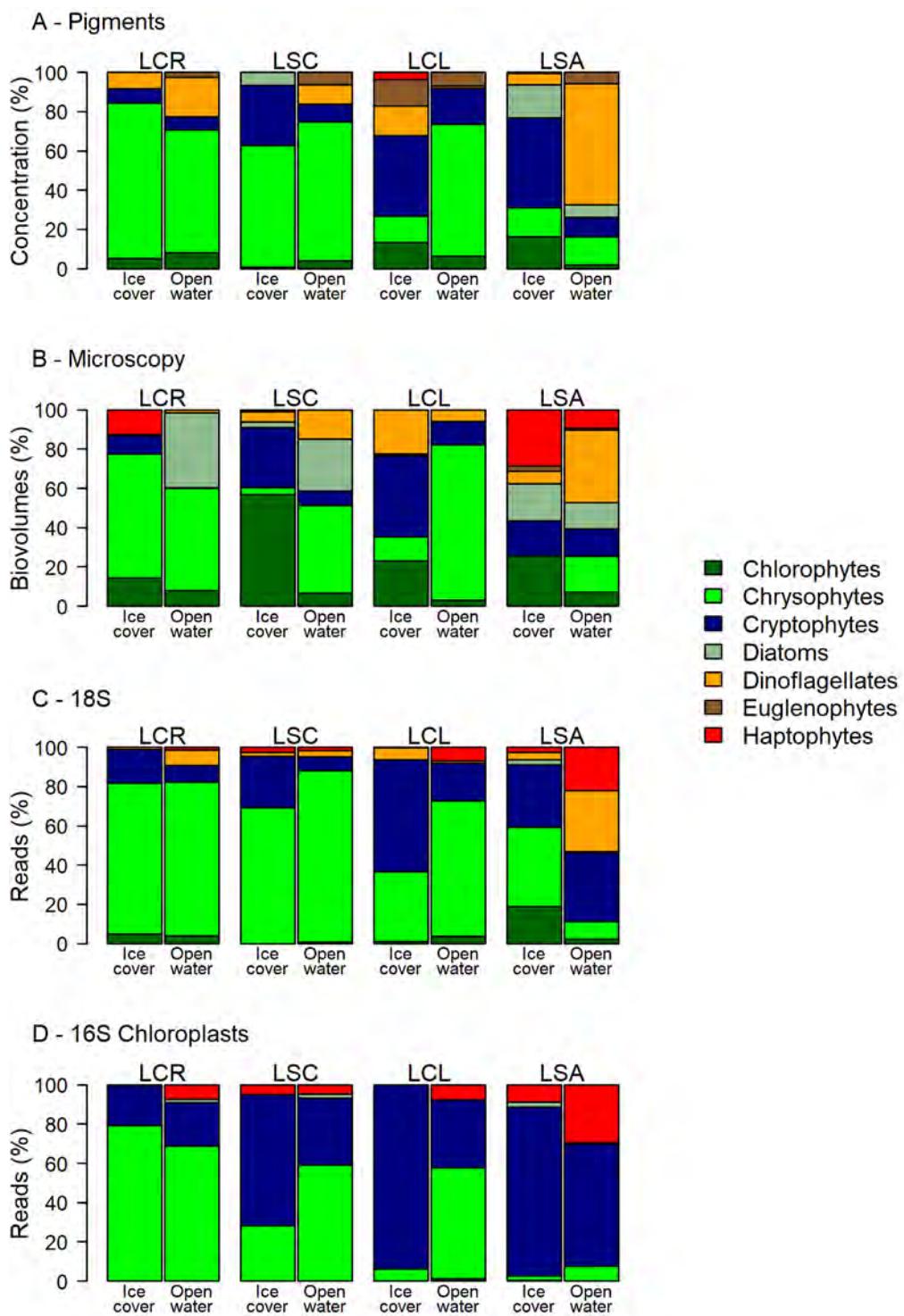
Sources: 1976-1978 Meunier and Alain 1979; 1993-2009 Galvez-Cloutier et al. 2012 and reference therein; 2015 OBV de la Capitale 2018; 2016-2017 present study; 13 September 2020, RBR-620-CTD, W.F. Vincent.



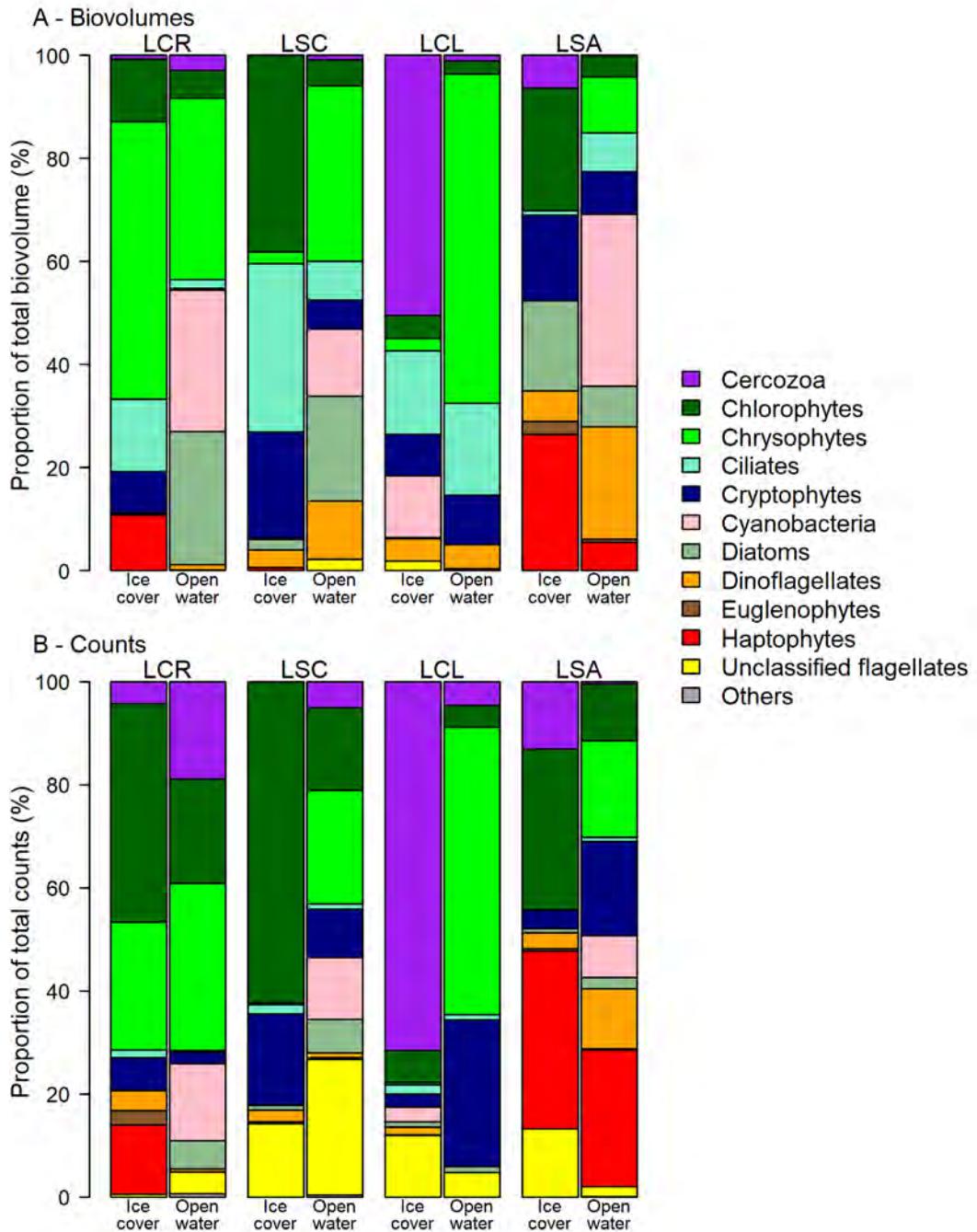
**Figure S2-2.** Temperature, oxygen and conductivity profiles of Lake Clair, Lake Saint-Charles, Lake Clément and Lake Saint-Augustin in 2017.



**Figure S2-3.** Taxonomic composition at the phylum level for A) bacteria and B) microbial eukaryotes from the rRNA analysis for Lake Clair, Lake Saint-Charles, Lake Clément, and Lake Saint-Augustin during the ice-cover and the open water periods.



**Figure S2-4.** Taxonomic composition at the phylum level for phytoplankton from A) photosynthetic pigments, B) microscopy, C) 18S rRNA and D) 16S rRNA chloroplasts for Lake Clair, Lake Saint-Charles, Lake Clément, and Lake Saint-Augustin during the ice-cover and the open water periods.



**Figure S2-5.** Taxonomic composition at the phylum level for A) Biovolumes and B) counts derived from microscopy for Lake Clair, Lake Saint-Charles, Lake Clément, and Lake Saint-Augustin during the ice-cover and the open water periods.

**Table S2-1.** Sampling dates for Lake Clair, Lake Saint-Charles, Lake Clément, and Lake Saint-Augustin in 2017.

Season	Lake Clair	Lake Saint-Charles	Lake Clément	Lake Saint-Augustin
Winter	January 24	January 23	January 10	January 12
	February 21	February 23	February 9	February 7
	March 21	March 23	March 9	March 7
Spring	May 30	May 19	June 01	May 22
Summer	July 24	July 25	July 13	July 14
Autumn	September 18	August 24	September 26	September 13
		August 31		

**Table S2-2.** Photosynthetic pigment mass ratios to chlorophyll a for different groups of photosynthetic plankton, used as input to the HPLC analysis. Carotene is the sum of  $\beta,\alpha$ -carotene and  $\beta,\beta$ -carotene and Chl c is the sum of chlorophyll c1 and chlorophyll c2.

Pigments	Chlorophytes	Chrysophytes	Cryptophytes	Cyanobacteria	Diatoms	Dinoflagellates	Euglenophytes	Haptophytes
Alloxanthin	0	0	0.368	0	0	0	0	0
Antheraxanthin	0.016	0	0	0	0	0	0	0
Aphanizophyll	0	0	0	0.0054	0	0	0	0
Astaxanthin	0.004	0	0	0	0	0	0	0
Canthaxanthin	0	0	0	0	0	0	0.014	0
Carotene	0.003	0.003	0.004	0.097	0.003	0.0025	0.004	0.03
Chl b	0.356	0	0	0	0	0	0.198	0
Chl c	0	0.032	0.091	0	0.091	0.1555	0	0
Diadinoxanthin	0	0.016	0	0	0.064	0.2465	0.327	0.165
Diatoxanthin	0	0.025	0	0	0.083	0.0795	0	0.235
Dinoxanthin	0	0	0	0	0	0.053	0	0
Echinone	0	0	0	0.526	0	0	0.026	0
Fucoxanthin	0	0.283	0	0	0.458	0	0	0.637
Lutein	0.147	0	0	0	0	0	0	0
Myxoxanthophyll	0	0	0	0.136	0	0	0	0
Neoxanthin	0.04	0	0	0	0	0	0.034	0
Peridinin	0	0	0	0	0	0.5075	0	0
Violaxanthin	0.026	0.063	0	0	0.003	0	0	0
Zeaxanthin	0.036	0.016	0	0.28	0.007	0	0	0

**Table S2-3.** Conditions of the polymerase chain reaction (PCR) thermal cycling for the 515F/806R primers as modified by Aprill et al. (2015) and the 572F/1009R primers from Comeau et al. (2011).

Steps	515F/806R			1389F/1510R		
	Temperature (°C)	Time (seconds)	Cycles	Temperature (°C)	Time (seconds)	Cycles
Initial denaturation	None		NA	98	30	1
Denaturation	94	45		98	10	
Annealing	50	60	36	52	30	30
Extension	72	90		72	30	
Final extension	72	60	1	72	270	1

**Table S2-4.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Clair for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the open water period; %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Clair open water			Chloroplast 16S rRNA		
Taxonomy	Reads%	CV%	Taxonomy	Reads%	CV%	Taxonomy	Biovolume%	CV%	Taxonomy	Reads%	CV%
Unclassified	28.5	10	<i>Mesodinium</i> sp.	44.1	52	<i>Dinobryon bavaricum</i>	29.1	69	Unclassified	18.3	95
Unclassified	20.5	98	Chrysophyceae	7.7	75	Cyanobacteria unclassified	20.7	169	<i>Synura uvella</i>	13.8	118
Unclassified	11.0	99	Unclassified	4.4	146	<i>Chrysochromulina</i> sp.	15.9	173	<i>Cryptomonas curvata</i>	12.3	77
<i>Polynucleobacter</i> sp.	3.5	34	Litostomatea	4.3	82	Picocyanobacteria	12.1	120	<i>Florenciella parvula</i>	11.7	70
<i>Rhodovarius</i> sp.	3.5	69	StrombidiidaA	3.8	97	<i>Chlamydomonas</i> sp.	5.9	106	<i>Dinobryon LO226KS</i>	7.9	59
CL500-3	3.4	102	Chrysophyceae cladeD	3.5	69	Unclassified	4.2	136	<i>Epipyxis PR26KG</i>	7.6	56
<i>Sediminibacterium</i> sp.	1.9	77	Chrysophyceae cladeC	2.4	63	<i>Dinobryon divergens</i>	2.9	173	<i>Chrysochromulina CCMP291</i>	4.1	116
Actinobacteria unclassified	1.8	38	<i>Dinobryon</i> sp.	2.4	35	<i>Strobilidium</i> sp.	1.8	173	<i>Chrysosphaera</i> sp.	2.8	145
<i>Flavobacterium</i> sp.	1.6	135	Unclassified ochrophyta	2.1	110	<i>Peridinium</i> sp.1	1.4	146	<i>Cryptomonas erosa</i>	2.6	47
Unclassified	1.5	96	Pterocystida	2.0	171	<i>Oocystis</i> sp.	1.1	173	<i>Ochromonas CCMP1393</i>	2.0	45
<i>Emticicia</i> sp.	1.5	118	Unclassified	1.7	86	<i>Tabellaria fenestrata</i>	0.8	173	Rhodophytes unclassified	1.7	53
GKS98	1.3	10	<i>Cryptomonas pyrenoidifera</i>	1.7	81	<i>Uronema</i> sp.	0.5	173	<i>Hemiselmis</i> sp.	1.5	172
Actinobacteria hgcl clade	1.2	45	Unclassified	1.5	76	<i>Cryptomonas marssonii</i>	0.5	104	<i>Acanthoceras zachariasii</i>	1.3	161
<i>Dinghuibacter</i> sp.	1.2	102	<i>Chrysosphaerella</i> sp.	1.2	149	<i>Rabdoderma</i> sp.	0.5	110	Unclassified	1.0	87
<i>Caulobacter</i> sp.	0.9	62	<i>Dinobryon crenulatum</i>	0.9	67	<i>Merismopedia</i> sp.	0.3	173	Unclassified	0.6	55
<i>Ferruginibacter</i> sp.	0.9	75	Chrysophyceae cladeE	0.9	39	Unclassified flagellate	0.2	173	<i>Mallomonas splendens</i>	0.5	126
<i>Arcicella</i> sp.	0.7	172	<i>Askenasia</i> sp.	0.8	56	<i>Staurastrum</i> sp.	0.2	173	<i>Chromulina</i> sp.	0.4	85
IMCC26134	0.7	167	Mamiellophyceae	0.8	118	<i>Peridinium</i> sp.2	0.2	173	<i>Pavlova gyrans</i>	0.3	105
SM1A02	0.7	155	Centroheliozoa	0.8	157	<i>Urosolenia</i> sp.	0.1	173	<i>Pseudopedinella elastica</i>	0.3	73
Roseomonas sp.	0.6	94	StrobiliidaeC	0.7	114	<i>Mallomonas tonsurata</i>	0.1	87	<i>Oophila amblystomatis</i>	0.1	87

Legend 16S rRNA		Legend 18S rRNA, microscopy and chloroplast 16S rRNA					
Alphaproteobacteria	Gammaproteobacteria	Ciliophora	Katablepharidophyta				
Bacteroidetes	Planctomycetes	Chlorophyta	Telomemia				
Betaproteobacteria	Verrucomicrobia	Cryptophyta	Ochrophyta				
Cyanobacteria	Others	Dinoflagellata	Others				
		Haptophyta	Unknown				

**Table S2-5.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Clair for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the ice-cover period (Jan-Feb-Mar); %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Clair ice-cover			Chloroplast 16S rRNA		
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolume %	CV %	Taxonomy	Reads %	CV %
Unclassified	21.1	13	<i>Mesodinium</i> sp.	39.8	60	<i>Dinobryon divergens</i>	46.0	132	<i>Synura uvella</i>	68.6	81
Unclassified	15.5	3	<i>Synura mollispina</i>	35.8	72	<i>Strobilidium</i> sp.	12.0	173	<i>Cryptomonas erosa</i>	10.6	37
Unclassified	13.1	30	Unclassified cryptophyceae	5.2	90	<i>Chlamydomonas</i> sp.	11.2	84	<i>Floreniella parvula</i>	5.4	96
<i>Chthoniobacter</i> sp.	6.4	37	<i>Hamakko caudatus</i>	2.4	69	<i>Chrysochromulina</i> sp.	10.8	173	<i>Cryptomonas curvata</i>	4.6	48
<i>Sediminibacterium</i> sp.	5.4	71	<i>Cryptomonas pyrenoidifera</i>	2.9	81	<i>Cryptomonas ovata</i>	7.9	120	Unclassified	4.1	63
SH3-11	3.9	60	Novel clade 10	1.2	28	<i>Dinobryon bavaricum</i>	5.1	145	Unclassified	3.0	127
Unclassified	3.2	46	<i>Cryptomonas curvata</i>	1.1	68	<i>Mallomonas caudata</i>	2.8	173	<i>Teleaulax amphioxiae</i>	1.0	130
BSV13	2.6	86	<i>Strombidiida</i> A	0.9	139	<i>Uronema</i> sp.	1.7	93	<i>Epipyxis PR26KG</i>	0.8	120
<i>Luteolibacter</i> sp.	2.5	152	Cryptomonadales	0.8	131	Unclassified	0.8	173	Unclassified	0.4	49
<i>Cephaloticroccus</i> sp.	1.9	74	Katablepharidales	0.8	64	<i>Cryptomonas marssonii</i>	0.6	107	<i>Dinobryon LO226KS</i>	0.4	68
<i>Flavobacterium</i> sp.	1.8	21	Ochrophyta	0.8	85	Unclassified flagellate 2	0.5	112	<i>Heterosigma akashimo</i>	0.4	59
Unclassified Actinobacteria unclassified	1.8	65	Chrysophyceae	0.7	87	<i>Mesodinium</i> sp.	0.4	173	<i>Chrysochromulina CCMP291</i>	0.1	147
<i>Polynucleobacter</i> sp.	1.6	48	<i>Mallomonas</i> sp.	0.6	78	<i>Chloromonas</i> sp.	0.4	173	Rhodophytes unclassified	0.1	124
Actinobacteria hgcI clade	1.5	33	Group 2	0.6	94	<i>Peridinium</i> sp.1	0.3	173	<i>Pavlova gyrans</i>	0.1	87
<i>Caulobacter</i> sp.	1.0	96	Pseudodendromonadales	0.6	80	<i>Eutreptia</i> sp.	0.1	100	<i>Chrysosphaera</i> sp.	0.1	136
Flexibacteraceae	0.9	87	Chrysophyceae cladeD	0.5	110	Unclassified	0.1	173	<i>Ochromonas CCMP1393</i>	0.1	173
<i>Rhodovastum</i> sp.	0.9	57	<i>Cryptomonas tetrapyrenoidosa</i>	0.5	101	<i>Cymbella</i> sp.	0.0	173	<i>Cryptomonas ovata</i>	0.1	87
Marine group	0.8	119	Unclassified	0.4	121				<i>Chromulina</i> sp.	0.1	173
<i>Polaromonas</i> sp.	0.8	57	Unclassified	0.4	134				<i>Mallomonas splendens</i>	0.0	173
			Choanoflagellata	0.4	155				Unclassified	0.0	173

Legend 16S rRNA

- Alphaproteobacteria
- Bacteroidetes
- Betaproteobacteria
- Cyanobacteria

Gammaproteobacteria  
Planctomycetes  
Verrucomicrobia  
Others

Legend 18S rRNA, microscopy and chloroplast 16S rRNA

- Cercozoa
- Chlorophyta
- Ciliophora
- Cryptophyta
- Dinoflagellata
- Haptophyta
- Katablepharidophyta
- Telomelia
- Ochrophyta
- Others
- Unknown

**Table S2-6.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Saint-Charles for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the open water period; %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Saint-Charles open water			Chloroplast 16S rRNA		
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolume %	CV %	Taxonomy	Reads %	CV %
Unclassified	31.1	104	Litostomatea	28.6	103	<i>Synura uvella</i>	17.2	148	<i>Florenciella parvula</i>	18.6	122
Unclassified	11.2	40	<i>Mesodinium</i> sp.	6.2	124	<i>Picocyanobacteria</i>	12.1	72	Unclassified	15.7	65
Phycisphaerae CL500-3	10.7	85	<i>Dinobryon sociale</i>	3.6	72	<i>Chryschromulina</i> sp.	10.2	136	<i>Cryptomonas eros</i>	11.1	32
Unclassified	8.9	63	<i>Pseudoholophrya</i> sp.	3.5	135	<i>Ceratium hirundinella</i>	9.3	200	<i>Cryptomonas curvata</i>	8.3	60
<i>Polynucleobacter</i> sp.	3.8	132	Unclassified chrysophyceae	3.2	51	<i>Asterionella formosa</i>	7.7	130	<i>Epipyxis PR26KG</i>	7.8	55
<i>Sediminibacterium</i> sp.	2.5	185	<i>Dinobryon divergens</i>	3.1	122	<i>Dinobryon bavaricum</i>	6.9	70	<i>Synura uvella</i>	7.1	56
Actinobacteria unclassified	2.3	154	Chrysophyceae cladeD	2.8	84	<i>Strobilidium</i> sp.	6.6	71	<i>Dinobryon LO226KS</i>	6.6	46
Unclassified	1.7	80	StrombidiidaA	2.7	106	<i>Chlamydomonas</i> sp.	4.1	132	<i>Ochromonas CCMP1393</i>	3.8	175
<i>Microcystis</i> sp.	1.7	170	Didiniidae	2.3	189	<i>Peridinium</i> sp. 1	3.1	62	<i>Chryschromulina CCMP291</i>	2.3	102
<i>Flavobacterium</i> sp.	1.6	185	<i>Sphaeroeca leprechaunica</i>	2.3	190	<i>Tabellaria fenestrata</i>	3.0	155	Unclassified	2.0	133
GKS98	1.4	172	<i>Chrysosphaerella</i> sp.	2.0	133	<i>Dinobryon divergens</i>	3.0	80	<i>Cryptomonas ovata</i>	1.7	89
SH3-11	1.2	88	Unclassified	1.8	104	<i>Cryptomonas ovata</i>	2.9	155	Rhodophytes unclassified	1.5	60
Unclassified	1.1	112	Chrysophyceae cladeC	1.7	74	Unclassified flagellate	2.3	118	<i>Acanthoceras zachariasii</i>	1.3	75
<i>Caulobacter</i> sp.	0.9	128	Unclassified ochrophyta	1.6	36	<i>Cryptomonas</i> sp.	2.0	89	Unclassified	1.0	108
Babeliae unclassified	0.8	158	<i>Uroglena americana</i>	1.6	162	<i>Uronema</i> sp.	1.4	99	<i>Heterosigma akashimo</i>	1.0	175
<i>Phenyllobacterium</i> sp.	0.7	133	Unclassified	1.6	42	Unclassified	1.0	81	<i>Synura LO234KE</i>	0.9	115
<i>Polaromonas</i> sp.	0.7	166	<i>Dinobryon bavaricum</i>	1.4	114	<i>Cryptomonas marssonii</i>	0.9	49	<i>Hemiselmis</i> sp.	0.9	152
IMCC26134	0.7	100	<i>Dinobryon</i> sp.	1.3	94	Cyanobacteria unclassified	0.9	144	<i>Mallomonas splendens</i>	0.6	133
<i>Methylotenera</i> sp.	0.7	196	<i>Askenasia</i> sp.	1.3	102	<i>Pediastrum tetras</i>	0.9	200	<i>Pseudopedinella elastica</i>	0.4	58
<i>Aphanizomenon</i> sp.	0.7	84	StrobiliidaeB	1.1	181	<i>Aulacoseira c.f. italica</i>	0.5	200	<i>Chromulina</i> sp.	0.4	98

Alphaproteobacteria	Gammaproteobacteria
Bacteroidetes	Planctomycetes
Betaproteobacteria	Verrucomicrobia
Cyanobacteria	Others

Ciliophora	Katablepharidophyta
Chlorophyta	Telomelia
Cryptophyta	Ochrophyta
Dinoflagellata	Others
Haptophyta	Unknown

**Table S2-7.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Saint-Charles for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the ice-cover period (Jan-Feb-Mar); %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Saint-Charles ice-cover			Chloroplast 16S rRNA		
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolume %	CV %	Taxonomy	Reads %	CV %
Unclassified	26.3	67	<i>Synura petersenii</i>	21.3	126	<i>Chlamydomonas</i> sp.	34.9	118	Unclassified	25.9	135
Unclassified	7.0	72	<i>Strombidiida</i> A	9.9	50	<i>Strobilidium</i> sp.	27.6	89	<i>Cryptomonas erosa</i>	22.6	45
Unclassified	6.9	39	<i>Cryptomonadales</i>	6.9	118	<i>Cryptomonas ovata</i>	14.9	92	<i>Synura uvella</i>	19.0	79
<i>Sediminibacterium</i> sp.	4.6	66	Unclassified	4.6	61	<i>Cryptomonas marssonii</i>	5.6	173	<i>Teleaulax amphioxiae</i>	10.9	147
<i>Methyllobacter</i> sp.	4.5	81	<i>Protaspida</i> lineage	4.2	110	<i>Uronema</i> sp.	5.1	97	Unclassified	6.7	120
<i>Nitrosospira</i> sp.	4.4	163	<i>Chrysophyceae</i> cladeE	3.9	126	<i>Peridinium</i> sp. 2	2.8	88	<i>Cryptomonas curvata</i>	5.0	91
<i>Polaromonas</i> sp.	4.0	155	<i>Chrysophyceae</i>	3.0	94	<i>Dinobryon divergens</i>	2.4	118	<i>Chrysochromulina</i> CCMP291	4.9	118
Unclassified	3.9	65	<i>Vorticella</i> sp.	2.8	81	<i>Oocystis</i> sp.	2.1	173	<i>Epipyxis</i> PR26KG	2.0	161
Unclassified	3.6	134	<i>Synura</i> sp.	2.7	111	<i>Asterionella formosa</i>	2.1	173	<i>Cryptomonas ovata</i>	1.4	87
<i>Polynucleobacter</i> sp.	3.3	63	<i>Chrysophyceae</i> cladeF	2.3	80	Unclassified flagellate	1.0	97	<i>Florenciella parvula</i>	0.8	66
Unclassified	2.4	68	<i>Chrysophyceae</i> 1	2.1	67	<i>Peridinium</i> sp. 1	0.7	173	<i>Guillardia theta</i>	0.2	60
<i>Nitrotoga</i> sp.	2.4	156	<i>Cryptomonas pyrenoidifera</i>	2.1	76	<i>Chrysochromulina</i> sp.	0.6	173	<i>Dinobryon</i> LO226KS	0.2	85
SH3-11	1.8	72	<i>Chrysotrichida</i>	1.3	79	<i>Picocyanobacteria</i>	0.2	173	<i>Chroomonas caudata</i>	0.1	82
CL500-3	1.7	124	<i>Chrysophyceae</i> cladeC	1.2	99				Rhodophytes unclassified	0.1	129
<i>Luteolibacter</i> sp.	1.5	173	<i>Litostomatea</i>	1.0	164				<i>Acanthocera zachariasii</i>	0.0	88
Acidobacteria subgroup 3	1.5	129	Unclassified	1.0	49				<i>Chromalina</i> sp.	0.0	138
Actinobacteria unclassified	1.4	64	<i>Askenasia</i> sp.	0.9	68				<i>Pseudopedinella elastica</i>	0.0	132
<i>Rhodovastum</i> sp.	1.3	97							<i>Chrysosphaera</i> sp.	0.0	100
Margulisbacteria unclassified	1.3	84							Unclassified	0.0	100
<i>Flavobacterium</i> sp.	1.0	50							<i>Hemiselmis</i> sp.	0.0	87

Legend 16S rRNA	Legend 18S rRNA, microscopy and chloroplast 16S rRNA
Alphaproteobacteria	Gammaproteobacteria
Bacteroidetes	Planctomycetes
Betaproteobacteria	Verrucomicrobia
Cyanobacteria	Others
	Cercozoa
	Chlorophyta
	Ciliophora
	Cryptophyta
	Dinoflagellata
	Haptophyta
	Katablepharidophyta
	Telomelia
	Ochrophyta
	Others
	Unknown

**Table S2-8.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Clément for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the open water period; %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Clément open water			Chloroplast 16S rRNA		
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolume %	CV %	Taxonomy	Reads %	CV %
Unclassified	28.62	41	Litostomatea	10.79	65	<i>Chrysochromulina</i> sp.	55.35	88	<i>Dinobryon</i> LO226KS	21.93	137
Unclassified	21.12	26	<i>Dinobryon divergens</i>	10.61	142	<i>Strobilidium</i> sp.	15.97	73	<i>Synura uvella</i>	12.48	72
<i>Polynucleobacter</i> sp.	5.21	72	<i>Chrysophyceae</i> cladeC	8.05	22	<i>Dinobryon divergens</i>	6.89	101	<i>Teleaulax amphioxiae</i>	10.96	80
CL500-3	5.05	94	<i>Dinobryon bavaricum</i>	6.29	144	<i>Cryptomonas marssonii</i>	6.64	79	<i>Cryptomonas erosa</i>	10.27	36
Unclassified	3.69	38	<i>Strombidiidae</i> A	5.94	42	<i>Ceratium hirundinella</i>	4.61	173	<i>Florenciella parvula</i>	10.02	65
Unclassified	2.99	140	<i>Halteriidae</i>	5.73	82	<i>Cryptomonas ovata</i>	1.66	155	Unclassified	7.14	90
<i>Dinghuibacter</i> sp.	2.34	73	<i>Mallomonas caudata</i>	4.72	173	<i>Uronema</i> sp.	1.48	173	<i>Chrysophytes unclassified</i>	6.62	47
SH3-11	2.27	56	<i>Chrysophyceae</i>	4.71	21	<i>Oocystis</i> sp.	1.15	173	<i>Epipyxis PR26KG</i>	5.61	92
Actinobacteria unclassified	2.21	85	<i>Chrysophyceae</i> B1	3.41	6	<i>Cryptomonas</i> sp.	1.15	173	<i>Chrysochromulina</i> CCMP291	4.16	83
Fimbriimonadia unclassified	1.99	111	<i>Diacronema</i> sp.	2.79	103	Unclassified	1.13	104	Rhodophytes unclassified	3.55	51
<i>Sediminibacterium</i> sp.	1.68	74	<i>Cryptophyceae</i> 1	2.49	81	<i>Chloromonas</i> sp.	0.54	109	<i>Pavlova gyrans</i>	1.89	115
Actinobacteria hgcl clade	1.53	44	<i>Chlamydomonadales</i>	2.36	75	<i>Chlamydomonas</i> sp.	0.43	141	<i>Ochromonas</i> CCMP1393	0.65	88
Delta proteobacteria unclassified	1.42	150	<i>Cryptomonas curvata</i>	2.10	104	<i>Mallomonas caudata</i>	0.37	58	<i>Oophila amblystomatis</i>	0.63	70
Delta proteobacteria OM27 clade	1.38	141	<i>Hypotrichia</i>	2.01	51	Unclassified flagellate	0.33	173	<i>Pseudopedinella elastica</i>	0.56	114
MWH-UniP1	1.35	171	<i>Katablepharidales</i>	1.97	105	Unclassified flagellate 2	0.19	90	<i>Chlamydomonas asymmetrica</i>	0.38	83
<i>Flavobacterium</i> sp.	1.10	137	Novel clade 2	1.65	109	<i>Asterococcus</i> sp.	0.06	88	Unclassified	0.34	111
<i>Polaromonas</i> sp.	1.05	66	<i>Chrysochromulina parva</i>	1.62	100	<i>Elakatothrix</i> sp.	0.04	173	<i>Cryptomonas curvata</i>	0.27	86
Unclassified	0.89	20	<i>Plagioselmis</i> sp.	1.86	56	<i>Kephryion</i> sp.	0.04	173	<i>Hemiselmis</i> sp.	0.25	25
<i>Hirschia</i> sp.	0.75	39	<i>Mallomonas</i> sp.	1.52	99	<i>Pseudanabaena</i> sp.	0.02	173	<i>Virgulinella fragilis</i>	0.15	94
<i>Fluviicola</i> sp.	0.71	44				<i>Ophiocytium</i> sp.	0.01	173	<i>Guillardia theta</i>	0.02	115

Legend 16S rRNA		Legend 18S rRNA, microscopy and chloroplast 16S rRNA	
Alphaproteobacteria	Gammaproteobacteria	Cercozoa	Katablepharidophyta
Bacteroidetes	Planctomycetes	Chlorophyta	Telonemia
Betaproteobacteria	Verrucomicrobia	Ciliophora	Ochrophyta
Cyanobacteria	Others	Cryptophyta	Others
		Dinoflagellata	Unknown
		Haptophyta	

**Table S2-9.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Clément for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the ice-cover period (Jan-Feb-Mar); %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			Lake Clément ice-cover			Chloroplast 16S rRNA					
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolume %	CV %	Taxonomy	Reads %	C V %
Unclassified	19.8	15	<i>Cryptomonas</i> sp.	28.1	89	Unclassified	50.5	115	<i>Cryptomonas erosa</i>	78.7	83
Unclassified	16.1	16	Chrysophyceae cladeF	16.1	49	<i>Uronema</i> sp.	16.3	173	Unclassified	8.7	86
Unclassified	13.1	26	Novel clade 10	9.3	86	Picocyanobacteria	12.0	173	<i>Teleaulax amphioxiae</i>	4.3	7
Unclassified	8.0	65	<i>Halteria grandinella</i>	7.7	162	<i>Cryptomonas</i> sp.	8.0	173	<i>Epipyxis PR26KG</i>	4.2	17
<i>Methylobacter</i> sp.	7.6	53	Group 2	7.4	117	<i>Peridinium</i> sp. 2	4.3	62	Chrysophytes unclassified	1.9	47
CL500-3	6.4	91	Cryptophyceae 1	4.8	82	<i>Chloromonas</i> sp.	2.9	173	<i>Florencella parvula</i>	1.1	80
Unclassified	4.2	13	Cryptomonadales	4.4	118	Unclassified choanoflagellate	1.9	173	<i>Cryptomonas curvata</i>	0.4	1
<i>Polaromonas</i> sp.	2.8	52	<i>Borghiella tenuissima</i>	3.7	171	<i>Dinobryon divergens</i>	1.2	173	<i>Pavlova gyrans</i>	0.1	12
<i>Polynucleobacter</i> sp.	2.6	33	Chrysophyceae cladeD	3.7	115	<i>Chrysochromulina</i> sp.	1.1	173	<i>Synura uvela</i>	0.1	2
<i>Sediminibacterium</i> sp.	2.0	40	Chrysophyceae	2.7	97	Unclassified flagellate 2	0.9	87	<i>Chrysochromulina CCMP291</i>	0.1	66
Unclassified	1.3	54	Parmales	1.3	42	<i>Cryptomonas marssonii</i>	0.8	173	<i>Dinobryon LO226KS</i>	0.0	0
CM1G08	1.2	46	StrombidiidaA	1.1	87	<i>Ophiciotium</i> sp.	0.5	173	<i>Chrysosphaera</i> sp.	0.0	13
<i>Fluviicola</i> sp.	1.2	68	Chrysophyceae cladeC	1.0	99	<i>Chlamydomonas</i> sp.	0.2	173	<i>Hemiselmis</i> sp.	0.0	5
SH3-11	1.1	114	Ochrophyta	0.9	158	<i>Cymbella</i> sp.	0.1	173	<i>Guillardia theta</i>	0.0	17
Actinobacteria hgcI clade	0.9	38	Halteriidae	0.9	103	<i>Synedra</i> sp.	0.1	173	<i>Ochromonas CCMP1393</i>	0.0	0
Actinobacteria unclassified	0.9	38	<i>Cryptomonas curvata</i>	0.7	107				<i>Pseudopedinella elastica</i>	0.0	17
<i>Nitrosospira</i> sp.	0.8	148	Chlamydomonadales	0.6	167				<i>Ophelia amblystomatis</i>	0.0	3
IMCC26134	0.7	133	Protaspida lineage	0.5	132				<i>Skeletonema pseudocostatum</i>	0.0	17
<i>Methylotenera</i> sp.	0.7	40	<i>Plagioselmis</i> sp.	0.5	161					0.0	3
<i>Rhodoferax</i> sp.	0.5	78	Philasterida	0.4	103						

Legend 16S rRNA				Legend 18S rRNA, microscopy and chloroplast 16S rRNA			
Alphaproteobacteria	Gammaproteobacteria	Ciliophora	Katablepharidophyta				
Bacteroidetes	Planctomycetes	Chlorophyta	Telomnia				
Betaproteobacteria	Verrucomicrobia	Ochrophyta	Ochrophyta				
Cyanobacteria	Others	Cryptophyta	Others				
		Dinoflagellata	Unknown				
		Haptophyta					

**Table S2-10.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Saint-Augustin for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the open water period; %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Saint-Augustin open water			Chloroplast 16S rRNA		
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolumen %	CV %	Taxonomy	Reads %	CV %
CL500-3	15.2	50	Litostomatea	17.8	40	Aphanocapsa sp.	31.5	173	<i>Chryschromulina</i>	28.7	52
Unclassified	14.0	13	<i>Chryschromulina parva</i>	7.4	85	<i>Peridinium</i> sp. 1	18.0	66	<i>CCMP291</i>	25.0	133
Unclassified	12.9	61	CONTH4 unclassified	6.8	122	<i>Chryschromulina</i> sp.	16.3	85	<i>Cryptomonas curvata</i>	22.7	36
Unclassified	8.0	73	<i>Gymnodinium</i> sp.	6.3	116	<i>Strobilidium</i> sp.	6.2	68	<i>Cryptomonas erosa</i>	12.2	89
<i>Cuspidothrix</i> sp.	4.7	91	<i>Obertrumia georgiana</i>	5.8	173	<i>Chryschromulina parva</i>	5.4	169	<i>Teleaulax amphioxeia</i>	3.8	109
Unclassified	4.2	75	<i>Cryptomonas</i> sp.	5.5	110	<i>Asterionella formosa</i>	5.0	173	<i>Chrysophytes</i> unclassified	2.2	93
<i>Polynucleobacter</i> sp.	3.0	20	Katablepharidales	4.7	70	<i>Peridinium</i> sp. 2	4.9	129	<i>Florenciella parvula</i>	1.9	165
Unclassified	2.7	106	CONThreeP unclassified	4.1	130	<i>Ceratium furca</i>	4.0	173	<i>Synura uvella</i>	1.8	162
SH3-11	2.6	109	Cryptophyceae 1	3.4	132	<i>Cryptomonas ovata</i>	4.0	58	Unclassified	0.8	82
<i>Flavobacterium</i> sp.	2.2	153	<i>Epistylis</i> sp.	3.3	173	<i>Cryptomonas</i> sp.	2.5	173	<i>Acanthoceras zachariasii</i>	0.4	169
<i>Cyanobium</i> sp.	2.0	167	<i>Askenasia</i> sp.	3.2	145	<i>Tabellaria fenestrata</i>	2.3	173	<i>Skeletonema pseudocostatum</i>	0.3	129
<i>Sediminibacterium</i> sp.	1.6	162	<i>Tintinnidium fluvatile</i>	2.7	167	<i>Chlamydomonas</i> sp.	1.7	126	<i>Pseudopedinella elastica</i>	0.1	102
Delta proteobacteria unclassified	1.6	82	Strombidiidae	2.6	161	<i>Pediastrum tetras</i>	1.7	108	<i>Chroomonas caudata</i>	0.0	125
Unclassified	1.6	87	<i>Vorticella microstoma</i>	1.9	112	<i>Cryptomonas marssonii</i>	1.6	132	Unclassified	0.0	173
Unclassified	1.2	64	Thoracosphaeraceae	1.9	173	<i>Aphanizomenon flos-aquae</i>	1.5	173	<i>Chrysosphaera</i> sp.	0.0	173
<i>Dolichospermum</i> sp.	1.1	85	Pseudoholophrya	1.5	83	<i>Dolichospermum planctonicum</i>	0.8	94	<i>Guillardia theta</i>	0.0	173
MWH-UniP1	1.1	125	Unclassified	1.4	127	c.f. <i>Glaucoma scintillans</i>	0.7	173			
<i>Reyranella</i> sp.	1.0	158	<i>Cryptomonas tetrapteryrenoidosa</i>	1.3	101	<i>Uronema</i> sp.	0.6	131			
<i>Fluviicola</i> sp.	0.7	73	CONTH5 unclassified	1.2	116	<i>Trachelomonas</i> sp. 1	0.5	173			
<i>Dinghuibacter</i> sp.	0.6	59	Novel clade 10	1.1	14	<i>Asterococcus</i> sp.	0.2	173			

Legend 16S rRNA			Legend 18S rRNA, microscopy and chloroplast 16S rRNA		
Alphaproteobacteria	Gammaproteobacteria	Ciliophora	Cercozoa	Katablepharidophyta	
Bacteroidetes	Planctomycetes	Chlorophyta	Chlorophyta	Telomelia	
Betaproteobacteria	Verrucomicrobia	Ciliophora	Ciliophora	Ochrophyta	
Cyanobacteria	Others	Others	Cryptophyta	Others	
			Dinoflagellata	Haptophyta	Unknown

**Table S2-11.** Most abundant genera, class or family (OTUs were pooled at their lowest taxonomic ranks) in Lake Saint-Augustin for 16S rRNA, 18S rRNA, microscopy, and chloroplast 16S rRNA during the ice-cover period (Jan-Feb-Mar); %reads: mean relative abundance in % of total reads, Biovolume%: mean relative abundance in % of total biovolume, CV%: coefficient of variation, SD as % mean.

16S rRNA			18S rRNA			Lake Saint-Augustin ice-cover			Chloroplast 16S rRNA		
Taxonomy	Reads %	CV %	Taxonomy	Reads %	CV %	Taxonomy	Biovolume %	CV %	Taxonomy	Reads %	CV %
CL500-3	21.5	52	Chlamydomonadales	15.7	129	<i>Chrysochromulina parva</i>	26.2	106	<i>Cryptomonas erosa</i>	38.1	59
Unclassified	18.5	12	Chrysophyceae cladeF	11.0	104	<i>Chlamydomonas</i> sp.	23.4	111	<i>Teleaulax amphioxeria</i>	25.0	87
Unclassified	9.3	75	Chrysophyceae	9.9	84	<i>Tabellaria fenestrata</i>	17.5	173	Unclassified	16.1	79
<i>Flavobacterium</i> sp.	7.6	130	Cryptomonadales	8.8	80	<i>Cryptomonas ovata</i>	15.9	89	<i>Chrysochromulina CCMP291</i>	8.3	101
<i>Undibacterium</i> sp.	7.4	139	Chrysophyceae cladeC	6.7	172	Unclassified	6.4	116	<i>Florenciella parvula</i>	3.4	97
<i>Polaromonas</i> sp.	4.0	117	<i>Cryptomonas curvata</i>	5.9	55	<i>Peridinium</i> sp. 2	5.7	76	<i>Skeletonema pseudocostatum</i>	2.5	108
Unclassified	3.7	78	Chrysophyceae cladeD	4.9	79	<i>Trachelomonas</i> sp. 2	1.3	173	<i>Cryptomonas curvata</i>	1.1	135
Unclassified	3.6	83	<i>Plagioselmis</i> sp.	4.3	113	<i>Trachelomonas</i> sp. 1	1.2	124	<i>Epipyxis PR26KG</i>	0.7	140
Deltaproteobacteria OM27 clade	2.7	91	Cryptophyceae 1	3.6	73	<i>Uronema</i> sp.	0.9	173	<i>Chromulina</i> sp.	0.7	109
<i>Aquirestis</i> sp.	1.6	130	Katablepharidales	2.6	95	<i>Cryptomonas marssonii</i>	0.8	173	<i>Ochromonas CCMP1393</i>	0.4	168
Unclassified	1.6	4	<i>Stephanodiscus</i> sp.	2.3	118	<i>Chrysochromulina</i> sp.	0.3	173	<i>Oophila amblystomatis</i>	0.4	80
<i>Dinghuibacter</i> sp.	1.5	83	<i>Prorocentrum</i> sp.	2.1	158	<i>Borghiella</i> sp.	0.2	173	Chrysophytes unclassified	0.3	71
Unclassified	1.0	78	<i>Chrysochromulina parva</i>	2.1	82	Unclassified flagellate 2	0.2	173	<i>Chrysosphaera</i> sp.	0.1	48
<i>Terrimicrobium</i> sp.	1.0	155	Unclassified	2.0	116	<i>Ophiocytium</i> sp.	0.0	173	<i>Dinobryon LO226KS</i>	0.0	173
<i>Polynucleobacter</i> sp.	0.9	54	<i>Spumella vulgaris</i>	1.8	173				<i>Synura uvela</i>	0.0	173
Actinobacteria hgclI clade	0.9	78	Protaspida lineage	1.5	16				Unclassified	0.0	173
<i>Rhodoferax</i> sp.	0.8	92	Parmales	1.4	71				<i>Guillardia theta</i>	0.0	173
<i>Methylotenera</i> sp.	0.7	166	Philasterida	0.7	60						
Actinobacteria unclassified	0.7	65	<i>Rimostrombidium</i> sp.	0.7	166						
<i>Nitrosospira</i> sp.	0.7	111	<i>Askenasia</i> sp.	0.6	108						

Legend 16S rRNA

- Alphaproteobacteria
- Bacteroidetes
- Betaproteobacteria
- Cyanobacteria

Gammaproteobacteria

- Planctomycetes
- Verrucomicrobia
- Others

Legend 18S rRNA, microscopy and chloroplast 16S rRNA

Cercozoa

- Chlorophyta
- Ciliophora
- Cryptophyta
- Dinoflagellata
- Haptophyta

Katablepharidophyta

- Telomonia
- Ochrophyta
- Others
- Unknown

**Table S2-22.** Genera or family identified by the DESeq analysis to discriminate between the lakes with the lower conductivity/urbanization (Lake Clair and Lake Saint-Charles) and the lakes with the higher conductivity/urbanization (Lake Clément and Lake Saint-Augustin) for 16S rRNA, 18S rRNA, microscopy and chloroplast 16S rRNA. For the data resulting from the rRNA analysis, the OTUs were pooled at their lowest taxonomic ranks. The DESeq analysis was conducted on the two periods (ice-cover and open water) combined. L2FC: Average log<sup>2</sup> fold change between the two groups of lakes. CV%: coefficient of variation, SD as % mean. Low: mean relative (%) abundance (in reads for 16S, 18S and chloroplasts 16S) or biovolume (microscopy) in the lakes with the lower conductivity/urbanization. Genera or family with a higher relative abundance (in reads) or biovolume in this group of lakes are in **bold**. High: mean relative (%) abundance (in reads for 16S, 18S and chloroplasts 16S) or biovolume (microscopy) in the lakes with the higher conductivity/urbanization.

16S rRNA			18S rRNA			Microscopy			Chloroplast 16S rRNA						
Taxonomy	L2FC	Low	High	Taxonomy	L2FC	Low	High	Taxonomy	L2FC	Low	High				
<i>Chthoniobacter</i> sp.	<b>7.2</b>	<b>0.9</b>	0.7	<i>Mesodinium</i> sp.	<b>8.4</b>	<b>7.6</b>	<0.1	<i>Dinobryon bavaricum</i>	<b>15.0</b>	<b>10.5</b>	<0.1	<i>Synura uvella</i>	<b>5.5</b>	<b>6.9</b>	<0.1
Unclas.	<b>6.6</b>	<b>0.9</b>	0.6	<i>Mesodinium</i> sp.	<b>7.0</b>	<b>3.4</b>	0.2	<i>Synura uvella</i>	<b>7.5</b>	<b>4.3</b>	<0.1	<i>Cryptomonas ovata</i>	<b>4.4</b>	<b>0.9</b>	<0.1
Soil bacterium	<b>5.9</b>	<b>0.4</b>	<0.1	<i>Mesodinium</i> sp.	<b>6.8</b>	<b>2.6</b>	<0.1	<i>Eutreptia</i> sp.	<b>7.4</b>	<b>0.0</b>	<0.1	<i>Synura uvella</i>	<b>3.2</b>	<b>14.8</b>	14.8
Unclas.	<b>5.8</b>	<b>0.2</b>	0.1	<i>Mesodinium</i> sp.	<b>5.8</b>	<b>1.3</b>	0.1	<i>Rabdoderma</i> sp.	<b>6.4</b>	<b>0.2</b>	<0.1	<i>Synura uvella</i>	<b>2.8</b>	<b>1.3</b>	<0.1
<i>Phenyllobacterium</i> sp.	<b>5.6</b>	<b>0.6</b>	<0.1	<i>Mesodinium</i> sp.	<b>5.4</b>	<b>3.8</b>	0.1	<i>Cyanobacteria</i> unclas.	<b>6.0</b>	<b>4.1</b>	<0.1	<i>Rhodophytes</i> unclas.	<b>2.8</b>	<b>0.5</b>	<0.1
<i>Chthoniobacter</i> sp.	<b>5.5</b>	<b>1.0</b>	0.8	<i>Dinobryon sociale</i>	<b>4.7</b>	<b>0.6</b>	<0.1	<i>Urosolenia</i> sp.	<b>5.4</b>	<b>0.1</b>	<0.1	Unclas.	<b>2.3</b>	<b>4.6</b>	1.7
Unclas.	<b>5.5</b>	<b>1.0</b>	<0.1	<i>Strobiliidiidae</i> H	<b>4.5</b>	<b>0.3</b>	<0.1	<i>Kephyrion</i> sp.	<b>4.9</b>	<b>&lt;0.1</b>	<0.1	Unclas.	<b>2.3</b>	<b>2.6</b>	0.2
<i>Dinghuibacter</i> sp.	<b>5.3</b>	<b>0.4</b>	<0.1	<i>Mesodinium</i> sp.	<b>4.5</b>	<b>2.0</b>	0.4	<i>Dinobryon petiolatum</i>	<b>4.8</b>	<b>&lt;0.1</b>	<0.1	<i>Epipyxis PR26KG</i>	<b>2.2</b>	<b>0.4</b>	<0.1
Unclas.	<b>5.1</b>	<b>0.3</b>	<0.1	<i>Mesodinium</i> sp.	<b>4.4</b>	<b>1.3</b>	<0.1	<i>Crucigenia</i> sp.	<b>4.5</b>	<b>&lt;0.1</b>	<0.1	Unclas.	<b>2.2</b>	<b>0.5</b>	<0.1
Unclas.	<b>5.0</b>	<b>0.2</b>	0.1	<i>Strombidiida</i> A	<b>4.2</b>	<b>0.4</b>	<0.1	c.f. <i>Cyclotella</i> sp.	<b>4.5</b>	<b>&lt;0.1</b>	<0.1	<i>Synura LO234KE</i>	<b>2.1</b>	<b>0.5</b>	<0.1
CL500-3	-6.6	<0.1	1.2	<i>Plagioselmis</i> sp.	-5.2	<0.1	1.4	<i>Merismopedia</i> sp.	<b>2.5</b>	<b>0.1</b>	<0.1	<i>Cryptomonas curvata</i>	-6.8	<0.1	3.2
<i>Algorigphagus hongiella</i>	-6.6	<0.1	0.3	<i>Strombidiida</i> A	-5.1	<0.1	1.9	<i>Chroococcus</i> sp.	<b>2.3</b>	<b>&lt;0.1</b>	<0.1	<i>Pavlova gyrans</i>	-5.4	<0.1	0.1
CL500-3	-6.3	<0.1	0.6	<i>Cryptomonas</i> sp.	-4.9	<0.1	7.5	<i>Dolichospermum plancticum</i>	-11.8	<0.1	0.4	<i>Cryptomonas erosa</i>	-5.2	0.1	16.4
CL500-3	-6.1	<0.1	0.7	<i>Katablepharidales</i>	-4.6	<0.1	0.9	<i>Chloromonas</i> sp.	-8.8	0.1	1.0	<i>Teleaulax amphioxiae</i>	-5.0	0.1	0.9
Unclas.	-5.9	<0.1	0.5	<i>Cryptomonadales</i>	-4.3	<0.1	0.6	c.f. <i>Polytomella</i> sp.	-6.9	0.4	0.5	<i>Teleaulax amphioxiae</i>	-5.0	1.8	5.7
MWH-UniP1	-5.8	<0.1	0.3	<i>Cryptomonas</i> sp.	-4.0	<0.1	0.6	<i>Peridinium</i> sp. 2	-5.7	0.8	6.1	<i>Cryptomonas curvata</i>	-4.8	<0.1	0.6
Unclas.	-5.8	<0.1	0.3	<i>Diacronema</i> sp.	-4.0	<0.1	0.7	Ochrophyte unclas.	-5.5	5.9	13.2	<i>Teleaulax amphioxiae</i>	-4.8	0.5	2.8
<i>Cuspodothrix</i> LMECYA-163	-5.8	<0.1	0.8	<i>Diacronema</i> sp.	-3.8	<0.1	0.5	Choanoflagellate unclas.	-4.0	<0.1	0.5	<i>Skeletonema pseudocastatum</i>	-4.7	0.1	0.6
OM190	-5.7	<0.1	0.6	<i>Litostomatea</i>	-3.5	<0.1	0.5	<i>Ophiocytium</i> sp.	-3.3	<0.1	0.2	Rhodophytes unclas.	-4.2	<0.1	<0.1
Fimbriimonadia unclas.	-5.5	<0.1	<0.1									<i>Chrysochromulina CCMP291</i>	-3.6	<0.1	0.1

#### Legend 16S rRNA

Alphaproteobacteria  
Bacteroidetes  
Betaproteobacteria  
Cyanobacteria

Gammaproteobacteria  
Planctomycetes  
Verrucomicrobia  
Others

#### Legend 18S rRNA, microscopy and chloroplast 16S rRNA

Ciliophora  
Chlorophyta  
Cryptophyta  
Dinoflagellata  
Haptophyta

Katablepharidophyta  
Telomelia  
Ochrophyta  
Others  
Unknown

**Table S2-33.** Genera or family identified by the DESeq analysis to discriminate between the ice-cover (Jan-Feb-Mar) and the open water (rest of the year) periods for 16S rRNA, 18S rRNA, microscopy and chloroplasts 16S rRNA. For the data resulting from the rRNA analysis, the OTUs were pooled at their lowest taxonomic ranks. The DESeq analysis was conducted on the four lakes combined. L2FC: Average  $\log_2$  fold change between the two periods. CV%: coefficient of variation, SD as % mean. IC: mean relative (%) abundance (in reads for 16S, 18S and chloroplast 16S) or biovolume (microscopy) during the ice-cover period. Genera or family with a higher relative abundance (in reads) or biovolume in this group of lakes are in **bold**. OW: mean relative (%) abundance (in reads for 16S, 18S and chloroplast 16S) or biovolume (microscopy) during the open water period.

16S rRNA				18S rRNA				Microscopy				Chloroplast 16S rRNA			
Taxonomy	L2FC	IC	OW	Taxonomy	L2FC	IC	OW	Taxonomy	L2FC	IC	OW	Taxonomy	L2FC	IC	OW
<b>Nitrosospira</b> sp.	<b>7.8</b>	<b>1.0</b>	<0.1	<b>Synura mollispina</b>	<b>6.3</b>	<b>8.5</b>	<0.1	<b>c.f. Polytomella</b> sp.	<b>7.1</b>	<b>0.8</b>	<b>0.1</b>	<b>Synura uvella</b>	<b>8.5</b>	<b>14.8</b>	<0.1
Unclas.	<b>7.1</b>	<b>1.3</b>	<0.1	<b>Chrysophyceae_cladeF</b>	<b>6.3</b>	<b>4.7</b>	<0.1	<b>Chloromonas</b> sp.	<b>5.7</b>	<b>0.9</b>	<b>0.1</b>	<b>Epipyxis PR26KG</b>	<b>7.1</b>	<b>1.4</b>	<0.1
<b>Flavobacterium</b> sp.	<b>7.0</b>	<b>1.0</b>	<0.1	<b>StrombidiidaA</b>	<b>5.9</b>	<b>2.1</b>	<0.1	<b>Pediastrum tetras</b>	-13.9	<0.1	0.9	<b>Florenciella parvula</b>	<b>6.8</b>	<b>1.7</b>	<0.1
<b>Methylobacter</b> sp.	<b>6.2</b>	<b>2.5</b>	<0.1	<b>Halteria grandinella</b>	<b>5.9</b>	<b>1.1</b>	<0.1	<b>Ochrophyte</b> unclas.	-13.7	0.3	18.7	<b>Synura uvella</b>	<b>4.7</b>	<b>6.8</b>	<b>0.1</b>
Unclas.	<b>6.1</b>	<b>0.7</b>	<0.1	<b>Chrysophyceae_cladeF</b>	<b>5.6</b>	<b>0.8</b>	<0.1	Unclassified flagellate 1	-11.9	<0.1	0.5	<b>Cryptomonas erosa</b>	<b>4.4</b>	<b>16.5</b>	<b>0.2</b>
Unclas.	<b>6.0</b>	<b>1.6</b>	<0.1	<b>Halteria grandinella</b>	<b>5.2</b>	<b>0.6</b>	<0.1	<b>Mallomonas tonsurata</b>	-10.6	<0.1	0.1	<b>Teleaulax amphioxiae</b>	<b>4.4</b>	<b>5.4</b>	<b>4.2</b>
<b>Nitrotoga</b> sp.	<b>5.0</b>	<b>0.4</b>	<0.1	<b>Synura petersenii</b>	<b>5.0</b>	<b>3.7</b>	<0.1	<b>Bitrichia</b> sp.	-9.1	<0.1	<0.1	<b>Cryptomonas erosa</b>	<b>4.2</b>	<b>6.8</b>	<b>1.1</b>
CL500-3	<b>5.0</b>	<b>0.8</b>	<0.1	<b>Chrysophyceae_cladeD</b>	<b>4.8</b>	<b>0.6</b>	<0.1	<b>Peridinium</b> sp. 1	-8.0	0.3	3.5	Unclas.	<b>3.9</b>	<b>12.3</b>	<b>2.4</b>
Unclas.	<b>4.9</b>	<b>1.0</b>	<0.1	<b>Chrysophyceae_cladeC</b>	<b>4.5</b>	<b>0.4</b>	<0.1	<b>Scenedesmus</b> sp.	-7.8	<0.1	<0.1	<b>Teleaulax amphioxiae</b>	<b>3.4</b>	<b>2.5</b>	<b>1.5</b>
<b>Subgroup 6</b>	<b>4.7</b>	<b>0.1</b>	<0.1	<b>Chrysophyceae_cladeD</b>	<b>4.4</b>	<b>0.3</b>	<0.1	Unclas.	-7.5	<0.1	0.2	<b>Chromulina</b> sp.	<b>3.0</b>	<b>0.1</b>	<0.1
Unclas.	-7.0	<0.1	0.7	<b>Dinobryon divergens</b>	-6.3	<0.1	2.0	<b>Kephryion</b> sp.	-7.3	<0.1	<0.1	<b>Synura uvella</b>	-5.2	<0.1	1.8
Unclas.	-6.1	<0.1	0.4	Litostomatea	-6.2	<0.1	1.7	<b>Synura uvella</b>	-7.1	<0.1	4.3	<b>Epipyxis PR26KG</b>	-4.6	<0.1	1.4
CL500-3	-5.8	<0.1	0.8	<b>Dinobryon bavaricum</b>	-5.3	<0.1	1.2	<b>Rabdoderma</b> sp.	-6.0	<0.1	0.2	<b>Dinobryon LO226KS</b>	-4.3	<0.1	1.2
Delta proteobacteria unclas.	-5.7	<0.1	0.3	Litostomatea	-4.8	<0.1	0.6	<b>Cyanobacteria</b> unclas.	-5.6	<0.1	4.1	Rhodophytes unclas.	-4.2	<0.1	0.9
Unclas.	-5.5	<0.1	0.3	Litostomatea	-4.8	<0.1	0.6	<b>Urosolenia</b> sp.	-5.0	<0.1	0.1	<b>Epipyxis PR26KG</b>	-3.7	0.2	3.0
Unclas.	-5.3	<0.1	0.2	Litostomatea	-4.8	<0.1	0.6	Choanoflagellate unclas.	-4.7	0.5	0.1	<b>Epipyxis PR26KG</b>	-3.6	<0.1	0.8
Unclas.	-5.2	<0.1	0.2	CONThreeP_uncl	-4.4	<0.1	0.6	<b>Picocyanobacteria</b> unclas.	-4.6	3.0	5.6	<b>Synura uvella</b>	-3.6	<0.1	0.5
Unclas.	-5.2	<0.1	0.3	<b>Chrysophyceae_cladeC</b>	-4.4	<0.1	0.5	<b>Kephryion</b> sp. 2	-4.5	<0.1	<0.1	<b>Hemiselmis tepida</b>	-3.4	<0.1	0.7
Dinghuibacter sp.	-4.9	<0.1	0.5	CONThreeP_uncl	-4.4	<0.1	0.5	<b>Dinobryon petiolatum</b>	-4.4	<0.1	<0.1	Unclas.	-3.1	<0.1	0.6
Rhizorhapis sp.	-4.8	<0.1	0.1	Litostomatea	-4.2	<0.1	0.4	<b>Strobilidium</b> sp.	-4.1	8.1	12.2	<b>Synura uvella</b>	-2.9	<0.1	1.3

Legend 16S rRNA

- Alphaproteobacteria
- Bacteroidetes
- Betaproteobacteria
- Cyanobacteria

Legend 18S rRNA, microscopy and chloroplast 16S rRNA

- Gammaproteobacteria
- Planctomycetes
- Verrucomicrobia
- Others
- Cercozoa
- Chlorophyta
- Ciliophora
- Cryptophyta
- Dinoflagellata
- Haptophyta
- Katablepharidophyta
- Telomelia
- Ochrophyta
- Others
- Unknown

## **Chapitre 3**

### Supporting Information

#### Methods

##### Lake and snow sampling

March 11<sup>th</sup> 2016, microbial plankton was sampled in Lake Saint-Charles, more precisely in Echo Bay. This particular location was chosen because it is known for water quality problem, it is notably the place in the lake with the highest occurrence of cyanobacterial blooms during summer (APEL 2014). Surface water (0-50 cm) was sampled through three 8" ice holes distributed in a 5 m radius around the middle of the Bay. Approximately 10 L were collected at each location with a 1 L Nalgen bottle and transfer into acid washed (HCl, 0.1 N) Aqua-Paks (Reliance, Manitoba, Canada). This water was used for chemical analysis and the experiment. Water was also collected in sterile Nalgene bottles for RNA characterization of the initial microbial plankton community and in pre-washed glass containers for PAHs analysis. All containers were rinse 3 times with sample. Water from the Nalgene bottles was filtered through a 0.2 µm Sterivex unit (Millipore), which was filled with RNA later (Life Technologies) and froze at -80°C until RNA analysis.

Urban snow was sampled at the disposal site de la Colline (46°52'36.0"N, 71°21'23.9"W), which is now closed. At this site, snow was sampled at three locations in acid washed (HCl 0.1 N) storage box (Rubbermaid, Atlanta, USA). The snow was left to melt in the dark at 4°C and was use for the experiment and the chemical analysis. To compose the melted snow use in the experiment the three triplicates were pooled in equal volume.

##### Chemical analyses

Total nitrogen (TN) and total phosphorus (TP) samples were acidified (H<sub>2</sub>SO<sub>4</sub> 0.1% final), then kept at 4°C until concentrations were determined by colorimetric method with, respectively, sulfanilamide and ascorbic acid reduction after a persulfate digestion. Ions and dissolved organic carbon (DOC) samples were filtered through Milli-Q water pre-rinsed cellulose acetate filters (0.2 µm pore size, Advantec Micro Filtration Systems), then acidified (for cations only, HNO<sub>3</sub> Trace Metal Grade 0.2% final) and kept at 4°C until analysis. Anions

concentrations were measured by ionic chromatography (ICS-2000, Dionex), major cations by atomic emission spectroscopy (ICP-AES, Varian Vista AX), trace cations by mass spectroscopy (ICP-MS, Thermo X Series) and DOC by combustion catalytic oxidation (Shimadzu TOC-5000A carbon analyzer calibrated with potassium biphenylate). Alkalinity (calcium carbonate equivalent) was determined with a titration by sulfuric acid 0.02 N (Wetzel and Likens 2000). Polycyclic aromatic hydrocarbons concentrations were determined by Maxxam Analytique Inc. under the QUE SOP-00207 protocol.

### Experimental design

During the acclimation and the experiment, cubitainers were rotated once a day in the incubator to ensure equal light exposure over time as well as manually inverted to resuspend plankton and renew oxygen. The cubitainers were left open during the experiment.

### RNA analysis

The RNA was extracted from the Sterivex units using the AllPrep DNA/RNA Mini Kit (Qiagen) and the Qiagen protocol modified as follows: (1) RLT<sup>+</sup>+β-ME was added in the Sterivex units, which were incubated at 37°C for 45 min. Lysozyme and proteinase k were then added in the units and they were incubated at 65°C for 15 min. Those steps were done replacing Qiagen steps 1 and 2 and (2) in the RNA purification phase, a DNA digestion step was added between Qiagen steps 8 and 9: 10 μL of DNase (Qiagen RNase free DNase set) diluted in 55 μL RDD Buffer was added in the RNeasy spin columns and left for 15 min at room temperature. RNeasy spin columns were then rinsed with Buffer RW1 as in step 8.

Extracted RNA was tested for DNA contamination by a PCR and then converted to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems-Ambion). The transcription was carried out in 20 μL (10 μL RNA template, 2 μL RT Buffer 5X, 0.8 μL dNTPs, 2 μL RT primers, 1 μL transcriptase and 4.2 μL water) with the following thermal cycle: 25°C for 10 min, 37°C for 120 min and 85°C for 5 min. The cDNA was stored at -80°C until further analysis.

The cDNA was amplified using two sets of primers modified with illumina adaptors: (1) 515F/806R which target the V4 region of the 16S rRNA gene for prokaryotes (bacteria and

archaea) as modified by Apprill *et al.* (2015) and (2) 572F/1009R from Comeau *et al.* 2011 which target the V4 region of the eukaryotes 18S rRNA gene. PCR was carried out in a total volume of 25 µL (1 µL cDNA template, 5 µL PCR buffer (New England Biolabs), 1.25 µL reverse and forward primers, 0.5 µL mix dNTP, 0.25 µL Q5 High-Fidelity DNA Polymerase (New England Biolabs) and 15.75 µL water). Conditions of the PCR thermal cycling for each set of primers are presented in Table S3-1. PCR products were purified with ethanol and magnetic beads (Agencourt AMPure XP) and a second PCR was run to introduce samples tags. This reaction had an initial denaturation temperature of 98°C for 30 sec, then 13 cycles of 10 sec denaturation at 98°C, 30 sec annealing at 55°C and 30 sec elongation at 72°C followed by a final extension of 4.5 min at 72°C. The second amplicons were purified with beads as previously described, quantified with the Nanodrop 1000 (Thermo Fisher Scientific), pooled in equimolar ratio and then sequenced with illumina MiSeq at the IBIS/Laval University Plate-forme d'analyses génomiques (Quebec City, QC).

Forward and reverse reads pairs were merged using bbmerge v37.36 (Bushnell *et al.* 2017), and the obtained merged reads were filtered with maximum expected errors of 1 (Rognes *et al.* 2016). Unique reads were identified as well as abundance- and size-filtered to discard chimera (300 bp) and singletons using vsearch (Edgar 2013). USEARCH was then used to clustered reads at 98% similarity level (Operational Taxonomic Units, OTUs) for 18S and at 87% similarity level for 16S. We used mothur v1.39 (Schloss *et al.* 2009) to assign the taxonomy of the most abundant sequence of each OTU based on the Protist Ribosomal Reference database (PR<sup>2</sup>, Guillou *et al.* 2013) for 18S and SILVA 132 (Quast *et al.* 2013) for 16S. OTU tables were constructed with the number of reads per OTU in each sample.

Sequences identified as *Phragmoplastophyta* (Lecointre and Le Guyader 2018), rotifer, chloroplasts, and mitochondria were removed from further analysis. When relevant, unidentified OTUs were submitted to a BLAST search to the nr database of NCBI GenBank and identified to the closest match. The nucleotide sequence data reported are available in the NCBI database under the project number PRJNA681563.

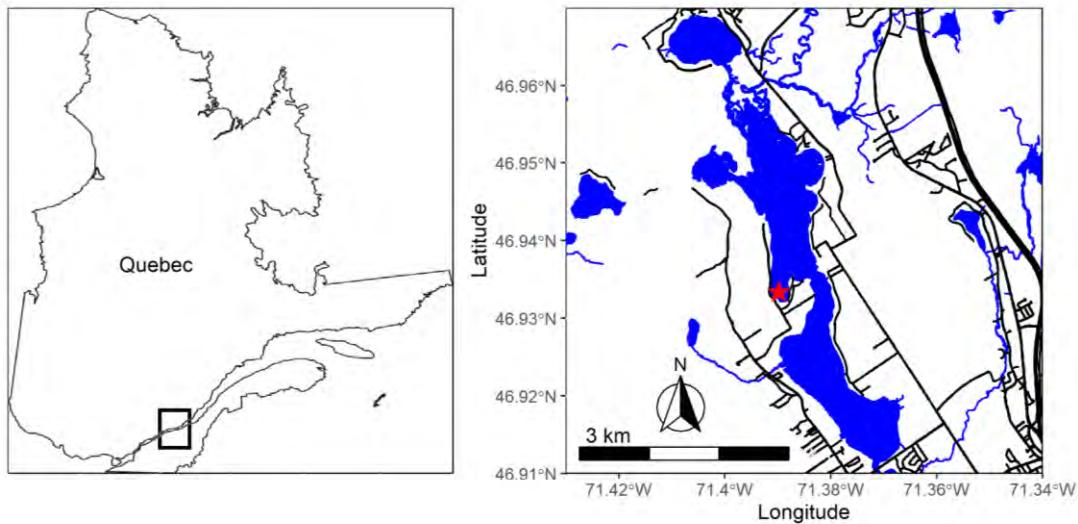
## Statistical analysis

The effects of the experimental factors (phosphorus enrichment and increase sodium chloride in either water or waste snow) on microbial plankton community structure were tested by a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), with a dummy variable representing the treatments, using the adonis function of the vegan package (v2.4.6, Oksanen et al. 2019). Principal coordinate analysis (PCoA) was conducted to visualize possible clustering of the samples with the pcoa function of the ape package (v5.1, Paradis and Schliep 2019). This PCoA was based on a Bray–Curtis dissimilarity matrix using the vegdist function of the vegan package. The effects of experimental factors on individual taxonomic groups were tested with an Analysis of Variance using the aov function of the stats package (v3.5.1) on the square root of the relative abundance. When significant, differences were further investigated using the Tukey's Honest Significant Difference test with the TukeyHSD function of the stats package. All analyses were performed using the R software (v3.4.3, R Core Team 2017).

## Results

### Properties of the lake water and urban snow

The following PAHs were below the  $0.06 \mu\text{g L}^{-1}$  detection limit in the lake water and the urban snow: benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, while acenaphptene, anthracene, benzo(a)anthracene, dibenz(a,h)anthracene, fluorene, indeno(1,2,3-cd)pyrene and naphthalene were below the  $0.03 \mu\text{g L}^{-1}$  detection limit (data not shown). Benzo(a)pyrene (detection limit of  $0.008 \mu\text{g L}^{-1}$ ), chrysene ( $0.03 \mu\text{g L}^{-1}$ ), fluoranthene ( $0.03 \mu\text{g L}^{-1}$ ) phenanthrene ( $0.03 \mu\text{g L}^{-1}$ ) and pyrene ( $0.03 \mu\text{g L}^{-1}$ ) were also below detection limits in the lake water, while they were detected in the urban snow at respectively  $0.02 \pm 0.01$ ,  $0.07 \pm 0.01$ ,  $0.12 \pm 0.09$ ,  $0.21 \pm 0.19$  and  $0.10 \pm 0.05 \mu\text{g L}^{-1}$  (mean $\pm$ SD from the three sampling sites, data not shown).



**Figure S3-1.** Localization of Lake Saint-Charles and of the sampling site (red star) of the overwintering microbial plankton.

**Table S3-1.** P-value for the ANOVA and the Tukey HSD tests for the differences in relative abundance of prokaryotes among the experimental treatments.

Taxa	ANOVA		Tukey HSD				
	sn-c	p-c	s-c	p-sn	s-sn	s-p	
<b>Control</b>							
<i>Arcicella</i> sp.	<b>0.01</b>	<b>0.01</b>	<b>0.03</b>	<b>0.04</b>	0.96	0.87	0.99
<i>Asticcacaulis</i> sp.	<0.01	<0.01	<0.01	<0.01	0.16	0.66	0.65
<b>Snow</b>							
<i>Hirschia</i> sp.	<0.01	<0.01	0.95	0.35	<b>&lt;0.01</b>	<b>0.01</b>	0.62
<i>Planctomyces</i> sp.	<0.01	<0.01	0.09	0.45	<b>0.02</b>	<0.01	0.63
<i>Psychrobacter</i> sp.	<0.01	<0.01	1.00	1.00	<0.01	<0.01	1.00
<b>Salt</b>							
<i>Chthoniobacter</i> sp.	<b>0.01</b>	0.99	0.89	<b>0.02</b>	0.98	<b>0.03</b>	<b>0.04</b>
Deltaproteobacteria unclass.	<0.01	1.00	1.00	<b>0.01</b>	1.00	<b>0.01</b>	<b>0.01</b>
<i>Pirellula</i> sp.	<b>0.01</b>	1.00	1.00	<b>0.02</b>	0.99	<b>0.02</b>	<b>0.03</b>
<i>Pseudanabaena</i> sp.	<0.01	1.00	1.00	<0.01	1.00	<0.01	<0.01
<i>Roseococcus</i> sp.	<b>0.01</b>	0.84	0.97	<b>0.04</b>	0.97	<b>0.01</b>	<b>0.02</b>
<i>Synechococcus</i> sp.	<0.01	0.10	0.54	<0.01	0.58	<b>0.02</b>	<0.01
TK10 unclass.	<0.01	0.80	1.00	<0.01	0.85	<b>0.01</b>	<0.01

sn: snow, c: control, p: P-Only, s: salt

**Table S3-2.** P-value for the ANOVA and the Tukey HSD tests for the differences in relative abundance of eukaryotes among the experimental treatments.

Taxa	ANOVA	Tukey HSD					
		sn-c	p-c	s-c	p-sn	s-sn	s-p
<b>Salt</b>							
Intramacronucleata unclass.	<b>0.01</b>	0.99	0.90	<b>0.04</b>	0.98	<b>0.03</b>	<b>0.02</b>
<i>Mallomonas</i> sp.	<b>0.01</b>	0.94	0.99	<b>0.01</b>	0.84	<b>0.01</b>	<b>0.02</b>
<i>Oikomonas</i> sp.	< <b>0.01</b>	1.00	1.00	<b>0.01</b>	1.00	<b>0.01</b>	<b>0.01</b>
Cryptophyta SA1-3C06	<b>0.02</b>	1.00	0.96	<b>0.04</b>	0.95	<b>0.05</b>	<b>0.02</b>

sn: snow, c: control, p: P-Only, s: salt