Supplementary Material

Changes in the Community Structure of Under-Ice and Open-Water Microbiomes in Urban Lakes Exposed to Road Salts

Isabelle B. Fournier, Connie Lovejoy, Warwick F. Vincent

Supplementary Figures and Tables

**Supplementary Figure 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.
Supplementary Figure 2. Temperature, oxygen and conductivity profiles of Lake Clair, Lake Saint-Charles, Lake Clément and Lake Saint-Augustin in 2017.
Supplementary Figure 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.
Supplementary Figure 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.
Supplementary Figure 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.
**Supplementary Table 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        |
|        |            |                    |              |                     |
**Supplementary Table 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 | Dinoflagellates | Euglenophytes | Haptophytes |
|-------------------|--------------|--------------|--------------|---------------|----------------|---------------|-------------|
| Alloxanthin       | 0.016        | 0            | 0            | 0             | 0              | 0             | 0           |
| Antheraxanthin    | 0            | 0            | 0            | 0             | 0              | 0             | 0           |
| Aphanizophyll     | 0            | 0            | 0            | 0.0054        | 0              | 0             | 0           |
| Astaxanthin       | 0.004        | 0            | 0            | 0             | 0              | 0             | 0           |
| Canthaxanthin     | 0            | 0            | 0            | 0             | 0              | 0             | 0.014       |
| Carotene          | 0.003        | 0.003        | 0.004        | 0.004         | 0.0025         | 0.004         | 0.03        |
| Chl $b$           | 0.356        | 0            | 0            | 0             | 0              | 0             | 0.198       |
| Chl $c$           | 0            | 0.032        | 0.091        | 0.091         | 0.1555         | 0             | 0           |
| Diadinoxanthin    | 0            | 0.016        | 0            | 0             | 0.064          | 0.2465        | 0.327       |
| Diatoxanthin      | 0            | 0.025        | 0            | 0             | 0.083          | 0.0795        | 0.235       |
| Dinoxanthin       | 0            | 0            | 0            | 0             | 0              | 0.053         | 0           |
| Echinonene        | 0            | 0            | 0            | 0.526         | 0              | 0             | 0.026       |
| Fucoxanthin       | 0            | 0.283        | 0            | 0             | 0.458          | 0             | 0           |
| Lutein            | 0.147        | 0            | 0            | 0             | 0              | 0             | 0.637       |
| Neoxanthophyll    | 0            | 0            | 0            | 0             | 0.136          | 0             | 0           |
| Neoxanthin        | 0.04         | 0            | 0            | 0             | 0              | 0             | 0.034       |
| Peridinin         | 0            | 0            | 0            | 0             | 0              | 0.5075        | 0           |
| Violaxanthin      | 0.026        | 0.063        | 0            | 0             | 0.003          | 0             | 0           |
| Zeaxanthin        | 0.036        | 0.016        | 0            | 0.28          | 0.007          | 0             | 0           |
Supplementary Table 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          | 36      | 98           | 10          | 1      |
| Annealing           | 50        | 60          | 36      | 52           | 30          | 30     |
| Extension           | 72        | 90          |         | 72           | 30          |        |
| Final extension     | 72        | 60          | 1       | 72           | 270         | 1      |
### Supplementary Table 4.

| Taxonomy                              | 16S rRNA | 18S rRNA | Lake Clair open water | Microscopy | Chloroplast 16S rRNA |
|---------------------------------------|----------|----------|-----------------------|------------|----------------------|
|                                       | Reads%   | %reads    | Taxonomy              | Biovolume% | CV%                  |
|                                       | CV%      |           |                       |            |                      |
| Unclassified                          | 28.5     | 10        | Mesodinium sp          | 44.1       | 52                   |
| Unclassified                          | 20.5     | 98        | Chrysophyceae         | 7.7        | 75                   |
| Unclassified                          | 11.0     | 99        | Unclassified           | 4.4        | 146                  |
| Polynucleobacter sp                   | 3.5      | 34        | Litotomata             | 4.3        | 82                   |
| Rhodovarius sp                        | 3.5      | 69        | Strombidiida          | 3.8        | 97                   |
| CLS08-3                               | 3.4      | 102       | Chrysophyceae cladeD  | 3.5        | 69                   |
| Sediminibacterium sp                  | 1.9      | 77        | Chrysophyceae cladeC  | 2.4        | 63                   |
| Actinobacteria unclassified           | 1.8      | 38        | Dinobryon sp          | 2.4        | 35                   |
| Flavobacterium sp                    | 1.6      | 135       | Unclassified ochrophyta| 2.1        | 110                  |
| Unclassified                          | 1.5      | 96        | Pericrostida           | 2.0        | 171                  |
| Enticicia sp                          | 1.5      | 118       | Unclassified           | 1.7        | 86                   |
| GKS99                                 | 1.3      | 10        | Cryptomonas pyroidifera| 1.7        | 81                   |
| Actinobacteria hgcI clade             | 1.2      | 45        | Unclassified           | 1.5        | 76                   |
| Dinghuibacter sp                      | 1.2      | 102       | Chrysophyceae sp      | 1.2        | 149                  |
| Condobacter sp                        | 0.9      | 62        | Dinobryon crenulatum  | 0.9        | 67                   |
| Ferruginibacter sp                   | 0.9      | 75        | Chrysophyceae cladeE  | 0.9        | 39                   |
| Arcicella sp                          | 0.7      | 172       | Asterinaeia           | 0.8        | 56                   |
| IMCC26134                             | 0.7      | 167       | Mamilliphycaceae      | 0.8        | 118                  |
| SMEA102                               | 0.7      | 155       | Centroheliozoa        | 0.8        | 157                  |
| Roseomonas sp                         | 0.6      | 94        | StrobilididaC         | 0.7        | 114                  |
| **Legend 16S rRNA**                   |          |           |                       |            |                      |
| Alphaproteobacteria                   |          |           |                       |            |                      |
| Gammaproteobacteria                   |          |           |                       |            |                      |
| Betaproteobacteria                    |          |           |                       |            |                      |
| Cyanobacteria                         |          |           |                       |            |                      |
| **Legend 18S rRNA**                   |          |           |                       |            |                      |
| Alphaproteobacteria                   |          |           |                       |            |                      |
| Gammaproteobacteria                   |          |           |                       |            |                      |
| Bacteroidetes                         |          |           |                       |            |                      |
| Planctomycetes                        |          |           |                       |            |                      |
| Bacteroidetes                         |          |           |                       |            |                      |
| Planctomycetes                        |          |           |                       |            |                      |
| Chloroflexi                           |          |           |                       |            |                      |
| Other                                  |          |           |                       |            |                      |
| **Legend Chloroplast 16S rRNA**       |          |           |                       |            |                      |
| Alphaproteobacteria                   |          |           |                       |            |                      |
| Gammaproteobacteria                   |          |           |                       |            |                      |
| Betaproteobacteria                    |          |           |                       |            |                      |
| Cyanobacteria                         |          |           |                       |            |                      |
| **Legend Microscopy**                 |          |           |                       |            |                      |
| Alphaproteobacteria                   |          |           |                       |            |                      |
| Gammaproteobacteria                   |          |           |                       |            |                      |
| Bacteroidetes                         |          |           |                       |            |                      |
| Planctomycetes                        |          |           |                       |            |                      |
| Bacteroidetes                         |          |           |                       |            |                      |
| Planctomycetes                        |          |           |                       |            |                      |
| Chloroflexi                           |          |           |                       |            |                      |
| Other                                  |          |           |                       |            |                      |
| **Legend Chloroplast 16S rRNA**       |          |           |                       |            |                      |
| Alphaproteobacteria                   |          |           |                       |            |                      |
| Gammaproteobacteria                   |          |           |                       |            |                      |
| Bacteroidetes                         |          |           |                       |            |                      |
| Planctomycetes                        |          |           |                       |            |                      |
| Bacteroidetes                         |          |           |                       |            |                      |
| Planctomycetes                        |          |           |                       |            |                      |
| Chloroflexi                           |          |           |                       |            |                      |
| Other                                  |          |           |                       |            |                      |

*Note: CV%: coefficient of variation, SD as % mean.*
**Supplementary Table 5.** Most abundant taxa (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.

| Taxonomy | 16S rRNA Reads | 16S rRNA CV% | 18S rRNA Reads | 18S rRNA CV% | Lake Clair ice-cover Microscopy Biovolume | Lake Clair ice-cover Chloroplast 16S rRNA Reads | Lake Clair ice-cover Chloroplast 16S rRNA CV% |
|----------|---------------|--------------|---------------|--------------|-----------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Unclassified | 21.1 | 13 | Unclassified | 39.8 | 60 | Unclassified | 46.0 | 132 |
| Unclassified | 15.5 | 3 | Synura mollispina | 35.8 | 72 | Unclassified | 12.0 | 173 |
| Unclassified | 13.1 | 30 | Unclassified cryptophyceae | 5.2 | 90 | Chlamydomonas sp. | 11.2 | 84 |
| Clathniobacter sp. | 6.4 | 37 | Cryptomonas pyrenoidifera | 46.0 | 132 | Chrysochromulina sp. | 10.8 | 173 |
| Sediminibacterium sp. | 5.4 | 71 | Synura curvata | 2.9 | 81 | Cryptomonas ovata | 7.9 | 120 |
| SH-11 | 3.9 | 60 | Dinobryon bavaricum | 1.2 | 28 | Dinobryon bavaricum | 5.1 | 145 |
| Unclassified | 3.2 | 46 | Unclassified | 1.1 | 68 | Unidentified | 2.8 | 173 |
| BSV13 | 2.6 | 86 | Stroma | 0.9 | 139 | Uronema sp. | 1.7 | 93 |
| Lateolabbelus sp. | 2.5 | 152 | Unclassified | 0.8 | 131 | Unclassified | 0.8 | 173 |
| Cephaloticoccus sp. | 1.9 | 74 | Cryptomonas marssonii | 0.8 | 85 | Unclassified flagellate 2 | 0.5 | 112 |
| Flexibacterium sp. | 1.8 | 21 | Mesodinium sp. | 0.7 | 87 | Unidentified | 0.4 | 173 |
| Unclassified | 1.8 | 65 | Mallomonas sp. | 0.6 | 78 | Unidentified | 0.4 | 173 |
| Actinobacteria unclassified | 1.6 | 44 | Pseudodendromonadales | 0.6 | 78 | Peridinium sp.1 | 0.3 | 173 |
| Polynucleobacter sp. | 1.6 | 48 | Group 2 | 0.6 | 94 | Enteroxps | 0.1 | 100 |
| Actinobacteria hgcI clade | 1.5 | 33 | Unclassified | 0.6 | 94 | Unidentified | 0.1 | 173 |
| Caulobacter sp. | 1.0 | 96 | Cryptomonas tetraptymnodosa | 0.6 | 110 | Unidentified | 0.1 | 173 |
| Flexibacteraceae | 0.9 | 87 | Chrysochromulina cladeD | 0.5 | 101 | Unidentified | 0.1 | 173 |
| Rhodovibrio sp. | 0.9 | 57 | Unclassified | 0.5 | 101 | Unclassified | 0.1 | 173 |
| Marine group | 0.8 | 119 | Unclassified flagellates | 0.4 | 121 | Unidentified | 0.1 | 173 |
| Polaronomonas sp. | 0.8 | 57 | Chlorella | 0.4 | 134 | Unidentified | 0.1 | 173 |

**Legend 16S rRNA**
- Alphaproteobacteria
- Betaproteobacteria
- Cyanobacteria
- Gammaproteobacteria
- Planctomycetes
- Verrucomicrobia
- Others

**Legend 18S rRNA, microscopy and chloroplast 16S rRNA**
- Chlorophyta
- Cryptophyta
- Dinoflagellata
- Haptophyta
- Katablepharidophyta
- Telonemia
- Ochrophyta
- Others
- Unknown
### Supplementary Table 6

Most abundant taxa (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.

| Taxonomy | 16S rRNA | Lake Saint-Charles open water | Taxonomy | 18S rRNA | Lake Saint-Charles open water | Taxonomy | Microscopy | Lake Saint-Charles open water | Taxonomy | Chloroplast 16S rRNA | Lake Saint-Charles open water |
|----------|----------|-------------------------------|----------|----------|-------------------------------|----------|------------|-------------------------------|----------|------------------------|-------------------------------|
|          | Reads %  | CV%                           |          | Reads %  | CV%                           |          | Biovolume% | CV%                           |          | Reads %  | CV%                           |
| Unclassified | 31.1     | 104                           | Litostomatea | 28.6     | 103                           | Synagococcus | 17.2     | 148                       |          |          | Floresiella parvula | 18.6 | 122                        |
| Unclassified | 11.2     | 40                            | Mesodinium sp. | 6.2      | 124                           | Picocyanobacteria | 12.1     | 72                        |          |          | Unclassified | 15.7 | 65                        |
| Phylochaetae CL500-3 | 10.7     | 85                            | Dinobryon sociale | 3.6      | 72                            | Chrysochromulina sp. | 10.2     | 136                       |          |          | Cryptomonas curvata | 11.1 | 32                        |
| Unclassified | 8.9      | 63                            | Pseudoholophyta sp. | 3.5      | 135                           | Ceratium hirundinella | 9.3     | 200                       |          |          | Epiphippia P25K | 8.3 | 60                        |
| Polymorphlscheracter sp. | 3.8      | 132                           | Unclassified chrysophyceae | 3.2      | 103                           | Asterionella formosa | 7.7     | 130                       |          |          | Synagococcus | 7.1 | 56                        |
| Sediminibacterium sp. | 2.5      | 185                           | Dinobryon divergens | 3.1      | 122                           | Dinobryon bavaricum | 6.9     | 70                        |          |          | Dinobryon LO226KS | 6.6 | 46                        |
| Actinobacteria unclassified | 2.3      | 154                           | Chrysophyceae cladeD | 2.8      | 84                            | StrombidiaidaA | 6.6     | 71                        |          |          | Ochromonas CCMP1393 | 3.8 | 175                      |
| Unclassified | 1.7      | 80                            | Litostomatea | 2.7      | 106                           | Chlamydomonas sp. | 4.1     | 132                       |          |          | Chrysophyceae cladeC | 2.3 | 102                      |
| Microcystis sp. | 1.7      | 170                           | Didiniidae | 2.3      | 189                           | Peridinium sp. 1 | 3.1     | 62                        |          |          | Unclassified | 2.0 | 133                      |
| Flavobacterium sp. | 1.6      | 185                           | Sphaerocoea lepdesignica | 2.3      | 190                           | Tabellaria fenestrata | 3.0     | 155                       |          |          | Cryptomonas curvata | 1.7 | 89                        |
| GKS98 | 1.4      | 172                           | Chrysophaearella sp. | 2.0      | 133                           | Cryptomonas ovata | 2.9     | 155                       |          |          | Rhodophyta unclassified | 1.5 | 60                        |
| SH3-11 | 1.2      | 88                            | Unclassified chrysophyceae | 1.7      | 74                            | Unclassified flagellate | 2.3     | 118                       |          |          | Acanthoceras zaccharisi | 1.3 | 75                        |
| Unclassified | 1.1      | 112                           | Chrysophyceae cladeC | 1.6      | 36                            | Cryptomonas sp. | 2.0     | 89                        |          |          | Unclassified | 1.0 | 108                      |
| Caulobacter sp. | 0.9      | 128                           | Unclassified ochrophyta | 1.6      | 36                            | Uronema sp. | 1.4     | 99                        |          |          | Heterosigma akashiino | 1.0 | 175                      |
| Bacteria unclassified | 0.8      | 158                           | Uroplana americana | 1.6      | 162                           | Unclassified | 1.0     | 81                        |          |          | Synagococcus LO234KE | 0.9 | 115                      |
| Phenylobacterium sp. | 0.7      | 133                           | Unclassified | 1.6      | 42                            | Unclassified ochrophyta | 1.0     | 81                        |          |          | Hemieclnus sp. | 0.9 | 152                      |
| Polaronas sp. | 0.7      | 166                           | Dinobryon bavaricum | 1.4      | 114                           | Cryptomonas marssonii | 0.9     | 49                        |          |          | Mallomonas splendidus | 0.6 | 133                      |
| IMCC26134 | 0.7      | 100                           | Dinobryon sp. | 1.3      | 94                            | Cyanobacteria unclassified | 0.9     | 144                       |          |          | Pseudopedinella elastica | 0.4 | 58                        |
| Methylomonas sp. | 0.7      | 196                           | Askenasia sp. | 1.3      | 102                           | Pediasrium teras | 0.9     | 200                       |          |          | Chromulina sp. | 0.4 | 98                        |
| Aphaniotomina sp. | 0.7      | 84                            | StrombidiaidaB | 1.1      | 181                           | Ascosuctera cf. italic | 0.5     | 200                       |          |          | Katablepharidophyta |                  |            |

**Legend 16S rRNA**
- Alphaproteobacteria
- Betaproteobacteria
- Cyanobacteria
- Gammaproteobacteria
- Planctomycetes
- Verrucomicrobia
- Others

**Legend 18S rRNA, microscopy and chloroplast 16S rRNA**
- Coccozoa
- Chlorophyta
- Cryptophyta
- Dinoflagellata
- Haptophyta
- Katablepharidophyta
- Telonemia
- Ochrophyta
- Others
- Unknown
Supplementary Table 7. Most abundant taxa (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.

| Taxonomy | 16S rRNA | Lake Saint-Charles ice-cover | Microscopy | Chloroplast 16S rRNA |
|----------|----------|-------------------------------|------------|----------------------|
|          | Reads %  | CV%                           | Reads %    | Biovolume %          | Reads % |
| Unclassified | 26.3 67  | Synura petersenii | 21.3       | 126                  | 34.9 118 |
| Unclassified | 7.0 72   | Strombidium sp. | 9.9        | 50                   | 27.6 89 |
| Unclassified | 6.9 39   | Cryptomonadales | 6.9        | 118                  | 14.9 92 |
| Sediminibacterium sp. | 4.6 66   | Unclassified | 4.6        | 61                   | 5.6 173 |
| Methyllobacter sp. | 4.5 81   | Prototaxa lineage | 4.2       | 110                  | 5.1 97 |
| Nitrosospira sp. | 4.4 163  | Chrysophyceae cladeE | 3.9       | 126                  | 2.8 88 |
| Polaronomas sp. | 4.0 155  | Chrysophyceae | 3.0        | 94                   | 2.4 118 |
| Unclassified | 3.9 65   | Chrysophyceae cladeD | 2.8       | 68                   | 2.1 173 |
| Unclassified | 3.6 134  | Vorticella sp. | 2.8        | 81                   | 2.1 173 |
| Polymicrobacter sp. | 3.3 63   | Synura sp. | 2.7        | 111                  | 1.0 97 |
| Unclassified | 2.4 68   | Cryptomonas curvata | 2.3       | 26                   | 0.7 173 |
| Nitrospira sp. | 2.4 156  | Chrysophyceae cladeF | 2.3       | 80                   | 0.6 173 |
| SH3-11 | 1.8 72   | Chrysophyceae 1 | 2.1        | 67                   | 0.2 173 |
| CL300-3 | 1.7 124  | Cryptomonas pyriformis | 2.1       | 76                   |          |
| Lutobacter sp. | 1.5 173  | Chrochromatina pura | 1.3       | 79                   |          |
| Acidobacteria subgroup 3 | 1.5 129  | Choreotrichia | 1.3        | 79                   |          |
| Actinobacteria unclassified | 1.4 64   | Chryseomonadales | 1.2        | 99                   |          |
| Rhodovulum sp. | 1.3 97   | Chryseomonadales | 1.2        | 99                   |          |
| Margulisibacteria unclassified | 1.3 84   | Litostomata | 1.0        | 164                  |          |
| Flavobacterium sp. | 1.0 50   | Unclassified | 1.0        | 49                   |          |
|          |          | Askinia sp. | 0.9        | 68                   |          |

Legend 16S rRNA
Alphaproteobacteria
Betaproteobacteria
Cyanobacteria
Gammaproteobacteria
Planctomycetes
Verrucomicrobia
Others

Legend 18S rRNA, microscopy and chloroplast 16S rRNA
Cercozoa
Chlороphyta
Cryptophyta
Dinoflagellata
Haptophyta
Katablepharidophyta
Telonemia
Ochrophyta
Others
Unknown
Supplementary Table 8. Most abundant taxa (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.

| Taxonomy                          | 16S rRNA | 18S rRNA | Lake Clément open water | Chloroplast 16S rRNA |
|-----------------------------------|----------|----------|-------------------------|----------------------|
|                                   | Reads %  | CV%      | Reads %                 | Biovolume %          |
| Unclassified                      | 28.62    | 41       | Litostomatea            | 5.35                 |
|                                  |          |          | 10.79                   |                      |
| Unclassified                      | 21.12    | 26       | Dinobryon divergens     | 15.97                |
|                                  |          |          | 10.61                   |                      |
| Polynucleobacter sp.              | 5.21     | 72       | Chrysochromulina cladeC | 6.89                 |
|                                  |          |          | 8.05                    |                      |
| CL500-3                           | 5.05     | 94       | Dinobryon hexasporum    | 6.64                 |
|                                  |          |          | 6.29                    |                      |
| Unclassified                      | 3.69     | 38       | StrobilididaA           | 4.61                 |
|                                  |          |          | 5.94                    |                      |
| Unclassified                      | 2.99     | 140      | Dinobryon LO226KS       | 1.66                 |
|                                  |          |          | 21.93                   |                      |
| Unclassified                      | 2.34     | 73       | Cryptomonadales         | 1.48                 |
|                                  |          |          | 4.90                    |                      |
| SH3-11                            | 2.27     | 56       | Mallomonas caudata      | 1.51                 |
|                                  |          |          | 4.72                    |                      |
| Actinobacteria unclassified       | 2.21     | 85       | Chrysophyceae           | 0.54                 |
|                                  |          |          | 4.71                    |                      |
| Pinnotheriomadia unclassified     | 1.99     | 111      | Chrysochromulina cladeA | 0.37                 |
|                                  |          |          | 3.41                    |                      |
| Sediminibacterium sp.             | 1.68     | 74       | Cryptomonadales         | 0.33                 |
|                                  |          |          | 2.79                    |                      |
| Actinobacteria hgC clade          | 1.53     | 44       | Cryptophyceae 1         | 0.19                 |
|                                  |          |          | 2.49                    |                      |
| Deltaproteobacteria unclassified  | 1.42     | 150      | Chlorophyceae           | 0.19                 |
|                                  |          |          | 2.36                    |                      |
| Deltaproteobacteria OM27 clade    | 1.38     | 141      | Cryptomonadales         | 0.19                 |
|                                  |          |          | 2.10                    |                      |
| MWH-UniP1                        | 1.35     | 171      | Hypotrichia             | 0.19                 |
|                                  |          |          | 2.01                    |                      |
| Flavobacterium sp.                | 1.10     | 137      | Katatelpharidales       | 0.19                 |
|                                  |          |          | 1.97                    |                      |
| Polaronomonas sp.                 | 1.05     | 66       | Novel clade 2           | 0.19                 |
|                                  |          |          | 1.65                    |                      |
| Unclassified                      | 0.89     | 20       | Chrysochromulina parva  | 0.19                 |
|                                  |          |          | 1.62                    |                      |
| Hirschia sp.                      | 0.75     | 39       | Phagellimusi            | 0.19                 |
|                                  |          |          | 1.86                    |                      |
| Flavisciola sp.                   | 0.71     | 44       | Mallomonas              | 0.19                 |
|                                  |          |          | 1.52                    |                      |

Legend 16S rRNA
- Purple: Alphaproteobacteria
- Blue: Gammaproteobacteria
- Green: Bacteroidetes
- Dark green: Cyanobacteria
- Orange: Bacteroidetes
- Yellow: Betaproteobacteria
- Gray: Others

Legend 18S rRNA, microscopy and chloroplast 16S rRNA
- Red: Chrysophyceae
- Green: Cryptophyceae
- Yellow: Dinoflagellata
- Blue: Haptophyta
- Gray: Others
- Orange: Unknown
Supplementary Table 9. Most abundant taxa (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.

| Taxonomy                  | Lake Clément ice-cover | Microscopy | Chloroplast 16S rRNA |
|---------------------------|------------------------|------------|---------------------|
|                           | 16S rRNA  | 18S rRNA  |                      |                      |
| **Unclassified**          | 19.8      | 16.1      | 13.1                | 8.0                  |
| **Unclassified**          | 11.3      | 9.6       | 8.4                 | 6.4                  |
| **Unclassified**          | 6.4       | 7.6       | 5.6                 | 4.1                  |
| **Cryptomonas sp.**       | 28.1      | 16.1      | 9.3                 | 7.7                  |
| **Cryptomonas curvata**   | 0.7       | 0.9       | 0.9                 | 0.7                  |
| **Cryptomonadales**       | 4.4       | 2.7       | 3.7                 | 2.7                  |
| **Cryptophyceae 1**       | 4.8       | 2.7       | 2.7                 | 2.7                  |
| **Chrysochromulina sp.**  | 1.1       | 0.9       | 0.9                 | 0.9                  |
| **Chytridiomycetes**      | 1.2       | 0.9       | 1.0                 | 0.9                  |
| **Amphiprora**            | 1.0       | 0.9       | 1.0                 | 1.0                  |
| **Strombidia**            | 1.1       | 0.9       | 1.0                 | 1.0                  |
| **StrombidiidaA**         | 1.1       | 0.9       | 1.0                 | 1.0                  |
| **Chromulina**            | 1.0       | 0.9       | 1.0                 | 1.0                  |
| **Ophiocytum**            | 0.5       | 0.9       | 0.9                 | 0.9                  |
| **Synedra**               | 0.1       | 0.9       | 0.9                 | 0.9                  |
| **Cymbella**              | 0.1       | 0.9       | 0.9                 | 0.9                  |
| **Chlamydomonas**         | 0.2       | 0.9       | 0.9                 | 0.9                  |
| **Chlorophyta**           | 1.0       | 0.9       | 1.0                 | 1.0                  |
| **Chlamydomonadales**     | 0.6       | 0.9       | 0.9                 | 0.9                  |
| **Porphyridium**          | 0.5       | 0.9       | 0.9                 | 0.9                  |
| **Rhodomonas**            | 0.5       | 0.9       | 0.9                 | 0.9                  |

Legend 16S rRNA
- **Alphaproteobacteria**
- **Bacteria**
- **Betaproteobacteria**
- **Cyanobacteria**
- **Gammaproteobacteria**

Legend 18S rRNA, microscopy and chloroplast 16S rRNA
- **Corophia**
- **Chlorophyta**
- **Cryptophyta**
- **Dinoflagellata**
- **Haptophyta**
- **Katablepharidophyta**
- **Telenemia**
- **Ochromyta**
- **Others**
- **Unknown**
### Supplementary Table 10.

Most abundant taxa (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.

| Taxonomy                  | 16S rRNA | 18S rRNA | Microscopy | Chloroplast 16S rRNA |
|---------------------------|----------|----------|------------|---------------------|
|                           | Reads    | CV%      | Reads      | Biovolume%          | CV%      |
| **Lake Saint-Augustin open water** |          |          |            |                     |          |
| CL500-3                   | 15.2     | 50       | 17.8       | 40                  | 31.5     | 173  |
| Unclassified              | 14.0     | 13       | 7.4        | 85                  | 18.0     | 66   |
| Unclassified              | 12.9     | 61       | 6.8        | 122                 | 16.3     | 85   |
| Unclassified              | 8.0      | 73       | 6.3        | 116                 | 6.2      | 68   |
| Cuspidithrix sp.          | 4.7      | 91       | 5.8        | 173                 | 5.4      | 169  |
| Unclassified              | 4.2      | 75       | 5.5        | 110                 | 5.0      | 173  |
| Polynucleobacter sp.      | 3.0      | 20       | 4.7        | 70                  | 4.9      | 129  |
| Unclassified              | 2.7      | 106      | 4.1        | 130                 | 4.0      | 173  |
| SH3-11                    | 2.6      | 109      | 3.4        | 132                 | 4.0      | 58   |
| Flavobacterium sp.        | 2.2      | 155      | 3.3        | 173                 | 2.5      | 173  |
| Cyanobium sp.             | 2.0      | 167      | 3.2        | 145                 | 2.3      | 173  |
| Sediminibacterium sp.     | 1.6      | 162      | 2.7        | 167                 | 1.7      | 126  |
| Deltaproteobacteria       | 1.6      | 82       | 2.6        | 161                 | 1.7      | 108  |
| Unclassified              | 1.6      | 87       | 1.9        | 112                 | 1.6      | 132  |
| Unclassified              | 1.2      | 64       | 1.9        | 173                 | 1.5      | 173  |
| Dolichospermum sp.        | 1.1      | 85       | 1.5        | 83                  | 0.8      | 94   |
| MWH-UniP1                 | 1.1      | 125      | 1.4        | 127                 | 0.7      | 173  |
| Reyvanella sp.            | 1.0      | 158      | 1.3        | 101                 | 0.6      | 131  |
| Filovicola sp.            | 0.7      | 73       | 1.2        | 116                 | 0.5      | 173  |
| Dinghuiibacter sp.        | 0.6      | 59       | 1.1        | 14                  | 0.2      | 173  |

**Legend 16S rRNA**
- Alphaproteobacteria
- Bacteroidetes
- Betaproteobacteria
- Cyanobacteria
- Gammaproteobacteria
- Planctomycetes
- Verrucomicrobia
- Others

**Legend 18S rRNA, microscopy and chloroplast 16S rRNA**
- Cerezoa
- Chlorophyta
- Ciliophora
- Cryptophyta
- Dinoflagellata
- Haptophyta
- Katablepharidophyta
- Telonemia
- Ochrophyta
- Others
- Unknown
**Supplementary Table 11.** Most abundant taxa (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.

| Taxonomy                     | 16S rRNA | 18S rRNA | Lake Saint-Augustin ice-cover | Chloroplast 16S rRNA |
|------------------------------|----------|----------|-------------------------------|---------------------|
|                              | Reads %  | CV%      | Biovolume %                  | CV%                |
| CL500-3                      | 21.5     | 52       | Chlamydomonadales             | 15.7                |
| Unclassified                 | 18.5     | 12       | Chrysophyceae cladeF          | 11.0                |
| Unclassified                 | 9.3      | 75       | Cryptomonadales               | 8.8                 |
| Flavobacterium sp.           | 7.6      | 130      | Cryptophyceae                 | 6.7                 |
| Undibacterium sp.            | 7.4      | 139      | Cryptomonas curvata           | 5.9                 |
| Polaronomas sp.              | 4.0      | 117      | Chrysophyceae cladeD          | 4.9                 |
| Unclassified                 | 3.7      | 78       | Plagioselmis sp.              | 4.3                 |
| Unclassified                 | 3.6      | 83       | Cryptothecaceae 1             | 3.6                 |
| Aquirestis sp.               | 1.6      | 130      | Katablepharidales             | 2.6                 |
| Unclassified                 | 1.6      | 4        | Stephanodiscus sp.            | 2.3                 |
| Dinghuiibacter sp.           | 1.5      | 83       | Procentrum sp.                | 2.1                 |
| Unclassified                 | 1.0      | 78       | Chlamydomonadales             | 2.1                 |
| Terrimicrobium sp.           | 1.0      | 155      | Unclassified                  | 2.0                 |
| Polynucleobacter sp.         | 0.9      | 54       | Spumella vulgaris             | 1.8                 |
| Actinobacteria lgc1 clade    | 0.9      | 78       | Protaurolineage               | 1.5                 |
| Rhodovibrio sp.              | 0.8      | 92       | Parmales                      | 1.4                 |
| Methylotenera sp.            | 0.7      | 166      | Philasterida                  | 0.7                 |
| Actinobacteria unclassified  | 0.7      | 65       | Rickettsiobium sp.            | 0.7                 |
| Nitrosocaproy sp.            | 0.7      | 111      | Askemania sp.                 | 0.6                 |

**Legend 16S rRNA**
- Alphaproteobacteria
- Bacteroidetes
- Betaproteobacteria
- Cyanobacteria
- Gammaproteobacteria
- Planctomycetes
- Verrucomicrobia
- Others

**Legend 18S rRNA, microscopy and chloroplast 16S rRNA**
- Cercozoa
- Chlorphyta
- Chlorophora
- Cryptophyta
- Dinoflagellata
- Haptophyta

**Legend 18S rRNA**
- Catalapharidophyta
- Teknomeia
- Ochrophyta
- Others
- Unknown
Supplementary Table 12. Taxa 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 log² 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. Taxa 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.

| Taxonomy | 16S rRNA L2FC | Low | High | 18S rRNA L2FC | Low | High | Microscopy L2FC | Low | High | Chloroplast 16S rRNA L2FC | Low | High |
|----------|--------------|-----|------|--------------|-----|------|----------------|-----|------|----------------------------|-----|------|
| **Cyanobacteria** | 7.2 | 0.9 | 0.7 | 8.4 | 7.6 | <0.1 | 15.0 | 10.5 | <0.1 | 5.6 | 6.9 | <0.1 |
| **Betaproteobacteria** | 5.9 | 0.4 | <0.1 | 6.8 | 2.6 | <0.1 | 7.4 | 0.0 | <0.1 | 3.2 | 14.8 | 14.8 |
| **Unclas.** | 5.8 | 0.2 | 0.1 | 5.4 | 3.8 | 0.1 | 6.0 | 4.1 | <0.1 | 2.8 | 0.5 | <0.1 |
| **Alphaproteobacteria** | 5.5 | 1.0 | 0.8 | 4.7 | 0.6 | <0.1 | 5.4 | 0.1 | <0.1 | 2.3 | 4.6 | 1.7 |
| **Unclas.** | 5.5 | 1.0 | <0.1 | 4.5 | 2.0 | 0.4 | 4.8 | 0.1 | <0.1 | 2.3 | 2.6 | 0.2 |
| **Dinghaibacter sp.** | 5.3 | 0.4 | <0.1 | 4.4 | 1.3 | <0.1 | 4.5 | 0.1 | <0.1 | 2.2 | 0.5 | <0.1 |
| **Unclas.** | 5.1 | 0.3 | <0.1 | 4.4 | 1.0 | <0.1 | 4.5 | 0.1 | <0.1 | 2.1 | 0.5 | <0.1 |
| **Unclas.** | 5.0 | 0.2 | 0.1 | 4.5 | 2.4 | <0.1 | 3.5 | 0.0 | <0.1 | 5.0 | 0.1 | 0.9 |
| **CL500-3** | -6.6 | <0.1 | 1.2 | -5.2 | <0.1 | 1.4 | 2.5 | 0.1 | <0.1 | -6.8 | <0.1 | 3.2 |
| **Mesorrhagias hongiella** | -6.6 | <0.1 | 0.3 | -5.1 | <0.1 | 1.9 | 2.3 | <0.1 | <0.1 | -5.4 | <0.1 | 0.1 |
| **CL500-3** | -6.3 | <0.1 | 0.6 | -4.9 | <0.1 | 7.5 | -11.8 | <0.1 | 0.4 | -5.2 | 0.1 | 16.4 |
| **CL500-3** | -6.1 | <0.1 | 0.7 | -4.6 | <0.1 | 0.9 | -8.8 | 0.1 | 1.0 | -5.0 | 0.1 | 0.9 |
| **Unclas.** | -5.9 | <0.1 | 0.5 | -4.3 | <0.1 | 0.6 | -6.9 | 0.4 | 0.5 | -5.0 | 1.8 | 5.7 |
| **MWH-UniP** | -5.8 | <0.1 | 0.3 | -4.0 | <0.1 | 0.6 | -5.7 | 0.8 | 0.6 | -4.8 | <0.1 | 0.6 |
| **Unclas.** | -5.8 | <0.1 | 0.3 | -4.0 | <0.1 | 0.8 | -5.5 | 5.9 | 13.2 | -4.8 | 0.5 | 2.8 |
| **Craspedothrix LMECA-163** | -5.7 | <0.1 | 0.8 | -4.0 | <0.1 | 0.7 | -4.0 | <0.1 | 0.5 | -4.7 | 0.1 | 0.6 |
| **Craspedothrix LMECA-163** | -5.7 | <0.1 | 0.6 | -3.8 | <0.1 | 0.5 | -3.3 | <0.1 | 0.2 | -4.2 | <0.1 | 0.1 |

**Legend:**
- **16S rRNA:** Alphaproteobacteria, Betaproteobacteria, Cyanobacteria, Deltaproteobacteria, Firmicutes, Proteobacteria
- **18S rRNA:** Chlorophyta, Cyanobacteria, Cryptophyta, Chrysophyta, Dinoflagellata, Eustigmatophyta, Haptophyta, Ochrophyta, Ochrophyta, Haptophyta
- **Chloroplast 16S rRNA:** Katablepharidophyta, Chlamydomonadales, Chrysophyta, Chrysophyta, Cryptophyta, Dinoflagellata, Unknown.
Supplementary Table 13. Taxa 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 log² 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. Taxa 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.

| Taxonomy                      | 16S rRNA L2FC | 18S rRNA L2FC | Microscopy L2FC | Chloroplast 16S rRNA L2FC |
|-------------------------------|--------------|--------------|----------------|--------------------------|
|                               | IC           | OW           | IC             | OW                       |
|                               |              |              |                |                          |
| Nitrosospira sp.              | 7.8          | 1.0          | <0.1           |                           |
| Unclas.                       | 7.1          | 1.3          | <0.1           |                           |
| Flavobacterium sp.            | 7.0          | 1.0          | <0.1           |                           |
| Methylobacter sp.             | 6.2          | 2.5          | <0.1           |                           |
| Unclas.                       | 6.1          | 0.7          | <0.1           |                           |
| Nitrotoga sp.                 | 6.0          | 0.4          | <0.1           |                           |
| CL500-3                       | 5.0          | 0.8          | <0.1           |                           |
| Unclas.                       | 4.9          | 0.1          | <0.1           |                           |
| Subgroup 6                    | 4.7          | 0.1          | <0.1           |                           |
| Unclas.                       | -7.0         | <0.1         | 0.7            |                           |
| Unclas.                       | -6.1         | <0.1         | 0.4            |                           |
| CL500-3                       | -5.8         | <0.1         | 0.8            |                           |
| Unclas.                       | -5.7         | <0.1         | 0.3            |                           |
| Unclas.                       | -5.5         | <0.1         | 0.3            |                           |
| Unclas.                       | -5.3         | <0.1         | 0.2            |                           |
| Unclas.                       | -5.2         | <0.1         | 0.2            |                           |
| Dinobryon bavaricum           | -5.7         | <0.1         | 0.3            |                           |
| Dinobryon divergens           | -6.3         | <0.1         | 2.0            |                           |
| Dinobryon sp.                 | -6.4         | <0.1         | 0.3            |                           |
| Dinobryon stoloniferum        | -6.4         | <0.1         | 0.5            |                           |
| Dinobryon petiolatum          | -6.4         | <0.1         | 0.5            |                           |
| Diatom sp.                    | -5.2         | <0.1         | 0.3            |                           |
| Dinobryon sp.                 | -4.9         | <0.1         | 0.5            |                           |
| Dinobryon sp.                 | -4.8         | <0.1         | 0.1            |                           |
| Dinobryon sp.                 | -4.8         | <0.1         | 0.1            |                           |
| Dinobryon sp.                 | -4.8         | <0.1         | 0.1            |                           |