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Excess chloride and impervious surfaces reduce over-winter quality of stream algal assemblages

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Abstract

Winter road salt use leads to the salinization of freshwater habitats. Freshwater organisms experience negative effects due to increased chloride ions, including algae in periphyton biofilms. This study examines the effects of road salt on algal assemblage composition, lipid production, and enzyme activity. Five streams throughout Erie County, New York, US were sampled monthly from October 2018 to March 2019. Chloride concentrations in all streams averaged 203.6 Cl^- mg/L throughout the winter and had a highly significant relationship ($r_s = 0.82$) with developed land use. Algal biodiversity scores decreased with elevated salinity ($r_s = 0.11$). Algae exhibited the greatest total lipids in January (2.92 mg/m^2) and the lowest in March (1.03 mg/m^2). Similar trends were observed with $\omega 3$ and $\omega 6$ compounds. Overall desaturase $\Delta 6$ activity trended with stream Cl^- concentrations, mainly along $\omega 6$ pathways, suggesting an inflammatory stress response. Algal assemblages exhibited evidence of chronic salt exposure through impaired taxonomic composition and patterns of lipid production that followed trends in stream water salinity. These effects suggest road salt applications have negative effects on stream primary producers with consequences for higher trophic levels.

Keywords: fatty acids

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Introduction

Combating the forces of winter is a seasonal challenge. For temperate regions, the application of deicers such as road salt is a common practice during winter months. Around 20 million tons of road salt are applied annually in the United States alone (Hintz and Relyea 2017), and New York state has the highest rate of salt use at 13 metric tons/lane km (Kelting and Laxson 2010, Hintz et al. 2021). Road salt is typically a combination of sodium chloride (NaCl) and inorganic anti-clumping agents such as potassium ferrocyanide, though variations are gaining wider use. For example, calcium magnesium acetate is an effective deicer at far lower temperatures than NaCl, although it is much more expensive (Kelly et al. 2019). Chloride ions (Cl^-) are highly soluble and often leach into nearby rivers and streams following road salt application, a key factor in freshwater salinization. While elevated salinity in freshwater bodies is often attributed to agricultural or industrial practices, transportation applications such as road salt runoff are considerably less studied among anthropogenic causes of increased freshwater habitat salinity (Dugan et al. 2017).

Inland freshwater lakes, streams, and wetlands naturally contain low levels of salt, and increases in salt ion concentrations (namely chloride) can have drastic consequences on aquatic organisms and the trophic structure of the ecosystems they occupy. A variety of negative effects related to increases in freshwater salinity have been documented in a few recent studies, such as the formation of dense saline layers in lakes which can threaten aquatic life by preventing oxygen transfer to the lake bed (Tenebaum 2008). For example, a study by Hintz and Relyea (2017) found that the mass and length of rainbow trout (*Oncorhynchus mykiss*) decreased at an elevated salt concentration of 4,000 mg Cl^-/L. They observed that zooplankton (*Daphnia pulex*) was reduced in abundance yet increased ephippia production. *D. pulex* generally produce between 10-50 eggs under normal environmental conditions, but produce a single hard-shelled unfertilized egg called an ephippium when conditions become unfavorable (van Meter and Swan 2014; Hintz and Relyea 2017). According to Hintz and Relyea (2017), concentrations as low as 230 mg Cl^-/L triggered the production of resting ephippia in *D. pulex*. Hintz and Relyea (2017) also recorded the abundance of *D. pulex* at four increasing salt concentrations between 30 and 1300 mg Cl^-/L, and found that abundance decreased as salt concentration increased. Two additional studies by Petranka and Doyle (2010) and van Meter and Swan (2014) found that freshwater salinization limits community composition to salt

Table 1 Location and watershed land use areas (Stroud Water Resource Center 2017) of Ellicott, Ransom, Spring, Plum Bottom, and S. Branch Smoke Creek in Erie County, NY (location coordinates from Google Maps).

Stream Site	Ellicott	Ransom	Spring	Plum	Smoke
Dominant habitat	Suburban	Rural	Rural	Urban	Suburban
Municipality	Williamsville	Clarence	Alden	Lancaster	Blasdell
HUC-12	41201040404	41201040303	41201040402	41201030105	41201030401
Lat (Y)	42.965525	42.987935	42.908396	42.902114	42.796593
Long (X)	-78.745811	-78.593225	-78.492225	-78.669591	-78.803345
Total agriculture (km^2)	8.10	14.73	23.81	14.93	12.70
Total aquatic (km^2)	9.47	6.70	10.05	7.41	8.49
Total developed (km^2)	31.84	11.03	4.11	41.49	38.60
Total forest (km^2)	6.01	7.60	17.47	14.11	23.36
Total grasslands (km^2)	0.73	0.32	0.48	1.42	2.50

tolerant zooplankton and macroinvertebrates capable of withstanding and adapting to foreign stressors. In this case, salinity acts as an environmental constraint by presenting stressful conditions that exceed many species' tolerance thresholds. The presence of road salt in pond communities alters community composition to favor salt-tolerant insects and zooplankton, such that salt-intolerant aquatic organisms are easily outcompeted at elevated salt concentrations, which results in a reduction of community diversity (Petranka and Doyle 2010; van Meter and Swan 2014).

The previous studies mainly focused on heterotrophic organisms. By comparison, there is limited research investigating the potentially harmful effects of elevated freshwater salinity on primary producers in aquatic ecosystems, especially algae (Steinman et al. 2011) associated with periphyton biofilms. Periphyton is a mixture of photosynthetic algae, fungal hyphae, and other heterotrophic bacteria. Algae are a crucial group of organisms and support multiple levels of life (Chapman 2013). It is estimated that all the algae on earth, both marine and freshwater, produce about half of the total oxygen produced on the planet (Chapman 2013). As a rare example evaluating the effects of road salt on algae, a study by Cook and Francoeur (2013) found that the maximum photosynthetic efficiency of photosystem II in periphyton was significantly reduced when exposed to stream water that had an elevated conductivity of 35,320 μS/cm^2 due to road salt runoff. Besides supplying oxygen, algae are more nutritious than terrestrial plants due to their low carbon-nitrogen and carbon-phosphorus ratios (O'Brien and Wehr 2010). Algae also produce high concentrations of polyunsaturated essential fatty acids (PUFAs), which are large lipid molecules containing more than one double bond in their carbon backbone. Most heterotrophs, such as aquatic invertebrates, cannot synthesize their own PUFAs; therefore, algae are considered high-quality food sources (Torres-Ruiz et al. 2007; Guo et al. 2016). For this reason, algal abundance is not only an acceptable indicator of ecological impairment and water quality in freshwater ecosystems, but it also provides insight concerning the growth and fitness potential of consumers (Charles et al. 2018).

Few algal species have developed methods to combat drastic increases in salinity. Some select microalgae species like *Neochloris oleoabundans* use mechanisms such as glycerol or sucrose production, and amino acid accumulation to increase salt tolerance (de Jaeger et al. 2018), but many freshwater algae species lack the ability to conform to extreme changes in their environment and may experience adverse effects as a result. The goal of this study is to determine how elevated salinity due to winter road salt application affects fatty acid enzyme activity and algal taxonomic composition in freshwater algae found in periphyton assemblages. To do this, we quantified activity of the Δ6 desaturase enzyme, expressed in the ω3 and ω6 pathways. The Δ6 desaturase enzyme was selected because it appears in both pathways, and because it is involved in multiple steps in each pathway (Meesapyodsuk and Qiu 2012). These pathways lead to the production of polyunsaturated essential fatty acids in autotrophs. We predicted that elevated salinity would have a negative effect on periphyton biomass, stoichiometry, and lipid enzyme activity.

Materials and Methods

We visited five streams in Erie County, NY, once a month from October 2018 to March 2019 (Table 1). These streams were chosen because they interact with roadways in a mixture of urban and rural habitats. Land use in each stream's HUC-12 drainage area was collected from Model My Watershed (https://wikiwatershed.org/). At each stream, a Hach Hydrolab multisonde (Hach, Loveland, CO USA) was placed upstream from our collection site and used to determine salinity (PSS) and specific conductance (mS/cm^2). A syringe filter was used to collect 10 mL of water from each stream. Filtered water was stored at 4°C and preserved with 12.5% H_2SO_4. This water was analyzed for Cl^- (mg/L) concentrations following a mercuric thiocyanate reaction (Astoria Pacific 2020). Attached periphyton was collected from each stream by scrubbing the exposed surfaces of three cobbles with a stiff bristle brush; typical volume of collected biomass from three cobbles was 150 mL. The dimensions (cm) of each cobble were recorded before returning them to the streams, allowing biochemical measurements to be reported on the bases of cobble surface area (m^2). In February 2019, only three streams, Ellicott, Ransom, and Plum Bottom, were sampled due to storm events; Ellicott was the only stream sampled for periphyton.

Periphyton samples were stored in a 4°C refrigerator with a loose cap no longer than 48 hours following collection. These were ultimately analyzed for total nitrogen, total phosphorous, ash-free dry mass (AFDM), and fatty acid content. Total dissolved nitrogen was analyzed using a potassium persulfate digest (Solórzano and Sharp 1980a). Total phosphorus was analyzed by magnesium carbonate digest, combustion at 450°C, and resuspension in 0.2 M HCl. Samples were then diluted and processed as soluble reactive phosphorus (SRP; Solórzano and Sharp 1980b). AFDM was determined following filtration onto pre-ashed and preweighed 47 mm glass fiber filters. The contents were dried (24 hours) at 80°C, weighed, then ashed at 450°C for 2-3 hours and re-weighed to achieve AFDM (O'Brien and Wehr 2010).

We filtered 30 mL of periphyton onto pre-ashed 47 mm GF/F filters (GE/Whatman, Buckinghamshire, UK) and stored the filters at -20°C in chloroform-washed borosilicate test tubes after flushing samples with N_2 gas. Lipids were extracted with a chloroform:methanol (2:1) solution, methylated using BF_3, and transferred to a hexane solvent (Parrish 1999; Whorley and Wehr 2018). An internal standard of nonadecanoic acid (19:0) and blank hexane samples were used to test methylation efficiency, and to assess consistency among sample runs. Samples were analyzed and quantified on a Shimadzu (Tokyo, Japan) GC-2014 fitted with a capillary column (Omegawax-320, 30 m x 0.32 mm x 0.25 ωm; Supelco®, Bellefonte, Pennsylvania). The temperature program had an initial injection into a splitless inlet at a temperature of 100°C, followed by 1 h ramping to 260°C with He as the carrier gas to a flame ionization detector. The FAME-37 mix (Supelco) was used for standard series. Quantification was limited to compounds ≥16C because many biologically important FAs are derived from 16C base molecules (Stanley-Samuelson 1994).

The Fadhlaoui and Couture (2016) method was adopted to quantify fatty acid enzyme activity, in which the activity of the Δ6 desaturase enzyme in the ω3 and ω6 pathways was estimated as the product-over-precursor ratio of individual fatty acids. Enzyme activity of Δ6 desaturase was calculated by dividing concentrations of 20:4ω6/18:2ω6, 20:5ω3/18:3ω3, and 22:6ω3/20:5ω3. The activity of additional enzymes Δ9 desaturase, Δ5 desaturase, and elongase 5, which appear in the ω6 and ω7 pathways, were investigated further. Enzyme activity of Δ9 desaturase was calculated by dividing concentrations of 16:1ω7/16:0, while enzyme activity of Δ5 desaturase and elongase 5 (E5) were calculated by dividing concentrations of 20:4ω6/20:3ω6 and 20:3ω6/18:3ω6 respectively.

A 20 mL portion of each periphyton sample were preserved using Lugol's iodine solution. A wet mount was created for each of the samples by mixing thoroughly and placing 2 drops onto a slide covered by a 24 × 50 mm coverslip. A minimum of 150 algal cells across a minimum of 10 fields of view were identified for each sample using a Leica DM500 compound light microscope and the Freshwater Algae of North America taxonomic key (Wehr et al. 2015). Cells were identified to genus level as well as categorized as either cyanobacteria, chlorophytes, or diatoms for later statistical analysis. Shannon's H' taxonomic diversity metrics were calculated for total and divisional periphyton taxa.

Data collected for each variable were highly heterogenous in terms of normality, therefore all statistics performed were non-parametric. Kruskal-Wallis comparisons were conducted across months on all reported water chemistry, periphyton elemental measurements, periphyton fatty acid concentrations, and fatty acid enzyme activity ratios. Spearman's rank-based correlation coefficient were conducted on comparisons of Cl$^-$ (mg/L) to fatty acid enzyme activity ratios and biodiversity H' scores. All statistics were performed in SigmaPlot v.14 (Systat Software, Inc., San Jose, CA) with a critical $\alpha = 0.05$.

Results

Throughout the winter season, measurements of stream salinity and conductivity were consistently elevated, although highly variable (Table 2). Measurements of stream water PSS and specific conductance were collected *in situ*, but did not reveal any significant differences based on the month collected. Stream watershed developed land area (km^2) included paved roads and was highly correlated with chloride concentration ($r_s = 0.816$, $p < 0.001$). No similar correlations were observed with PSS and specific conductance. Salt measured as Cl$^-$ ion concentration similarly revealed no significant differences based on month collected, although notable peaks occurred. The waterbody S. Branch Smoke Creek achieved high Cl$^-$ values in December 2018 (715.8 mg/L) and March 2019 (875.1 mg/L). Other waterbodies in the study did not experience these monthly spikes. The S. Branch Smoke Creek watershed contained the second highest amount of developed land (83.6 km^2) and is downstream of the Buffalo Bills NFL stadium.

Table 2 Mean (±SE) values of salinity and conductivity measurements obtained from October 2018 through March 2019 (Kruskal-Wallis H values (*p* value) follow each variable).

Variable	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	KW Results
Practical Salinity Scale (PSS)	0.63 (0.17)	0.44 (0.07)	0.52 (0.08)	0.52 (0.08)	0.43 (0.03)	0.47 (0.10)	3.185 (0.672)
Specific Conductance (SpC mS/cm^2)	1.29 (0.23)	0.92 (0.15)	1.08 (0.17)	1.08 (0.15)	0.96 (0.04)	0.97 (0.20)	2.618 (0.759)
Chloride ion (Cl^- mg/L)	166.92 (21.72)	167.25 (32.04)	253.92 (118.77)	163.74 (46.98)	166.60 (46.44)	280.92 (153.18)	0.643 (0.986)

Periphyton biochemical measurements also showed no difference across the time span of this study (Table 3). Several algal measurements reached their peak in December and January. These months were the maximum for average AFDM (2.13 g/m^2), phosphorus (143.31 mg/m^2), total fatty acids (FA; 2.92 mg/m^2), and total ω6 FA (0.14 mg/m^2). Both AFDM and phosphorus high averages occurred in December, followed by the high average FA measurements in January. Total ω3 fatty acid compound peaked earlier in the winter (November) with a high average of 0.08 mg/m^2, as did nitrogen (0.58 g/m^2). Values of biochemical metrics did not correspond with extreme Cl^- values. Spearman's correlations between periphyton biochemical properties and various measures of salinity were not statistically significant ($p > 0.15$).

Fatty acid enzyme activity exhibited interesting relationships with stream chloride concentrations, although these relationships were not significant (Figs. 1 and 2). The activity of the Δ6 desaturase enzyme (Fig. 1A) followed chloride concentration most closely ($r_s = 0.353$, $p = 0.082$) compared to other fatty acid enzymes considered. When the activity of Δ6 was broken down into its ω3 and ω6 components, the relationship between Δ6's activity creating ω3 FAs became more variable (Fig. 1B) and there was no clear relationship to chloride concentration ($r_s = 0.214$, $p = 0.301$). The relationship between the ω6 creating activity of Δ6 (Fig. 1C) appears more similar to the overall Δ6 relationship with chloride concentration, however this relationship was not significant ($r_s = 0.256$, $p = 0.215$). Relationships between chloride concentrations and Δ5 enzyme activity ($r_s = -0.166$, $p = 0.423$), Δ9 enzyme activity ($r_s = 0.027$, $p = 0.813$), and E5 elongase ($r_s = -0.091$, $p = 0.662$) were also highly variable and did not show a statistically significant correlation (Fig. 2).

A total of 27 different algal genera were identified (Table 4). The most common diatom genus was *Navicula* (21.99% of identified cells); *Stigeoclonum* was the most common chlorophyte genus (10.13%) and *Leptolyngbya* was the most common cyanobacteria (20.27%). *Navicula* spp. were present in all streams throughout the winter season. *Stigeoclonum* sp. occurred mainly in October and November. Instances of *Leptolyngbya* were notable because they only appeared in Plum Bottom Creek, which had the highest amount of developed land in its watershed (Table 1). Spring Creek was the most rural stream and reported the greatest number of observed taxa. Over the different months of the study, October and December each reported the greatest number of observed taxa (Table 4). Overall algal diversity within the periphyton did not correlate with chloride concentration ($r_s = 0.111$, $p = 0.594$). Similarly, algal divisional diversity was not well-related to stream water chloride concentrations (Fig. 3). The diatom taxa presented the greatest overall diversity to all algal assemblages, but was not significantly correlated with chloride concentration ($r_s = 0.143$, $p = 0.493$). Chlorophyte diversity ($r_s = -0.178$, $p = 0.391$) and cyanobacterial diversity ($r_s = 0.024$, $p = 0.908$) were also not related to chloride concentration. There also were no significant correlations between algal divisional diversity and measured fatty acid concentration ($p > 0.10$).

Table 3 Periphyton biochemical composition obtained from October 2018 through March 2019 (metric mean (±SE) reported, followed by Kruskal-Wallis H values (*p* value) for comparison among months).

Variable	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19*	Mar-19	KW stats
Carbon as AFDM (g/m^2)	2.13 (0.43)	0.66 (0.28)	2.13 (0.36)	1.81 (0.20)	0.34 (n/a)	1.61 (0.71)	9.821 (0.080)
Nitrogen as NO_3 (g/m^2)	1.13 (0.50)	0.58 (0.13)	0.11 (0.04)	0.24 (0.16)	0.92 (n/a)	0.48 (0.23)	6.477 (0.263)
Phosphorus as PO_4 (mg/m^2)	25.83 (13.47)	79.05 (29.80)	143.31 (39.81)	101.36 (43.48)	48.59 (n/a)	51.49 (29.41)	5.109 (0.403)
Total FA (mg/m^2)	1.59 (0.32)	1.40 (0.38)	2.36 (0.45)	2.92 (0.77)	0.27 (n/a)	1.03 (0.39)	9.253 (0.099)
Total w3 FA (mg/m^2)	0.07 (0.02)	0.08 (0.03)	0.07 (0.02)	0.07 (0.01)	0.01 (n/a)	0.02 (0.01)	8.877 (0.114)
Total w6 FA (mg/m^2)	0.12 (0.02)	0.11 (0.03)	0.12 (0.02)	0.14 (0.03)	0.05 (n/a)	0.06 (0.01)	6.805 (0.236)

* February means do not have an SE due to having only one replicate.

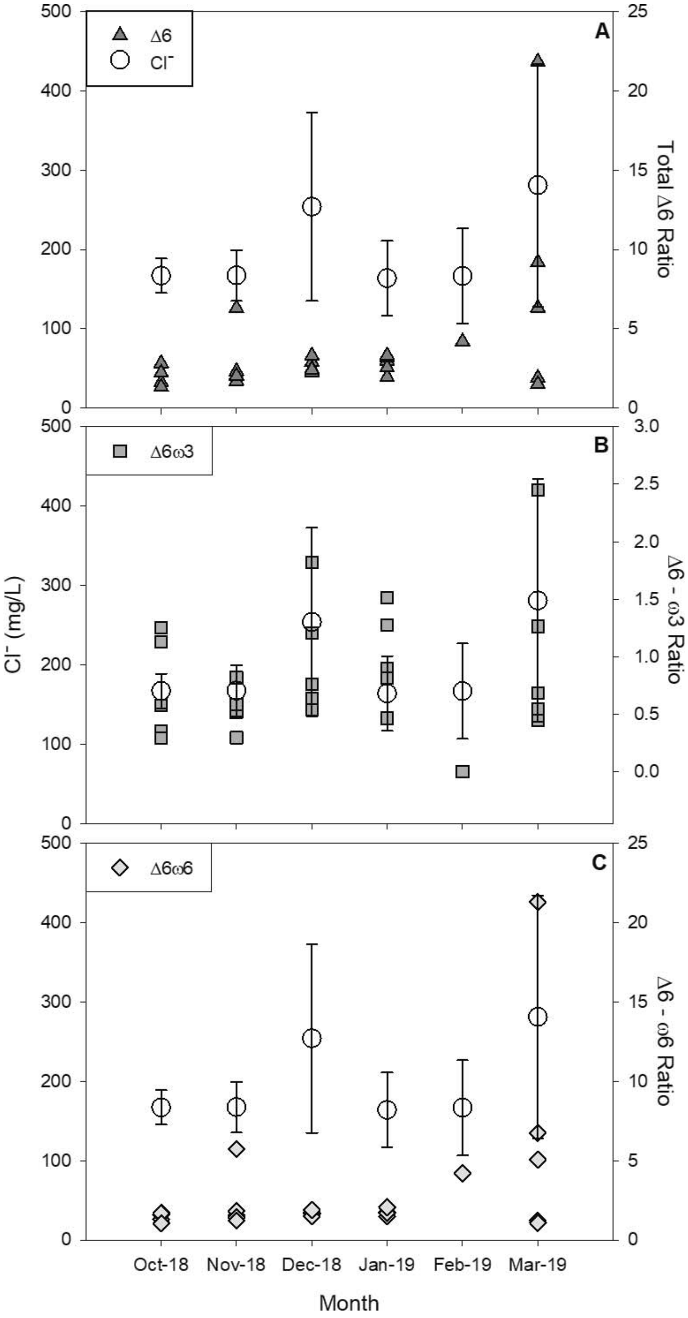

Figure 1 Amount of enzyme activity in each stream sampled during each month of the study for (A) total desaturase Δ6, (B) Δ6 enzymes creating ω3 fatty acids, and (C) Δ6 enzymes creating ω6 fatty acids. Compared alongside monthly Cl^- (mg/L) means (±SE).

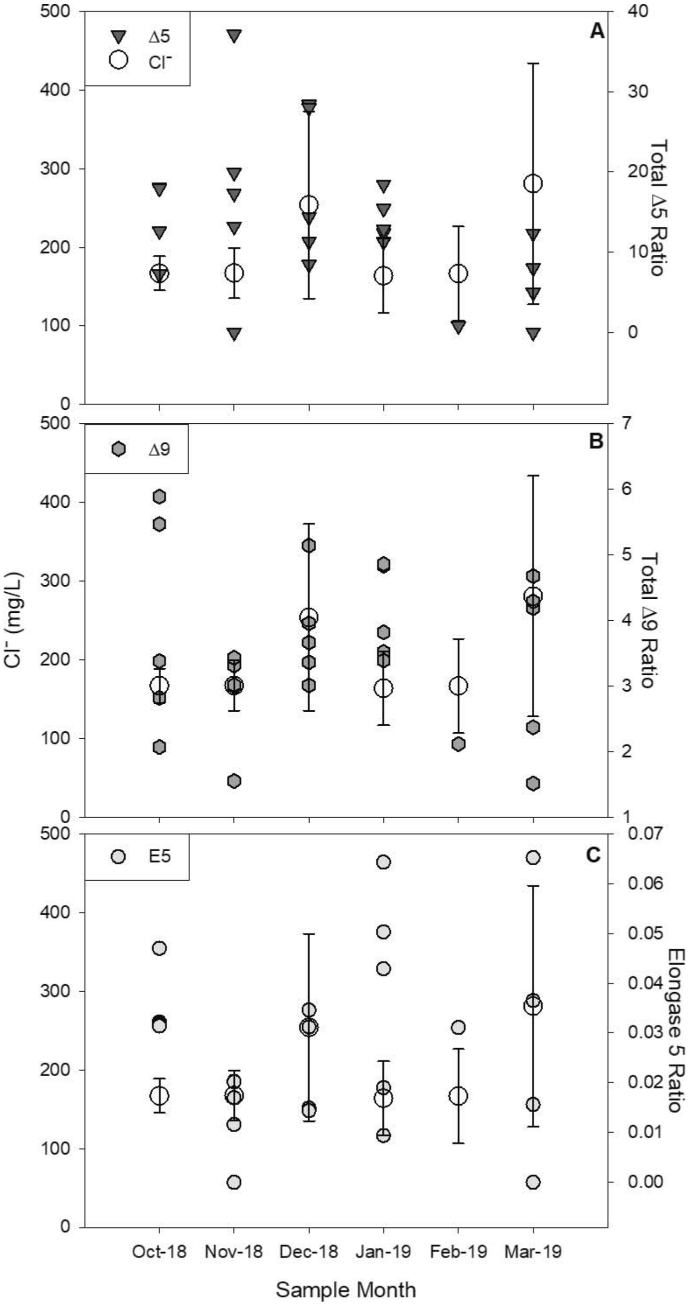

Figure 2 Amount of enzyme activity in each stream sampled during each month of the study for (A) desaturase Δ5, (B) desaturase Δ9, and (C) elongase E5. Compared alongside monthly Cl^- (mg/L) means (±SE).

Discussion

A comparison of average freshwater stream salinity by month reveals that no significant trend exists from October 2018 to March 2019 despite recording elevated levels of salinity (Fig. 2). These results suggest that stream salinity and related effects on stream algae are already in effect before deposition of winter road salt happens. A recent analysis of watershed salinity and chloride concentrations in Toronto, Canada observed elevated levels of salinity in several watersheds during summertime months (Lawson and Jackson 2021). Our findings agree with their findings that excessive winter-time road salt use continues to have chronic effects on freshwater habitats outside of the winter months. While overall city and town measurements of salt usage are available, records are not kept on a street-by-street basis, so it is difficult to know how much salt is deposited at each location. Despite this, the

Table 4 Percentages of algal taxa observed in periphyton samples (total stream algal assemblages is presented for each stream for all samples from October 2018 through March 2019).

		Total stream algal assemblage (%)					**Total monthly algal assemblage (%)***					
Division	**Genera**	**Ellicott**	**Ransom**	**Spring**	**Plum**	**Smokes**	**Oct-18**	**Nov-18**	**Dec-18**	**Jan-19**	**Feb-19**	**Mar-19**
Diatom	*Achnathidium*	2.1	1.1	7.5	0.4	7.7	4.4	4.4	2.1	3.4	0.6	5.4
	Amphora	0.8	*n/o*	0.1	*n/o*	0.4	1.2	*n/o*	*n/o*	*n/o*	*n/o*	*n/o*
	Cocconeis	5.2	2.0	2.0	2.9	4.2	2.7	4.8	3.7	3.3	4.5	7.9
	Cymbella	3.8	2.5	3.2	2.4	5.4	3.8	4.5	3.2	1.7	5.2	5.3
	Diatoma	5.5	1.3	1.7	1.0	3.4	3.4	0.8	2.6	2.4	*n/o*	1.1
	Fragillaria	2.9	*n/o*	0.1	*n/o*	0.7	3.5	0.2	*n/o*	*n/o*	*n/o*	*n/o*
	Gomphonema	22.7	10.5	19.4	10.2	11.1	3.6	2.7	17.1	8.3	11.0	13.5
	Gyrosigma	1.0	0.1	0.1	0.3	*n/o*	1.3	0.2	0.1	0.1	*n/o*	*n/o*
	Melosira	0.5	4.7	0.4	4.7	0.7	9.9	0.6	2.7	0.1	*n/o*	0.1
	Meridion	0.1	1.4	0.7	0.1	0.2	*n/o*	*n/o*	1.4	0.5	*n/o*	1.1
	Navicula	30.1	18.5	22.9	9.9	35.6	14.8	22.7	34.8	18.4	9.1	37.4
	Nitzschia	3.5	2.1	1.0	0.6	0.8	4.3	0.8	1.7	0.6	0.0	1.8
	Rhoicosphenia	1.1	1.7	0.4	1.2	1.5	2.3	1.5	1.1	1.0	0.6	0.4
	Staurosirella	0.4	*n/o*	*n/o*	*n/o*	*n/o*	0.4	*n/o*	*n/o*	*n/o*	*n/o*	*n/o*
	Synedra	3.9	4.4	4.2	2.2	2.3	7.9	3.0	5.2	1.4	1.3	0.3
	Unknown Diatom	0.9	*n/o*	*n/o*	*n/o*	*n/o*	*n/o*	*n/o*	0.2	*n/o*	4.5	*n/o*
Cyanobacteria	*Chroococcus*	0.5	0.5	0.7	*n/o*	0.6	0.7	0.8	0.5	0.2	*n/o*	0.4
	Leptolyngbya	8.0	3.1	8.8	49.5	4.0	*n/o*	19.8	6.6	41.3	51.9	7.0
	Lyngbya	1.4	1.2	3.1	9.7	1.5	0.5	*n/o*	0.2	9.4	*n/o*	9.7
	Oscillatoria	4.1	5.5	5.1	*n/o*	*n/o*	*n/o*	6.8	2.7	2.9	11.0	6.4
	Spirulina	*n/o*	*n/o*	1.1	*n/o*	*n/o*	*n/o*	*n/o*	*n/o*	0.6	*n/o*	*n/o*
	Unknown Cyanobacteria	*n/o*	6.0	10.0	1.1	8.9	10.9	10.7	5.3	*n/o*	*n/o*	*n/o*
Chlorophyte	*Chlorella*	*n/o*	*n/o*	0.1	0.1	*n/o*	0.0	0.1	*n/o*	0.1	*n/o*	0.1
	Cladophora	*n/o*	*n/o*	1.9	*n/o*	*n/o*	1.9	*n/o*	*n/o*	*n/o*	*n/o*	*n/o*
	Stigeoclonum	1.1	33.3	4.3	3.5	4.7	21.7	15.4	3.3	4.2	*n/o*	1.7
	Ulothrix	*n/o*	*n/o*	0.6	*n/o*	5.9	0.6	*n/o*	5.3	*n/o*	*n/o*	*n/o*
	Unknown Chlorophyte	0.3	0.3	0.4	0.2	0.2	*n/o*	*n/o*	0.1	0.1	*n/o*	0.3
	Total taxa observed	**22**	**19**	**25**	**18**	**20**	**21**	**18**	**21**	**20**	**11**	**18**

* Total monthly algal assemblages are presented for each month inclusive of all five streams. February 2019 only has one replicate (Ellicott). When taxa were not observed, they were marked as "n/o". Total taxa observed for each stream and month is totaled at the bottom.

average salinity of the five streams examined during this experiment was much greater than the average salinity typical of unaffected freshwater streams, as described by Hintz and Relyea (2019). North American governments have criteria for chronic stream Cl^- mg/L concentrations as high as 120 mg Cl^- / L (Hintz et al. 2021). Average stream Cl^- concentration observed here were regularly above this threshold (Table 2). The lack of a significant trend in urban freshwater stream salinity from October to March does not devalue the fact that salinity was much greater than is typical of unaffected freshwater streams.

The relationship between salinity and algal AFDM (as a proxy for carbon), nitrogen, and phosphorus content, as well as the relationship between specific conductance and algal AFDM, nitrogen, and phosphorus content were not significant. The freshwater streams examined during this experiment are located in both urban and rural areas and, for this reason, were expected to exhibit high and low concentrations of nitrogen and phosphorus respectively; human activity is largely responsible for the abundance of nitrogen and phosphorus in urban streams, differentiating them from rural or suburban streams (Walsh et al. 2005). Physiologically speaking, algae cells have a direct relationship with the water in their environment, such that an increase in phosphorus or nitrogen concentration in freshwater causes an increase in algal cell phosphorus or nitrogen content (Dugdale 1967). An increase in freshwater phosphorus concentration is also known to overstimulate the growth of algae, the effect of which could be measured through comparison of AFDM. Regardless, algal AFDM and nitrogen content increased with greater salinity and specific conductance while phosphorus content decreased with greater salinity and specific conductance.

Remote, unsalted winter streams have been found to achieve periphytic fatty acid concentrations ranging 3-6 g/m^2 (Whorley and Wehr 2018). Total fatty acid values achieved during this experiment were far below this, constituting a range of 0.34-2.13 g/m^2, which indicates that periphyton assemblages in these disturbed streams were entering the winter season with sustained stress. This chronic stress may explain why

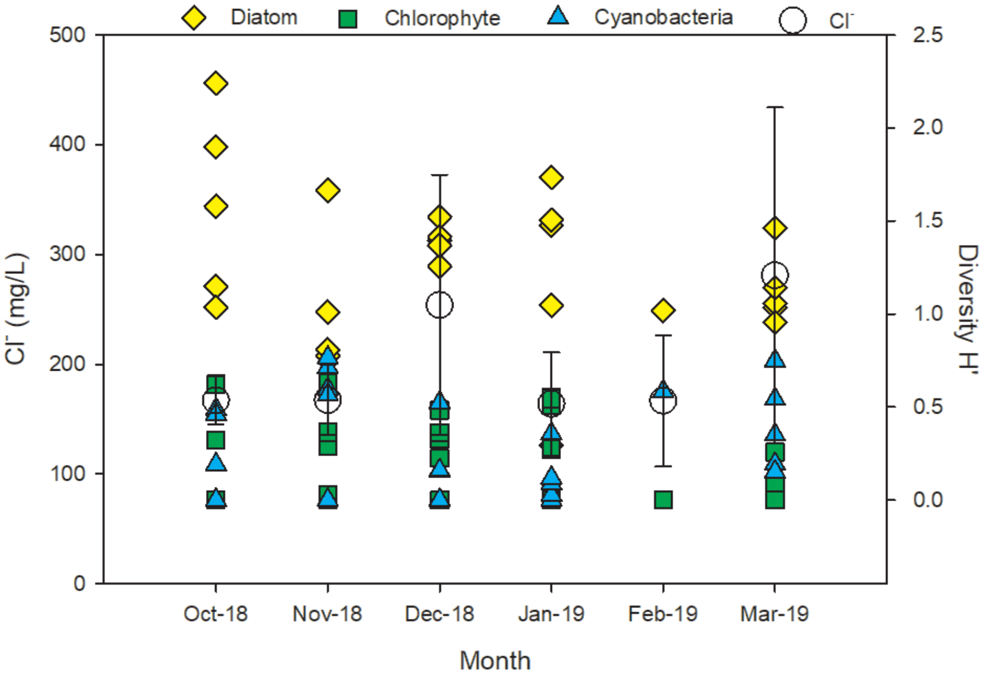

Figure 3 Shannon diversity H′ scores for each algal division within each stream across the duration of the winter season compared with mean (±SE) stream Cl- (mg/L).

within-season salinity variation did not result in significant differences. Product-over-precursor ratios and activity varied among enzymes, yet similar trends are visible. In the case of Δ6 desaturase (20:5n3/18:3n3 and 18:3n6/18:2n6), the activity of this enzyme increased overall from relative consistency in October 2018 through January 2019 to increasing in February and March 2019 in both the ω3 and ω6 pathways (Fig. 1). Considering average monthly stream salinity, October 2018 had the highest measurement and fluctuated over a five month period, such that salinity maintained somewhat of an indirect relationship with enzyme activity (Figs. 1 and 2). The relationship of Δ6 activity appears to be driven by increased activity on the ω6 side of fatty acid production; during the synthesis of ω3 and ω6 fatty acids, the presence of unfavorable environmental conditions results in a competition for desaturase and elongase enzymes which generally favors ω6 fatty acid production (Hixson and Arts 2016). Our data reveal the highest amount of ω6 fatty acids was produced in both December and January (Table 2). This is further evidence of stream algae experiencing chronic stress since an increase in ω6 fatty acid production is associated with inflammatory response pathways (Hixson and Arts 2016). These findings may support the idea of eventual seasonal recovery; the first few months of winter are generally the harshest and warrant greater use of road salt, and enzyme activity may be affected negatively by this process. The rate of Δ6 desaturase activity has also been found to increase in the presence of metals at both warm and low temperatures (Fadhlaoui and Couture 2016). While our results do not show a statistically significant relationship, this trend demonstrates overall concentrations of algal lipids increasing through the greater part of the winter season (Table 2) despite a lack of enzyme activity.

Additionally, the biodiversity of cyanobacteria began to increase towards the end of winter, corresponding with a decrease in diatom biodiversity (Fig. 3). This shift in algal assemblage composition may explain the increasing variability of Δ6 enzyme activity in later winter months. The importance of this finding suggests the algal community in these streams is heavily influenced by cyanobacteria and other heterotrophic bacteria (Ahlgren et al. 1992). Certain species of algae are better at making specific fatty acids than others, and those that contain many 16-carbon compounds are typically associated with bacteria and cyanobacteria (Ahlgren et al. 1992). These prokaryotes produce lower-quality lipids, which may provide insight regarding the future of urban freshwater stream health. Furthermore, Δ5 desaturase (20:4ω6/20:3ω6) and elongase 5 (20:3ω6/18:3ω6) showed no significance and did not produce any interesting trends (Fig. 2).

The results provide insight as to what anthropogenic and environmental factors may contribute to a decrease in health of freshwater ecosystems, and to what degree. Changes to aquatic ecosystems brought about by stream location and urban practices can have a wide variety of negative effects, a concept referred to as "urban stream syndrome" by Meyer et al. (2005).

Freshwater bodies suffering from urban stream syndrome display consistent symptoms; for our purposes, these include low species richness and elevated concentrations of nutrients and contaminants (Walsh et al. 2005). As described by Petranka and Doyle (2010), as well as van Meter and Swan (2014), elevated salinity in pond communities alters community composition by favoring salt-tolerant organisms. All five urban streams in this study possessed unsafe salinity levels that may have negatively impacted species diversity, as well as changed community composition. In turn, the total periphyton biochemical properties and low levels of fatty acid production observed may influence the health and integrity of freshwater ecosystems.

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References

Ahlgren G, Gustafsson IB, Boberg M. 1992. Fatty acid content and chemical composition of freshwater microalgae. Journal of Phycology. 28:37–50.

Astoria Pacific. 2020. Chloride in water and wastewater 1.0 – 50.0 mg/L Cl^-. Method Chloride T090. Astoria Pacific, Clackamas, OR, USA.

Chapman RL. 2013. Algae: the world's most important "plants"—an introduction. Mitigation and Adaptation Strategies for Global Change. 18:5–12.

Charles DF, Tuccillo AP, Belton TJ. 2018. Use of diatoms for developing nutrient criteria for rivers and streams: A biological condition gradient approach. Ecological Indicators. 96:258–269.

Cook LJ, Francoeur SN. 2013. Effects of simulated short-term road salt exposure on lotic periphyton function. Journal of Freshwater Ecology. 28:211–223.

de Jaeger L, Carreres BM, Springer J, Schaap PJ, Eggink G, Martins Dos Santos VAP, Wijffels RH, Martens DE. 2018. *Neochloris oleoabundans* is worth its salt: Transcriptomic analysis under salt and nitrogen stress. PLoS ONE. 13:1–22.

Dugan HA, Bartlett SL, Burke SM, Doubek JP, Krivak-Tetley FE, Skaff NK, Summers JC, Farrell KJ, McCullough IM, Morales-Williams AM, Roberts DC, Ouyang Z, Scordo F, Hanson PC, Weathers KC. 2017. Salting our freshwater lakes. Proceedings of the National Academy of Sciences. 114:4453–4459.

Dugdale RC. 1967. Nutrient limitation in the sea: dynamics, identification, and significance. Limnology and Oceanography. 47:685–695.

Fadhlaoui M, Couture P. 2016. Combined effects of temperature and metal exposure on the fatty acid composition of cell membranes, antioxidant enzyme activities and lipid peroxidation in yellow perch (*Perca flavescens*). Aquatic Toxicology. 180:45–55.

Guo F, Kainz MJ, Sheldon F, Bunn SE. 2016. The importance of high-quality algal food sources in stream food webs – current status and future perspectives. Freshwater Biology. 61:815–831.

Hintz WD, Relyea RA. 2017. A salty landscape of fear: responses of fish and zooplankton to freshwater salinization and predatory stress. Oecologia. 185:147–156.

Hintz WD, Relyea RA. 2019. A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. Freshwater Biology. 64:1081–1097.

Hintz WD, Fay L, Relyea RA. 2021. Road salts, human safety, and the rising salinity of our fresh waters. Frontiers in Ecology and the Environment. https://doi.org/10.1002/fee.2433.

Hixson SM, Arts MT. 2016. Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. Global Change Biology. 22:2744–2755.

Kelly VR, Findlay SEG, Weathers KC. 2019. Road salt: the problem, the solution, and how to get there. Cary Institute of Ecosystem Studies. pp. 16.

Kelting DL, Laxson CL. 2010. Review of effects and costs of road de-icing with recommendations for winter road management in the Adirondack Park. Paul Smiths, NY: Adirondack Watershed Institute.

Lawson L, Jackson DA. 2021. Salty summertime streams – road salt contaminated watersheds and estimates of the proportion of impacted species. FACETS. 6:317–333.

Meesapyodsuk D, Qiu X. 2012. The front-end desaturase: structure, function, evolution and biotechnological use. Lipids. 47:227–237.

Meyer JL, Paul MJ, Taulbee WK. 2005. Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society. 24:602–612.

O'Brien PJ, Wehr JD. 2010. Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. Hydrobiologia. 657:89–105.

Parrish CC. 1999. Determination of total lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC, editors. Lipids in freshwater ecosystems. New York (US): Springer. p. 4-20.

Petranka JW, Doyle EJ. 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.

Solórzano L, Sharp JH. 1980a. Determination of total dissolved nitrogen in natural waters. Limnology and Oceanography. 25:751-754.

Solórzano L, Sharp JH. 1980b. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnology and Oceanography. 25:754–758.

Stanley-Samuelson DW. 1994. Prostaglandins and related eicosanoids in insects. Advances in Insect Physiology. 24:115–147.

Steinman AD, Ogdahl ME, Wessell K, Biddanda B, Kendall S, Nold S. 2011. Periphyton response to simulated nonpoint source pollution: local over regional control. Aquatic Ecology. 45:439-454.

Stroud Water Research Center. 2017. Model My Watershed [Software]. Available from https://wikiwatershed.org/.

Tenebaum D. 2008. Transportation: De-icers add sweet to salt. Environmental Health Perspectives. 116:475–476.

Torres-Ruiz M, Wehr JD, Perrone AA. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. Journal of the North American Benthological Society. 26:509–522.

van Meter RJ, Swan CM. 2014. Road salts as environmental constraints in urban pond food webs. PLoS ONE. 9:1–13.

Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society. 24:706–723.

Wehr JD, Sheath RG, Kociolek PJ. 2015. Freshwater algae of North America, 2nd ed. Amsterdam (ND): Elsevier/Academic Press.

Whorley SB, Wehr JD. 2018. Multiyear patterns in benthic algal fatty-acid compounds under agricultural stress. Freshwater Science. 37:534–550.

Author contribution statement:

CM and SW conducted all fieldwork. JB identified and analyzed algal taxa. All authors contributed equally to sample analysis, data analysis, and manuscript preparation.

Conflict of interest statement:

The authors declare no conflict of interest regarding the contents of this study.

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