

Effects of partial-catchment helicopter liming on stream water chemistry in West River Sheet Harbour, Nova Scotia

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1 Introduction

1.1 Problem Statement

Atlantic salmon (*Salmo salar L.*) populations in Nova Scotia are decreasing; the Inner Bay of Fundy, Outer Bay of Fundy, Eastern Cape Breton, and Nova Scotia Southern Uplands (NSSU) populations are designated as “endangered” by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC, 2010). The NSSU Atlantic salmon populations in particular has decreased by 88-99% from the maximum recorded abundance in four rivers (Bowlby, Horsman, Mitchell, & Gibson, 2013), with as few as 1,427 adult life stage individuals remaining in 2008 (COSEWIC, 2010). Freshwater acidification has been identified as a major cause of the population decline, as a result of reduced freshwater survival and fitness of juveniles (COSEWIC, 2010; Dennis, Clair, & Kidd, 2012; Nilsen, et al., 2013).

The decline and potential extirpation of Atlantic salmon populations from Nova Scotia has cultural, economic and biodiversity repercussions. The Mi'kmaq indigenous peoples of Nova Scotia historically consumed Atlantic salmon as a staple food, due to the abundance and predictable migration patterns of the fish species (Parenteau, 1998). Although Mi'kmaq communities have generally limited their consumption of Atlantic salmon to culturally significant events in recent years, extirpation of this species would result in cultural and heritage losses for these peoples (Parenteau, 1998). Furthermore, 49 First Nations organizations, commercial fisheries and recreational fisheries are dependent on Atlantic salmon as a source of income (COSEWIC, 2010). Atlantic Salmon angling supported 3,872 full-time equivalent (FTE), and 10,500 seasonal jobs in Canada in 2010 (Gardner Pinfold, 2011). In Nova Scotia specifically, the economic value of Atlantic salmon angling was assessed at \$255 Million in 2011 (Gardner Pinfold, 2011); however, these numbers reflect only those populations still robust enough to support limited recreational fisheries. The full potential economic value of Atlantic salmon in Nova Scotia is expected to be substantially larger considering that a large number of populations are not currently fished for conservation reasons (COSEWIC, 2010). Therefore, further population decline and potential extirpation of Atlantic salmon would result in substantial economic losses to the province, conversely a significant potential economic benefit could arise from recovery of imperilled populations.

Population modeling conducted by the Canadian Department of Fisheries and Oceans (DFO) for two of the largest NSSU Atlantic salmon populations indicates a high probability of extirpation within the next 45 years (87%, and 73% likelihood respectively) (DFO, 2013). Nova Scotian Atlantic salmon populations are genetically dissimilar from other Atlantic salmon populations, displaying local adaptation for survival in lower-pH waters; therefore, their abundance cannot be increased via outbreeding with other populations (COSEWIC, 2010). However, by increasing freshwater habitat quality by only 20%, it is estimated that the extirpation risk of these populations would be decreased to 21% within the next 45 years (DFO, 2013).

Chronic acidification has been determined to be a primary driver of habitat quality loss in the NSSU (COSEWIC, 2010); therefore, mitigating freshwater acidification is a potentially effective method for reducing the extirpation risk for Atlantic salmon populations within Nova Scotia. Beginning in 2016, a pilot project has been conducted to evaluate terrestrial liming via helicopter (i.e., helicopter liming) as a freshwater acidification mitigation technique in the NSSU. In this study, I aim to evaluate the efficacy of this project at mitigating the chemical effects of freshwater acidification in three tributaries to the Keef Brook watershed in West River, Sheet Harbour, Nova Scotia.

1.2 Background

1.2.1 Freshwater acidification in Nova Scotia

Freshwater acidification is characterised by low in-stream pH and low acid neutralization capacity (ANC) resulting from a higher concentration of acid anions than base cations or a depletion of base cations relative to the original state of the freshwater system, and presence of naturally-occurring dissolved organic carbon (DOC) (Clair, Dennis, Scruton, & Gillis, 2007; Dennis et al., 2012). Freshwater acidification resulting from industrial emissions of sulphur dioxide (SO₂) and nitrogen oxides (NO_x) was identified as an environmental issue in Europe and North America during the 1970's (Likens, Bormann, & Johnson, 1972; Lükewille, Jeffries, Raddum, Stoddard, & Traaen, 1997; Environmental Protection Agency, 2014). In Nova Scotia, pH in freshwater lakes has decreased by at least 0.4 pH units within the last 160 years alone (Ginn, Cumming, & Smol, 2007). Multiple programs aiming to reduce sulphur emissions contributing to freshwater acidification have been introduced since that time, such as the Canada-United States

Clean Air Act amendments in 1990 (Environmental Protection Agency, 2014). Although these programs have been effective at reducing sulphate deposition the effects of freshwater acidification in Southwestern Nova Scotia have not improved to the same extent as elsewhere in North America and Europe (Clair et al., 2007), and continued decrease in freshwater pH has been identified at nine monitoring sites across Nova Scotia (Clair, et al., 2002). Although the current sulphur-reducing legislation limits sulphate deposition, deposition rates are still above the carrying capacity of the environment for numerous sites across Nova Scotia (Whitfield, Aherne, Watmough, Dillon, & Clair, 2006), as evidenced by decreasing calcium and magnesium trends (Clair, et al., 2002; Macleod, 2016). Furthermore, with recent coal burning policies and pressure from the United States government to decrease Environmental Protection Agency (EPA) oversight (Government of the United States, 2017), there is concern of increased sulphur deposition, which may exacerbate chronic acidification in Nova Scotia.

1.2.2 Mobilization of aluminium due to acidification

Hydrogen ions (H^+) from the reaction of water and acid anions in atmospheric pollutants can be exchanged with cations in the soil at cation exchange sites (CEC) during both wet atmospheric deposition of acidifying compounds, (i.e., precipitation), and dry deposition (i.e., settling) (Seybold, Grossman, & Reinsch, 2005). Cations are exchanged in order of weight; from the lightest base cations to heaviest, where heavy ions are not mobilized from the soil until lighter ions are depleted (Watt W. , Scott, Zamora, & White, 2000). Chronic acidification of Nova Scotian watersheds has caused depletion of the lighter cations such as calcium (Ca), resulting in the mobilization of heavier cations such as aluminum (Al) (Clair, Dennis, & Vet, 2011). Furthermore, 98% of monitored watersheds in Nova Scotia were found to have increasing Al trends; although aluminum trends in other rivers in Nova Scotia are unknown, the Macleod (2016) study highlights that increasing Al levels due to chronic acidification are an environmental problem which needs to be addressed (Macleod, 2016).

1.2.3 Aluminium speciation in acidified waters

Al speciation is driven by water pH and temperature (Figure 1) (Helliweli, Batley, Florence, & Lumsden, 1983). At the standardized 25°C and pH 6.4, the precipitated $Al(OH)_4$ species is most prevalent, while the three positively charged inorganic aluminum (Al_i) species (Al_i^{3+} , $Al(OH)_2^{1+}$, and $Al(OH)^{2+}$) are dominant between pH 4.0 and 6.0 (Helliweli, 1983).

Additionally, the dominant Al species associated with a given pH range is also influenced by water temperature, where the positively charged Al_i species are dominant at $pH < 6.0$ at colder temperatures ($2^\circ C$), and at $pH < 5.5$ at warmer temperatures ($25^\circ C$) (Lydersen, 1990) (Figure 1). Furthermore, Al_i species and DOC form an organoaluminum complex (Al_o) in freshwater (Tipping, Rey-Castro, Bryan, & Hamilton-Taylor, 2002). It has been demonstrated that although Nova Scotian freshwater systems tend to have high DOC levels, these levels are not correlated with the extent to which Al_o complexes form, and some Al_i particles remain unbound from organics, resulting in free Al_i particles (Tipping et al., 2002; Dennis et al., 2012). At pH values below 6.0, Al^{3+} is the dominant Al_i specie forming the Al_o complex, while at pH values greater than 6.0, $Al(OH)^{2+}$ is the dominant Al_i specie forming organoaluminum complexes (Tipping et al., 2002).

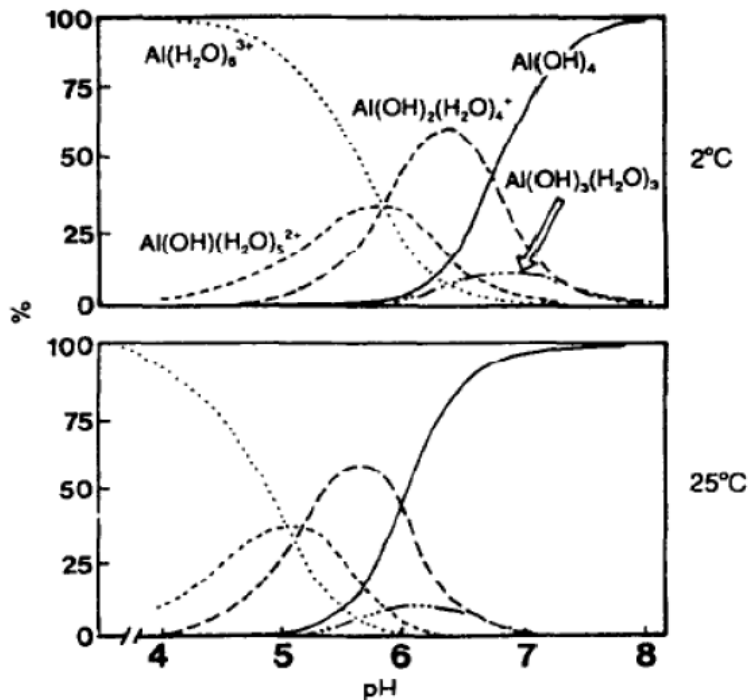


Figure 1 Graphs of aluminium speciation as a percentage of total aluminium at pH 4 to 8 for colder waters ($2^\circ C$) and warmer waters ($25^\circ C$). From (Lydersen, 1990).

1.2.4 Effects of chronic freshwater acidification on Atlantic salmon

Chronic freshwater acidification, and presence of mobilized Al_i in particular, has been associated with negative morphological and physiological changes to Atlantic salmon (Kroglund

F., et al., 2007), including asphyxiation and mortality (Exley, Chappell, & Birchall, 1991; Lacroix & Townsend, 1987), delays and prevention in egg laying (Peterson, et al., 1989), reduced nutrient intake via gills (Nilsen, et al., 2013), microRNA changes (Kure, 2013) and a loss of osmoregulatory ability (e.g., Staurnes et al., 1996, Kroglund and Staurnes, 1999). These negative responses are due to both the acidity of the water and the presence of mobilized Al_i , where the response of the fish to Al_i is further dependant on river pH due to altered concentrations of Al_i species (Howells, Dalziel, Reader, & Solbe, 1990). At pH 5.0 to 6.0 Al toxicity in Atlantic salmon results from Al_i concentrations as low as $15\mu\text{g}\cdot\text{L}^{-1}$ due to the presence of $Al(OH)_2^{1+}$ and $Al(OH)^{2+}$ which bind readily to the -1 charged fish gills, whereas toxicity levels increase to $30\mu\text{g}\cdot\text{L}^{-1}$ at pH levels below 5.0 due to increased presence of Al^{3+} (Howells, 1990). Al toxicity above pH 6.0 is negligible due to reduced Al solubility at lower acidity (Dennis et al., 2012). Using a the $15\mu\text{g}\cdot\text{L}^{-1}$ threshold suggested by Dennis et al. (2012), Macleod (2016) identified multiple rivers in Nova Scotia with Al_i concentrations above this level; indicating the need for freshwater acidification mitigation studies in Nova Scotia.

1.2.5 Mitigating freshwater acidification

It has been predicted that Nova Scotian freshwater systems will not naturally recover from chronic acidification in the next 60 years (Clair T. A., Dennis, Amiro, & Cosby, 2004) due to the presence of high concentrations of DOC in Nova Scotian watersheds (Kerekes, Beauchamp, Tordon, Tremblay, & Pollock, 1986) which reduces the capacity of the system to chemically recover from chronic acidification (Hruška, Krám, McDowell, & Oulehle, 2009). Therefore, alongside emission reductions, the addition of buffering materials to acidified watersheds (liming) has been suggested as a freshwater acidification mitigation strategy (Olem, 1991). Calcite is the most commonly used buffering material as it is readily available, and therefore less expensive than other materials, and can be purchased at varying particle sizes due to use in the agricultural industry (Clair & Hindar, 2005). Liming is recommended for chronic freshwater acidification mitigation in Nova Scotia, as emission reductions have not resulted in significant recovery of acidified systems (Clair et al., 2007), and liming has proven effective across Europe and other areas of North America (Stoddard, 1999). Four common methods of lime application exist: in-stream, river-bed, lake, and terrestrial liming (Clair & Hindar, 2005). Unlike the first three methods, terrestrial liming applies base cations (e.g., Ca^{2+}) directly to the soils of the catchment, allowing exchange with H^+ ions during deposition events, mitigating the acidification which would otherwise result (Dennis

et al., 2012). Furthermore, terrestrial liming is the only liming method which addresses metal leaching from soils, making it preferential for freshwater acidification initiatives targeting Atlantic salmon habitat improvement, and improving freshwater quality in acidified regions with dissolved metal problems (Clair & Hindar, 2005). Additionally, if an adequate dose is applied, reapplication may not be necessary for 15 to 50 years after the initial application; a much longer effectivity effectiveness than other methods (Hindar, Wright, Nilsen, Larssen, & Hogberget, 2003; Clair & Hindar, 2005; Hindar & Wright, 2005). Terrestrial liming is usually conducted using a helicopter (helicopter liming) (Clair & Hindar, 2005)

1.3 Knowledge Gap

1.3.1 Absence of helicopter liming attempts in Nova Scotia

Helicopter liming has been successful in Sweden, Norway and the United States in raising pH and Ca (Driscoll, et al., 1996; Fransman & Nihlgård, 1995; Hindar & Wright, 2005), and decreasing in-stream Fe and Al concentrations (Andersson, 1995). However, there have been no helicopter liming project in Nova Scotia; previous studies in Europe and elsewhere in North America indicate that helicopter liming is effective at mitigating freshwater acidification, with mitigative effects lasting for 20 years, and being predicted to last for 50 years (Hindar & Wright, 2005). Liming projects in Nova Scotia have been limited to in-stream liming via a lime doser in West River, Sheet Harbour (Halfyard, 2003), and manual application terrestrial liming (Sterling, et al., 2014).

1.3.2 Poor understanding of water chemistry responses to terrestrial liming in Nova Scotia

Due to the small number of studies examining freshwater chemical responses to terrestrial liming in Nova Scotia, the effects of helicopter liming are poorly understood. Recent studies examining freshwater acidification in Nova Scotia have indicated the need for more research examining pH and Al levels and temporal variation thereof in Nova Scotia (Dennis et al., 2012; Macleod, 2016; Sterling, et al., 2014). Additionally, no studies have been identified on evaluating effectiveness of terrestrial liming at mitigating seasonal Al concentration highs in the fall (Macleod, 2016). Determining if helicopter liming reduces seasonal high values for Al_i, and other

seasonal variation of water chemistry, will add to the body of knowledge used to develop freshwater acidification mitigation projects and studies.

1.4 Study Introduction

1.4.1 Study objectives

My research aimed to evaluate a partial-catchment helicopter liming method, which had not been used before in Canada. Compared to previous methods, this acidification mitigation method was relatively low-cost and has the potential for long term mitigation (Hindar & Wright, 2005). To evaluate this method, my research aimed to document the changes caused by the NSSA partial-catchment helicopter liming project to increase our understanding of the effects of helicopter application of powdered dolomitic limestone (CaMgCO_3) at a concentration of $10 \text{ t}\cdot\text{ha}^{-1}$ on land and $2 \text{ t}\cdot\text{ha}^{-1}$ on lakes/ponds to 2.0 m Wet Area Model buffer zone on in-stream pH, total Ca (Ca_t), total Al (Al_t), and Al_i . A Wet Area Model (WAM) identifies areas which have a proportionately larger influence on stream chemistry based on the proximity of the water table to the land surface (Sterling, et al., 2014).

1.4.2 Research questions

- 1) Does helicopter application of powdered CaMgCO_3 improve freshwater acidification conditions during the first year post-liming (between November 19th, 2016 and September 16th, 2017) within the Keef Brook watershed in West River, Sheet Harbour, Nova Scotia, Canada, as measured at the watershed outlet?
 - a. Is Ca_t concentration significantly increased?
 - b. Is in-stream pH significantly increased?
 - c. Is Al_i concentration significantly decreased?
 - d. Is mean Ca_t concentration increased to $\geq 2.0 \text{ mg}\cdot\text{L}^{-1}$?
 - e. Is mean in-stream pH raised to $\geq 6.0 - 6.5$ units?
 - f. Is mean Al_i concentration decreased to $\leq 15 \text{ }\mu\text{g}\cdot\text{L}^{-1}$?
- 2) What are the temporal trends in water chemistry parameters associated with helicopter liming during the first year post liming within the Keef Brook watershed in West River, Sheet Harbour, Nova Scotia, Canada, as measured at the watershed outlet?

- a. When was the first notable change in Ca_t concentration observed?
 - b. When was the first notable change in pH level observed?
 - c. When was the first notable change in Al_i concentration observed?
- 3) Can watershed characteristics, and other water chemistry parameters measured in the Upper Killag- and Keef Brook watersheds in West River, Sheet Harbour, Nova Scotia, Canada, predict pre-treatment Al_t concentration, as measured at the watershed outlet?
- a. Does the highest Al_t concentration occur in the sub-catchment with the highest percent cover of wetlands?
 - b. Does the highest Al_t concentration occur in the sub-catchment with the highest percent cover of mature forest?
 - c. Does the highest Al_t concentration occur in the sub-catchment with the highest TOC concentration?
 - d. Does the highest Al_t concentration occur in sub-catchment with the lowest Ca_t concentration?

1.4.3 Study scope

This study has temporal limitations associated with the time period of data collection; this thesis will only document the changes in water chemistry resulting from the helicopter liming project in the first year following lime application. Since results from helicopter liming have been shown to last 20 years post-application (Clair & Hindar, 2005; Hindar et al., 2003; Hindar & Wright, 2005), the results of this study will not fully represent the effects of the helicopter liming project. Furthermore, although this study has a before/after, control/impact (BACI) design, pre-liming water chemistry data are limited, ranging from three to eight samples depending on site. Additionally, the results of this study may not be generalizable to the rest of Nova Scotia, and will be limited to other watersheds sharing similar watershed characteristics to the study sites. Finally, when comparing the results of this study with other studies examining Al_i ; Al_i has been identified as inorganic Al, inorganic monomeric Al, labile Al, or cationic Al in prior studies. For the purposes of this study, Al_i refers to all three Al_i species (Al^{3+} , $Al(OH)^{2+}$, and $Al(OH)_2^+$) following Poléo (1995) and MacLeod (2016).

1.4.4 Methodology overview

The Nova Scotia Salmon Association is conducting freshwater acidification mitigation projects in West River Sheet Harbour to evaluate stream acidification mitigation strategies, including a terrestrial liming via helicopter application project (the helicopter liming project) which is under the scientific lead from Dr. Shannon Sterling (Earth Sciences Department, Dalhousie University), Dr. Edmund Halfyard (Nova Scotia Salmon Association), and Dr. Kevin Keys (Forestry Division, Nova Scotia Department of Natural Resources). During the helicopter liming project, dolomitic limestone (CaMgCO_3) was applied to the forested catchment of Keef Brook, a highly acidified tributary to the West River, located in West River, Sheet Harbour, Nova Scotia. The liming activities took place between October 3rd, 2016 and November 19th, 2016. This study has a BACI design; freshwater chemistry data, including pH, Ca_t , Al_t , and Al_i were collected prior, during, and after the limestone application, resulting in a dataset spanning a 16-month period. A BACI study design has data for both control and treatment sites, before and after treatment, so change causal relationships can be determined and attributed to treatment (Palys & Atchison, 2007). Pre-liming data were limited three to eight samples, depending on site, taken between April 29th, 2016 and October 2nd, 2016. Generalized Linear Mixed Modeling (GLMM) and post-hoc Tukey multiple comparisons analysis were conducted in R version 3.4.2 to determine statistical significance of changes to water chemistry parameters caused by the helicopter liming project. Akkaki Information Criterion (AIC) was used to compare models, and select the most parsimonious model. Timeseries analysis was conducted on water chemistry data in Excel 2013 to examine for temporal trends and variation following limestone application. Finally, watershed characteristics were calculated using ArcGIS version 10.3 mapping software to determine if pre-treatment water chemistry values could be predicted using watershed characteristics and other chemistry parameters, as identified by Rotteveel, Sterling, Heubach, and Takala (2018, *in prep*).

The research conducted in this thesis was descriptive, quantitative, and analytic in nature. Research question one (sub-questions a through f), were explanatory and interventional in nature, as these questions sought to determine if the dependent variables could be manipulated via experimental design (Rudestam & Newton, 2014). Research question two (sub-questions a through c) was characterized as descriptive and observational, as these questions sought to describe a change in the dependant variable over time (Rudestam & Newton, 2014). Research question three (sub-questions a through d) was characterized as relational and observational, as this question

sought to determine if the pre-treatment explanatory variables could be predicted based on independent variables (Rudestam & Newton, 2014). All research questions addressed in this research were be cross sectional in nature, as the data were both collected and analyzed during the 16-month study period (April 29, 2016 to September 16, 2017) (Rudestam & Newton, 2014).

2 Literature Review

2.1 Introduction

This chapter will review freshwater acidification research and mitigation efforts published from the 1970's onward. This literature review will focus on research conducted in Southwest Nova Scotia, or NSSU ecoregion, but will also include research conducted in other Temperate Broadleaf, Temperate Coniferous, and Mixed Forest ecoregions across the globe, including the Appalachian Ecoregion in the United States, and the Scandinavian coastal conifer forest ecoregion of Norway and Sweden. These regions have been selected for comparison, as they are the most similar to the NSSU ecoregion (WWF, 2017a) (WWF, 2017b). The temporal scope of this literature review has been limited to the 1970's onward, as this is when freshwater acidification was recognized as a threat to ecosystems (EPA, 2014; Stoddard, 1999). The articles examined in this literature review were predominantly published in the Journal of Hydrology, Canadian Journal of Fisheries and Aquatic Sciences and Water, Air and Soil Pollution. In this literature review, the effects of freshwater acidification, and aluminum mobilization resulting therefrom, on Atlantic salmon (*Salmo salar*) will be synthesized, and discrepancies in the research will be discussed and evaluated. Furthermore, liming (freshwater acidification mitigation) techniques and efforts will be evaluated and reviewed, including key terrestrial liming studies conducted in Norway and Sweden, the leading countries in liming research. These efforts will then be compared to liming efforts conducted in the NSSU ecoregion. This literature review aims to evaluate and discuss the scientific knowledge base on freshwater acidification, and critically compare various mitigation techniques and efforts in the NSSU and similar ecoregions across the world. A review of the liming efforts conducted in the NSSU will be used to identify key knowledge gaps in freshwater acidification research within the area and demonstrate the need for more liming efforts and research within the ecoregion.

2.2 Freshwater Acidification

Acidification was first identified as a threat to freshwater ecosystems in Scandinavia and Eastern Canada in the 1970's and continues to be an area of focus and concern for conservation efforts in the NSSU ecoregion. ANC is defined as the ability of rock to neutralize acidic inputs

and is determined by the weathering rate and the concentration of base cations such as Ca and magnesium (Mg) within it, where bedrock with poor ANC weathers slowly and has low concentrations of Ca and Mg, and therefore does not contribute sufficient base cations to surface waters to mitigate acid anion input (Clair et al., 2007; Schnoor & Stumm, 1986). Chronic freshwater acidification therefore results in higher concentrations of acid anions than base cations within the freshwater system (Clair, Dennis, Scruton, & Gillis, 2007; Dennis, Clair, & Kidd, 2012; Watt, Scott, & Ray, 1979). Freshwater acidification resulting from industrial burning of coal, producing sulphur dioxide (SO₂) and nitrogen oxides (NO_x) emissions, was identified as an environmental issue in Europe and North America during the 1970's (Likens, Bormann, & Johnson, 1972; Lükewille, Jeffries, Raddum, Stoddard, & Traaen, 1997; Environmental Protection Agency, 2014). Since that time, mitigative measures have been implemented, such as the Canada-United States of America Clean Air Act Amendments in 1990 (Environmental Protection Agency, 2014). However, despite national and international efforts to address freshwater acidification, the threat has not been fully mitigated in the NSSU, and continues to be an area of focus for mitigation efforts within the ecoregion (Committee on the Status of Endangered Wildlife in Canada, 2010; Sterling, et al., 2014). In conclusion; although freshwater acidification was identified as a threat to ecosystems in Eastern Canada more than 45 years ago, the threat still persists, and is considered a primary concern for conservation efforts within the NSSU ecoregion.

2.2.1 Sources

Freshwater acidification can have both natural and anthropogenic sources; however, although the process may be caused by natural conditions and events, the process is exacerbated by inputs of anthropogenic-sourced pollutants. Natural sources of freshwater acidification can be either point source inputs in the form of acidic drainage from wetlands (Kerekes, Beauchamp, Tordon, Tremblay, & Pollock, 1986), or non-point source inputs. Natural non-point source freshwater acidification may occur in regions where sulphide-bearing rock is exposed to air or water, causing sulphur to be leached, producing Acid Rock Drainage (ARD) (Clair et al., 2007). Human activity may contribute to natural processes which expose sulphide-bearing rock, intensifying ARD (Clair et al., 2007). Despite natural inputs from wetlands and sulphide-bearing rocks, acid precipitation containing anthropogenic-sourced pollutants such as sulphate (SO₄) and nitrate (NO₃), generated primarily via the combustion of coal, are the predominant causes of

freshwater acidification (Clair et al., 2007; Environmental Protection Agency, 2014; Stoddard, 1999). Furthermore, regions overlying bedrock with poor ANC may be disproportionately negatively affected by acid precipitation or runoff (Clair et al., 2007; Schnoor & Stumm, 1986).

2.2.2 Large-Scope Mitigation Efforts

After the recognition of freshwater acidification as a threat to ecosystems in the 1970's and identification of sulphate emissions from the burning of coal as the main source, legislation was enacted to reduce sulphate emissions, and mitigation efforts were established to address freshwater acidification. In Europe, countries focussed primarily on acid mitigation initiatives; for example, the Integrated Studies on the Effects of Liming (ISEL) project in Sweden (Nyberg & Thørneløf, 1988), and the Monitoring Programme of Acidification in Norway (Raddum & Fjelheim, 1995). In North America, countries mainly focussed on enacting legislation aiming to reduce anthropogenic sulphate emissions contributing to acidification via the Canada-United States Clean Air Act Amendments (Environmental Protection Agency, 2014; Stoddard, 1999). Although these programs have been effective at limiting and mitigating freshwater acidification elsewhere across the globe, the effects of freshwater acidification in the NSSU have not improved to the same extent as elsewhere in North America and in Europe (Clair et al., 2007). Furthermore, with recent coal burning policies and pressure from the United States government to decrease Environmental Protection Agency oversight (Government of the United States, 2017), there is concern of increased sulphur deposition, which may exacerbate chronic acidification in Nova Scotia. In summary, multiple large-scope freshwater acidification mitigation projects and policies have been enacted across the globe; however they have not addressed the freshwater acidification problem in Nova Scotia.

2.2.3 Freshwater Acidification in Nova Scotia

Freshwater systems in the NSSU continue to be among the most acidified in North America, and while pH has stabilized in most rivers in recent decades, the problems of decreasing calcium and increasing aluminum concentrations remain (Clair et al., 2007; Clair, et al., 2002; Stoddard, 1999; Rotteveel et al., 2018, *in prep*). Following the enactment of the 1990 Canada-United States Clean Air Act Amendments, Canada's SO₂ emissions have decreased by 63% from 1980's levels, leading to improvements in freshwater acidity levels across North America

(Environmental Protection Agency, 2014). However, despite these reductions, pollutants are still produced at a level above the carrying capacity of Nova Scotian freshwater systems (Whitfield, Aherne, Watmough, Dillon, & Clair, 2006). Additionally, due to the low ANC of granite bedrock, alkaline soil, and large number of wetlands producing organic acids within the NSSU, the region is at risk from continued freshwater acidification resulting from insufficient emission reductions (Clair et al., 2007). Ginn, Cumming and Smol (2007) identified that Nova Scotian fresh water pH has decreased by at least 0.4 pH units within the last 160 years alone, with the majority of increases occurring since the 1970's. Furthermore, additional decreases in pH have been found at nine monitoring sites across Nova Scotia (Clair, et al., 2002).

The effects of freshwater acidification in the NSSU have been exacerbated by increased aluminum concentrations within freshwater systems due to the mobilization of aluminum from base-cation poor soils and bedrock. The chronic acidification of Nova Scotian watersheds has caused depletion of the lighter cations such as Ca (Clair, Dennis, & Vet, 2011), which is theorized to have resulted in the mobilization of heavier cations such as Al (Clair & Hindar, 2005). This theory has been supported by recent work conducted by Macleod (2016). Since the instatement of the Canada-United States Clean Air Act Amendments, aluminium levels were expected to decrease due to reduced freshwater acidity; however, recent research conducted by MacLeod (2016) found that 23 of 65 monitored watersheds in Nova Scotia had significantly increasing Al trends since the 1980's, and 25 of 65 sites had significantly decreasing calcium trends. Additionally, recent literature has found that aluminum levels in the NSSU often exceed the European Inland Fisheries Advisory Council (EIFAC) aluminum threshold for freshwater ecosystem health ($15 \mu\text{g}\cdot\text{L}^{-1}$) (Dennis et al., 2012; Macleod, 2016). Due to the chronic acidified state of watersheds within the NSSU, aluminum has been mobilized from the soils and has entered the freshwater systems; the levels of aluminum within the freshwater systems are significantly increasing and do not appear to be leveling off, contributing to the overall poor status of freshwater ecosystem health within the region.

2.2.4 Effects on Southern Upland Atlantic Salmon

The two largest Atlantic salmon populations in the NNSU have a high probability of extirpation within the next 50 years due to freshwater acidification; however, the likelihood of extirpation can be greatly reduced by improving freshwater habitat quality within the region. The

NSSU Atlantic salmon designatable unit has been evaluated as endangered by COSEWIC in 2010 (Committee on the Status of Endangered Wildlife in Canada, 2010). Poor marine survival rates due to negative effects from freshwater acidification and consequent aluminum mobilization have been identified as one of the primary factors limiting the freshwater production of NSSU Atlantic salmon population (Clair et al., 2007; Dennis et al., 2012; Department of Fisheries and Oceans, 2013; Lacroix & Townsend, 1987). Population viability modeling for the LaHave and St. Mary's Atlantic salmon populations, two of the largest populations within the NSSU and the DFO index rivers, has predicted a high probability of extirpation for both populations within the next 50 years (97%, and 73%, respectively) (Bowlby, Gibson, & Levy, 2013). However, the population viability models also indicate that a 20% increase in freshwater productivity (i.e., improved habitat quality or reduced acidification) within the LaHave river watershed will reduce the population extirpation risk to from 97% to 21% within the next 50 years (Bowlby et al., 2013). Although the current risk of extirpation for Atlantic salmon populations from the NSSU ecoregion is high, this risk can be greatly reduced with relatively small habitat quality improvements, indicating that habitat quality improvements will likely be required to prevent the extirpation of NSSU Atlantic salmon designatable unit.

Research published in the 1980's indicated that aluminum toxicity was not an issue for fish in the NSSU, as the high concentration of organic matter originating from the abundant wetlands in the region facilitated the formation of biologically unavailable colloidal complexes (Clair et al., 2011). Thus, prior to 1990, an Al_i toxicity threshold was set at $50 \mu\text{g}\cdot\text{L}^{-1}$ and it was generally believed that the high fish mortality rates in Nova Scotia were the direct result of the low river pH conditions (Lacroix & Townsend, 1987). However, after the identification of Al_i as a threat to aquatic ecosystems in 1990, the EIFAC decreased the Al_i toxicity threshold to $15 \mu\text{g}\cdot\text{L}^{-1}$ (European Inland Freshwater Fisheries Advisory Council, 1990). Following this reduction, prior studies on fish mortality due to low pH conditions were re-examined to determine if Al_i toxicity may have been a contributing factor. For example, Howells, Dalziel, Reader, & Solbe (1990) examined the 1987 Lacroix et al. study which concluded that Atlantic salmon parr (juvenile fish) mortality in Nova Scotia was the result of low pH conditions, and identified that Al_i concentration exceeded the new $15 \mu\text{g}\cdot\text{L}^{-1}$ limit in 75% of the test sites, and therefore concluded that Al_i toxicity was likely a contributing factor to fish death in Nova Scotia, contrary to prior scientific opinion.

Both chronic and acute freshwater acidification and consequent mobilization of ionic aluminum (Al_i) cause adverse morphological and physiological changes leading to morbidity and mortality in Atlantic salmon (Kroglund F., et al., 2007). These changes include asphyxiation and mortality (Exley, Chappell, & Birchall, 1991; Lacroix & Townsend, 1987), delay and prevention of egg production in rivers with pH levels of 4.0 to 5.5 (Peterson, et al., 1989), reduced gill nutrient intake (Nilsen, et al., 2013), excess mucus production leading to respiratory issues (Dennis et al., 2012), negative microRNA changes (Kure, 2013), immunosuppression (Kroglund F., et al., 2007), loss of fright response (Åtland & Barlaup, 1996), and reluctance to enter full strength sea water (Kroglund, et al., 2008). Furthermore, exposure to Al_i during the smoltification period of the salmon (i.e., preparation for marine survival) reduces marine survival by inhibiting the activity of sodium-potassium-adenosine triphosphate-ase ($\text{Na}^+\text{-K}^+\text{-ATPase}$) which limits the hypo-osmoregulation ability of the fish in salt water (Kroglund, et al., 2007). Freshwater acidification also indirectly influences Atlantic salmon by reducing survival of freshwater invertebrates (Burton & Allan, 1986; Havas & Hutchinson, 1982; Mackie, 1989), thereby reducing a key food source for salmon, and limiting growth, production, and survival rates of the fish. Atlantic salmon parr mortality has also been observed following acute freshwater acidification events, such as after rainfall and snowmelt (Lacroix & Townsend, 1987). In the study conducted by Lacroix and Townsend (1987), total Atlantic salmon parr mortality was observed in river systems where pH dropped below 4.7 units, while no mortality was observed in systems where pH remained above 4.8 units. These values are substantially lower than previously identified thresholds, an explanation for which may relate to Al_i speciation at varying river pH (Howells et al., 1990). At pH 5.0 to 6.0 Al toxicity in Atlantic salmon results from Al_i concentrations as low as $15\mu\text{g}\cdot\text{L}^{-1}$ due to the presence of $\text{Al}(\text{OH})_2^+$ and $\text{Al}(\text{OH})^{2+}$ which bind readily to the -1 charged fish gills. Whereas toxicity levels increase to $30\mu\text{g}\cdot\text{L}^{-1}$ at pH levels below 5.0 due to increased presence of the less readily attached Al^{3+} (Howells, 1990). Al toxicity above pH 6.0 is negligible due to reduced Al solubility at lower acidity (Dennis et al., 2012). Furthermore, a recent study conducted on the United Kingdom Acid Monitoring Network (AWMN) identified aluminum concentrations as the dominant water chemistry parameter predicting fry (juvenile fish which are capable of feeding themselves) presence in freshwater systems (Malcolm, et al., 2014). Al levels exceeding the $15\mu\text{g}\cdot\text{L}^{-1}$ toxicity threshold used in Dennis et al. (2012) were recently measured in multiple rivers

in Nova Scotia (Macleod, 2016; Sterling et al., 2017), indicating that ionic aluminum is a threat within the province and the need for freshwater acidification mitigation.

2.3 Liming

Liming is the addition of buffering materials, most commonly limestone, to acidified watersheds. Liming to mitigate freshwater acidification has proven effective, and is the preferred method for freshwater acidification mitigation in North America and Europe (Stoddard, 1999). Concurrently reducing acidifying emissions and liming has been suggested as a freshwater acidification mitigation strategy (Olem, 1991). Since the Canada-United States Air Quality agreement was enacted in 1990 to reduce sulphate emissions (Environmental Protection Agency, 2014), a lack of significant recovery of acidified systems in Nova Scotia has been observed (Clair et al., 2007). This suggests that liming may be required to facilitate chronic freshwater acidification recovery within the region; particularly on timelines relevant to the recovery of acid-sensitive freshwater species, such as Atlantic salmon. In a study conducted by Lacroix et al. (1985) on Atlantic salmon fry survival rates in acidic water, found that fry held in pH 5.0 has a cumulative mortality rate of 70%, while fry held in water from the same source, but which was treated with limestone to raise the pH to 6.1 only had a 4% mortality rate. The Lacroix et al. (1985) study further supports the conclusion that liming is an effective method for increasing freshwater pH and increasing Atlantic salmon survival rates.

2.3.1 Liming Methods

Various buffering materials with varying efficacy, availability, and cost are used in freshwater acidification mitigation. The effectiveness of various buffering materials is measured in Theoretical Neutralization Equivalents (TNE), in terms of limestone (CaCO_3), which is assigned a value of 100 % (Olem, 1991) (Table 1). Limestone is the most commonly used buffering material, as it is readily available, and therefore less expensive than other materials, and can be purchased at varying particle sizes due to widespread use in the agricultural industry (Clair & Hindar, 2005). Although various types of buffering materials with varying effectiveness can be used in liming initiatives, the choice of material is often dependant on local availability.

Table 1 Common names, chemical formulas, and TNE values of frequently used liming materials (Olem, 1991).

Common name	Formula	TNE (%)
Limestone	CaCO ₃	100
Dolomite	CaCO ₃ -MgCO ₃	109
Sodium carbonate	Na ₂ CO ₃	94
Sodium bicarbonate	NaHCO ₃	119
Calcined lime	CaO	179
Calcined dolomite	CaO-MgO	207
Hydrated lime	Ca(OH) ₂	135
Dolomitic hydrate	Ca(OH) ₂ -MgO	175
Pressure dolomitic hydrate	Ca(OH) ₂ -Mg(OH) ₂	151
Caustic soda	NaOH	125

There are several methods used to apply lime, each with associated benefits and challenges, therefore the choice of application method used is project and location specific. There are four common methods of lime application: in-stream, river-bed, lake, and terrestrial liming (Clair & Hindar, 2005).

Table 2 provides a brief description of these methods, identifies the expected acidification mitigation duration, and provides examples of projects in which the method has been used.

Table 2 Brief explanation and expected duration of acidification mitigation effects of the four most commonly used liming methods (Clair & Hindar, 2005).

Liming Method	Description	Expected Mitigation Duration
In-stream liming	Addition of buffering materials directly to running waters, typically through the use of a lime doser.	Improvement immediate, and lasting as long as the lime doser dispenses buffering materials.
River-bed liming	Addition of gravel sized particles of buffering materials to stream sediments.	Improvement immediate, often decreasing within weeks (Hindar et al., 2003; Watt et al., 1984). Frequent reapplication required.
Lake liming	Addition of slurried or fine grain buffering materials directly onto lake surface.	Improvement immediate. Duration of improvement dependant on lake flushing rate; reapplication necessary when liming products are lost, which may be as frequent as every 1-2 years (Hindar et al., 2005) or as infrequent as 4-16 years (Yan, Keller, & Gunn, 1995).
Terrestrial liming	Addition of buffering material to the catchment or drainage area of a freshwater system.	Improvements immediate. Usually long lasting with low reapplication frequency; reapplication can be as infrequent as every 15-50 years (Dalziel, Wilson, & Proctor, 1994; Hindar, Wright, Nilsen, Larssen, & Hogberget, 2003).

Each liming method has associated benefits and challenges. For example, a challenge associated with river-bed liming is that lime gravel and particles rapidly become encased in an organometallic coating, resulting in a loss of effectiveness soon after application (Clair & Hindar, 2005; Watt & Farmer, 1984). Furthermore, particles are transported downstream with the river bedload, removing the buffering materials from the target areas (Clair & Hindar, 2005; Watt & Farmer, 1984). Additionally, with in-stream liming buffering material applications must be ongoing and mitigation lasts only for as long as the material is applied to the river, as it is directly transported downstream (Clair & Hindar, 2005). Lake liming is less costly than in-stream liming, as no lime doser is required; however, buffering material is lost via lake discharge and sedimentation. These losses are dependent on the flushing rate of the lake, and therefore the reapplication frequency associated with this method varies greatly, between 1 to 16 years (Clair & Hindar, 2005; Yan et al., 1995). In Nova Scotia, flushing rates tend to be high, with most flushing in less than 1 year due to high precipitation rates and small lake sizes, suggesting frequent reapplication would be necessary. Additionally, buffering agents may not be well-mixed within the lake due to ice cover or stratification of lake water, with cold, acidic water near the surface

(Clair & Hindar, 2005). Unlike the other three methods, terrestrial liming applies base cations directly to the soils of the catchment, allowing exchange with H^+ ions during acid deposition events, increasing the ANC and mitigating the acidification which would otherwise result (Dennis et al., 2012). Additionally, if an adequate dose is applied, reapplication may not be necessary for 15 to 50 years after the initial application; a much longer effectiveness than other methods (Hindar et al., 2003; Clair & Hindar, 2005; Hindar & Wright, 2005). However, the front-end cost of terrestrial liming, particularly when conducted with a conventional helicopter, is often greater than other methods (Clair & Hindar, 2005). Due to the varying effectiveness and associated benefits, the liming methods used for a particular freshwater acidification mitigation project will depend on lake and catchment characteristics, and project budget constraints.

Within the NSSU, terrestrial liming would be the preferred freshwater acidification mitigation method, as it is uniquely capable of mitigating both chronic freshwater acidification, and acute acidification events, which often occur during precipitation and snowmelt events in the region. Furthermore, terrestrial liming is the only liming method which addresses metal leaching from soils, making it a preferred method for freshwater acidification initiatives targeting Atlantic salmon habitat improvement and improving freshwater quality in acidified regions with dissolved metal problems (Clair & Hindar, 2005). Also, since terrestrial liming is usually conducted using a helicopter (helicopter liming) (Clair & Hindar, 2005), this method allows access to remote catchments of key freshwater systems for Atlantic salmon which would otherwise not be accessible. However, the front-end investment cost of helicopter liming is often greater than other methods, but this cost is a trade-off with much reduced application rates (up to 15 – 20 years between applications), and no maintenance costs such as those associated with in-stream lime dosers (Andersson, 1995; Clair & Hindar, 2005; Fransman & Nihlgård, 1995). An attempt to reduce costs of terrestrial liming was made by Sterling, et al. (2014); where high school students spread powdered limestone throughout the Maria Brook experimental liming catchment in New Ross, Nova Scotia. However, since the correct dosage of buffering materials must be applied to the catchment to provide effective freshwater acidification mitigation (Clair & Hindar, 2005), more precise methods are preferred.

An additional draw-back of terrestrial liming is the scope of the area needed to treat a meaningful amount of freshwater habitat relative to more direct liming methods, such as lime dosing. For example, lime dosers are capable of treating fluvial habitats with moderate discharges

(e.g., $5\text{-}10\text{ m}^3\cdot\text{s}^{-1}$), whereas the spatial area required to be treated with terrestrial liming to elicit an equivalent response may be cost, and time, prohibitive (E.A. Halfyard, *pers. comm.*).

Helicopter liming is a highly suitable freshwater acidification mitigation method in the NSSU, as this method may improve the poor ANC of the region, addressing acute acid deposition events as well as chronic acidification, and the front-end investment cost is traded-off with reduced reapplication rates and lack of maintenance costs.

2.3.2 Prior Terrestrial Liming Efforts

Sweden has among the most acidified freshwater systems in the world, and also has some of the most developed liming programs in the world, which include large-scale, government-subsidized acidification recovery programmes (Nyberg & Thørneløf, 1988). Through this programme, Fransman & Nihlgård (1995) conducted two applications of dolomitic limestone $\text{CaMg}(\text{CO}_3)_2$ in spring of 1985 and 1988 at a dose of $3\text{ t}\cdot\text{ha}^{-1}$ to three 20 ha catchments in Hagfors, Sweden. This project was successful at increasing the in-stream pH, Ca, and ANC. Research conducted by Anderson (1995) between 1984 and 1990 applied CaCO_3 via helicopter every two years to catchments of the Prästvallsbäcken stream in Sweden at a dosage rate of $6.4\text{ t}\cdot\text{ha}^{-1}$ to $315\text{ t}\cdot\text{ha}^{-1}$. The study found an increase in pH, and decrease in metal (aluminum and iron) concentrations, resulting in increases in Brown Trout fry production and habitat range (Andersson, 1995).

Since identifying freshwater acidification as a threat to ecosystems, Norway has also implemented multiple large-scale liming operations to mitigate this threat, demonstrating that helicopter liming produces long-lasting acidification mitigation. For example, helicopter liming of CaCO_3 at a rate of $3\text{ t}\cdot\text{ha}^{-1}$ was conducted in the Tjønnstrond catchment (25 ha) in Norway resulted in a stream pH increase from 4.5 to 7.0 (Traaen, et al., 1997). This project provided mitigation for 20 years. In a different study conducted by Hindar and associates (2003), helicopter liming of $\text{CaMg}(\text{CO}_3)_2$ at a rate of $3\text{ t}\cdot\text{ha}^{-1}$ to an 80 ha forested catchment in Southern Norway resulted in an increase of pH, Ca, Mg, and ANC, with a corresponding decrease in Al_i . This project has provided 8 years of freshwater acidification mitigation and is expected to do so for the next 49 years. In conclusion, research conducted in Norway has also demonstrated that helicopter liming is an effective and long-lasting mitigation method for freshwater acidification.

2.3.3 Liming Efforts in Nova Scotia

Although significant research and liming efforts have been conducted in Europe and other regions of North America, very little liming research has been conducted in Canada, and specifically, Nova Scotia. In North America, helicopter liming has been conducted in New York State, where CaCO_3 was applied at a rate of $6.3 \text{ t}\cdot\text{ha}^{-1}$ to two catchments within the Woods Lake watershed (100 ha, and 200 ha, respectively), resulting in an increase in pH and base cations, with a corresponding decrease in metal ions (Driscoll, et al., 1996). Within Canada, and Nova Scotia specifically, liming efforts aiming to improve freshwater quality have been limited to in-stream liming, river-bed liming, and manual-application terrestrial liming. In-stream liming via a lime doser has been conducted in West River, Sheet Harbour, Nova Scotia, which is located in the NSSU, since 2005 (Halfyard, 2003). This project was expanded in the fall of 2017 to include a secondary lime doser to expand the spatial footprint of acid mitigation. Sterling et al. (2014) conducted manual-application terrestrial liming within the Maria Brook experimental catchment in New Ross, Nova Scotia. During the Sterling et al. (2014) experiment, powdered CaCO_3 was applied manually on an annual basis over the course of three years, the experiment did not result in significant water quality improvements in the first three years (Sterling, et al., 2014). The lack of improvement was attributed to clumping of the limestone powder associated with chronic wet conditions and precipitation during application (Sterling, et al., 2014). Clearly, in contrast to liming efforts in European countries such as Norway and Sweden, liming efforts in North America, and specifically in the NSSU are minimal. Additionally, previous terrestrial liming efforts in the region have had mixed success, indicating a lack of understanding of NSSU freshwater system response to liming or insufficient resources

Recently, several studies have identified the need for more research examining pH and Al levels, and temporal variation thereof in Nova Scotia (Dennis et al., 2012; Macleod, 2016; Sterling, et al., 2014). Amongst these studies, Sterling et al. (2014) identified five areas of freshwater acidification mitigation research including recommendations for further examination of temporal patterns of freshwater chemistry response to terrestrial liming. Additionally, no studies have been identified on evaluating effectiveness of terrestrial liming at mitigating seasonal Al concentration highs in the fall (Macleod, 2016). Recent studies have indicated the need for more research on pH and Al in the NSSU, and specifically, the effect of helicopter-applied terrestrial liming on these parameters.

2.4 Knowledge gaps

2.4.1 Absence of Helicopter Liming Attempts in Nova Scotia

Although helicopter liming has proven very successful in Sweden, Norway, and other regions of North America, such efforts have not been conducted in Nova Scotia. In Sweden, Norway and the United States, helicopter liming has led to increased pH and Ca (Driscoll, et al., 1996; Fransman & Nihlgård, 1995; Hindar & Wright, 2005), and decreased in-stream Fe and Al concentrations (Andersson, 1995). These mitigative effects were found to last 20 years, and were predicted to last for 50 years (Hindar & Wright, 2005). Furthermore, terrestrial liming applies base cations directly to the soils of the catchment, uniquely allowing exchange with H^+ ions during deposition events, increasing the ANC and mitigating the acidification which would otherwise result, making it an ideal candidate for freshwater quality improvement efforts in Nova Scotia (Dennis et al., 2012). Although helicopter liming has the potential to greatly improve freshwater chemistry in NSSU, and the mechanisms through which the mitigative effects occur are well suited to the geology and lithology of the province, liming projects in Nova Scotia have been limited to in-stream liming *via* a lime doser in West River Sheet Harbour, Sheet Harbour (Halfyard, 2003), and manual application terrestrial liming (Sterling, et al., 2014).

2.4.2 Poor Understanding of Water Chemistry Responses to Terrestrial Liming in Nova Scotia

Due to the small number of studies examining freshwater chemical responses to terrestrial liming in Nova Scotia, the effects of helicopter liming to watersheds within the region are still poorly understood. Recent studies examining freshwater acidification in Nova Scotia have indicated the need for more research examining pH and Al levels and temporal variation thereof in Nova Scotia (Dennis et al., 2012; Macleod, 2016; Sterling, et al., 2014). Determining if helicopter liming reduces seasonal high values for Al_i , and other seasonal variations in water chemistry, will add to the body of knowledge used to develop freshwater acidification mitigation projects and studies.

2.5 Conclusion

This literature review has examined the threat of freshwater acidification, and has identified mitigation measures undertaken to reduce this issue. This review has also examined liming methods used in countries leading freshwater acidification mitigation, and has contrasted the efforts taken within those countries to the limited efforts in Nova Scotia, and specifically the NSSU. After evaluating the existing research on freshwater acidification mitigation in Nova Scotia, this literature review has identified knowledge gaps and areas of research focus required to fully understand and mitigate this threat in this highly acid sensitive ecoregion. Research indicates that although freshwater acidification was identified as a threat to ecosystems in the 1970's; Canada, and specifically Nova Scotia, has undertaken limited initiatives to assist in the recovery of acid-sensitive systems beyond emission reduction initiatives. Following this, a knowledge gap exists in liming research within the NSSU, particularly on terrestrial liming. Furthermore, prior studies have identified the need for further research and restoration initiatives, as restoration of the habitat of the endangered Atlantic salmon will greatly reduce the likelihood of extirpation for this species. By researching this topic I will aid in reducing the risk of extirpation of the NSSU designatable unit of Atlantic salmon from this ecoregion and contribute to the body of knowledge on liming in Nova Scotia and aid in the understanding of the temporal variability of chemistry responses to this procedure.

3 Methods

3.1 Overview

This study aimed to evaluate the effects of a pilot helicopter liming project on freshwater acidification conditions in the NSSU. To evaluate this pilot project, purposive, non-probabilistic water quality samples were collected approximately every three weeks for two months prior to liming (September and October, 2016), and during the first year post-liming (November 2016 to September 2017). Purposive sampling entails selectively collecting data which captures the phenomenon which the researcher aims to evaluate (Rudestam & Newton, 2014), in this case, samples were collected in such a way to capture the effects of liming on water quality. Additional pre-liming water quality data were obtained from the Dalhousie Hydrology Research Group (DHRG), and the Nova Scotia Salmon Association (NSSA). This study was located within the Keef Brook, Brandon Lake Brook, and Upper Killag watersheds in West River, Sheet Harbour, Nova Scotia, Canada. Water quality samples were analyzed by Maxxam Analytics Laboratory in Bedford, Nova Scotia, AGAT Laboratory in Dartmouth, Nova Scotia, and the Dalhousie Health and Environment Research Center (HERC) in Halifax, Nova Scotia. Generalized Linear Mixed Modeling (GLMM) and post-hoc Tukey multiple comparisons analysis were conducted in R version 3.4.2 to determine statistical significance of changes to water chemistry parameters caused by the helicopter liming project. Akkaki Information Criterion (AIC) was used to compare models, and select the most parsimonious model. Timeseries analysis was conducted on water chemistry data in Excel 2013 to examine for temporal trends and variation following limestone application. Finally, watershed characteristics were calculated using ArcGIS version 10.3 mapping software to determine if pre-treatment water chemistry values could be predicted using watershed characteristics and other chemistry parameters, so knowledge gained during this study regarding the effectiveness of helicopter catchment liming can be extrapolated for future freshwater acidification mitigation initiatives in similar watersheds.

3.2 Study Sites

This study was conducted in West River, Sheet Harbour, Nova Scotia, Canada within the Brandon Lake Brook watershed, the Upper Killag River watershed, and three sub-catchments of the Keef Brook watershed (Figure 2). All study sites were located in the West River Sheet Harbour watershed secondary watershed (Nova Scotia Environment, 2011a), within the Eastwest Sheet Harbour primary watershed (Nova Scotia Environment, 2011b). The study sites were not located within any surficial aquifer identified by the Nova Scotia Department of Natural Resources (Kennedy, 2014).

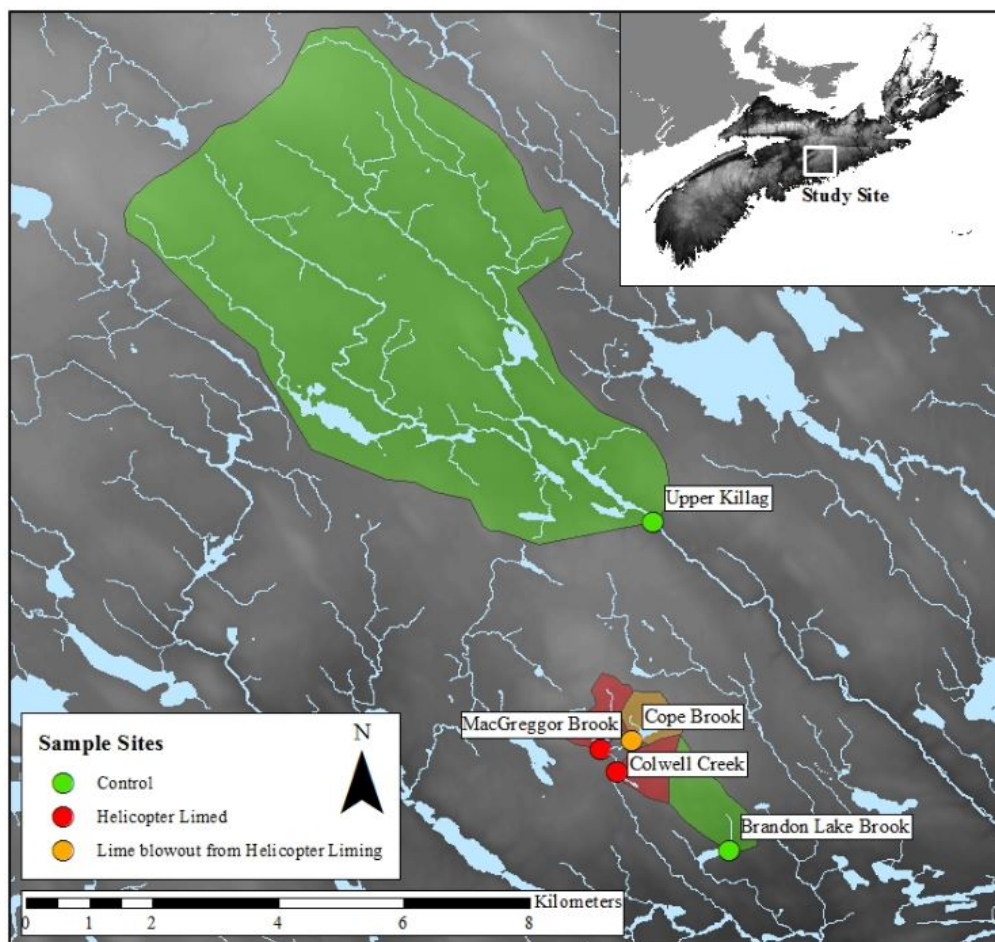


Figure 2 Map of the study sites in West River Sheet Harbour, Sheet Harbour, Nova Scotia, Canada. Watershed boundaries manually delineated in ArcGIS 10.3 using contour lines from the Nova Scotia Topographic Database (Appendix 8.2).

This BACI study used two treatment sites and two control sites (Table 3). A BACI study design observes both control and treatment sites, before and after treatment, so change causal relationships can be determined and attributed to treatment (Palys & Atchison, 2007). A fifth site was added: Cope Brook (CB), which was not intentionally limed, but was located adjacent to the area where lime was loaded into the helicopter, thus totalling five study sites (Table 3). Due to the high volume of lime dust blow out during loading, an unknown volume of lime dust was unevenly deposited in this watershed during treatment of Colwell Creek (L_CC) and MacGregor Brook (L_MGB). The CB site was added to examine the effects of small volumes of lime deposition within the catchment on water chemistry.

The study sites are underlain by the Goldenville formation; metamorphic greywacke, quartzite and slate containing gold-bearing metasediments, the Halifax formation; metamorphic slate containing sulphide-bearing metasediments, and middle to late Devonian granitoid intrusives of consisting of granite, granodiorite, diorite, diabase, and gabbro (Nova Scotia Department of Natural Resources, 2006) (Table 3). Of these intrusives granite and granodiorite also contain sulphide minerals (Clarke, Erdmann, Samson, & Jamieson, 2009). The sulphide-bearing metasediments of the Halifax formation, and the sulphide minerals within the granite and granodiorite, are known to produce acid drainage (ARD) when exposed to water or air (Clarke, Erdmann, Samson, & Jamieson, 2009; Fox, Robinson, & Zentilli, 1997).

Surficial geology of the study sites is predominantly stony till plain. This material originated from the Wisconsinan Quaternary period and was deposited in the area from the base of ice sheets centered over Nova Scotia during the last glaciation period approximately 10,000 ybp. Soils present are a stony, sandy matrix, derived from local bedrock sources, characterised by rapid drainage, high erodability, a high water table, and poor acid rain buffering capacity (Nova Scotia Department of Natural Resources, 2006). The Upper Killag River (C_UKR) control site is also underlain by silty drumlins of four to 30 m thickness also originating from the Wisconsinan Quaternary period. This surficial geology is typified by a higher percentage of distant source material including red clay, and characterised by moderate drainage and stoniness, and moderate to good acid rain buffering capacity (Nova Scotia Department of Natural Resources, 2006). Additionally, the C_UKR site has organic deposits forming bogs, fens, and swamps of one to five meter thickness. These areas originated from the Holocene Quaternary period, and were deposited after the last glaciations (Nova Scotia Department of Natural Resources, 2006).

Table 3 Site name, abbreviation, treatment type, location, size, and geological characteristics of study sites.. Site abbreviations with L_ prefix indicate a limed site, while sites denoted with C_ prefix indicate a control site. CB not assigned a prefix, as this site was not used in analysis.

Site Name	Site Abb.	Treatment Type	Latitude	Longitude	Catchment Size (km²)	Dominant Bedrock	Secondary Bedrock	Surficial Geology
Colwell Creek	L_CC	Treatment	45.0279	-62.7127	0.930	Goldenville formation	Halifax formation	Stony till plain
MacGregor Brook	L_MGB	Treatment	45.0312	-62.7160	0.727	Middle-late Devonian granitoid intrusives	Goldenville formation	Stony till plain
Cope Brook	CB	n/a	45.0324	-62.7096	0.633	Goldenville formation	n/a	Stony till plain
Brandon Lake Brook	C_BLB	Control	45.0166	-62.6900	0.760	Halifax formation	Goldenville formation	Stony till plain
Upper Killag River	C_UKR	Control	45.0638	-62.7051	38.445	Goldenville formation	Halifax formation and middle-late Devonian granitoid intrusives	Stony till plain (dominant), with areas of silty drumlin, organic deposits, and bedrock

The study sites were selected by Dr. Edmund A. Halfyard (NSSA), Dr. Shannon M. Sterling (Dalhousie University), and Dr. Kevin Keys (Nova Scotia Department of Natural Resources (NS DNR), with mapping support from Marley Geddes, a Dalhousie BSc of Environmental Science graduate. The site was selected based on hydrological connectivity, soil type, land use impact, topography, basin size, surficial materials, likelihood of detectable change in stream chemistry from terrestrial application of powdered limestone, ease of accessibility and potential for Atlantic salmon reintroduction. This site is believed to be representative of geology, forest and land use for much of the acidified streams in the NSSU.

3.3 Catchment Liming

The pilot catchment liming project is a partnership between the NSSA and the province of Nova Scotia, with project oversight from Dr. Edmund Halfyard (NSSA) and funding from the Nova Scotia Department of Natural Resources (NS DNR), Nova Scotia Department of Fisheries and Aquaculture, Nova Scotia Salmon Association, Atlantic Canada Opportunities Agency, Recreational Fisheries Conservation Partnerships Program (Fisheries and Oceans Canada), Northern Pulp and Paper, Dalhousie University, the Nova Scotia Sportfish Habitat Fund, and the Atlantic Salmon Conservation Foundation.

Powdered dolomitic limestone (CaMgCO_3) was applied at a concentration of $10 \text{ t}\cdot\text{ha}^{-1}$ on land and $2 \text{ t}\cdot\text{ha}^{-1}$ on lakes/ponds to 2.0 m WAM buffer zone (treatment area) using a MD 500E helicopter flown by NS DNR pilots. A 2.0 m WAM identifies watershed areas which have a proportionately larger influence on stream chemistry, as the water table lies within 2.0 meters of the surface (Sterling, et al., 2014). Limestone was applied in approximately 340 kg loads using a hopper suspended beneath the helicopter. The CaMgCO_3 was supplied by Mosher Limestone, located in Upper Musquodoboit, Nova Scotia. The limestone products supplied by this manufacturer range in chemical make-up from 50 to 95 % calcium carbonate, four to 40 % magnesium carbonate, and 0.5 to 2.5 % silica dioxide (Mosher Limestone Company Limited, 2015). The entire treatment area of L_CC was limed, 21.548% of the total catchment (Table 4). One small 0.23 ha pond in L_CC was limed as a landmass; at $10 \text{ t}\cdot\text{ha}^{-1}$, although its direct connection with L_CC is ephemeral. One section of L_MGB did not receive treatment because the hopper broke and could not be repaired within the allotted application period. The unlimed-treatment section represented 11.004 % of the total watershed, while the limed treatment area represented 61.991 % of the L_MGB watershed. It is unknown what portion of CB watershed received limestone or the mass of limestone deposited within the watershed resulting from blow out. Catchment liming was conducted between October 3rd, 2016 and November 19th, 2016.

Table 4 Catchment area treated with powdered dolomitic limestone, and quantity of limestone applied.

Sub-catchment	Sub-catchment Area (km²)	Limed Treatment Area (km²)	Unlimed Treatment Area (km²)	Proportion Catchment Area Limed (%)	Lime Applied (t)
Colwell Creek	0.930	0.200	0	21.548	200.42
MacGregor Brook	0.727	0.458	0.080	62.991	424.40
Cope Brook	0.633	unknown	unknown	unknown	unknown
Total	2.290	0.658	0.080	n/a	624.82

3.4 Data Collection

3.4.1 Sampling schedule

Water chemistry samples were collected on an approximately monthly interval in the fall of 2016, and approximately every three weeks during the spring and summer of 2017 (Table 5). Sampling frequency was increased during the snowmelt season because seasonal pH decrease and Al_i increase have been observed during this season within the NSSU, and we wished to observe the effects of the helicopter liming on these events (Dennis et al., 2012; MacLeod, 2016). Sample events were influenced by weather, as sampling targeted the falling limb of the hydrograph. The falling limb of the hydrograph was targeted, as accumulated contaminants on the soil surface are washed into the surface and subsurface waters during precipitation events, decreasing pH and increasing Al_i concentrations, biasing samples (Driscoll, Baker, Bisogni, & Schofield, 1980).

3.4.2 Contributed Water Chemistry Data

Additional baseline data for Brandon Lake Brook (C_BLB) and L_CC were provided by the Dalhousie Hydrology Research Group, and additional data for all sites were provided by the Nova Scotia Salmon Association (Table 5). Although the supplementary pre-liming data provided by the DHRG and NSSA provide additional data points for statistical analysis, this study is delimited by the time frame of available data, i.e., one year. It is known that water chemistry, particularly pH and Al levels, seasonally fluctuate in Nova Scotia (Dennis, Clair, & Kidd, 2012; Sterling, et al., 2014; Sterling, et al., in progress). Therefore, the analysis will be influenced by the

inherent fluctuation of water chemistry which occurs. This delimitation will be considered and discussed during interpretation of results.

Table 5 Collected samples.

Date	Sites Sampled	Liming stage	Source
April 29, 2016	C_BLB		DHRG
June 3, 2016	C_BLB, L_CC		DHRG
June 16, 2016	C_BLB, L_CC		DHRG
June 28, 2016	C_BLB, L_CC	Before	DHRG
July 15, 2016	C_BLB, L_CC		DHRG
August 5, 2016	All sites		NSSA
September 10, 2016	All sites		Collected
October 2, 2016	All sites		Collected
November 17, 2016	All sites	During	Collected
April 19, 2017	All sites		Collected
May 14, 2017	All sites		Collected
May 30, 2017	All sites		Collected
June 21, 2017	All sites	After	Collected
August 1, 2017	All sites		Collected
August 23, 2017	All sites		Collected
September 16, 2017	All sites		Collected

3.4.3 Meteorological and Hydrograph Data

Air temperature data were collected for each sampling event from the EC Malay Falls climate monitoring station (Climate ID: 8203405, Latitude: 45.5900, Longitude: -62.2900), located approximately 70 km from the study sites. Hydrograph data were approximated from EC St. Mary's River at Stillwater hydrometric monitoring station (Station ID: 01EO001, Latitude: 45.1027, Longitude: -61.5837), located approximately 89 km from the study sites. Although this hydrometric monitoring site represents a different watershed, it is assumed that the station is near enough to be subject to the same weather patterns, and therefore, hydrographic stage. Both the monitoring stations were selected because they were the closest monitoring stations to the study sites.

3.4.4 Sample Collection

During sampling events, in-stream measurements of water temperature, pH, and specific conductivity were measured using an YSI Pro Plus sonde. The sonde used for in-stream measurements is known to measure an average of 0.47 ± 0.44 pH units below true in-stream pH, based on simultaneous side-by-side measurements with a calibrated YSI Ecosense pH Pen. A calibration curve was created based on the side-by-side measurements ($n = 69$ pairs) and the in-stream pH data were adjusted accordingly (Equation 1).

$$\text{true pH} = 0.595(\text{measured pH}) + 2.3868 \quad (1)$$

Water samples were also collected for laboratory analysis. Grab samples were collected in sterilized polyethylene bottles and analysed for total organic carbon (TOC), pH and sulphate (SO_4). Metals, cation, and anion samples were collected in a sterilized polyethylene bottle containing nitric acid (HNO_3) to preserve metals using a sterilized polyethylene syringe. Samples used for dissolved metal, cation, and anion analysis were passed through a $0.45 \mu\text{m}$ glass fibre filter prior to collection. To approximate organically-complexed colloidal aluminium (Al_o), the samples were passed through a 3 cm negatively-charged cation exchange column (Bond Elut Jr. Strong Cation Exchange Column), in addition to the $0.45 \mu\text{m}$ glass fibre filter following the method used by Dennis et al. (2012) and Sterling et al. (2018, *in progress*). Water was passed through the column at a low rate ($\sim 60 \text{ drops} \cdot \text{min}^{-1}$) to ensure cations were taken up by the column, as inorganic aluminum (Al_i) will be underestimated if Al_o contains residual cations.

After collection, samples were immediately placed in a cooler with ice to be cooled to 7°C before delivery to Maxxam Analytics Laboratory in Bedford, Nova Scotia, AGAT Laboratory in Dartmouth, Nova Scotia, and the Health and Environmental Research Centre (HERC) in Halifax, Nova Scotia. Samples were delivered to the laboratories within 48 hours of sample collection. When samples could not be delivered to the laboratories within five hours of collection, the samples were stored at 7°C until delivery. Due to the warm temperature of the stream water between June and October, samples delivered within five hours of collection had not been completely cooled to 7°C at the time of delivery.

Data provided by the DHRG and the NSSA were also collected following the procedures outlined above.

Sampling assistants were used for data collection. The assistants were students of Dalhousie University enrolled in the Bachelors of Science or Engineering programmes. Assistants received training in sample collection and handling techniques prior to handling samples and were supervised throughout.

3.4.5 Laboratory Analysis

Upon delivery to the laboratories, the samples were further cooled to approximately 4°C prior to analysis. Data provided by the DHRG were analyzed by Maxxam Analytics Laboratory and the Dalhousie University Health and Environments Research Center (HERC). Data provided by the NSSA were analyzed by AGAT Laboratories. Data collected directly for this study were analyzed by AGAT Laboratories.

Maxxam Laboratory

Metal, cation, anion, DOC, and pH samples were analyzed at Maxxam Analytics Laboratory in Bedford, Nova Scotia. Maxxam Laboratory protocols adhere to the United States of America Environmental Protection Agency (US EPA) approved methods for identifying trace elements in water (United States Environmental Protection Agency, 1994) and analyzing samples using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (United States Environmental Protection Agency, 1998).

Metal, cation, and anion samples were analyzed using ICP-MS, DOC samples were analyzed using a Continuous Flow Analyzer, and laboratory pH was measured using a standard hydrogen electrode and reference electrode. All samples were prepared and analyzed in accordance with US EPA (1994; 1998) guidelines.

HERC Laboratory

SO₄ samples were analyzed at HERC in Halifax, Nova Scotia. This laboratory was selected by the DHRG as this laboratory provides a lower detection limit for SO₄ analysis. Samples were filtered using a 0.45 µm glass fiber filter and analyzed using an Ion-Chromatography System (ICS) 5000 Dionex detector.

AGAT Laboratory

Metal, cation, anion, TOC, pH, and SO₄ samples were analyzed at AGAT Laboratories in Dartmouth, Nova Scotia. AGAT Laboratories are accredited by the International Organization for Standardization, and hold both the 9001:2015, and 17025:2005 accreditations (AGAT Laboratory, 2017).

Metal and cation samples were analyzed using ICP-MS, TOC samples were analyzed using Infrared Combustion (IR Combustion), laboratory pH was measured using a standard hydrogen electrode and reference electrode, and SO₄ and anions was measured using ICS (Canadian Association for Laboratory Accreditation Inc., 2017)

Quality Assurance and Control

To ensure results for samples analyzed at Maxxam Analytics and HERC laboratories were comparable to results for samples analyzed at AGAT laboratory, three duplicate samples were taken at C_BLB and Upper Killag River (C_UKR) sample sites on April 19th, 2017, May 14th, 2017, and May 30th, 2017 and analyzed by both laboratories. Results were analyzed using the Wilcoxon Rank Sum statistical test in Python 3.6 using the SciPy 0.19 package. This analysis showed a significant difference in laboratory pH results ($T = 1$, $p = 0.04$). Therefore, analysis of pH was conducted using the corrected Sonde pH data. Comparison of the total, dissolved and organically-complexed colloidal aluminum results indicated a non-significant difference between laboratory results ($T = 8.5$, $p = 0.674$; $T = 5.0$, $p = 0.249$; and $T = 8.0$, $p = 0.600$, respectively), indicating Al results are comparable between laboratories. After adjusting for difference in accuracy of Ca_t detection limits (100 µg·L⁻¹ for Maxxam, and 0.1 mg·L⁻¹ for AGAT, Appendix 8.1), no significance difference was found between samples ($T = 4.0$, $p = 0.173$), indicating that Ca_t results are also comparable between laboratories. Due to a difference in SO₄ detection limit (10 µg·L⁻¹ for HERC, and 2 mg·L⁻¹ for AGAT), all duplicate samples analyzed by AGAT resulted in values below the detectable limit, indicating that these results are not comparable. Lastly, the organic carbon samples analyzed by AGAT were analyzed as total organic carbon (TOC), while samples from Maxxam Analytics were analyzed as dissolved organic carbon (DOC), and the results are therefore not comparable.

To ensure measurement accuracy, the YSI Pro Plus Sonde was calibrated within 36 hours of in-stream data collection. Additionally, both Maxxam Analytics and AGAT laboratories

independently conducted duplicate, blanks, reference material, and matrix spike analysis to ensure result accuracy in accordance with their respective accreditations.

3.4.6 Post Laboratory Analysis

Inorganic aluminum was estimated as the difference between Al_d and Al_o (Equation 2):

$$Al_i = Al_d - Al_o \quad (2)$$

Where Al_d is quantity of dissolved aluminum (filtered sample) and Al_o is the quantity of non-labile organically-completed colloidal aluminum (eluate of the cation exchange column).

Al_i is defined as all positive ionic species of Al in this approach; consistent with Dennis & Clair (2012) and Poléo (1995).

3.4.7 Spatial Analysis

Topographic, and landuse data were obtained from a variety of government and non-government sources, as indicated in Appendix 8.2.

Spatial analysis was conducted using the ArcGIS 10.3.1 platform. Study site watersheds were manually delineated using the NS Topographic Database. The accuracy of the delineated watersheds was limited by the 20 m resolution of this database. The GIS model developed by Rotteveel et al. (2017) was then used to overlay the landuse data with the delineated watersheds to determine the percentage of watershed underlain by a 2.0 m Wet Area Model (WAM), 0.5 m WAM, wetlands, roads, roads located within 30 m of streams, mature forest, forestry lots, agricultural land, urban land, and corridors (Appendix 8.3). The model was also used to identify the dominant bedrock underlying the sites, and the average geological Ca level.

In addition to accuracy limitation due to data resolution, spatial analysis was further limited by the creation date of the shapefiles used in the analysis, as identified in Appendix 8.2.

3.4.8 Statistical Analysis

LMM/GLMM statistical analysis was selected to evaluate liming efficacy as opposed to standard parametric statistics because repeated measures were taken of the same subjects (i.e., sites). By controlling for within-site variability/nested structure of samples via LMM/GLMM, a violation of the independent assumption was avoided, more reliable p-values were calculated, and the chance of type 1 errors was reduced (Burnham & Anderson, Model selection and multimodel inference: practical information - theoretical approach, 2002). Furthermore, given the limitation of

few data points ($n = 10$ to $n = 15$, depending on site), if a significant effect was demonstrated using LMM/GLMM, this indicated that there were sufficient samples, while reducing the probability that the test incorrectly identified a false positive as much as possible.

LMM/GLMM models were assessed using Akkaki Information Criterion (AIC) to verify the most parsimonious model was used (Burnham & Anderson, 2004). A random-intercept model was used, with fixed effects of treatment (control vs. treatment), time (before vs. after), and the interaction thereof (effect of treatment over time), and the random effect of site (L_CC, L_MGB, C_BLB, and C_UKR).

To evaluate if LMM/GLMM models were appropriate for our data, the variability due site-specific traits was calculated following the methods outlined by Nakagawa and Schielzeth (2012) (Equation 3)

$$R_c^2 - R_m^2 = R_r^2 \quad (3)$$

where R_c^2 are conditional effects (fixed effects plus random effects), R_m^2 are marginal effects (fixed effects), and R_r^2 are the random effects.

Tukey post-hoc multiple comparisons analysis was used to determine water chemistry parameter differences between sites.

All statistical analyses were conducted excluding the sample collected during liming procedures (November 17th, 2016), and was conducted in R version 3.4.2 using the car, MASS, bbmle, MuMIn, lme4, and lsmeans packages (Appendix 8.4).

3.5 Limitations

The main limitation of this study is the temporal scope, and subsequent small data set ($n = 11$ to $n = 16$ depending on site, before any data removal). Since water chemistry is influenced by season (Clair et al., 2002; Clair et al., 2011; Sterling et al., 2017), and this research will only document the changes which occur during the first year post-liming, the results will be influenced by seasonal variability in water chemistry, which may mask some of the effects of the liming. Additionally, sampling frequency was increased during seasons known to have lower water quality in Nova Scotia (snowmelt), which may bias results. Furthermore there is inherent spatial representativeness in topographic, geologic, and hydrographic characteristics between study sites, which may bias results, as helicopter liming may be more effective in watersheds with certain characteristics; however, this effect was minimized through use of LMM/GLMM for statistical analysis.

4 Results

4.1 Statistical Analysis

4.1.1 Effects of Liming

LMM/GLMM Findings

While control sites showed no change in calcium concentration following liming (Tukey adjusted comparison, $z = 0.00$, $p = 1.00$), limed sites showed a significant increase calcium concentration (improved water quality) following liming (Tukey adjusted comparison, $z = 5.49$, $p < 0.0001$), with estimated increases of $1.57 \pm 0.29 \text{ mg}\cdot\text{L}^{-1}$ (Figure 3).

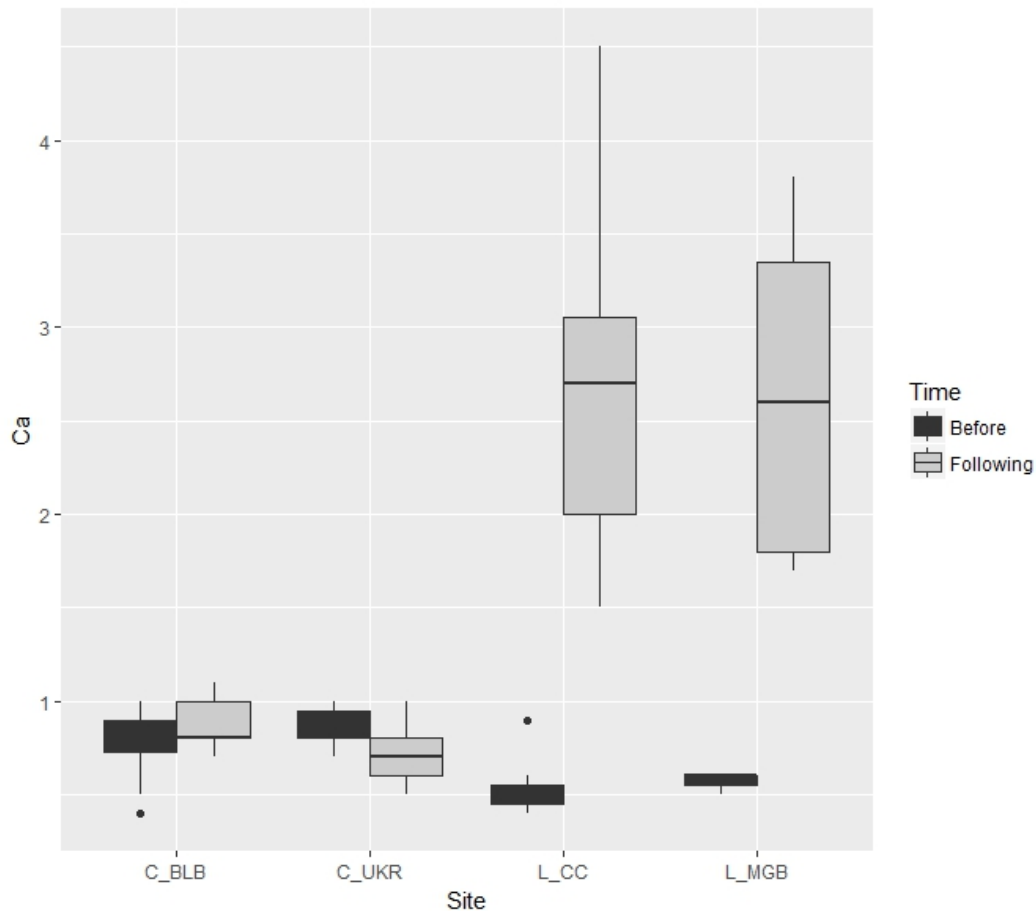


Figure 3 Effect of limestone application on Ca_i concentration, where y-axis displays total calcium concentration in $\text{mg}\cdot\text{L}^{-1}$, and x-axis study site. Sites denoted with “C_.” are control sites, while sites denoted with “L_.” are limed/treatment sites. Sample taken during limestone application not included (sites: all, date: 2016-11-17).

Control sites also had significantly lower pH (worse water quality) following liming (Tukey adjusted comparison, $df = 39.31$, $t = -3.21$, $p = 0.013$), with estimated decreases of 0.25 ± 0.08 pH units. Conversely, limed sites had significantly higher pH (improved water quality) following liming (Tukey adjusted comparison, $df = 39.24$, $t = 8.06$, $p < 0.001$), with estimated increases of 0.65 ± 0.08 pH units (Figure 17).

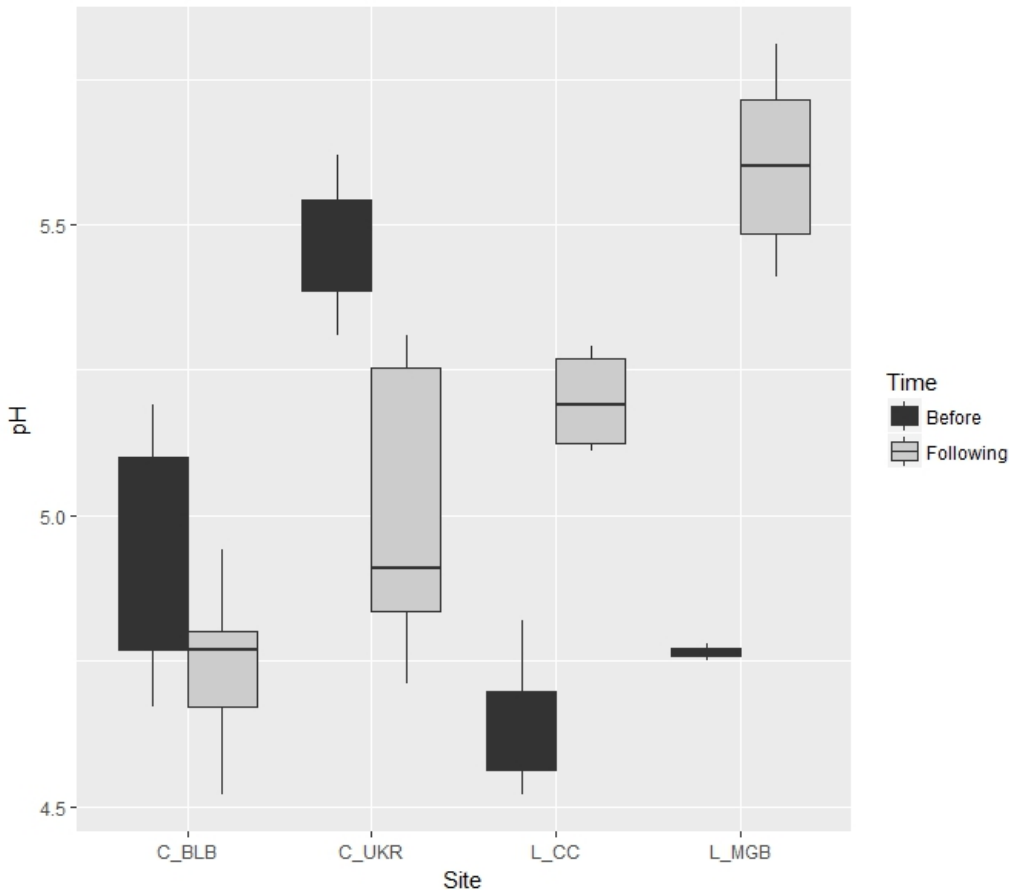


Figure 4 Effect of limestone application on pH, where y-axis displays calibrated pH in units, and x-axis study site. Sites denoted with “C_” are control sites, while sites denoted with “L_” are limed/treatment sites. Sample taken during limestone application not included (sites: all, date: 2016-11-17).

Additionally, control sites had non-significant increases in Al_i (worse water quality) following liming (Tukey adjusted comparison, $df = 42.74$, $t = 1.76$, $p = 0.303$), with estimated increases of $19.80 \pm 11.21 \mu\text{g}\cdot\text{L}^{-1}$. Conversely, limed sites showed a non-significant decrease in Al_i (improved water quality) following liming (Tukey adjusted comparison, $df = 42.74$, $t = -1.04$, $p = 0.73$), with estimated decreases of $11.69 \pm 11.21 \mu\text{g}\cdot\text{L}^{-1}$.

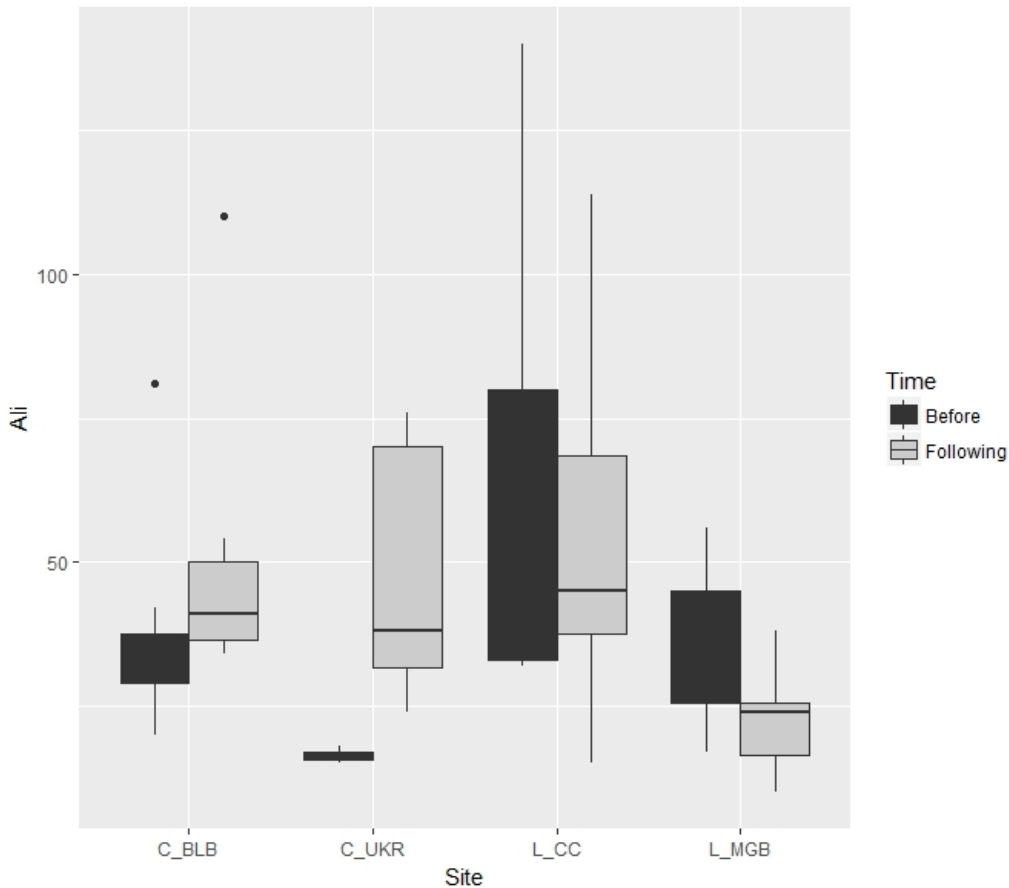


Figure 5 Effect of limestone application on Al_i concentration, where y-axis displays Al_i concentration in $\mu\text{g}\cdot\text{L}^{-1}$, and x-axis study site. Sites denoted with “C_” are control sites, while sites denoted with “L_” are limed/treatment sites. One unrealistic data point removed (site: C_BLB, date: 2016-08-05, value: $-6 \mu\text{g}\cdot\text{L}^{-1}$). Sample taken during limestone application not included (sites: all, date: 2016-11-17).

Freshwater Quality Targets

Catchment liming improved mean Ca_t concentration to above the minimum recommended level of $1.5 \text{ mg}\cdot\text{L}^{-1}$ (Dennis et al., 2012) for both treatment sites (L_CC: $2.7 \text{ mg}\cdot\text{L}^{-1}$, L_MGB: $2.6 \text{ mg}\cdot\text{L}^{-1}$) (Table 6). Mean pH level was raised for both treatment sites (L_CC: 5.20 units, L_MGB: 5.60 units), but has not yet met the suggested minimum target of 6.0 to 6.5 (Dennis et al., 2012). Catchment liming decreased mean Al_i concentration for both treatment sites (L_CC: $55 \mu\text{g}\cdot\text{L}^{-1}$, L_MGB: $22 \mu\text{g}\cdot\text{L}^{-1}$) but has not yet met the minimum target of $15 \mu\text{g}\cdot\text{L}^{-1}$ (European Inland Freshwater Fisheries Advisory Council, 1990).

Table 6 Mean water chemistry values before and following limestone application. Sample collected during liming operations not included (sites: all, date: 2016-11-17). pH displayed as calibrated pH value. One unrealistic data point removed for Al_i concentration (site: C_BLB, date: 2016-08-05, value: $-6 \mu\text{g}\cdot\text{L}^{-1}$).

Site	Mean Before			Mean Following		
	Ca_t ($\text{mg}\cdot\text{L}^{-1}$)	pH (unit)	Al_i ($\mu\text{g}\cdot\text{L}^{-1}$)	Ca_t ($\text{mg}\cdot\text{L}^{-1}$)	pH (unit)	Al_i ($\mu\text{g}\cdot\text{L}^{-1}$)
C_BLB	0.8	4.91	38	0.9	4.74	51
C_UKR	0.9	5.47	16	0.7	5.02	49
L_CC	0.5	4.64	63	2.7	5.20	55
L_MGB	0.6	4.77	36	2.6	5.60	22

4.1.2 Study Site Selection Evaluation

Correlation of fixed effects found no difference in Ca_t concentration between study sites prior to liming (intercept: $-0.2231436 \text{ mg}\cdot\text{L}^{-1}$ for all sites). This correlation was supported by post-hoc Tukey multiple comparisons analysis, which no significant difference in Ca_t concentration between sites prior to liming (Tukey adjusted comparison, $z = 1.109$, $p = 0.6841$). Similarly, correlation of fixed effects for pH levels found that treatment and control sites were similar prior to limestone application; C_BLB and L_CC were very similar (intercept of 4.705634 units and 4.725468 units, respectively) as were L_MGB and C_UKR (intercept of 5.028817 units and 5.048651 units, respectively). Additionally, Al_i concentration was found to be distributed without bias amongst sites prior to liming, with extreme high and low Al_i intercepts occurring in treatment sites (L_CC: $61.66841 \mu\text{g}\cdot\text{L}^{-1}$, and L_MGB: $38.18873 \mu\text{g}\cdot\text{L}^{-1}$, respectively), and moderate intercepts in control sites (C_BLB: $53.60701 \mu\text{g}\cdot\text{L}^{-1}$, and C_UKR: $46.25013 \mu\text{g}\cdot\text{L}^{-1}$, respectively).

4.1.3 LMM/GLMM Model Evaluation

For all three chemistry parameters examined via LMM or GLMM (method depending on data distribution, Appendix 8.5), the maximum effects model, including “time” (before vs. after), “treatment” (control vs. treatment), and the interaction effect of treatment over time as fixed effects, and “site” as a random effects term, was the most parsimonious (Ca_t : AIC = 85.5, weight = 0.996, pH: AIC = 4.0, weight = 1.000, and Al_i : AIC: 440.5, weight = 0.983). The use of LMM and GLMM was further supported by the finding that 36% of the overall variability in pH, and 20% of overall variability in Al_i was attributed to site-specific traits.

4.2 Timeseries Analysis

4.2.1 Initial Response to Liming

No effect of liming on in-stream pH was apparent until the first rain event after limestone was applied to the catchment. Beginning on October 10th, 2016 at 02:15, water levels in C_BLB rose by 0.605 m within 33 hours, which was accompanied by a pH increase in L_CC of 0.18 units within the same time period, with an additional increase of 1.5 units occurring in the hours afterward, reaching a peak of 5.54 units by 18:00 on October 11th, 2016. As water levels dropped following the rain storm, pH decreased again by less than 0.5 units; however, following the second substantial rain event (water level increase of 0.341 m within 21 hours), pH levels increased by 0.45 units, increasing by a further 0.6 units and peaking at 5.66 units by 15:30 on October 23rd, 2016. Following these two initial decreases in acidity, in-stream pH remained steady around 5.5 units until NSSA in-stream Sonde was removed on December 21st, 2016.

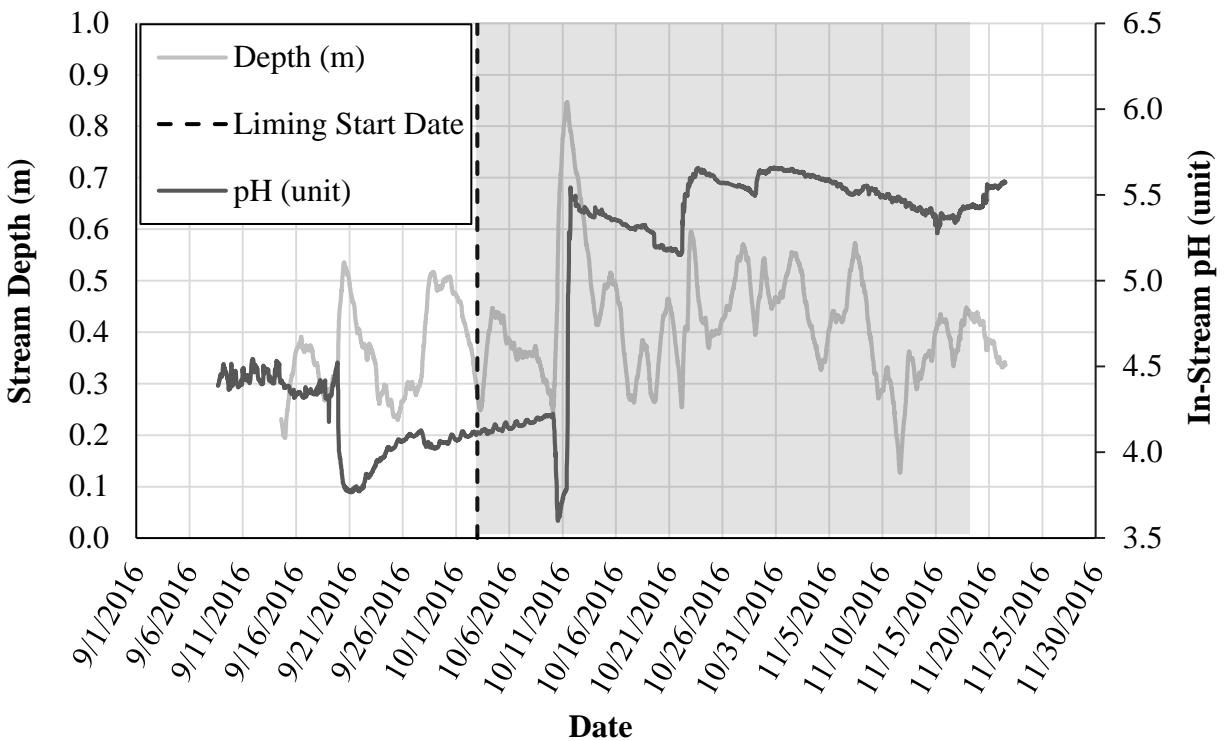


Figure 6 Effect of precipitation events, i.e., change in water level, on pH level following limestone application. Liming began on October 3rd, 2016 and ended on November 19th, 2016, as indicated by light grey area. Stream depth measured in C_BLB, data available between 14:15 on September 14th, 2016 and 12:45 on November 11th, 2016. pH level measured in L_CC, data included from 16:00 on September 8th, 2016 to 11:15 on November 11th, 2016.

4.2.2 Ongoing Response to Liming

After the initial increase of Ca_t following limestone application in 2016, Ca_t decreased to approximately $2.0 \text{ mg}\cdot\text{L}^{-1}$ for both L_CC and L_MGB during April and May of 2017, and increased again during summer and fall of 2017 to between $3.5 \text{ mg}\cdot\text{L}^{-1}$ and $4.5 \text{ mg}\cdot\text{L}^{-1}$ for L_CC, and $3.5 \text{ mg}\cdot\text{L}^{-1}$ and $4.0 \text{ mg}\cdot\text{L}^{-1}$ for L_MGB (Figure 7).

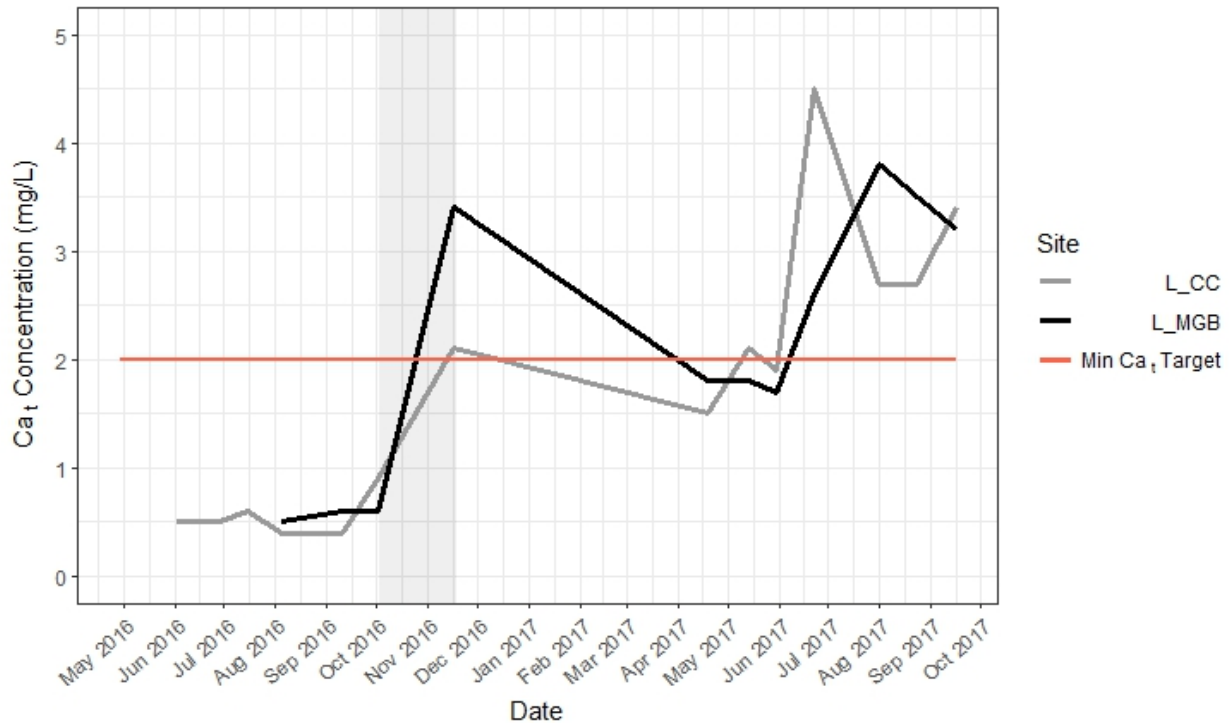


Figure 7 Timeseries of total calcium concentration in L_CC and L_MGB before, and after liming (October 3rd, 2016). Light grey area indicates limestone application period. Red line indicates minimum Ca_t concentration target ($2 \text{ mg}\cdot\text{L}^{-1}$). Samples collected between July 3rd, 2016, and September 16th, 2017. Samples taken before August 5th, 2016 analysed at Maxxam Laboratories, remaining samples analysed at AGAT Laboratories.

Furthermore, after the initial stabilization of pH in L_CC around 5.5 units in 2016 after limestone application seen in Figure 6, pH dropped slightly and was stable around 5.2 units for the remainder of the study period (Figure 8). However, L_MGB maintained a stable average of 5.6 pH units after liming.

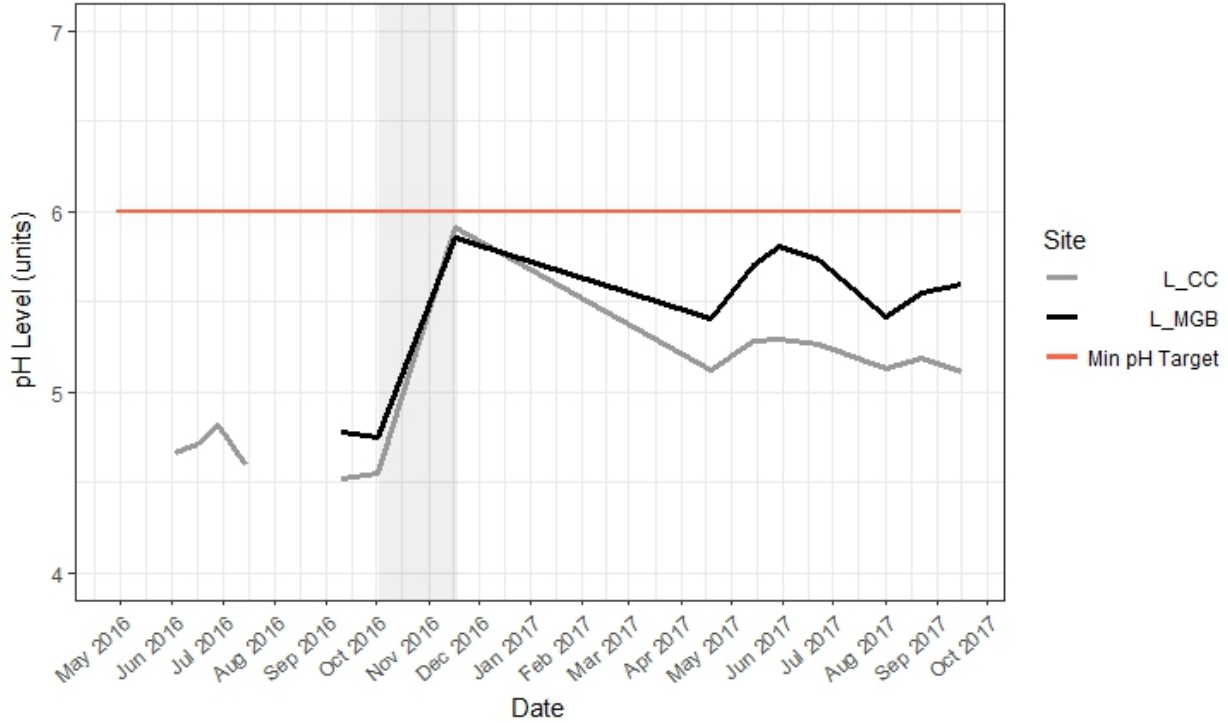


Figure 8 Timeseries of calibrated pH level in L_CC and L_MGB before, and after liming (October 3rd, 2016), as measured by handheld Sonde. Light grey area indicates limestone application period. Red line indicates minimum pH level target (6.0 units). Samples collected between July 3rd, 2016, and September 16th, 2017.

Lastly, after limestone application in 2016, Al_i concentration in L_CC was stable around $35 \mu\text{g}\cdot\text{L}^{-1}$, omitting the two spikes of $114 \mu\text{g}\cdot\text{L}^{-1}$ and $91 \mu\text{g}\cdot\text{L}^{-1}$ on June 22nd, 2017 and August 23rd, 2017 (Figure 9). After initial drop in Al_i concentration in L_MGB, levels were stable around $22 \mu\text{g}\cdot\text{L}^{-1}$, with indication of slight increases in fall of 2017.

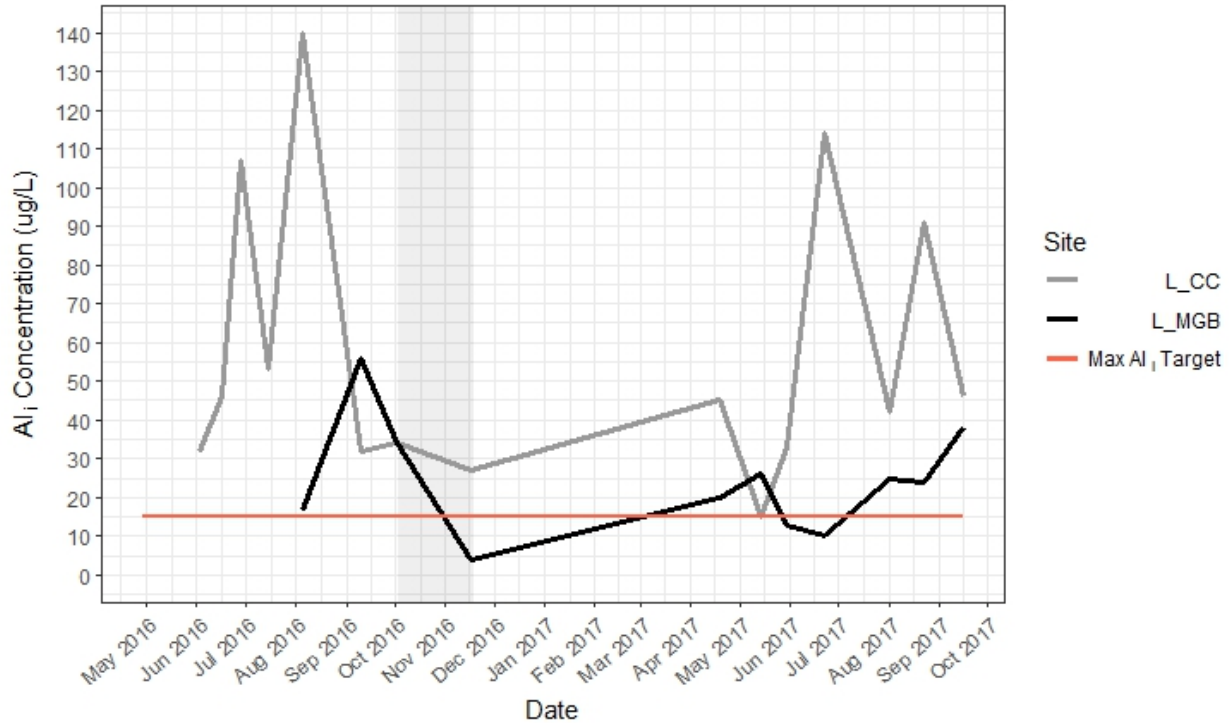


Figure 9 Timeseries of ionic aluminium concentration in L_CC and L_MGB before, and after liming (October 3rd, 2016). Samples taken between July 3rd, 2016, and September 16th, 2017. Light grey area indicates limestone application period. Red line indicates maximum Al_i concentration target ($15 \mu\text{g}\cdot\text{L}^{-1}$). Samples collected before August 5th, 2016 analysed at Maxxam Laboratories, remaining samples analysed at AGAT Laboratories.

Interestingly, temporal variation of Al_i concentration in L_CC varied similarly to C_BLB and C_UKR, which all displayed Al_i concentration spikes on June 22nd, 2017 and August 23rd, 2017. Conversely during these high Al_i events at other sites, Al_i levels in L_MGB remained stable. Similar co-variation between C_BLB, C_UKR, and L_CC were not found for pH and Ca_t levels.

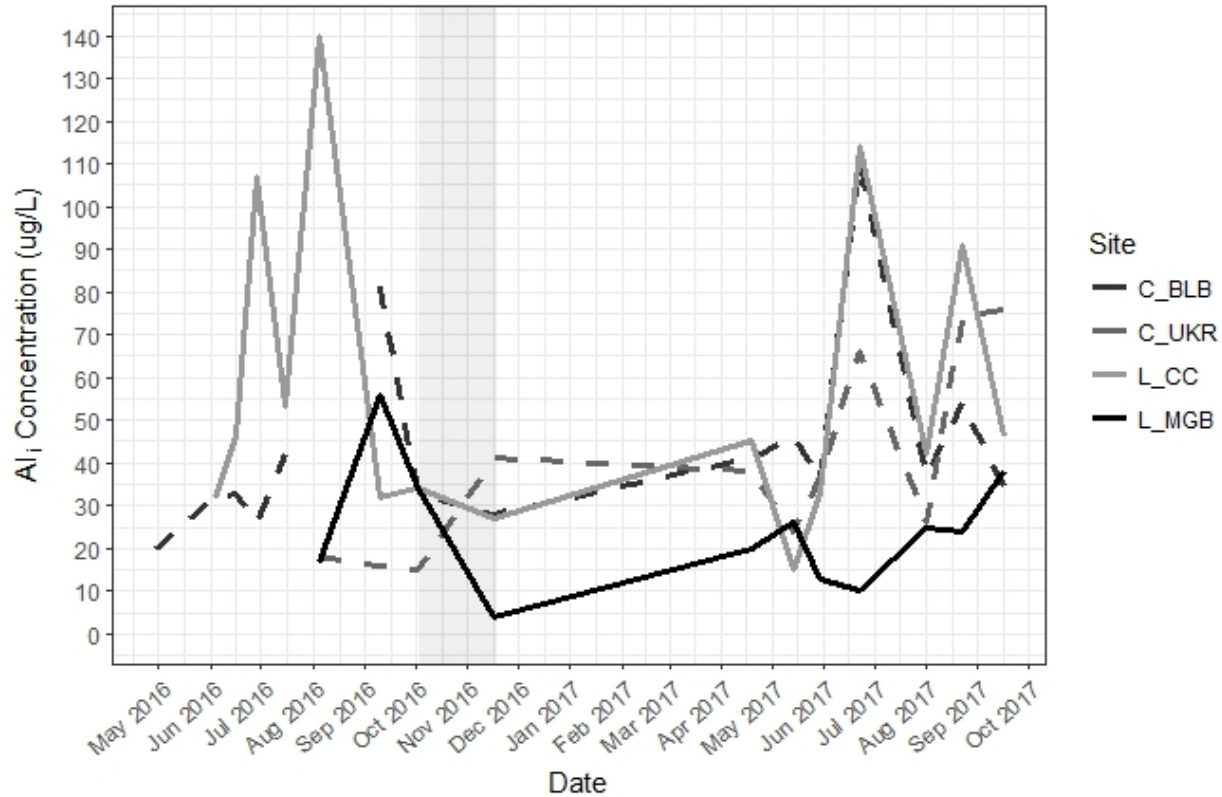


Figure 10 Timeseries of ionic aluminium concentration in C_BLB, C_UKR, L_CC, and L_MGB before, and after liming (October 3rd, 2016). Samples taken between July 3rd, 2016, and September 16th, 2017. Samples taken before August 5th, 2016 analysed at Maxxam Laboratories, remaining samples analysed at AGAT Laboratories.

4.3 Spatial Analysis

The catchment with the largest percent wetland area was C_UKR (59.73 %), which did not correspond with the study site with the highest Al_t concentration, namely C_BLB with a mean Al_t concentration of $413 \mu\text{g}\cdot\text{L}^{-1}$ (Figure 11). However, C_UKR had second highest Al_t concentration ($397 \mu\text{g}\cdot\text{L}^{-1}$) (Appendix 8.6). Despite the difference in site characteristics between the Rotteveel et al. (2018, *in prep*) study and the current study, the Al_t vs. percent wetland area trend does appear to be similar between the two studies; Al_t concentration increases with percent wetland area within the watershed.

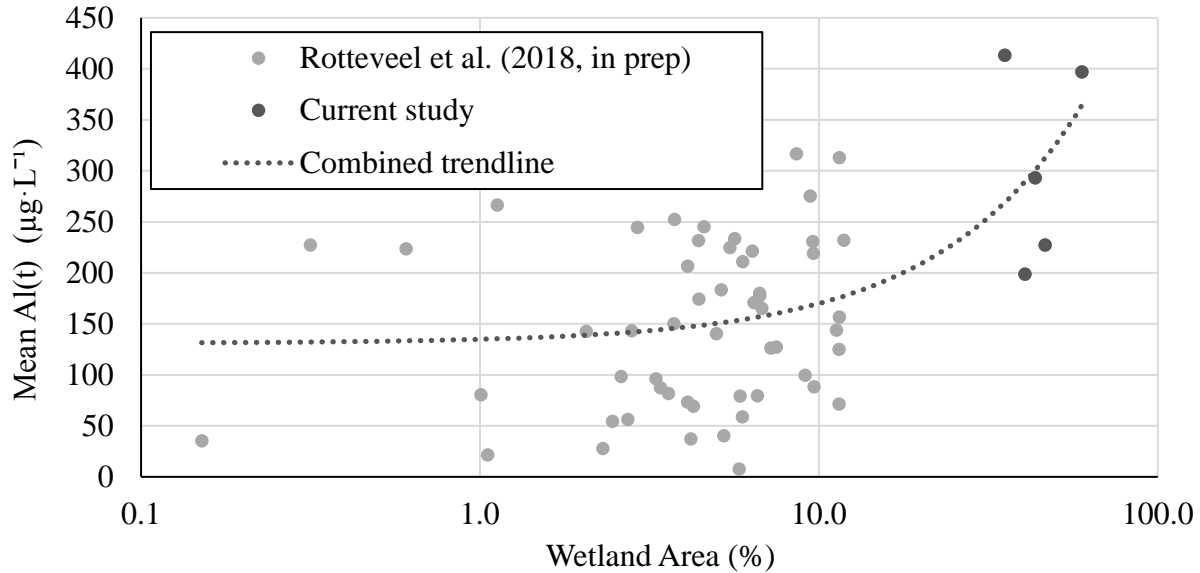


Figure 11 Agreement between results from Rotteveel et al. (2018, in prep) study and current study regarding correlation between mean Al_t concentration and percent wetland area within watershed. X-axis displayed in logarithmic scale, base 10. Same outliers removed from Rotteveel et al. (2018, in prep) data as in their study (percent wetland area for EC NS01ED0082 site (latitude: 44.3567, longitude: -65.2064, value: 35.6648 %), and zero values removed to facilitate logarithmic display ($n = 6$). Mean Al_t concentration calculated between August 5th, 2016 and October 2nd, 2016.

Maximum percent mature forest area also occurred in the C_UKR catchment (39.57 %), and did not correspond with the study site with the highest Al_t concentration (C_BLB, with a mean Al_t of 413 $\mu\text{g}\cdot\text{L}^{-1}$) (Figure 12). However, C_UKR had second highest Al_t concentration (397 $\mu\text{g}\cdot\text{L}^{-1}$). The correlation trend identified by this study is not in agreement with the trend identified by Rotteveel et al. (2018, in prep), which may be attributable to the overall higher freshwater Al_t concentration in the current study than the Nova Scotia-wide study conducted by Rotteveel et al. (2018, in prep). The higher Al_t concentration observed in the current study may be attributable to the higher percent wetland area, as discussed above; or the fact that the Rotteveel et al. (2018, in prep) study averaged Al_t values across the 2000's decade, which was found to have on average lower Al_t levels than the 2010's decade.

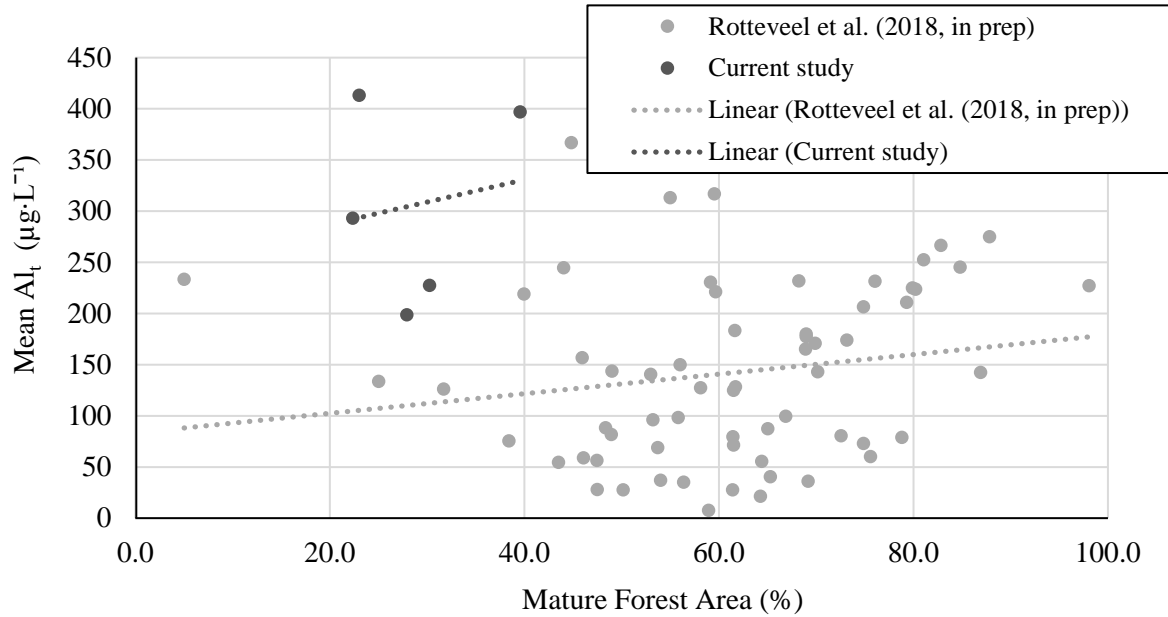


Figure 12 Comparison of Rotteveel et al. (2018, in prep) study and current study regarding correlation between mean Al_t concentration and percent mature forest area within watershed. Mean Al_t concentration calculated between August 5th, 2016 and October 2nd, 2016.

The maximum mean TOC concentration was found in C_BLB (31.0 mg·L⁻¹), which corresponds with the highest mean Al_t concentration, which was also found in C_BLB (413 µg·L⁻¹) (Figure 13). Furthermore, the trendline identified by Rotteveel et al. (2018, in prep), is in close agreement with the trend found in the current study. There is an indication of a diminishing effect of TOC on Al_t as TOC increases.

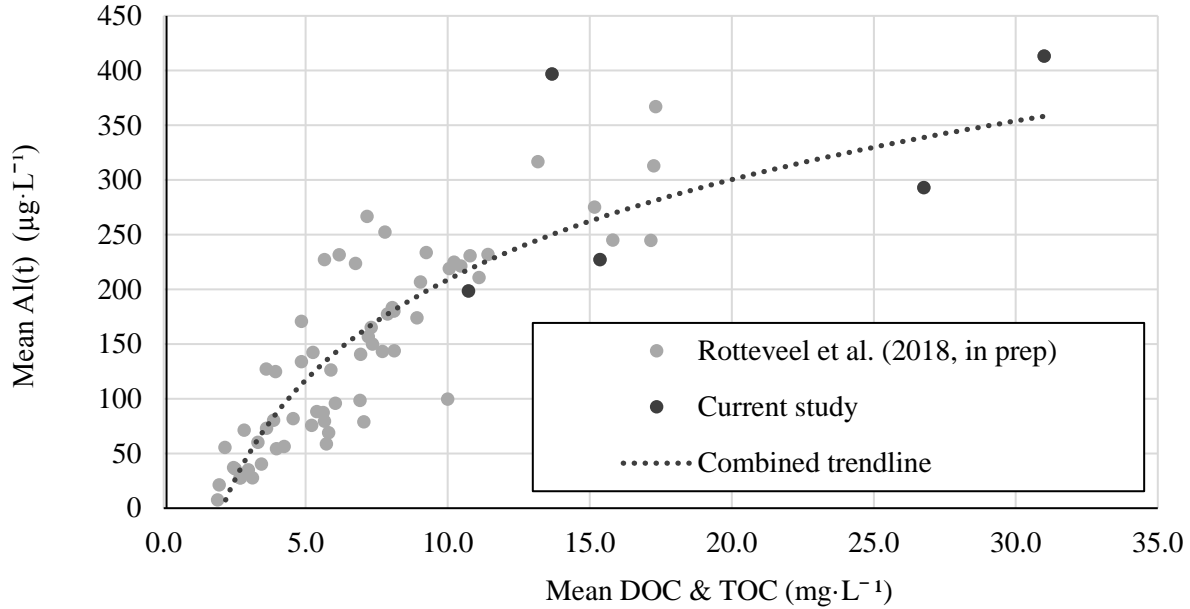


Figure 13 Strong agreement between results from Rotteveel *et al.* (2018, in prep) study and current study regarding correlation between mean Al_t - and DOC/TOC concentration. Both trendlines from Rotteveel *et al.* (2018, in prep) study and current study logarithmically, base 10, transformed. Rotteveel *et al.* (2018, in prep) data as DOC and current study data as TOC. Mean Al_t and TOC concentration calculated between August 5th, 2016 and October 2nd, 2016.

Maximum mean Ca_t concentration was found in C_UKR (0.87 mg·L⁻¹), while maximum mean Al_t concentration was found in C_BLB (413 µg·L⁻¹) (Figure 14). However, the second-highest Al_t concentration was found in C_UKR (397 µg·L⁻¹). The correlation trend between Ca_t and Al_t concentration found by this study is in complete disagreement with the trend identified by Rotteveel *et al.* (2018, in prep), as the slope of the trend in this study is increasing, while the slope of the Rotteveel *et al.* (2018, in prep) is decreasing. However, note that three datapoints fall within the same distribution as the prior study, while two sites in the current study fall outside of the range of data points identified by Rotteveel *et al.* (2018, in prep). The higher Al_t concentration observed in the current study may be attributable to the higher percent wetland area, as discussed above; or the fact that the Rotteveel *et al.* (2018, in prep) study averaged Al_t values across the 2000's decade, which was found to have on average lower Al_t levels than the 2010's decade.

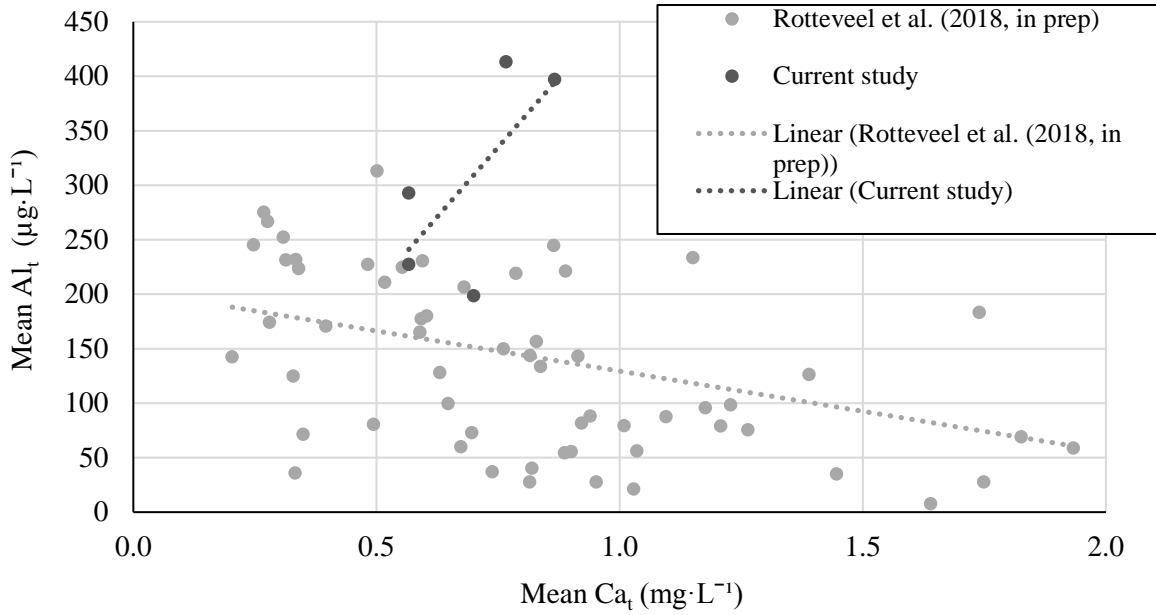


Figure 14 Comparison of results from Rotteveel et al. (2018, in prep) study and current study regarding correlation between mean Al_t and mean Ca_t concentration. Same outliers removed from Rotteveel et al. (2018, in prep) data as in their study (Ca_t concentration for EC site NS01DC001 (latitude: 44.9536, longitude: -65.0025, value: 11.1352 mg·L⁻¹) and EC site NS01FB0001 (latitude: 46.3556, longitude: -60.9750, value: 10.3417 mg·L⁻¹). Mean Al_t and Ca_t concentration calculated between August 5th, 2016 and October 2nd, 2016.

5 Discussion

5.1 Statistical Analysis

5.1.1 Study Site Selection Evaluation

The selected study sites were well suited for this research, as no significant differences were found between Ca_t concentrations of control and study sites, similar pH levels were identified at control and study sites, and Al_i concentration was distributed without bias between control and study sites. Therefore, the BACI study design used in this research is valid, and differences in water chemistry before and after liming can be attributed to the effects of helicopter liming, and not inter-site variation. However, due to the short duration of this study (i.e., one year post-liming), inter-annual water quality variability may still obscure some of the effects of liming, this limitation will be discussed further below. Additionally, caution should be used when extrapolating the results of this study to the rest of the NSSU region, as Al_i levels were found to be higher in these study sites than other sites in Nova Scotia (e.g., those examined by Rotteveel et al. (2018, *in prep*). Lower Al_i levels in other sites across NSSU may result in stronger decreases in Al_i and increases in pH if liming is conducted in these sites, as seen in the L_MGB site compared to L_CC.

5.1.2 LMM/GLMM Model Evaluation

Statistical error in interpreting results was reduced through the use of LMM and GLMM models as opposed to standard parametric statistics. The most parsimonious model of variation in water chemistry parameters was selected: the maximum effects model, including variables for treatment, time, and the interaction of the two. By accounting for site-specific traits, which accounted for up to 36% of observed water chemistry variability within sites, the conclusions drawn regarding the effects of helicopter liming are more likely to pertain to the effects of liming, as opposed to site-specific variability. A potential limitation of using this design was the small sample size. However, lack of samples should bias the results toward not finding an effect (Burnham & Anderson, 2002); therefore, the findings of significant effects indicate that there were likely sufficient samples.

5.1.3 Effects of Liming

Helicopter liming significantly improved water quality in both treatment sites, even though water quality significantly worsened (i.e., decreased pH and increased Al_i) at control sites

over the same time period. Ca_t was significantly increased in treatment sites, and mean Ca_t concentration remains above the minimum water quality target of $2.0 \text{ mg}\cdot\text{L}^{-1}$ set by EIFAC (1990) for both treatment sites. The significant increase in Ca_t concentration after terrestrial limestone application has been observed in most prior liming studies (e.g., Hindar & Wright, 2005; Howells, 1995; Miller, Anderson, Harriman, & Collen, 1995; Traaen, et al., 1997; Waters, Jenkins, Staples, & Donald, 1991).

pH levels also significantly improved in treatment sites, while levels significantly decreased in control sites. However, despite the significant increase in pH, levels did not reach the minimum target of to $\geq 6.0 - 6.5$ units. Although most prior studies have seen pH increases to levels near 6.0 units soon after liming (as did this study), some studies have reported variation in pH levels during the first few years after liming (e.g., Traaen et al., 1997), with some reporting long-term stabilization around pH 5.5 similar to results seen in L_MGB (Hindar & Wright, 2005). The Hindar and Wright (2005) study was similar to this study (dolomite application at whole-catchment average of approximately $2 \text{ t}\cdot\text{ha}^{-1}$ to a 0.8 km^2 catchment) and reported similar results of rapid increases in pH after initial application to pH 6.0, with continued averages of pH 5.5. Work by Jenkins, et al., (2003) demonstrated that liming of discharge areas alone can improve surface water quality, while further work by Fransman and Nihlgård (1995) in Sweden demonstrated that a minimum dosage of $5 \text{ t}\cdot\text{ha}^{-1}$ applied to discharge areas was required to produce stable pH levels above 6.0 for more than one year. Although this study used the 2.0 WAM definition of discharge area, and exceeded the minimum dosage identified by Fransman and Nihlgård (1995), the limestone applied may have not been sufficient to mitigate the chronic freshwater acidification. Alternatively, pH response may not have been as strong during the first year post-liming due to climatic conditions reducing the effectivity of the limestone. During the post-liming time period pH decreased further in control sites, likely due to extreme dry conditions in the Spring (Government of Canada, 2018), which have been demonstrated to drive low pH conditions (Clair et al., 2007; Macleod, 2016). These same factors also influenced the treatment sites, where reduced precipitation would have led to reduced volumes of limestone being washed into the freshwater system, reducing the quantity of Ca_t available to mitigate acidification. Potentially, under normal climatic conditions, pH in treatment sites would have increased further, and potentially have exceeded the minimum threshold recommended for freshwater health.

No significant changes were observed in Al_i concentration; however, Al_i non-significantly increased in control sites, and non-significantly improved in treatment sites. Subsequently, Al_i levels have not yet decreased to the maximum level recommended for freshwater health by the EIFAC ($\leq 15 \mu\text{g}\cdot\text{L}^{-1}$). This is likely related to pH, as Al_i speciation is pH dependant, with most positively charged Al_i precipitating into bio-unavailable species at pH levels above 6.0 in colder temperatures (2°C), and at $\text{pH} > 5.5$ at warmer temperatures (25°C) (Lydersen, 1990). Water temperatures ranged from 3.0°C to 16.5°C in L_CC, and 5.2°C to 22.4°C in L_MGB, indicating that pH levels would have to be increased to between 5.5 to 6.0 units in these streams before stronger decreases in Al_i occur. L_MGB pH did remain stable above the 5.5 pH unit threshold after liming, and consequently also had a lower average Al_i level than L_CC.

Preliminary work by Dr. Kevin Keys with the NS DNR shows that although there was a significant increase in soil water Ca concentration in the B horizon, there was no change in the A horizon, and that some of the increase in the B horizon could be attributed to lateral flow. Therefore, it is predicted that as limestone further percolates into the soil, and precipitation has more time to interact with the buffering base cations before entering the waterbodies, pH levels will further increase and Al_i concentration will further decrease in treatment catchments. This prediction is supported by findings of similar results across Sweden (Holmgren, et al., 2016).

5.2 Timeseries analysis

5.2.1 Initial Response to Liming

Notably, the first response in pH level to liming was not observed until after the first substantial rain event, indicated by a rapid increase in stream depth. The peak effect of liming on freshwater acidity was not observed until after the second substantial rain event. The abrupt increase in pH indicates that the majority of limestone was indeed deposited on soil and vegetation, where precipitation washed the limestone dust out of the upper stories of the canopy onto the soils and into the surface waters. The second rain event likely washed the remainder of the limestone out of the canopy, as indicated by a further pH increase. Following the initial response of pH level to helicopter liming during the first two precipitation events, pH levels remained relatively stable throughout the fine-scale data series, with slight decreases occurring during low-flow events. Similar decreases in pH during low-flow events have been observed in multiple prior studies

(Kopáček, Hejzlar, Borovec, Porcal, & Kotorova, 2000; Macleod, 2016; Rodushkin, Moiseenko, & Kudravsjeva, 1995; Sterling, et al., in progress).

Although Ca_t and Al_i levels were not measured directly in the fine-scale data series, their levels can be inferred based on known relations between Ca, pH, and Al_i relationships. Ca^{2+} is a driver of freshwater acidity, as it is a base cation and therefore acts as a pH buffer, reducing acidity (Olem, 1991). Based on this relationship, it can be inferred that the sudden increase in pH after limestone application is due to Ca^{2+} ions being washed into the surface waters, and therefore that the first response to in-stream Ca_t levels did not occur until during the first substantial rain event after limestone application. Similarly, Al_i species can be predicted based on pH and temperature; at 2°C $\text{Al}(\text{OH})_2^+$ is dominant at pH 6.5, $\text{Al}(\text{OH})^{2+}$ between 6.5 and 5.5, and Al^{3+} below pH 5.5 (Lydersen, 1990). Since average water temperature for the fine-scale pH and water depth data was 5.41°C post liming (from October 3rd, 2016 to December 21st, 2016), it is assumed that Al_i speciation in relation to pH level is comparable to that observed by Lydersen (1990). After the first substantial precipitation event, pH in L_CC rose from between 3.7 to 4.6, to approximately 5.5, after which it decreased again to approximately 5.2. Then, following the second substantial rain event, pH increased again and remained stable around 5.5 for the remainder of 2016. Based on the pH dependent Al_i speciation observed by Lydersen (1990), the dominant Al_i species before helicopter liming was likely Al^{3+} , whereas after liming, $\text{Al}(\text{OH})^{2+}$ was likely the dominant species. pH was sufficiently raised for portion of toxic Al_i species to precipitate into the insoluble $\text{Al}(\text{OH})_4$ form, which occurs predominantly above pH 7 at 2°C (Lydersen, 1990); however, pH was not sufficiently raised for Al_i to be decreased to sub-lethal levels for juvenile Atlantic salmon. Based on these factors, the first observable *change* in Al_i species likely occurred during the first substantial rain event with small changes between the first and second rain events, with relatively stable concentrations of each species following the second rain event.

5.2.2 Ongoing Response to Liming

Post limestone application, Ca_t levels were consistently above the minimum threshold concentration of 2 mg·L⁻¹, even during the decrease in concentration seen in the April of 2017. During this month, the province of Nova Scotia received an average of 44% less rain than long-term (1981-2010) climate normal prediction (Government of Canada, 2018). Since the limestone was predominantly applied to the terrestrial area of the treatment catchments, precipitation was required for the limestone to be either washed into the surface water system, or into the soil to

percolate through to the groundwater system. Without adequate precipitation input, the limestone likely remained on the soils, with reduced interaction with surface- and ground waters, reducing mitigative potential. Following the exceptionally dry month of April, precipitation rates returned to approximately equal to, or above, long-term climate normal levels (Government of Canada, 2018). As water input increased, so did calcium concentration in both L_CC and L_MGB, indicating that limestone is continually washed into the system during precipitation events.

Similarly, after the initial increase in pH post-liming, levels dropped in spring 2017 for both treatment sites. The decrease in pH was less pronounced in L_MGB than in L_CC, additionally, following the drop, the pH of L_MGB increased more strongly and remained above 5.5 for the majority of the remainder of the sampling period. Two factors have been identified which may have influenced the lesser response of the L_CC watershed to helicopter limestone application. Firstly, a smaller percentage of watershed area was limed in L_CC than L_MGB, namely approximately 21% as opposed to 63%. Therefore, the quantity of limestone applied to L_CC may have been inadequate to promote the extent of mitigation seen in the L_MGB watershed. An overview of multiple catchment liming initiatives across Europe conducted by Brown (1988) found that liming success rates were higher with greater application rates or proportion of limed catchment area. Specifically, success rate was highest when averaged whole-catchment application dosage was $> 1 \text{ t}\cdot\text{ha}^{-1}$, which could either be achieved through local liming at higher dosage or increased treatment area (Brown, 1988). For L_MGB, the averaged whole-catchment application rate was $5.84 \text{ t}\cdot\text{ha}^{-1}$, compared to $2.16 \text{ t}\cdot\text{ha}^{-1}$ in L_CC. Although both these application rates exceed the minimum estimated levels required for success, the application dosage in L_CC may not have been sufficient to fully mitigate the acidified state of the catchment. Additionally, the inherent catchment characteristics of L_CC may have made increased the minimum dosage required to fully mitigate its acidified state. Namely, the second-most dominant bedrock type in the L_CC watershed is the Halifax Formation, which underlays approximately 22.6% of the watershed. The Halifax formation contains sulphide-bearing metasediments, which are known to produce acid drainage (ARD) when exposed to water or air (Fox, Robinson, & Zentilli, 1997). The area of L_CC underlain by the Halifax formation has multiple logging roads crossing it, thereby increasing the disturbance of the thin soil layers (Nova Scotia Department of Natural Resources, 2006), and potentially exposing the bedrock, producing ARD. Any ARD produced within the catchment would interact with either the surface-, or ground water, reducing

pH levels. This hypothesis is supported by the lower pre-liming pH of L_CC, and the findings of prior research, where pH levels as low as 3.6 attributable to ARD were recorded in Nova Scotia (Kerekes, Freedman, Howell, & Clifford, 1984). Additionally, pH levels in L_CC tended to be lower in samples collected during base flow periods, which may indicate that water with a lower pH, due to interaction with ARD-producing bedrock, is entering the streams via groundwater.

As predicted, Al_i concentration was inversely related to pH level in L_MGB, with average levels slightly above the desired maximum level of $15 \mu\text{g}\cdot\text{L}^{-1}$. Although the average Al_i concentration in L_MGB was above the desired minimum threshold, the reduction in concentration likely had positive effects on the freshwater ecosystem, such as increased productivity and survival of invertebrates (Burton & Allan, 1986; Havas & Hutchinson, 1982), a key food source for young salmon, and less sensitive fish species (Holmgren, Degerman, Petersson, & Bergquist, 2016). However, this predictable variation was not seen in L_CC. Of especial concern were the two very high Al_i spikes post-liming of $114 \mu\text{g}\cdot\text{L}^{-1}$ and $91 \mu\text{g}\cdot\text{L}^{-1}$ on June 22nd, 2017 and August 23rd, 2017. Although the average post-liming Al_i concentration was $35 \mu\text{g}\cdot\text{L}^{-1}$ in L_CC, these spikes were 7.6 and 6.1 times higher than the recommended maximum concentration. Al_i levels would have been high enough to not only cause negative morphological and physiological changes to Atlantic salmon (Kroglund F., et al., 2007), but cause asphyxiation and mortality in even the less sensitive adult fish (Exley, Chappell, & Birchall, 1991; Lacroix & Townsend, 1987). Interestingly, when Al_i levels of control sites were compared to those of the treatment sites, variation seen in L_CC was similar to C_BLB and C_UKR, where high Al_i spikes were measured on the same dates. The similar Al_i concentration variation of the L_CC site and control sites indicates that the quantity of limestone applied to the catchment was not sufficient to mitigate seasonal Al_i highs, as these peaks did not occur in the L_MGB catchment which received more limestone. Prior studies have found that Al_i peaks occur in periods of reduced precipitation; however, no meteorological or hydrological monitoring stations within sufficient proximity to the study sites had precipitation or flow data for the duration of the study, therefore, no covariation between water input events and water chemistry response could be determined. Prior work examining seasonal Al_i variation in Nova Scotia found that the lowest concentrations occurred in the spring, followed by steady increases in concentration with decreased flow and increased temperatures, with peaks in the summer and fall (Clair et al., 2007; Macleod, 2016). However, the work by Macleod (2016) contrasts with research conducted in other areas of the world, where seasonal Al_i maximums

occurred during the spring freshet in the Kola peninsula, Russia (Rodushkin, Moiseenko, & Kudravsjeva, 1995), and the winter along the Czech-German border (Kopáček, Hejzlar, Borovec, Porcal, & Kotorova, 2000). The lack of consistency in the literature regarding seasonal Al_t variation indicates that more research is needed to understand the drivers of seasonal Al_t maximums.

5.3 Spatial Analysis

The watersheds examined in this study were substantially smaller, and had Al_t levels in the than the majority of watersheds studied by Rotteveel et al. (2018, *in prep*). The higher Al_t concentration observed in the current study may be attributable to the higher percent wetland area within the studied watersheds, or the fact that the Rotteveel et al. (2018, *in prep*) study averaged Al_t values across the 2000's decade, which was found to have on average lower Al_t levels than the 2010's decade. However, despite this dissimilarity of study sites, the positive correlation between wetland area and Al_t found in the prior study was very similar to that identified by the current study. Similar observations of this correlation have been observed as far back as the 1950's in Nova Scotia, where Hayes and Anthony (1958) found that high DOC, originating from wetlands, was a stronger driver of the natural acidity of surface waters in Nova Scotia than elsewhere in North America and Europe. These natural acids are partly responsible for the complexation of metals such as Al (Altman & Buffle, 1988), and subsequently increase Al concentrations. Additionally, increased acidity, through increased DOC originating from wetlands causes increased Al solubility, resulting in increased Al transport from cation exchange sites within the soil which inevitably enter the freshwater system (Cronan & Schofield, 1979).

The similarity of the correlation between percent mature forest area and Al_t concentration of the Rotteveel (2018, *in prep*) study and this study was less pronounced, as the sites in this study had a relatively small percent mature forest area and high Al_t values compared to the prior study. Consequently, the intercept and slope of the correlation in this study are higher than the Rotteveel (2018, *in prep*) study. However, both studies indicate that Al_t increases with percent mature forest area. Higher nutrient presence in surface waters in mature forest could be indicative of increased nutrient loss from soils (Feller, 2005). Feller (2005) found that nutrient uptake increases with forest age before reaching a peak and declining in old growth forests, leading to increased nutrient presence in stream waters. Due to over 500 years of intense forestry practices in Nova Scotia

following European settlement, more than 91% of forests within the province consist of even-aged stands less than 100 years old (Nova Scotia Department of Natural Resources, 2000), indicating that the majority of the study sites in both the Rotteveel et al. (2018, *in prep*) study and this study likely fall within the establishment and thinning stages of forest succession (Spies, 1997). Additionally Lynds and Leduc (1995) define an old growth Acadian forest as older than 150 to 200 years, depending on species. The definition for mature forest used in the Rotteveel et al. (2018, *in prep*) is a stand where a minimum of 50% of trees are between 60 to 150 years old, dependant on species. Based on this categorization, the forests would not be classified as old growth, and would still be considered in the increased stages of increased nutrient absorption. Ca is an essential nutrient for tree growth, and although xylem Ca absorption is reduced in acidic soils (Bilodeau-Gauthier, Houle, Gagnon, Côté, & Messier, 2011), mature trees retain more of this limiting nutrient than younger trees (Feller, 2005). The increased retention of Ca_t in vegetation leads to decreased base cation concentration in surface waters (Feller, 2005), thereby decreasing pH, and consequently increasing Al_t (Clair et al., 2007). The higher Al_t concentrations observed at lower percent mature forest in this study is likely indicative of worse pH conditions, leading to increased Al_t mobilization from soils, alongside the effects of increased Ca_t retention within the areas of mature forest within the catchments. Prior research found that watersheds in the study area had among the lowest pH conditions, and the highest Al_t concentrations within the province, supporting current findings.

The positive correlation found between DOC and TOC and Al_t in this study is in strong agreement with findings of Rotteveel et al. (2018, *in prep*), and prior research conducted in Sweden (Erlandsson, Cory, Köhler, & Bishop, 2010). Multiple processes relate DOC and Al_t , and multiple prior studies have identified DOC as a driver of freshwater Al_t concentration. For example, Baily et al. (1995) found that, at levels as low as < 4 mM DOC played an important role in seasonal pH and Al variability by increasing Al solubility and forming organometallic colloids. Furthermore, DOC and Al undergo cotransport from soils, leading to covariation in freshwater concentration (Bailey et al., 1995; Driscoll, Lehtinen, & Sullivan, 1994). However, a decreasing effect of DOC and TOC on Al_t was found at higher concentrations.

Lastly, the positive correlation between Ca_t and Al_t found in this research is inconsistent with the findings of Rotteveel et al. (2018, *in prep*), and contrary to predictions. As Ca and Al are related through their influence on pH, where Ca increases pH and Al decreases it, relationships are

assumed to be inverse (Clair et al. 2007; Cronan and Scholfield, 1979; Erlandson et al. 2010). This research found that in sites with highest Ca_t concentrations (C_BLB and C_UKR), Al_t concentrations were also highest, although both sites had moderate Al_i concentrations. The discrepancy between findings may be due to the severity of acidification within the study sites, which may be representative of the more acidified catchments within the NSSU, as opposed to the acidification conditions across the entire province as examined by Rotteveel et al., (2018, *in prep*).

6 Conclusion

In this study, I aimed to evaluate the efficacy of the NSSA and partners helicopter liming project at mitigating the chemical effects of freshwater acidification in two tributaries to the Keef Brook watershed in West River, Sheet Harbour, Nova Scotia. This research found that helicopter liming significantly improved water quality within the treatment catchments, demonstrated by significantly increased Ca_t concentrations to above the minimum threshold recommended for freshwater ecosystem health, significantly increased pH levels, and non-significantly decreased Al_i concentrations. The reduced effects of liming on pH and Al_i concentrations were attributed to climatic variation, namely an extremely dry spring, reducing volume of limestone washed into surface waters, and reducing soil percolation to Al-rich ground waters. Via time series analysis, it was determined that Ca_t , pH, and Al_i levels were tied to precipitation events, with poorer water quality during dry/low-flow periods. However, even during low-flow periods Ca_t concentration remained above the minimum threshold. After the dry period in spring 2017, pH levels in L_CC did not recover as strongly as in L_MGB. This response was attributed to site-specific watershed characteristics, namely, ARD from an area of Halifax formation bedrock within the L_CC catchment. Similarly, helicopter liming caused a more pronounced decrease in Al_i levels in L_MGB than L_CC, with Al_i concentration variation in L-CC remaining similar to that of unlimed sites. This reduced response was attributed to a lower catchment-average concentration of limestone. The seasonal Al_i spikes observed in the fall of 2017 are in agreement to some recent research, but in disagreement with others. The lack of consistency in the literature regarding seasonal Al_i variation indicates that more research is needed to understand the drivers of seasonal Al_i maximums. This research attempted to examine the relationship between Al_i concentration and precipitation events, but was unable to do so due to a lack of available precipitation/hydrograph data. As the NSSA and partners helicopter liming project is ongoing, it is recommended that future iterations focus on collecting this data so stronger associations between precipitation events and Al_i levels can be made.

The correlations between water chemistry parameters and watershed characteristics identified by this study are generally in agreement with prior research, and that of Rotteveel et al. (2018, *in prep*). The positive correlation between percent wetland area and Al_t concentration is likely due to the high volumes of DOC produced by Nova Scotian wetlands, promoting the mobilization of Al_t . The positive correlation between percent mature forest area and Al_t

concentration likely results from increased Ca_t retention in mature trees compared to younger trees, leading to decreased pH levels and therefore mobilized Al_t . The strong association between DOC and Al_t concentration found by this research is in agreement with previous literature. The finding that high Ca_t concentration sites also have high Al_t concentrations, although not lower Al_i concentrations was surprising, and indicative of other catchment-scale processes influencing water chemistry parameters. It is recommended that additional research be conducted examining the watershed-scale drivers of water chemistry, especially in catchments which seemingly defy the current model of water chemistry relations.

Based on these initial findings, helicopter liming seems to be an effective method for freshwater acidification mitigation, although further research is required to determine best practices for liming the unique watersheds of Nova Scotia. The reduced efficacy seen in the spring of 2017 and in the L_CC could be attributed to climatic and watershed-specific variation, with prior research suggesting that it may take additional time for the full effect of helicopter liming on water chemistry and the freshwater ecosystem to become apparent. Therefore, the reduced results in these circumstances should not discourage future liming initiatives, as failing to undertake freshwater acidification mitigation initiatives will likely lead to further decline and potential extirpation of the economically, ecologically, and culturally significant Atlantic salmon subpopulations in Nova Scotia. Furthermore, the 73% to 87% likelihood of extirpation within the next 45 years of the Atlantic salmon can be reduced to just 21% with a mere 20% improvement in habitat quality. By continuing to initiate and improve freshwater acidification mitigation initiatives the genetically unique Atlantic salmon subpopulations are more likely to survive in the naturally acidic waters of Nova Scotia, where other Atlantic salmon cannot survive. Thereby a culturally significant species to the Mi'kmaq people is retained, preventing further heritage losses for the marginalized communities. Lastly, preventing the extirpation-, and promoting the recovery of Atlantic salmon via liming projects such as this one, the multimillion dollar fishing market in Nova Scotia is maintained, and potentially further increased.

7 References

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8 Appendices

8.1 Laboratory Detection Limits

Table 7 identifies the respective chemistry parameter detection limits for Maxxam Analytic Laboratory, the Dalhousie University Health and Environment Research Center, and AGAT Laboratory.

Table 7 Laboratory detection limit comparison.

Chemistry Parameter	Units	Value		
		HERC	Maxxam	AGAT
pH	$\mu\text{g}\cdot\text{L}^{-1}$	n/a	n/a	n/a
DOC	$\text{mg}\cdot\text{L}^{-1}$	n/a	0.50	n/a
TOC	$\text{mg}\cdot\text{L}^{-1}$	n/a	n/a	0.5
SO ₄	$\mu\text{g}\cdot\text{L}^{-1}$	10.00	n/a	2000
Al _d	$\mu\text{g}\cdot\text{L}^{-1}$	n/a	5.00	5
Al _t	$\mu\text{g}\cdot\text{L}^{-1}$	n/a	5.00	5
Al _o	$\mu\text{g}\cdot\text{L}^{-1}$	n/a	5.00	5
Ca _t		n/a	100 $\mu\text{g}\cdot\text{L}^{-1}$	0.1 $\text{mg}\cdot\text{L}^{-1}$
Ca _d	$\mu\text{g}\cdot\text{L}^{-1}$	n/a	100	100

8.2 Data Sources

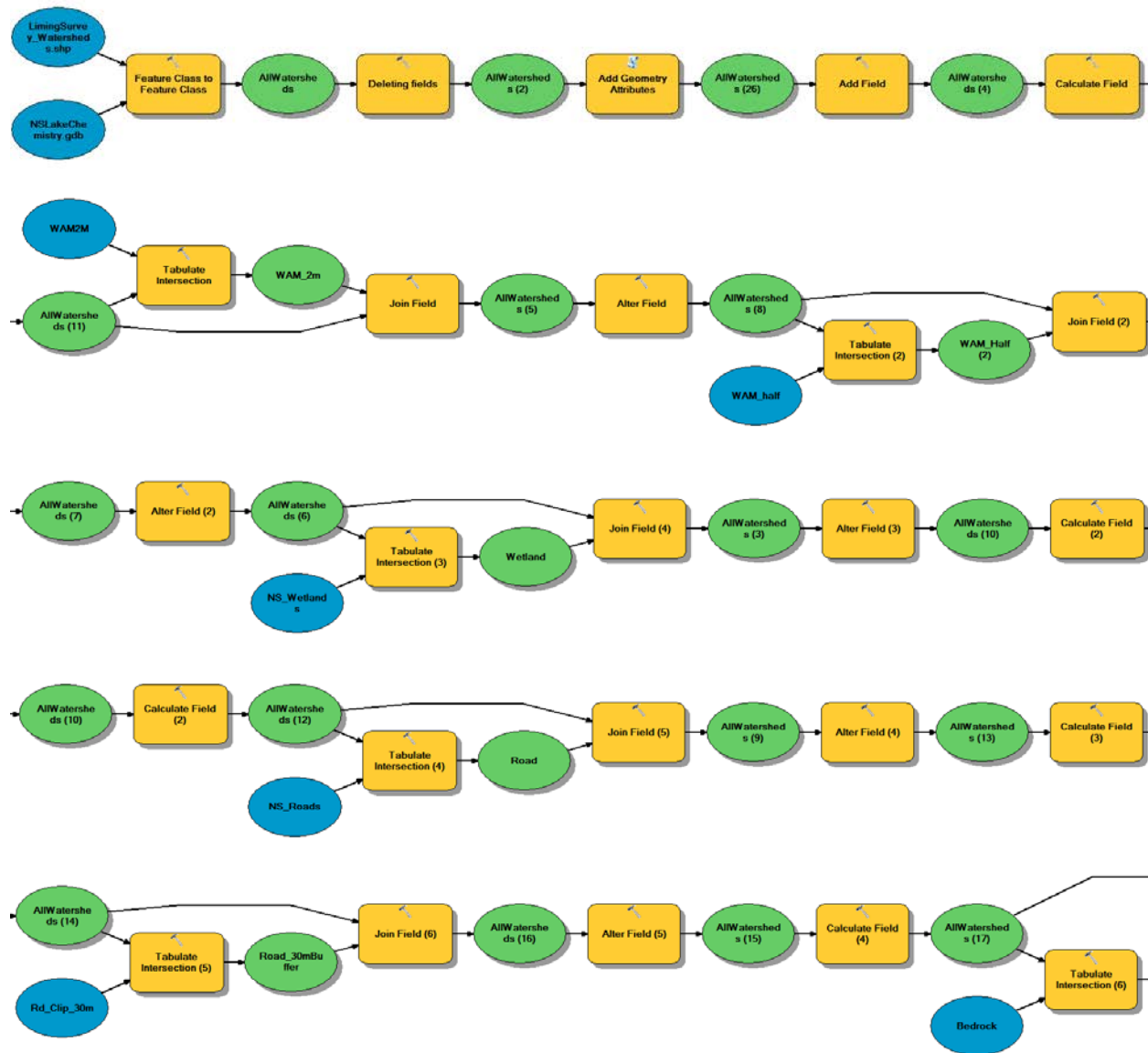
Table 8 identifies the sources of the geospatial data used for watershed characterization; table adapted from Sterling et al. (2014).

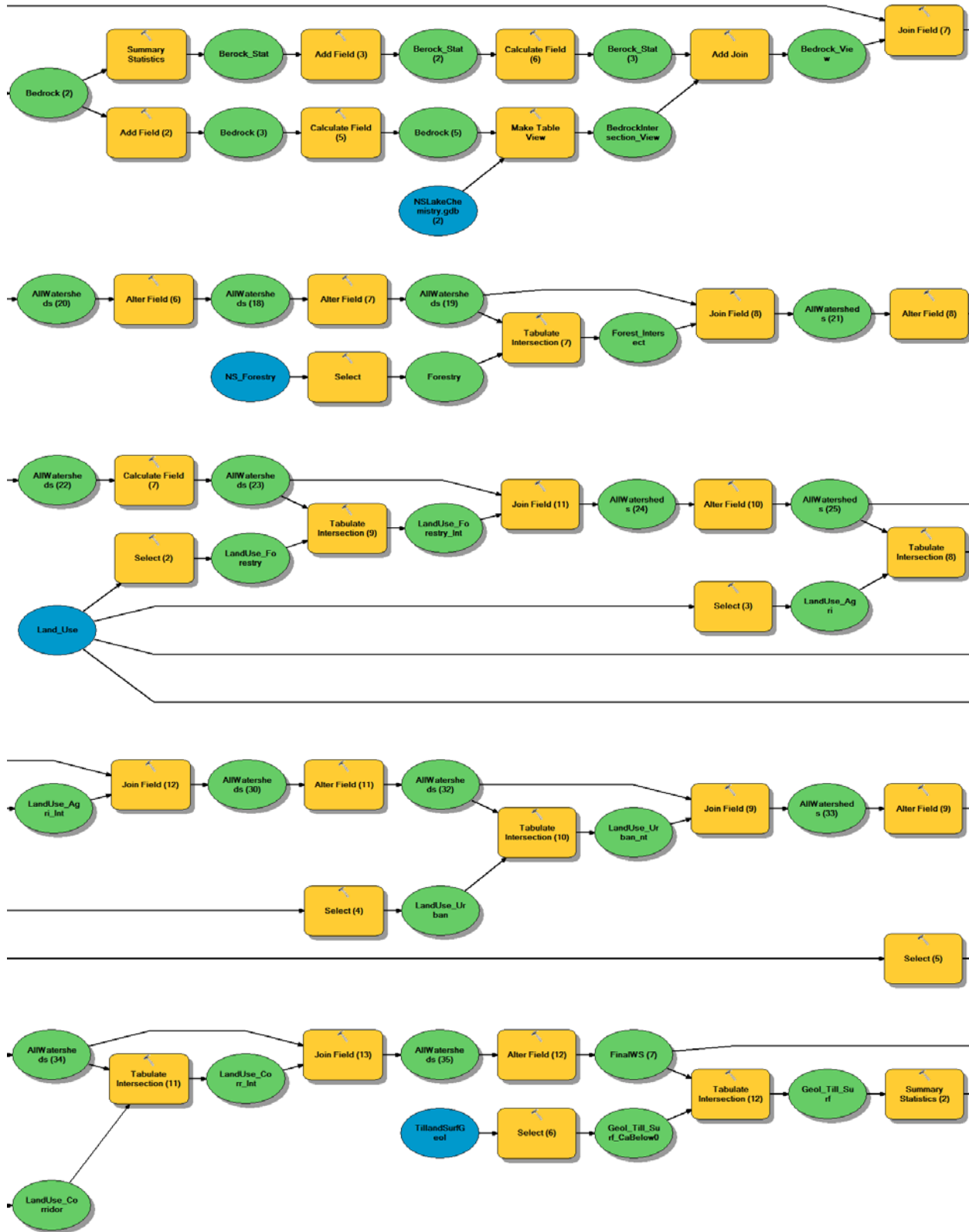
Table 8 Data sources used for spatial analysis; table adapted from Sterling et al. (2014).

Data Layer	Type	Description	Most Recent Update	Source
NS Topographic Database	Road network, contour lines, stream network, waterbodies	Elevation, roads, rails, rivers, streams, lakes, bogs, and swamps	2012	Nova Scotia Topographic Database (NSTDB) Copyright Her Majesty the Queen in Right of the Province of Nova Scotia
NS Forestry Landcover	Land cover	Land cover classes (e.g., Forested, Urban, Wetland, or Barren)	2006	Nova Scotia Department of Natural Resources
Wetland	Wetland	Marshes, swamps, saltmarshes, open waters, bogs, or fens	n/a	Nova Scotia Department of Natural Resources
NS_Agri_Inventory	Agricultural	Agricultural landuse within NS	1997	Agricultural Lands Inventory Program
Geology	Surficial	Surficial geology for the province of NS	2006	Province of Nova Scotia, compiled by R. R. Stea, H. Conley and Y. Brown, 1992
	Bedrock	Bedrock geology for the province of NS	2006	Province of Nova Scotia, compiled by J. D. Keppie, 2000
RLUL	Landuse	Multiple landuse types (e.g., parks, or wilderness sites)	n/a	Province of Nova Scotia
DEM_20m	Elevation raster	Digital elevation model of NS with 20m x 20m resolution	2006	Nova Scotia Environment
ns_satharv	Forestry	Forest harvesting data from the 1980s to 2006	2006	Nova Scotia Environment
IDW_Alk	Acid Neutralization Capacity (ANC)	Gran titration ANC interpolated from stream measurements throughout Nova Scotia	2007	Thomas A. Clair, Environment Canada

8.3 Spatial Analysis ArcGIS Model

GIS model developed by Rotteveel et al. (2018, *in prep*) was used to overlay landuse data with the delineated watersheds to determine the percentage of watershed underlain by a 2.0 m Wet Area Model (WAM), 0.5 m WAM, wetlands, roads, roads located within 30 m of streams, mature forest, forestry lots, agricultural land, urban land, and corridors (Figure 15). Model was also used to identify the dominant bedrock underlying the sites, and the average geological Ca level.





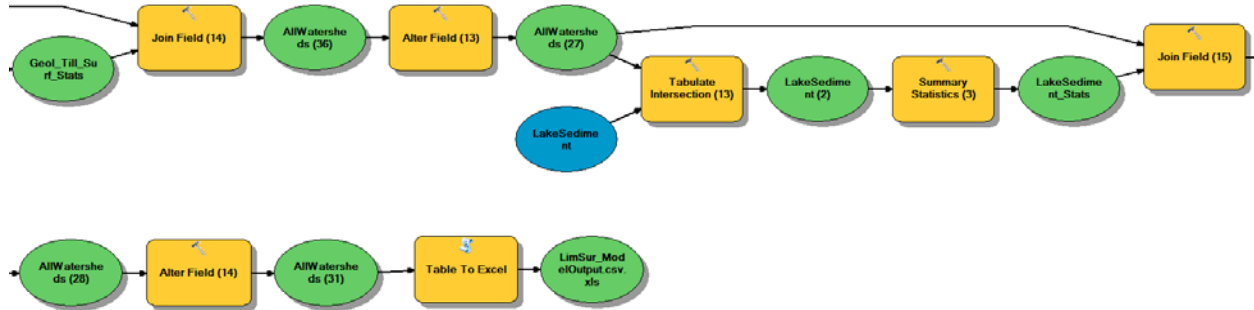


Figure 15 Spatial analysis model. Due to spatial limitations, model was split; each line of commands feeds into the following line of commands. Model executed in ArcGIS 10.3.

8.4 LMM/GLMM Analysis R Script

```

# OBJECTIVE
# examine variation in pH, Ca, Ali,
# and DOC in relation to explanatory
# variables
# and controlling for intrinsic
# variability associated with sampling
# sites.

require(car)
require(MASS)
require(bbmle)
library(MuMIn)
library(lme4)
library(lsmeans)

# _____
# Import and format data

setwd("C:/Users/Bouwien/Dropbox/DHRG
_New/LimingSurvey/LimSur_Data/LimSur
_LMMAnalysis/LimSur_Analysis_LMM")
dat <-
read.csv("LimSur_LMM_Input.csv")
# Ensure all fixed and random
# effects are defined as factors
str(dat)

# _____
# CALIBRATED pH
# _____
# STEP 1: Check Data distribution

par(mfrow=c(3,1),mar=c(1,1,1,1),oma=
c(1,1,1,1))
# Normal distribution
qqp(dat$Calib_pH, "norm")
# Decent fit

# Log-normal distribution
qqp(dat$Calib_pH, "lnorm")
# Not great fit

# Gamma distribution cannot be fit
# to a data matrix with missing
# values, so omit
# missing values
subset<-dat[complete.cases(dat[,
5]), ]
gamma <- fitdistr(subset$Calib_pH,
"gamma")
qqp(subset$Calib_pH, "gamma", shape
= gamma$estimate[[1]], rate =
gamma$estimate[[2]])

# Decent fit

# CONCLUSION:
# assume a normal fit given that the
# gamma and normal fit approximately
# the same
# modelling using the
# normal/Gaussian distribution is
# easier

# _____
# STEP 2 Modelling

# Because gaussian distribution with
# an identity link is assumed, use
# lmer()
# lmer() not applicable to other
# distribution types

h1<-lmer(formula = Calib_pH ~ Treat
+ Bef_Aft + Treat:Bef_Aft + (1 |
Site), data = dat)
# NOTE: this notation
# (Treat*Bef_Aft) of random effect is
# for common slope but
# varying intercept at each site
# (baseline differences in pH at each
# site but
# same response to treatment)

h2<-lmer(formula = Calib_pH ~ Treat
+ Bef_Aft + (1 | Site), data = dat)
h3<-lmer(formula = Calib_pH ~ Treat
+ (1 | Site), data = dat)
h4<-lmer(formula = Calib_pH ~
Bef_Aft + (1 | Site), data = dat)

null<-lmer(formula = Calib_pH ~ 1 +
(1 | Site), data = dat)

anova(null,h1)

# CONCLUSION: h1 model fits data
# better than null model

# _____
# STEP 3 Calculate R2 from max model

# Use built-in functionality of
# MuMIn() to calculate marginal and
# conditional
# R2 following Nakagawa and
# Schielzeth (2012): MEE 4(2).
r.squaredGLMM(h1)

```



```

# R2 of random effects =
[conditional R2]-[marginal R2]
# marginal R2 describes the
proportion of variance explained by
the fixed
# factor(s) alone
# conditional R2 describes the
proportion of variance explained by
both the
# fixed and random factors:

# CONCLUSION: 36% of random
variability due to inherent site
characteristics, so
# accounting for them is a good idea

# _____
# STEP #4 Model pool and model
averaging

#Create AIC table
AICctab(h1,h2,h3,h4,null, base = T,
weights = T, logLik=T)
# Creation of model-averaged
parameter estimates for all
candidate models within
# 5 AIC units (dAIC) of the most
parsimonious candidate model.

summary(h1)
coef(h1)

# CONCLUSION: maximal model (all
parameters and interactions) is the
best model
# Indication that BLB and CC were
very similar during 'pre-liming' as
were MacGB
# and UKR (treatment and control
sites were similar pre-liming)

# _____
# STEP 5 Post-Hoc Analyses

lsmeans(h1, pairwise ~ Treat *
Bef_Aft)

# CONCLUSION: Control sites showed
significantly lower pH following
liming
# (Tukey adjusted comparison,
df=39.31, t=-3.21, p=0.013) and was
on average
# 0.25 +/- 0.08 (estimate +/- se) pH
units lower following liming.

# Limed sites showed significantly
higher pH following liming (Tukey
adjusted
# comparison, df=39.24, t=8.06,
p<0.001)and was on average 0.65 +/-
0.08
# (estimate +/- se) pH units higher
following liming.

# _____
# Inorganic Aluminum

# _____
# STEP 1 Check Data distribution

par(mfrow=c(3,1),mar=c(1,1,1,1),oma=
c(1,1,1,1))
# Normal distribution
qqp(dat$Al_I, "norm")
# Not great

# Log-normal distribution
qqp(dat$Al_I, "lnorm")
# Terrible

# gamma distribution cannot be fit
to a data matrix with missing
values, omit
#missing values
subset<-dat[complete.cases(dat[,
10]), ]
gamma <- fitdistr(subset$Al_I,
"gamma")
qqp(subset$Al_I, "gamma", shape =
gamma$estimate[[1]], rate =
gamma$estimate[[2]])
# Not great

# CONCLUSION: Neither normal or
gamma distributions fit all that
great; roughly
# the same with the 9 highest values
deviating from the assumed
distribution.
# Therefore, assume a normal fit
given that the gamma and normal fit
approx the
# same and it is easier to model
normal/gaussian distribution

# _____
# STEP 2 Modelling

# Because Gaussian distribution with
an identity link is assumed, use
lmer()

```

```

# lmer() not applicable to other
distribution types

h1<-lmer(formula = Al_I ~ Treat +
Bef_Aft + Treat:Bef_Aft + (1 |
Site), data = dat)
h2<-lmer(formula = Al_I ~ Treat +
Bef_Aft + (1 | Site), data = dat)
h3<-lmer(formula = Al_I ~ Treat + (1
| Site), data = dat)
h4<-lmer(formula = Al_I ~ Bef_Aft +
(1 | Site), data = dat)

null<-lmer(formula = Al_I ~ 1 + (1 |
Site), data = dat)

anova(null,h1)

# CONCLUSION: There is no
significant difference between h1
and null model

# _____
# STEP 3 Calculate R2 from max.
model

r.squaredGLMM(h1)

# CONCLUSION: variability associated
with site-specific traits accounts
for 20%
# of overall variability

# _____
# STEP 4 Model pool and model
averaging

# Create AIC table
AICctab(h1,h2,h3,h4,null, base = T,
weights = T, logLik=T)

#CONCLUSION: maximal model (all
parameters and interactions) is the
best model

summary(h1)
coef(h1)

# CONCLUSION: CC was an extreme
site. UKR is right in between MacGB
and BLB.

# _____
# STEP 5 Post-Hoc Analyses

lsmeans(h1, pairwise ~ Treat *
Bef_Aft)

# CONCLUSION: The most parsimonious
model was that with full interaction
# between Bef_Aft and treatment
(AICc = 440.5, weight = 0.98, table
X), however
# there remains a large amount of
unexplained variability as evidenced
by the
# low overall conditional R2 (27%).

# Control sites showed a non-
significant increases inorganic Al
following liming
# (Tukey adjusted comparrison,
df=42.74, t=1.76, p=0.303)and was
estimated to
# increase 19.80 +/- 11.21 ug/L
(estimate +/- se) following liming.

#Limed sites showed a non-
significant decrease in inorganic Al
following liming
# (Tukey adjusted comparrison,
df=42.74, t=-1.04, p=0.73) and was
estimated to
# decrease by 11.69 +/- 11.21 ug/L
(estimate +/- se) following liming.

# _____
# CALCIUM
# _____
# STEP 1 Check Data distribution

par(mfrow=c(3,1),mar=c(1,1,1,1),oma=
c(1,1,1,1))
# Normal distribution
qqp(dat$Ca_TD, "norm")
# Not great

# Log-normal distribution
qqp(dat$Ca_TD, "lnorm")
# Good

gamma <- fitdistr(subset$Ca_TD,
"gamma")
qqp(subset$Ca_TD, "gamma", shape =
gamma$estimate[[1]], rate =
gamma$estimate[[2]])
# Not great

# CONCLUSION: Assume a log-normal
fit

# _____

```

```

# STEP 2 Modelling

h1<-glmer(formula = Ca_TD ~ Treat +
Bef_Aft + Treat:Bef_Aft + (1 |
Site), data = dat,
family=gaussian(link = "log"))

h2<-glmer(formula = Ca_TD ~ Treat +
Bef_Aft + (1 | Site), data = dat,
family=gaussian(link = "log"))
h3<-glmer(formula = Ca_TD ~ Treat +
(1 | Site), data = dat,
family=gaussian(link = "log"))
h4<-glmer(formula = Ca_TD ~ Bef_Aft
+ (1 | Site), data = dat,
family=gaussian(link = "log"))

null<-glmer(formula = Ca_TD ~ 1 + (1
| Site), data = dat,
family=gaussian(link = "log"))

anova(null,h1)

#CONCLUSION: h1 fits data better
than null model

#_____
# STEP 3 Calculate R2 from max model

#Not appropriate for glmer

#_____
# STEP 4 Model pool and model
averaging

# Create AIC table
AICctab(h1,h2,h3,h4,null, base = T,
weights = T, logLik=T)

# CONCLUSION: maximal model (all
parameters and interactions) is the
best model

summary(h1)
coef(h1)

# CONCLUSION: Shows no difference
between sites pre-liming (later
backed up by
# post-hoc Tukey multiple
comparrisons)

#_____
# STEP 5 Post-Hoc Analyses

lsmeans(h1, pairwise ~ Treat *
Bef_Aft)

# CONCLUSIONS: The most parsimonious
model was that with full interaction
# between Bef_Aft and treatment (AICc
= 85.5, weight = 0.996, table X),
and there
# was bit support for the null model
based on AIC ranking.

# Control sites showed no change in
calcium concentration following
liming
# (Tukey adjusted comparrrison,
z=0.00, p=1.00).
# Limed sites showed a significant
increase calcium concentration
following
# liming (Tukey adjusted comparrrison,
z=5.49, p <0.01) and was estimated
to
# increase by 1.57 +/- 0.29 ug/L
(estimate +/- se) following liming.

```

8.5 Water Chemistry Data Distribution

Ca_t data fit a lognormal distribution best (Figure 16), while calibrated pH data, and Al_i data fit a normal distribution best (Figure 17, and Figure 18, respectively).

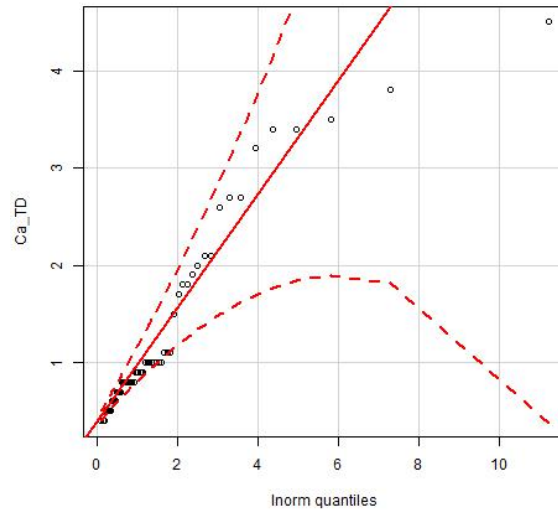


Figure 16 Ca_t data fit lognormal distribution best. Y-axis values displayed as $mg \cdot L^{-1}$.

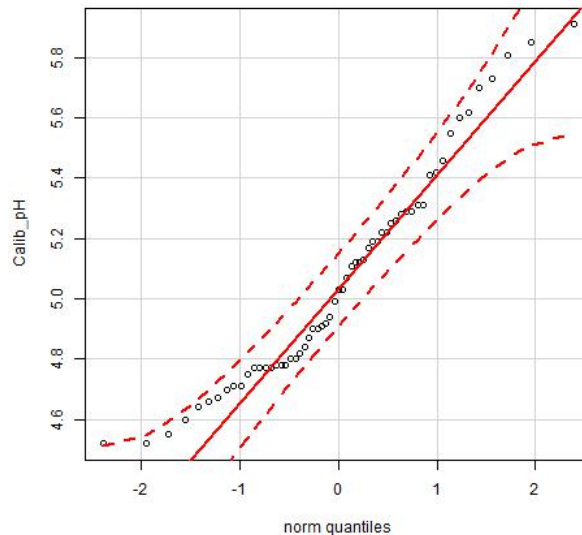


Figure 17 Calibrated pH data fit normal distribution best. pH data calibrated using the following calibration curve: $True\ pH = 0.595(Measured\ pH) + 2.3868$. X-axis values displayed as pH units.

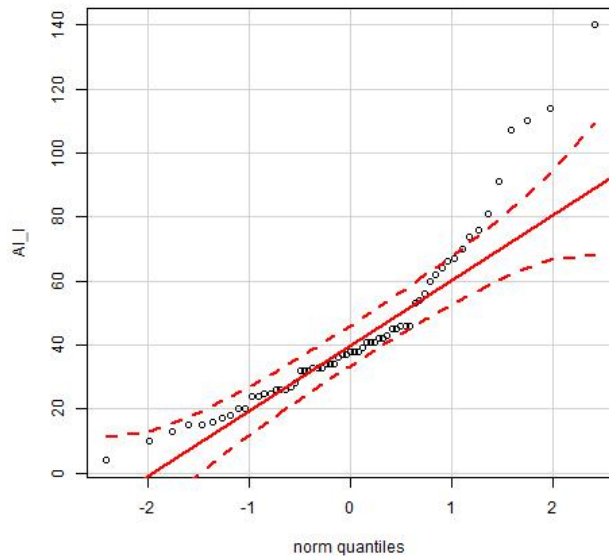


Figure 18 Al_i data fit normal distribution best. One unrealistic data point removed (Site: C_BLB, date: 2016-08-05, value: $-6 \mu\text{g}\cdot\text{L}^{-1}$). X-axis values displayed as $\mu\text{g}\cdot\text{L}^{-1}$.

8.6 Spatial Analysis Model Output

The spatial analysis model of the study sites was run in ArcGIS 10.3, output is displayed in Table 9.

Table 9 Spatial analysis model output

Site	Treatment	Main Watershed	Catchment Area (km ²)	Wetland Area (%)	WAM 2 m (%)	WAM 0.5 m (%)	Road Cover (%)	Roads Within 30 m of Streams (%)	Mature Forest Area (%)	Forestry Area (%)	Agricultural Area (%)	Urban Area (%)	Mean Stream Ca Concentration (ppm)
C_BLB	Control	C_BLB	1.29	35.39	13.02	2.72	0.00	0.00	23.04	31.04	0.00	0.00	1280
C_UKR	Control	C_BLB	36.79	59.73	26.94	15.32	0.04	0.02	39.56	19.01	0.00	0.00	1660
L_CC	Limed	KB	0.65	43.44	13.18	13.14	0.00	0.00	22.36	10.04	0.00	0.00	1980
L_MGB	Limed	KB	0.73	46.50	20.02	13.37	0.00	0.00	30.27	20.71	0.00	0.00	7040
CB	Blow-out	KB	0.61	40.57	18.38	9.33	0.00	0.00	27.93	6.59	0.00	0.00	2780