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<https://doi.org/10.37099/mtu.dc.etds/492>

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EFFECTS OF CHEMICAL DEICERS ON AMPHIBIAN COMMUNITIES

By
Meagan Leigh Harless

A DISSERTATION
Submitted in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
(Biological Sciences)

MICHIGAN TECHNOLOGICAL UNIVERSITY

2012

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This dissertation, "Effects of Chemical Deicers on Amphibian Communities," is hereby approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCES.

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Preface

The article presented in Chapter 2 of this dissertation is used with permission from John Wiley and Sons as a collaborative effort with co-authors Drs. Casey Huckins, Jacqueline Grant, and Thomas Pypker. All data was collected on lands owned by the Keweenaw Bay Indian Community (KBIC, with permission from P. Nankervis) and the campus of Michigan Technological University in the laboratories of Drs. Grant, Huckins, and Pypker. My personal contribution to the article included collection of test organisms, completing the experiments, data analysis, and interpretation. I had a significant role in the authorship of the article. My collaborators contributed to the article through providing assistance with experimental design, data analysis, and manuscript preparation. See Appendix A for documentation of permission to republish this material.

Acknowledgements

I could not have completed this work without the loving support of a number of amazing people in my life. Few people are as lucky as I am to have such a great support network in both their personal and professional lives. Firstly, my family has encouraged and supported me in all of my professional and academic endeavors. My parents, Lon and Joan, as well as my sister, Nicole, are always there to lend an ear to my struggles and to celebrate with me when I accomplish my goals. My family never ceased to encourage me in my progress, even though they may not understand what I am doing with my career. Thank you for all of your love and unwavering support over the years.

Secondly, I certainly would not be here if not for my fabulous committee members. I would like to thank Dr. Jackie Grant for bringing me to MTU to work on such a great research project and providing excellent advice in the early stages of my Ph.D. I learned a great deal about academia when working with Jackie. I would also like to commend Dr. Tom Pypker for stepping in to advise me on this project. Tom brought a fresh perspective to the project and has provided a wealth of professional advice. Dr. Casey Huckins has played a large role in the success of this project. He has given me the two most important components of this Ph.D.: lots of lab space to conduct my research and the best mentoring I have ever experienced. Dr. Huckins is a kind and thoughtful person with a quick wit and a wealth of knowledge on everything from music to ecological theory. His compassion for his students, coupled with his diplomatic approach to mentoring, creates one of the greatest Ph.D. advisors. Dr. Huckins has not only provided me with a wealth of teachable moments, but also with personal advice which I have learned a great deal from on this journey. I have him to thank in large part for my successes in completing my dissertation and becoming an effective instructor. I hope to one day be at least half of the mentor that he is. Last but not least, I would like to sincerely thank Dr. Amy Marcarelli for her contribution to this research in providing a broader perspective on the project, lab equipment, and honest answers to my many professional dilemmas. I am indebted to these wonderful mentors for their very kind and continued support. I feel awkward using “I” throughout this dissertation because it certainly was a team effort.

I would certainly not be here without the encouragement of my Master's advisor, Dr. Bill Hayes. Bill not only helped me a great deal (both professionally and personally) in completing my M.S., but he also completed a very hard feat: encouraging me and boosting my self-confidence enough to where I believed in myself. I am eternally grateful for our many conversations and owe this accomplishment to him.

My involvement in the NSF GK12 Global Watershed Program through the guidance of Dr. Alex Mayer was a breath of fresh air in my time here at MTU. I would like to thank Alex for inviting me to participate in this program. Under his leadership, I have dramatically improved my communication skills and have gained a better understanding of the importance of science communication. Alex was a great mentor in this program and my experiences have opened many doors for future career plans. In this program I worked closely with Jean Dunstan and the wonderful teachers at E.B. Holman School. Jean allowed me to find my footing in teaching middle school science. I owe her a great deal in demonstrating how to be an effective and compassionate instructor. Her dedication to her class and her continuing drive to improve the experiences of her students are commendable traits. I will always remember the lessons that she has taught me. The teachers at E.B. Holman were wonderfully supportive and took the time to teach me about classroom management. I could not have asked for a greater school to be involved with. The middle school students were a joy to work with and learn alongside. Being in their classroom has reminded me why what I do as a scientist is so valuable.

And there are also many friends, fellow graduate students, community partners, and faculty to thank for their contributions to my success. Dr. Datta, Kefeng Li, and Ramana Reddy provided lab equipment and expertise to complete the ion chromatography analysis. Pam Nankervis in the KBIC was integral in helping me locate study sites and very excited about my research. My best friend Michelle Holiday-Crandall provided invaluable advice and constant love to keep me afloat. She is a rare friend and I am fortunate to have her in my life. I am indebted to Dr. Sarah Kiemle, Stephanie Olgren, Marty Holtgren, Rita Koch, and Yiru Chen for their friendship and for working through

challenging times with me. Kim, Mark, and Lori Joki were my family away from home. Thank you for the many delicious dinners and for taking care of me when I needed it. Tim, Sandy, Sam, and Rose helped keep me sane with many meetings over board games and adult beverages. And finally, thanks to my four-legged friends Yukon and Tala for unconditional love, endless spirit, and for keeping me moving. You are the cutest stress relievers anyone could ask for. And for my three-legged friends, thank you to Gimpy, my box turtle, for your patience and standing (mostly brumating) by me over the past 17 years. I blame you for my fondness for the cold-blooded.

I am grateful to the following undergraduate research assistants for lending a hand in this project: E. Gorsalitz, K. Griffith, M. Mitchell, H. Erickson, E. Turos, N. DelPiccolo, D. Mudahl, E. Rogers, R. Alger, and many others who have braved formalin infused dead froglets and plenty of ticks in the name of science. The following organizations provided funding for this project: the DeVlieg Foundation, the Chicago Herpetological Society, the Western New York Herpetological Society, the Amphibian Specialist Group of the International Union for Conservation of Nature and Natural Resources, the Ecosystem Science Center at Michigan Technological University, Michigan Tech Transportation Institute, support from the MTU Global Watershed GK-12 program, and support from both the Forest Resources and Environmental Science as well as the Department of Biological Sciences at MTU.

List of Abbreviations

<i>A</i>	<i>Ambystoma</i>
ANOVA	analysis of variance
ASTM	American Society of Testing Materials
<i>B</i>	<i>Bufo</i>
BOD	biochemical oxygen demand
BPA	bisphenol A
COD	chemical oxygen demand
C ₈ H ₁₂ CaMgO ₈	calcium magnesium acetate
C ₄ H ₆ CaO ₄	calcium acetate
C ₄ H ₆ MgO ₄	magnesium acetate
CH ₄ N ₂ O	urea
CH ₃ OH	methanol
Ca ²⁺	calcium ion
CaCl ₂	calcium chloride
CALTRANS	California Department of Transportation
CH ₃ COOK	potassium acetate
CHNaO ₂	sodium formate
Cl ⁻	chloride ion
CMA	calcium magnesium acetate
DBP	dibutyl phthalate
HCO ₃	bicarbonate ion
K ⁺	potassium ion
KBIC	Keweenaw Bay Indian Community
KCl	potassium acetate
<i>L</i>	<i>Lithobates</i>
LC ₅₀ or LC ₅₀	median lethal concentration
LC ₅₀ _{24-h}	median lethal concentration after 24 hours of exposure
LC ₅₀ _{48-h}	median lethal concentration after 48 hours of exposure
LC ₅₀ _{72-h}	median lethal concentration after 72 hours of exposure

LC50 _{96-h}	median lethal concentration after 96 hours of exposure
Mg ²⁺	magnesium ion
MgCl ₂	magnesium chloride
MTU	Michigan Technological University
Na ⁺	sodium ion
NaCl	sodium chloride, road salt
NDOT	Nevada Department of Transportation
NOAA	National Oceanic and Atmospheric Administration
<i>R</i>	<i>Rana</i>
S.E.	standard error
SO ₄ ²⁻	sulfate ion
SVL	snout-vent length
TDS	total dissolved solids
TL	total length
USEPA	United States Environmental Protection Agency

Abstract

Roads and highways present a unique challenge to wildlife as they exhibit substantial impacts on the surrounding ecosystem through the interruption of a number of ecological processes. With new roads added to the national highway system every year, an understanding of these impacts is required for effective mitigation of potential environmental impacts. A major contributor to these negative effects is the deposition of chemicals used in winter deicing activities to nearby surface waters. These chemicals often vary in composition and may affect freshwater species differently. The negative impacts of widespread deposition of sodium chloride (NaCl) have prompted a search for an ‘environmentally friendly’ alternative. However, little research has investigated the potential environmental effects of widespread use of these alternatives.

Herein, I detail the results of laboratory tests and field surveys designed to determine the impacts of road salt (NaCl) and other chemical deicers on amphibian communities in Michigan’s Upper Peninsula. Using larval amphibians I demonstrate the lethal impacts of a suite of chemical deicers on this sensitive, freshwater species. Larval wood frogs (*Lithobates sylvatica*) were tolerant of short-term (96 hours) exposure to urea ($\text{CH}_4\text{N}_2\text{O}$), sodium chloride (NaCl), and magnesium chloride (MgCl_2). However, these larvae were very sensitive to acetate products ($\text{C}_8\text{H}_{12}\text{CaMgO}_8$, CH_3COOK) and calcium chloride (CaCl_2). These differences in tolerance suggest that certain deicers may be more harmful to amphibians than others.

Secondly, I expanded this analysis to include an experiment designed to determine the sublethal effects of chronic exposure to environmentally realistic concentrations of NaCl on two unique amphibian species, *L. sylvatica* and green frogs (*L. clamitans*). *L. sylvatica* tend to breed in small, ephemeral wetlands and metamorphose within a single season. However, *L. clamitans* breed primarily in more permanent wetlands and often remain as tadpoles for one year or more. These species employ different life history strategies in this region which may influence their response to chronic NaCl exposure. Both species demonstrated potentially harmful effects on individual fitness. *L. sylvatica* larvae had a

high incidence of edema suggesting the NaCl exposure was a significant physiologic stressor to these larvae. *L. clamitans* larvae reduced tail length during their exposure which may affect adult fitness of these individuals.

In order to determine the risk local amphibians face when using these roadside pools, I conducted a survey of the spatial distribution of chloride in the three northernmost counties of Michigan. This area receives a relatively low amount of NaCl which is confined to state and federal highways. The chloride concentrations in this region were much lower than those in urban systems; however, amphibians breeding in the local area may encounter harmful chloride levels arising from temporal variations in hydroperiods. Spatial variation of chloride levels suggests the road-effect zone for amphibians may be as large as 1000 m from a salt-treated highway.

Lastly, I performed an analysis of the use of specific conductance to predict chloride concentrations in natural surface water bodies. A number of studies have used this regression to predict chloride concentrations from measurements of specific conductance. This method is often chosen in the place of ion chromatography due to budget and time constraints. However, using a regression method to characterize this relationship does not result in accurate chloride ion concentration estimates.

Chapter 1: Introduction and Review of the Ecological Effects of Chemical Deicers

Introduction

Roads and highways are known to have a substantial impact on the surrounding ecosystem (Trombulak and Frissell 2000, Forman et al. 2002), influencing the ecological processes in an estimated one-fifth of the land area of the United States (Forman 2004). Roads negatively affect animal populations in a number of direct and indirect ways. Directly, wildlife mortality results from road construction and vehicular collisions. The establishment of a road also drastically alters the physical environment, acting as a barrier fragmenting habitat and animal populations. Indirectly, roads are known to modify animal behavior through shifts in home range, changes in movement patterns, alteration of the animal's physiological state, and decreased reproductive success. In addition, roads may facilitate the introduction of non-native species or act as a source of pollutants from vehicles and maintenance activities (Trombulak and Frissell 2000).

Road-based contaminants often have cascading effects through terrestrial and aquatic ecosystems. These contaminants are deposited onto roads primarily through vehicles and road management activities, but may come from diverse sources (Forman et al. 2002). Vehicles contribute a majority of road-based pollutants into the environment, including mineral nutrients, heavy metals, and organic compounds (Gales and VanderMeulen 1992). In addition, management activities such as winter maintenance (e.g., road deicing) and herbicide application contribute a substantial additional amount of pollutants. Of these pollutants, chemicals used in road deicing activities are applied heavily and recognized as a growing threat to ecosystem health (Trombulak and Frissell 2000; Environment Canada 2001; Ramakrishna and Viraraghavan 2005).

In cold climates, safe winter driving relies on the application of chemical products to remove and prevent snow and ice from adhering to road surfaces. Since the 1930s, the most common de-icing product used on roads in the United States is sodium chloride

(NaCl; henceforth, road salt) due to its low cost and widespread availability. In North America, an estimated 10 million tons of road salt is used each year in the United States (D'Itri 1992), ranging from 0.3 to 17.6 tons per lane mile across 26 states (National Research Council 1991), with an estimated 4.7 million tons used annually in Canada (Environment Canada 2001). When road salt mixes with water on a paved surface, it separates into its sodium and chloride ions to form a brine solution. This solution has a lower freezing point than pure water and penetrates through the ice and snow to prevent ice from bonding to the pavement surface (Gales and VanderMeulen 1992).

In addition to road salt, a number of different chemicals may be used or added to aid in the deicing process. For example, inorganic salts may also be used separately or in conjunction with road salt (e.g., CaCl_2 , MgCl_2 , and KCl ; Environment Canada 2001). Abrasives such as sand, dirt, and cinder are frequently added to road salt to increase friction between tires and the pavement surface (Jones et al. 1992). Maintenance often requires the addition of anti-caking agents, such as sodium ferrocyanide (Paschka et al. 1999), cyanide (Oberts 1986), chromium, and ferric ferrocyanide (Gales and VanderMeulen 1992). Because salt corrodes airplane components, deicing of airplanes is performed using large quantities of urea in Canada (Hassan et al. 2002) and different forms of glycol in the United States (D'Itri 1992; Ramakrishna and Viraraghavan 2005). Much like road salt, these materials often contain additional contaminants and adhesives such as ethylene oxide, dioxane, acetaldehyde, and rust inhibitors (Hassan et al. 2002).

Environmental contamination by road salt depends greatly on the amount used and the method of application. Road salt may enter the environment through the following methods (Burtwell 2001; *reviewed in* Forman 2004):

1. Traditional salting practices in which salt lands onto or bounces from the road onto the roadside vegetation;
2. By being splashed or thrown off to the roadside through the action of passing vehicle traffic;
3. The runoff of salt from roads directly into natural drainage systems;

4. The spraying of dissolved salt on roadside soil, vegetation, and surface waters by passing traffic;
5. By salted snow being blown or plowed onto the roadside by snowplows;
6. The leaching of salt from uncovered stockpiles;
7. The runoff from post-operational wash-down procedures of winter salting vehicles.

Apart from the direct costs of purchasing and applying road salt, there are a number of indirect costs associated with winter maintenance activities. Salt, when mixed with water, has a strong tendency to increase rusting of most ferrous based metals. This may cause corrosion of the reinforcing rebar in bridges and highways, as well as damage to motor vehicle materials (D'Itri 1992). In addition, road salt is known to cause a number of deleterious effects on road surfaces and environmental damage (Forman 2004; Ramakrishna and Viraraghavan 2005). Considering these indirect costs, separate studies estimated that the real cost of applying road salt is anywhere from \$35 to \$57 dollars in damage per dollar spent purchasing road salt (D'Itri 1992; Forman 2004). This negative economic impact of road salt application suggests that alternative solutions are warranted.

Of the indirect costs of road salt contamination, environmental damage is the most concerning. Environmental damage from road salt is particularly harmful due to the cumulative properties of chloride. Depending on its application, road salt may be contained at the road edge or transported far from its source. After initial application, the salt is temporarily contained within the interface between the soil surface and groundwater. Here the ions, namely sodium, are likely absorbed by roadside vegetation (Lunmark and Olofsson 2007). Sodium ions are typically involved in the chemical processes of the soil, whereas chloride is greatly conserved. Once deposited, chloride ions from these salts remain in the ecosystem in ground and surface waters (Environment Canada 2001).

With the intensive and widespread application of road salt on an annual basis over the past 60 years, it is not surprising that an increase in sodium and chloride concentrations in ground and surface waters is well documented (Williams et al. 2000; Benbow and Merritt

2004; *reviewed in* Ramakrishna and Viraraghavan 2005). Anthropogenic sources of NaCl have been shown to negatively affect ground and surface water quality at a number of sites in North America (Hoffman et al. 1981; Forman and Alexander 1998; Environment Canada 2001; Godwin et al. 2003). Road salt contamination also affects human health and population growth. Road salt contamination of drinking water sources is considered a serious threat to human health (Calabrese and Tuthill 1979), mainly through the development of hypertension (*reviewed in* Jones et al. 1992). In light of road salt as a threat to human health and its adverse impacts on the aquatic environment, Environment Canada identified road deicing chemicals as toxic (Environment Canada 2001). This designation prompted a new sense of urgency among scientists and managers to consider alternate deicing chemicals. In addition, road salt contamination is viewed as a constraint on urban growth in North America (Howard and Maier 2006). As population growth and urban sprawl contribute to an increasing mileage of road surfaces each year, the contamination from road salt and its associated pollutants are a major threat to the Earth's ecosystem. Understanding the effects of chemical deicers on freshwater and aquatic ecosystems will aid in the conservation of these important natural resources.

The goal of this paper is to examine the literature regarding the effects of chemical deicers on aquatic and terrestrial ecosystems. The physical, chemical, and biological impacts of common deicing products are discussed. In addition, alternatives to road salt are reviewed in terms of their level of environmental contamination.

Impacts on Surface and Ground Waters

Contamination of ground and surface waters by chemical deicers poses a major threat to freshwater ecosystems. The level of contamination is dependent on a number of spatial and temporal variables, including the area of treated roadway that drains to the water body, the frequency and rate of application of the deicer, the route of transport from the roadway to the water body, the extent of biodegradation along the route (McFarland and O'Reilly 1992), road and soil characteristics, type and amount of precipitation, and wind conditions (Blomqvist 1999; Lunmark and Olofsson 2007). Temporal variation of

chloride levels in surface waters is well-documented and depends on temperature and precipitation. Increasing temperatures in the spring are coupled with high chloride levels in surface water during the snowmelt, a result of the mobility of road deicers in contaminated soil and/or groundwater sources (Jones et al. 1992). Surface waters close to roads, or those that receive runoff from salted roads (i.e., via ditches and drainage systems), are at a greater risk of receiving road-based pollutants.

Groundwater supplies are most impacted by road salt contamination as the majority of road salt eventually reaches groundwater aquifers. Precipitation plays a large role in chloride transportation into the groundwater in mobilizing the ions in soil and surface waters (Fostad and Pedersen 1996; Pedersen et al. 2000; Lunmark and Olofsson 2007). Chloride may then accumulate in groundwater supplies as it is conserved during migration through soil and surface waters (Kincaid and Findlay 2009). Chloride accumulation in groundwater is irreversible, posing a threat to human health and sustainability.

The primary impact of road salt contamination is increased chloride or salinity levels. Surface waters may experience temporal fluctuations in salinity, however, these fluctuations are generally short term. Natural salinity of surface waters is a result of rock runoff from the drainage basin, atmospheric precipitation, and the balances between evaporation and precipitation (*reviewed in* Jones et al. 1992). Contributions from industrial sources, chemical deicers, and municipal waters may elevate chloride concentrations in surface waters. The level of road salt contamination in surface waters depends largely on the road drainage system, topography, discharge of the receiving stream, the degree of urbanization of the watershed, temperature, and precipitation (Scott et al. 1986).

Along with increased salinity, the secondary impacts of road salt contamination in surface waters can have grave impacts on the aquatic community. These include salt stimulation of algal growth, changes in density gradients, salt induced stratification, (*reviewed in* Ramakrishna and Viraraghavan 2005), and mobilization of heavy metals

from lake bottom sediments (Jones et al. 1992). Trace elements in road salt may leach into nearby water systems relieving the nutrient limitations for algae growth. This may further cause salt-induced stratification that prevent mixing and attribute to anoxic benthic conditions (Judd 1970; Hoffman et al. 1981; Benbow and Merritt 2004). Water run-off containing high density of salts may accumulate at the bottom of the lake, preventing the usual spring overturn (Jones et al. 1992). Without this essential annual mixing occurring, oxygen and nutrients within a lake are not distributed, making these important nutrients unavailable to aquatic organisms. This effect was observed in a study along a stretch of interstate in the Adirondack region of New York. Demers and Sage (1990) sampled chloride levels downstream of a heavily salted section of road, observing a dramatic increase in chloride concentrations up to 50 meters from the roadside. These streams carrying this dense contaminated water flowed into a nearby lake, forming a chemocline in the lake. The same road salt contamination induced chemocline was also observed in First Sister Lake, Michigan (Judd and Stegall 1982). In addition to chemoclines, changes in pH may mobilize heavy metals contained within lake-bottom sediments. Mercury contamination is known to occur in waters that experience high levels of road salt or pH fluctuations (Jones et al. 1992). Thus, although the salinity fluctuations in surface waters may be short-lived, these secondary effects can have long-term impacts on the freshwater ecosystem.

Modification of Soils

The initial impacts of road salt on soil may allow for greater distribution to surrounding water bodies as increased levels of sodium and chloride modify the structure of roadside soils. Sodium causes deflocculation in the soil leading to a loss of structure, making less air and water available for plant growth. Sodium ions may also exchange with nutrient ions such as calcium and magnesium, thereby reducing soil fertility (Burtwell 2001). The alteration of soil structure may also impact the degree of contamination from roadside runoff. More solid soils, such as those present during a frost, will be impermeable to water runoff. This would allow for greater mobility of the road salt contamination away from the road through transport by surface water (Fritzsche 1992; Lundmark and Olofsson 2007) and may be irreversible.

Impacts on Vegetation

Increased salt levels may drastically impact vegetation, though the severity of the damage depends in part on the method of salt application (Sucoff et al. 1976; Bryson and Barker 2002). Salt directly enters plants principally through spray to the foliage and absorption from the soil by root uptake (Jones et al. 1992). Deicing salt may accumulate on roadside vegetation, causing the browning of leaves and needles of trees through a salt burn (*reviewed in* Viskari and Karenlampi 2000). Salt damage to plants depends partly on the salinity of the soil and tolerance level of the species (Fostad and Pedersen 2000).

Indirect effects of roadside vegetation exposed to high levels of sodium chloride include increased susceptibility to disease and pests (Viskari and Karenlampi 2000), a loss of cold hardiness (Sucoff et al. 1976), and a reduction in competitive ability (Jones et al. 1992). In addition, saline soils may cause period of osmotic stress, in which the plant loses moisture into the surrounding soil. Destruction of soil structure following sodium deposition, as discussed above, may decrease the overall health of the associated plants (Hootman and Kelsey 1992).

The death of roadside vegetation may have greater implications for the localized road salt contamination of nearby surface waters. Roadside vegetation is important in preventing erosion of roadside soils, and likely acts as a buffer in absorbing contaminants. The removal of this vegetation would allow for greater mobility of both road salt and sediments into nearby surface waters. Increased sedimentation in freshwater systems is known to reduce habitat availability for fish (Newcombe and McDonald 1991) and amphibian species (Welsh and Olivier 1998).

Elevated salinity levels in surface waters, whether short or long-term, can have a drastic impact on the flora of the freshwater system. Salinity tolerance varies widely among plant species. Increases in salinity can affect plant growth by increased water stress in the root medium, the accumulation of ions or toxins in the soil, and/or the accumulation of ions in the plant tissue (*reviewed in* Howard and Mendelssohn 1999). Increased salinity levels

may also affect community composition either directly, through toxicity or osmotic effects, or indirectly through interspecific competition (*reviewed in* Miklovic and Galatowitsch 2005). This is particularly important in vegetative communities, where road salt may significantly affect seedling development and/or interspecific competition. With increasing salinity levels, diversity and species richness in freshwater wetlands decreased (Wilcox 1986; Panno et al. 1999; Latham et al. 1991; Miklovic and Galatowitsch 2005), seedling germination and survival decreased (Pearson et al. 1966; Zedler et al. 1990), and the biomass of the wetland communities decreased (Isabelle et al. 1987; McKee and Mendelssohn 1989). Excess salt in the roadside environment may also alter the vegetative community helping to facilitate the establishment of invasive species (e.g., *Phragmites*; Richberg et al. 2001). Runoff from uncovered, road salt storage piles has been directly linked to shifts in the plant community in which salt-sensitive species were replaced by salt tolerant species. In this setting, population shifts are likely to occur towards less desirable, non-native salt-tolerant species (Gales and VanderMeulen 1992).

Impacts on Terrestrial and Aquatic Wildlife

Plants and animals have an acute ability to respond to temporary changes in pH and acidity. Certain species of plants and animals are better adapted to saline environments than others (Freda 1991). However, when exposed to levels beyond their tolerance level, mortality may occur. Aquatic species are likely to be most impacted by road salt contamination through disruption of osmotic regulation. While many aquatic organisms are sensitive to salinity changes (e.g., invertebrates and fish), most freshwater bacteria and blue-green algae are less tolerant (Jones et al. 1992).

Invertebrates

Aquatic invertebrates may also be directly and indirectly affected by increases in environmental salt levels. Aquatic invertebrates are sensitive to changes in chloride levels (Demers and Sage 1990; Bridgeman et al. 2000; Benbow and Merritt 2004) as chloride ions are known to compromise osmoregulatory processes in aquatic insects (Sutcliffe 1961; Blasius and Merritt 2002). The sensitivity of certain aquatic insects to high levels of chloride threatens the community structure of wetland ecosystems (Benbow and

Merritt 2004). In a study on four streams in the Adirondack region of New York, Demers and Sage (1990) observed a significant decrease in the diversity of aquatic insects inhabiting artificial substrates downstream of a heavily salted section of a highway. Molles (1980) observed stream insects indirectly affected by road salt application following the death of roadside vegetation. Sedimentation loads in the rivers increased as a result, causing a reduction in invertebrate abundance and biomass. Increased sedimentation and the loss of aquatic insects as an important food source may drastically impact ecosystem integrity.

Mammals and Birds

During winter, many animals are attracted to natural and artificial salt deposits to satisfy a dietary need when food sources may be scarce. In Wisconsin, a number of small animals suffered mortality from salt poisoning during a hard winter of freezing temperatures and high snowfall. Trainer and Karstad (1960) observed wild rabbits, pheasants, quail, and pigeons exhibiting behavioral abnormalities arising from distress of the central nervous system from ingesting road salt prior to mortality.

The attraction to roadside sources of salt also poses a problem for larger mammals. In turn, human safety may be in danger from vehicle collisions when large mammals are attracted to roads and highways. In Canada, a study conducted by Fraser and Thomas (1982) examined the relationship between the salt content of roadside pools and the frequency of moose collisions along a stretch of highway. Fraser and Thomas (1982) found that moose concentrated around roadside pools with high levels of sodium and chloride. More than half of the moose-vehicle collisions on this stretch of highway were at or adjacent to these salty pools. In an effort to decrease the potential for moose-vehicle collisions, the Quebec Ministry of Transportation filled and drained these salty pools. LeBlond et al. (2007) documented a decrease in the number of moose visiting roadside pools after management was initiated.

Avian species are also attracted to roadside sources of salt putting them in danger of direct mortality from vehicle collisions (Schulkin 1991). Accidental ingestion may occur

when birds forage at the roadside in search of grit to aid in digestion. House sparrows, for example, are known to choose dietary grit with the same physical characteristics as road salt (Gionfriddo and Best 1995; Bollinger et al. 2005). This species demonstrated abnormal behavior, including depression, ataxia, inability to fly or perch, and death in as little as 45 minutes when consuming large doses of road salt (Bollinger et al. 2005). Behavioral changes associated with road salt toxicity, such as ataxia and the inability to fly, put birds at a greater risk of vehicular mortality.

In a recent review on birds, Mineau and Brownlee (2005) concluded that road salt is a major mortality factor. Their review included 12 published reports of bird kills associated with road salt, encompassing eight species. Birds that are readily observed ingesting road salt exhibit unusual behavior, appearing fearless and are easily approached, increasing their vulnerability to car strike. In all but one case the primary cause of poisoning was identified as sodium chloride, with calcium chloride (a popular road salt alternative) cited in the twelfth report.

Amphibians

The effect of road salt contamination on amphibians is also briefly studied (e.g., Turtle 2000; Dougherty and Smith 2006; Sanzo and Hecnar 2006; Karraker 2007a,b).

Amphibians are likely to be drastically effected by road salt contamination due to their explosive breeding at the peak of spring contamination, and breeding in small, ephemeral pools where salts are likely to be concentrated (Karraker 2007a). The by-products of road salt contamination, namely mercury contamination and low pH levels, are known to disrupt the osmotic balance in the membranes surrounding amphibian embryos, halting or impairing development (Freda 1991). Amphibians may also be greatly affected by the indirect effects of road salt pollution. As mentioned above, pollution from road salt may release heavy metals from lake-bottom sediments (Jones et al. 1992). The disruption or depletion of aquatic insect communities will greatly impact amphibians, due to their dependence on these organisms as a major food source. Amphibians are very dependent on water throughout their life cycle and will thus be consistently exposed to contaminants present in the water.

Herpetofaunal communities, which rely heavily on temporary and permanent sources of freshwater, are likely affected by road salt contamination. Turtle (2000) did not find evidence of localized adaptation to road salt by *Ambystoma maculatum* inhabiting roadside vernal pools. In comparing the tolerance levels of road salt on different amphibians, Karraker (2007a,b) observed distinct variations between species. *Rana* (*Lithobates*) *clamitans* tadpoles were found to be insensitive to low to moderate road salt contamination, while *R. sylvatica* tadpoles and *A. maculatum* larvae were negatively impacted (Karraker 2007a,b; Sanzo and Hecnar 2006). This has important implications for inter-specific competition, giving *R. clamitans* an advantage in the use of waters with higher salinity levels. To my knowledge, the community effect of increased salinization has not been studied in other vertebrates.

Alternatives to Road Salt

Due to the known negative environmental impacts and corrosive properties of NaCl, numerous alternatives are currently being developed to improve de-icing operations and limit the use of rock salt. State and municipal transportation agencies are evaluating these alternatives in an effort to maintain safe winter driving conditions while lessening environmental degradation (D'Itri 1992). The performance of these alternatives to traffic and environmental trials are reviewed in the following paragraphs.

Abrasives such as sand and cinders are thought to be a viable alternative to road salt in maintaining the safety of winter roads. The use of abrasives is an attractive alternative due to its low cost relative to road salt at over four times less expensive (Gales and VanderMeulen 1992). However, the application of vast quantities of these materials raises a number of environmental concerns. Primarily, highway runoff containing these materials will increase the sediment load in lakes and streams, hereby lowering the water quality and smothering roadside vegetation (Gales and VanderMeulen 1992).

A popular chemical alternative to road salt is calcium magnesium acetate, henceforth CMA. According to modeling efforts, the impacts of CMA on surface waters are believed

to be less than that of road salt (McFarland and O'Reilly 1992). Both calcium and magnesium ions are less mobile, tend to precipitate in water, and are thus less threatening in groundwater contamination (Bang and Johnston 1998). However, water hardness issues may occur through increasing concentrations of calcium and magnesium. CMA may also lower the amount of dissolved oxygen in surface water bodies, posing a problem for aquatic organisms. CMA is known to decrease algal and periphyton biomass in freshwater systems, while remaining relatively harmless in toxicity tests on invertebrates and fish (McFarland and O'Reilly 1992). Other studies noted a lack of significant changes in phytoplankton and bacterial communities in freshwater lakes following CMA contamination (Goldman et al. 1992). In soils, CMA may be beneficial in contributing nutrients (calcium and magnesium) and improving soil structure by increasing water and air movement (Gales and VanderMeulen 1992). Field trials indicated that direct application of CMA solutions did not affect the growth, foliage, strength, or rooting of plant seedlings or tree saplings (Horner 1988).

The Nevada Department of Transportation (NDOT) and the California Department of Transportation (CALTRANS) performed a detailed field and laboratory investigation comparing the effectiveness of CMA as a substitute for sodium chloride (Harris et al. 1993). The study found that CMA behaved differently as a deicer than rock salt, limiting its effectiveness in different combinations of road and atmospheric conditions. In addition, the chemical and physical properties of CMA made it difficult to handle and transport (Harris et al. 1993). While CMA does perform well in deicing roadways, its high cost (over twenty times more expensive than salt) may limit widespread implementation (D'Itri 1992).

Another popular alternative to road salt is calcium chloride, or CaCl_2 . Calcium chloride acts similarly to road salt in de-icing operations, and it is able to work effectively at lower temperatures than salt. Approximately 0.3 million tons of CaCl_2 are used in North America each year. Calcium chloride is thought to be less harmful to the environment than sodium chloride, and calcium poses no known health threat in drinking water (Gales and VanderMeulen 1992). Research on trees has shown that by increasing Ca

concentrations, the effects of NaCl may be reduced. In studying spruce trees, Bogemans et al. (1989) found that substituting 20 to 30% of CaCl_2 for NaCl resulted in a 50% decrease of Na in the needles. However valuable as an alternative, its high cost (over eight times that of road salt) may greatly limit its use (Gales and VanderMeulen 1992).

Other alternatives to road salt include, sodium formate (CHNaO_2), magnesium acetate ($\text{C}_4\text{H}_6\text{MgO}_4$), calcium acetate ($\text{C}_4\text{H}_6\text{CaO}_4$), glycol liquids, urea ($\text{CH}_4\text{N}_2\text{O}$), corrosion inhibiting salts (Gales and VanderMeulen 1992), methanol (CH_3OH ; Dougherty and Smith 2006), tetra potassium pyrophosphate (Gutiw 1998; Ramakrishna and Viraraghavan 2005) and Ice ShearTM (an equimolar mixture of sodium acetate and sodium formate; Bang and Johnson 1998). Of these, Ice ShearTM was found to be a viable alternative to road salt. Using a mixture of biochemical oxygen demand (BOD), chemical oxygen demand (COD), acute rainbow trout bioassays, and phytotoxicity tests on typical roadside vegetation, Ice ShearTM was shown to have minimal environmental disturbance in both aquatic and terrestrial ecosystems (Bang and Johnston 1998). However, Bang and Johnston did not address the impact of CMA on sensitive members of freshwater ecosystems. This information is crucial to understand before this deicer is recommended for widespread use.

Conclusions and Research Scope

Road deicing activities have cascading impacts on the surrounding terrestrial and aquatic ecosystems. The cumulative environmental damage from deicing activities is only now being realized, particularly as they threaten human health and population growth. The direct and indirect environmental impacts of chemical deicers on water sources, soils, vegetation, and wildlife should be considered a serious threat to ecosystem health. In light of the known environmental impacts of road salt contamination to aquatic and terrestrial ecosystems, a full understanding of the impacts of these alternatives is warranted prior to widespread application. Considerations of alternatives to road salt will likely gain in popularity as the negative impacts of road salt are more widely recognized.

At present, few alternatives are being implemented due to high cost and little incentive. However, cost as a limiting factor in application of alternative deicers may be less of a problem when the widespread impacts of intensive road salt application are considered. Confounded in this is the lack of knowledge concerning the behavior of these alternative deicing chemicals in the terrestrial and aquatic ecosystems. Deicing alternatives to road salt should be thoroughly investigated as to their impacts on the terrestrial and aquatic ecosystems, namely their effect on flora and fauna. In addition to chemical alternatives, management activities should aim to alter deicing practices. This may include limiting the amount of deicing chemicals that are applied to roadways (e.g., Pollock 1992) and/or identifying areas adjacent to highways of high environmental concern. Sensible deicing practices would greatly help to lessen the long-term impacts. As new roads are being added to the North American highway network each year, awareness of their potential environmental damage should evoke careful forethought in transportation planning.

Although previous research has addressed a number of questions concerning the ecological effects of road salt and road salt alternatives, we are left with many unanswered questions regarding this impact. For example, do sensitive organisms such as amphibians respond differently to one type of chemical deicers over another? In addition, we have knowledge of the lethal impacts of road salt exposure on a few populations of amphibians yet further analysis with different species and/or populations will help to determine the potential for local adaptation to road salt runoff. Furthermore, investigations into amount and spatial distribution of residual chloride from road salt (NaCl) application will help us to better understand the environmental impacts of its use in areas where application rates may be variable.

To address these research needs, I employed a mixture of laboratory, field, and geographical tools in the following chapters. Firstly, in Chapter 2, I examined the lethal response of local wood frog (*Lithobates sylvatica*) larvae to exposure to six chemical deicers in acute toxicity experiments. This analysis provided estimates of the lethal effects of six commonly used chemical deicers on a local population of wood frogs. The endpoint of this experiment is the estimation of the median lethal concentration or LC₅₀, a

measure of the effects of short term (96 hours) exposure on the survival of larval amphibians. These estimates are invaluable in that they allow for a direct comparison of the effects of this suite of chemical deicing products within a population. In addition, these estimates may be used to evaluate the potential risks amphibians using roadside habitats may encounter when attempting to breed in these water bodies.

Next, I built upon the knowledge gained through the acute toxicity tests by further examining the response of the larvae of two unique amphibians, wood frogs (*L. sylvatica*) and green frogs (*L. clamitans*), to long term (>90 days) exposure to low levels of NaCl in Chapter 3. This chronic toxicity approach allows for identification of the sublethal or developmental effects of this exposure that may have negative impacts on the fitness of these individuals. In using these two species, I was able to gain insight into the potential community level effects of this exposure on species that employ different life history strategies.

In Chapter 4, I characterize the environmental impacts of NaCl application in the three northernmost counties of Michigan in an effort to determine the risk local amphibians may face when using roadside water bodies in this area. I conducted a broad scale survey of water bodies both near and farther from the main arterial highway in this region, US 41, to determine the concentration and temporal variation in chloride. I used this data to spatially estimate the presence of a road salt effect zone in this region. This knowledge is important in that transportation managers may use this information to alter their application of NaCl so as to limit NaCl runoff in roadside water bodies. Finally, in Chapter 5, I address an issue in estimating chloride concentration from conductivity measurements. A number of researchers have used this method as an alternative to expensive and time consuming analytical chemistry, however, the methodology behind these estimates prohibits this calculation.

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Chapter 2: Effects of Six Chemical Deicers on Larval Wood Frogs (*Rana sylvatica*)¹

Abstract—Widespread and intensive application of road deicers, primarily road salt (NaCl), in North America threatens water quality and health of freshwater ecosystems. Intensive use of NaCl can be harmful to sensitive members of freshwater ecosystems such as amphibians. Detection of negative effects of NaCl application has prompted the search for alternative chemical deicers with lower environmental impacts. We conducted a series of 96 hour acute toxicity tests to determine the negative sensitivity of larval wood frogs (*Rana (Lithobates) sylvatica*) to six deicing chemicals: urea (CH₄N₂O), sodium chloride (NaCl), magnesium chloride (MgCl₂), potassium acetate (CH₃COOK), calcium chloride (CaCl₂), and calcium magnesium acetate (C₈H₁₂CaMgO₈). Acetates are sometimes touted as ‘environmentally friendly’ alternatives to NaCl, but have not been examined in enough detail to warrant this designation. When exposed to a range of environmentally realistic concentrations of these chemicals, larvae were least sensitive (i.e., lowest rate of mortality rate) to CH₄N₂O, NaCl, and MgCl₂ and most sensitive to acetates (C₈H₁₂CaMgO₈, CH₃COOK) and CaCl₂. Our observed LC50_{96-h} values for NaCl were over two times higher than values presented in previous studies, which suggests variability in tolerance among *R. sylvatica* populations. The deicers varied greatly in their toxicity and further research is warranted to examine the differential effects of this suite of deicers to other species.

Keywords—transportation, tadpoles, *Rana sylvatica*, highways, ecotoxicology

Introduction

¹ The material contained in this chapter was previously published in the journal *Environmental Toxicology and Chemistry* as Harless ML, Huckins CJ, Grant JB, Pypker TG. 2011. Effects of six chemical deicers on larval wood frogs (*Rana sylvatica*). *Environmental Toxicology and Chemistry*. 30: 1637-1641.

In cold climates, a myriad of chemicals may be used to remove or prevent the formation of ice on roads, highways, sidewalks, and runways. The most common deicing chemical used on roads is sodium chloride (NaCl) due to its low cost and widespread availability. In the United States, an amount of NaCl estimated at 10 million metric tons is used each year [1], ranging from 0.3 to 17.6 metric tons per lane mile across 26 states [2]. Roads in the State of Michigan receive more NaCl per lane mile (12.9 metric tons) than any other Great Lakes state [2]. The intensive and widespread application of NaCl on an annual basis over the past few decades has resulted in an increase in the salinity of ground and surface waters in North America [3-7]. Anthropogenic sources of NaCl have been shown to negatively affect ground and surface water quality [8-11]. Direct impacts of NaCl contamination in surface waters arise primarily from increased chloride concentrations, changes in water density gradients, salt induced stratification, and salt stimulation of algal growth leading to eutrophication [7]. Because of the threat NaCl poses to human health and the aquatic environment, Environment Canada identified road deicing chemicals as toxic [10].

Due to the known negative environmental impacts of NaCl, numerous alternatives are currently being evaluated to improve deicing operations and reduce the use of deicing chemicals. State and municipal transportation agencies are evaluating these alternatives in an effort to maintain safe winter driving conditions while avoiding the environmental degradation and potential harm to aquatic life caused by NaCl [1]. These chemicals include different inorganic salts that may be used separately or in conjunction with NaCl (e.g., CaCl_2 , MgCl_2 , and KCl) [10], sodium formate (CHNaO_2), calcium magnesium acetate (CMA, $\text{C}_8\text{H}_{12}\text{CaMgO}_8$), magnesium acetate ($\text{C}_4\text{H}_6\text{MgO}_4$), calcium acetate ($\text{C}_4\text{H}_6\text{CaO}_4$), glycol liquids, urea (NH_4CO) [12], methanol (CH_3OH) [13], tetra potassium pyrophosphate [14] and Ice ShearTM (an equimolar mixture of sodium acetate and sodium formate) [15]. Acetate chemicals, in particular, are often viewed as an environmentally friendly alternative to inorganic salts because they do not contain chloride [7]. Furthermore, not all chemical deicers are equally effective at deicing and may require the application of greater quantities to achieve the same results [1,16]. For example, NH_4CO , CaCl_2 , and CMA require up to 1.2-1.7 times as much deicer to achieve the same deicing

result as NaCl [1]. This higher application rate could exacerbate negative environmental impacts of these chemicals. Because each of these chemicals differ in their chemical make-up and expected environmental concentrations (i.e., resulting from differences in application, mobility, and decomposition rates), extensive testing of their ecotoxicological effects should predate their widespread use.

Few studies have addressed the effects of NaCl on wildlife species, but limited research has shown known road salt exposure to negatively affect mammals, birds, invertebrates, and amphibians that utilize roadside habitats [7]. Of these taxa, amphibians are likely to be the most affected by chemical deicer runoff. Amphibians possess highly permeable skin, aquatic larval stages, and many use roadside wetlands for breeding [17]. Embryonic and larval amphibians exposed to salinities beyond their natural range experience substantial negative impacts. For example, high salinity may decrease development rate and increase malformations in embryonic and larval amphibians [18-21]. In addition, exposure to NaCl can increase infection rate of a lethal water mold in embryonic amphibians [22]. Furthermore, amphibians experience elevated levels of NaCl contamination during spring melt and runoff, and many species breed in small, ephemeral pools where NaCl runoff is likely to be concentrated [20,21]. As such, amphibians are considered indicators of ecosystem health and are model organisms to investigate the environmental effects of contamination from NaCl and other chemical deicers. The lethal and sublethal effects of NaCl contamination on amphibians have been addressed in a few studies that suggest amphibians are negatively impacted at environmentally realistic concentrations [21,23,24]. In addition, previous research suggests that frogs and salamanders avoid salt-polluted pools and have not demonstrated local adaptation to high salinities when using roadside water bodies [21,25]. Furthermore, amphibian species may differ in their response to exposure to chemical deicers in runoff, which is predicted to influence demography and community structure [26].

Little is known about the relative population and ecosystem impacts of road salt alternatives and additives within the deicers. One study has directly compared the response of larval amphibians to exposure of NaCl and alternative deicers. Dougherty

and Smith [13] compared the lethal effects of NaCl and an alternative (MgCl_2) on two native amphibians, green frogs (*Rana (Lithobates) clamitans*) and American toads (*Bufo (Anaxyrus) americanus*). To our knowledge, no other studies have directly compared the relative lethal effects of NaCl and a suite of commonly proposed alternative deicers on North American amphibians. The objective of this study was to assess the direct acute toxicity of six deicing chemicals to native *R. sylvatica* larvae as a predictor of their relative toxicity in the environment.

Materials and Methods

Experimental design

We conducted a series of 96 hour acute toxicity tests using *R. sylvatica* larvae to determine the lethal effects of exposure to the following six chemical deicers: urea (pelleted fertilizer, $\text{CH}_4\text{N}_2\text{O}$; Garner Brothers Inc., Greenfield, TN), sodium chloride (coarse rock salt, NaCl; Morton Salt Inc., Chicago, IL), calcium chloride (pelleted, CaCl_2 ; Peladow[®], Dow Chemical Company, Midland, MI), magnesium chloride (anhydrous, MgCl_2 ; Schoenburg Salt Company, Lynbrook, NY), potassium acetate (liquid, KAc, CH_3COOK ; Cryotech CF7[®] Liquid Commercial Deicer, Cryotech Deicing Technology, Fort Madison, IA), and calcium magnesium acetate (pelleted, CMA, $\text{C}_8\text{H}_{12}\text{CaMgO}_8$; Cryotech CMA[®], Cryotech Deicing Technology, Fort Madison, IA).

On May 8, 2008 we collected nine recently deposited *R. sylvatica* egg masses from a palustrine wetland adjacent to a moderately traveled road in Baraga County, Michigan (latitude 46.796 N, longitude 88.390 W). This road receives a low amount of salt (4.06 tons per lane mile for the winter of 2007-2008) during winter maintenance activities (DJ Mills, personal communication). We transported the eggs to the lab and randomly assigned each egg masses to one of four aerated 78 L glass aquaria containing approximately 50 L of filtered water from Portage Lake, Houghton County, Michigan. The eggs began hatching five days later. The tadpoles were fed ad lib a 3:1 mixture of TetraFin Flake Goldfish Food (Tetra Werke, Melle, Germany) and pulverized Purina Rabbit Chow (Purina Mills, St. Louis, MO) from the time they hatched until they were

placed into the test chambers. Larvae in test chambers were not fed during the experiment.

Methods for the 96 hour acute toxicity tests strictly followed the protocols set forth by the American Society of Testing Materials (ASTM) [27] and the United States Environmental Protection Agency (USEPA) [28] for static toxicity tests. We chose the range of nominal test concentrations of chemical deicers in this experiment to encompass both known median lethal concentrations ($LC_{50_{96-h}}$) for NaCl exposure to larval *R. sylvatica*, and environmental concentrations of Cl^- in wetlands and vernal pools resulting from NaCl pollution ($0.002-10.3 \text{ g L}^{-1}$) [10,13,20,21,24,25,31]. Test chambers consisted of 44-2.84 L glass jars with loosely fitting glass lids to prevent evaporation and allow for sufficient oxygen exchange. We added two liters of filtered Portage Lake water and the appropriate amount of chemical deicer to obtain the following 11 nominal test concentrations: 0 (negative control), 0.19, 0.32, 0.54, 0.90, 1.50, 2.40, 3.84, 6.14, 9.83, and 15.73 g L^{-1} . We replicated the 11 nominal test concentrations four times for a total of 44 experimental units.

Deicer treatments were randomly assigned to each jar. We mixed the treatment solutions until the chemical deicer was completely dissolved in each jar. We pooled tadpoles from all egg masses and randomly assigned tadpoles to each test chamber (experimental units). Each experimental unit contained 10 tadpoles, except for $CaCl_2$ treatments which contained five tadpoles per replicate due to a limited supply of larvae. Test chambers were maintained in the laboratory on a 12:12h light to dark cycle using full spectrum lights. Water temperature averaged 20.7°C (range: $19.4 - 21.8^\circ\text{C}$) during all trials. Larvae were checked every 24 hours with mortality recorded at each interval. Larvae that were dead or unresponsive to probing with a small net were removed from the jar and preserved in a solution of 10% formalin. After 96 hours, we preserved all tadpoles remaining in the test chambers. From these data, we estimated the median lethal concentration (LC_{50}) value, or the concentration of the deicer at which mortality of the test population reached 50%. The methods for this calculation are described below.

Statistical analysis

Because survival data was not normally distributed, we used non-parametric statistics to examine differences in survival across test concentrations. For each chemical deicer, the proportion of larvae surviving at 96 hours among treatments was analyzed using the non-parametric Kruskal-Wallis one way analysis of variance test. To determine the lowest concentration that had significantly lower survival than in the control we used a Kruskal-Wallis test with multiple post-hoc comparisons. We used the Trimmed Spearman-Kärber Program (Version 1.5) to calculate median lethal concentrations (LC50) using untransformed data for each chemical deicer at 24, 48, 72, and 96 hours of exposure [30,31]. We pooled the survival data for each concentration across replicates when calculating the LC50 value. We used “R: A Language and Environment for Statistical Computing” to perform all statistical analyses with an alpha level of 0.05 [32].

Results

Survival of *R. sylvatica* tadpoles after 96 hours of exposure varied widely across deicers and concentrations (Fig. 2.1). Survival was 100% in all the control tanks except for the one assigned to the CH₄N₂O treatments and in this control survival was 95%. A significant effect of concentration on survival was detected for each deicer ($p \leq 0.038$). Tadpole survival was significantly lower in concentrations of CH₄N₂O at 9.83 g L⁻¹ or higher when compared to the control. Exposure to NaCl and MgCl₂ concentrations of 6.14 g L⁻¹ or above significantly reduced tadpole survival compared to the control. For CH₃COOK and CaCl₂, 3.84 g L⁻¹ was the lowest concentration to cause significantly lower survival than the control. Exposure to concentrations of C₈H₁₂CaMgO₈ at or above 1.50 g L⁻¹ caused significantly lower survival. Survival in test concentrations below those presented above for each deicer was not significantly lower than that in the control ($p > 0.05$).

Different toxicities of the six chemical deicers suggested by tadpole mortality were supported by estimated LC50_{96-h} values, which ranged from 3.23 g L⁻¹ (C₈H₁₂CaMgO₈) to 14.63 g L⁻¹ (CH₄N₂O; Table 2.1). The estimated LC50_{96-h} values were highest for CH₄N₂O, NaCl, and MgCl₂ and were much lower for acetate chemicals (C₈H₁₂CaMgO₈,

CH₃COOK) and CaCl₂. For each chemical, the LC50 values were highest after 24 hours of exposure and decreased by 2-40% through time until the end of the trial.

Discussion

Survival of *R. sylvatica* larvae was reduced by exposure to higher concentrations of all chemical deicers examined in this study; however, the response by larvae depended on the chemical deicer. In general, urea and several of the chloride compounds were less toxic than acetates (C₈H₁₂CaMgO₈, CH₃COOK) and CaCl₂. At each time step of the test, C₈H₁₂CaMgO₈ exposure induced 50% mortality at the lowest concentration compared with all other chemicals. In addition, LC50 values decreased with time indicating that either larval *R. sylvatica* were less able to tolerate or offset the physiological or toxic stress associated with these compounds as duration of exposure increased, or that there was a lag in the lethal effects of initial exposure. If the duration of exposure was critical, this suggests that the effects of exposure to winter season road maintenance involving deicers depends on the type of chemical that is applied, the concentration that builds up in the environment, and/or how long the chemical persists in the environment. If there was a lag response in mortality, then possibly even short-term exposure to the contamination could have lethal effects on amphibian larvae.

We are not aware of previous studies on amphibians that have investigated the toxicity of urea in the context of its use as an alternative chemical deicer. Urea is widely used in the United States as a source of fertilizer for both agricultural and forest lands, as well as an aircraft deicer. Adult amphibians avoid water bodies that receive urea runoff, even when concentrations are lower than the recommended terrestrial fertilization rates [33]. In this study, the high tolerance of *R. sylvatica* tadpoles for urea compared to the other five chemical deicers is expected given that tadpoles excrete urea as a waste product and may retain urea as an osmolyte to protect against salt stress and dehydration [19]. In addition, urea may be used a cryoprotectant by amphibians exposed to low temperatures and freeze/thaw cycles, as is likely during the early breeding cycle of *R. sylvatica* [34]. However, when exposed to high concentrations of urea in their environment, amphibians experience deleterious effects on protein structure and function [35]. Our results suggest

R. sylvatica larvae appear to tolerate relatively high concentrations ($<9.8 \text{ g L}^{-1}$) of urea during short-term exposure.

The $\text{LC50}_{96\text{-h}}$ values estimated in this study are over two times greater than values reported in previous studies with *R. sylvatica* (Table 2.2) [23,24]. The discrepancy between this and other studies suggests that this population may be more tolerant to short-term exposure to NaCl pollution than are other populations. The larvae tested in this experiment were collected adjacent to a road that receives a small amount of NaCl with sand as the primary winter maintenance product (4-5% NaCl; DJ Mills, personal communication). It is possible that localized adaptation or acquired tolerance to NaCl pollution could solely explain this difference in tolerance although this phenomenon has not been previously documented in *R. sylvatica* or spotted salamanders (*Ambystoma maculatum*) [20,25].

In addition, differences in experimental methodology and adherence to ASTM or EPA guidelines may confound comparisons between toxicity studies on chemical deicers with amphibians. The use of food grade salt, alternative methods of statistical analysis [24], purified or de-ionized water [13,21,24], and plastic [21] or glass containers may affect resultant $\text{LC50}_{96\text{-h}}$ values. Also, feeding tadpoles during exposure and conducted acute toxicity tests at a different room temperature may also influence results [24]. We would expect the use of coarse (non-purified) rock salt used in winter maintenance, a lack of food availability, and the use of glass in experimental chambers in this study (as outlined in ASTM guidelines) would have produced lower $\text{LC50}_{96\text{-h}}$ values when compared to previous studies. Plastic containers may have an interactive effect and interfere with estimates of toxicity endpoints if they leach additional chemicals. For example, plasticizers such as bisphenol A (BPA) and dibutyl phthalate (DBP) may cause adverse effects on embryonic or larval amphibians including malformations, early mortality, and sex reversal [36]. Strict standardization of experimental protocol is recommended in future toxicity tests to facilitate comparisons between studies on different populations and/or species.

MgCl₂ and CaCl₂ are used primarily as fugitive dust inhibitors on unpaved roadways and to a lesser degree as chemical deicers; however, they are commonly available in stores as consumer level sidewalk deicers. As a chemical deicer, these are slightly more efficient than NaCl in removing ice. Under the same application rates as NaCl, MgCl₂ and CaCl₂ application will contribute more detrimental Cl⁻ into the roadside environment, further expounding the negative impacts of chemical deicer application [1]. This raises serious concerns over the choice of either of these chemicals as an alternative to NaCl. In addition, our results and those of Dougherty and Smith [13] suggest that native larval amphibians are much more sensitive to both MgCl₂ and CaCl₂ than NaCl.

Variation in salt tolerance among North American amphibians has been described elsewhere (Table 2.2). Embryonic and larval *R. clamitans* tadpoles were found to be relatively insensitive to NaCl pollution with low mortality rates [21] and moderate LC50_{96-h} values [23]. However, Dougherty and Smith found larval *R. clamitans* to be intolerant of NaCl pollution [13]. Larval grey tree frogs (*Hyla versicolor*) [37] and *A. maculatum* [23] were also reported to be less tolerant of NaCl pollution than other native species. Larval *R. sylvatica* and spring peepers (*Pseudacris crucifer*) appear to be moderately tolerant of NaCl pollution with low LC50_{96-h} values [23]. *Bufo americanus* larvae were most tolerant of NaCl pollution [23] and had 100% survival in acute exposure up to 3.0 g L⁻¹ [13]. This tolerance exhibited by *Bufo* may stem from past selection to tolerate extreme and rapid drought conditions that could lead to rapid rises in solute concentrations. Different salt tolerance levels among species may influence demography and community structure of native amphibians, particularly for those using roadside breeding habitats [23,26].

To our knowledge, no other studies have investigated the acute effects of the range of NaCl alternatives on native amphibians as presented here. One study has investigated the acute toxicity of NaCl and an alternative deicer on amphibians. Results of Dougherty and Smith [13] suggest that *B. americanus*, *R. clamitans*, and *R. sylvatica* are much more sensitive to MgCl₂ exposure than NaCl pollution with LC50_{96-h} values ranging from 0.11-0.23 g L⁻¹ (Table 2.2). These estimates for MgCl₂ exposure are also much lower than

those estimated in this study suggesting that this population may be more tolerant of exposure than other populations. Knowledge of the MgCl_2 tolerance of other groups of native amphibians will help to determine the effect of this pollution source on native amphibian communities. The identification of the environmental effects of alternative chemicals to aquatic and terrestrial organisms is essential prior to implementation of these chemicals as a viable alternative to NaCl.

Knowledge of the potential and quantified environmental impacts of the other alternative deicing chemicals used in this study is limited. This is particularly important to consider for acetate chemicals, as they are generally considered an ‘environmentally friendly’ alternative to NaCl [7]. The behaviour of $\text{C}_8\text{H}_{12}\text{CaMgO}_8$ and CH_3COOK in the environment raises serious concerns about potential widespread use of these chemicals as a winter maintenance tool. When used as road deicer, average highway spray and runoff concentrations of CMA would likely range from 10 to 100 mgL^{-1} with average annual CMA loadings of 10 tons per mile [38]. In surface water, CMA (acetate) decomposition is predicted to occur in 100 days at 2°C and much faster at higher water temperatures [38]. Acetate products may also decrease the pH of roadside soils and lead to the mobilization of heavy metals. In aquatic environments, acetate products increase oxygen demand and may decrease the biomass of algae [1,16], which is a common food resource for developing larval amphibians. These potential environmental effects of $\text{C}_8\text{H}_{12}\text{CaMgO}_8$ and CH_3COOK may have grave implications for sensitive embryonic and larval amphibians as the availability of oxygen and algae is necessary for proper development. Results of this study showing low $\text{LC50}_{96\text{-h}}$ values for $\text{C}_8\text{H}_{12}\text{CaMgO}_8$ (3.23 g L^{-1}) and CH_3COOK (4.23 g L^{-1}) exposure demonstrates that this alternative deicer may indeed be more harmful than road salt and other deicing chemicals to amphibian communities.

Although the median lethal estimates of chemical deicers to amphibians in this study are above environmentally realistic concentrations of residual chloride from NaCl application in roadside water bodies, we cannot assume a lack of adverse effects of these chemicals on amphibians. The U.S. EPA categorizes substances with a LC50 above 0.10

g L⁻¹ to be practically non-toxic to aquatic organisms (<http://www.epa.gov/espp/litstatus/effects/redleg-frog/naled/appendix-i.pdf>). Similarly, Environment Canada considers prolonged exposure to Cl⁻ concentrations above 0.220 g L⁻¹ as harmful to approximately 10% of aquatic species [10]. However, previous studies indicate NaCl concentrations as low as 0.078 g L⁻¹ can cause significant sublethal effects including decreased survivorship over time, a decreased number of frogs that metamorphose, and delayed time to metamorphosis in *R. sylvatica* [24]. Residual chloride concentrations in roadside water bodies range from 0.002 to 10.3 g L⁻¹, and are highest in early spring and late summer [21,23,24]. Considering that inorganic salts other than NaCl used as chemical deicers will likely contribute more Cl⁻ into the environment, we can expect residual chloride levels to be higher in freshwater systems adjacent to roadways receiving MgCl₂ and CaCl₂. In addition, the higher application rate required for effective winter maintenance using C₈H₁₂CaMgO₈ and CH₃COOK suggests that the concentrations of these chemicals in roadside water bodies will be close to or above the LC50 estimates for amphibians.

The effects of chemical deicers in the environment depend on the rate at which they are applied and their persistence in the environment. Road salt alternatives may cause greater environmental degradation because, relative to NaCl, greater quantities need to be applied to achieve similar levels of road deicing [1,28]. Thus, the negative impacts of these chemicals to amphibian communities will likely be elevated. Future work should address the suitability of reduced salting practices or non-chemical deicing alternatives instead of alternative chemical deicers on roads adjacent to water bodies.

Acknowledgement—We are indebted to R. Alger, P. Nankervis, M. Mitchell, H. Erickson, and E. Rogers for assistance in both field and laboratory portions of the experiment. We thank three anonymous reviewers for their thorough review of this manuscript and helpful suggestions. Funding for this research was provided by grants from the DeVlieg Foundation, the Chicago Herpetological Society, the Western New York Herpetological Society, the Amphibian Specialist Group of the International Union

for Conservation of Nature and Natural Resources, the Ecosystem Science Center at Michigan Technological University, and support from the MTU Global Watershed GK-12 program to M. Harless. This research has also been supported by funding and infrastructure provided by Michigan Technological University. Embryos were collected under permissions granted by the Keweenaw Bay Indian Community (KBIC) Department of Natural Resources. We conducted laboratory research under permit by Michigan Technological University IUCAC permit L01401.

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Table 2.1

Median lethal concentration values (LC50, g L⁻¹) with their 95% confidence limits for larval *Rana sylvatica* at 24, 48, 72 and 96 hours of exposure to six chemical deicers during acute toxicity tests (n=4).

<i>Value</i>	<i>CH₄N₂O</i>	<i>NaCl</i>	<i>MgCl₂</i>	<i>CH₃COOK</i>	<i>CaCl₂</i>	<i>C₈H₁₂CaMgO₈</i>
LC50 _{24-h}	14.63 (12.83-16.69)	9.12 (8.53-9.82)	7.37 (6.81-7.99)	7.03 (6.22-7.95)	4.85 (4.16-5.65)	3.43 (3.13-3.81)
LC50 _{48-h}	14.37 (12.77-16.18)	7.82 (7.64-8.01)	7.28 (6.73-7.92)	5.42 (4.85-6.06)	4.72 (4.08-5.47)	3.39 (3.07-3.74)
LC50 _{72-h}	14.37 (12.77-16.18)	7.64 (7.46-7.82)	7.24 (6.64-7.82)	4.76 (4.27-5.31)	4.18 (3.69-4.73)	3.31 (2.97-3.68)
LC50 _{96-h}	14.29 (12.55-16.26)	7.56 (7.31-7.82)	7.11 (6.54-7.74)	4.23 (3.84-4.66)	3.98 (3.46-4.57)	3.23 (2.94-3.59)

Table 2.2

Median lethal concentration values ($LC_{50_{96-h}}$, $g\ L^{-1}$) and 95% C.I. where available for larval amphibians native to the Northern and Eastern United States during acute exposure to deicing chemicals.

Species	Deicer	LC_{50}
<i>Ambystoma maculatum</i>	NaCl	1.84 (1.42–2.39) ^d
<i>Bufo americanus</i>	NaCl	6.14 (5.94–6.48) ^d
	MgCl ₂	0.105 ^a
<i>Hyla versicolor</i>	NaCl	1.05 ^c
<i>Pseudacris crucifer</i>	NaCl	4.43 (3.89–5.05) ^d
<i>Rana clamitans</i>	NaCl	0.406 ^a
		4.86 (4.42–5.36) ^d
<i>Rana sylvatica</i>	MgCl ₂	0.116 ^a
	NaCl	2.64 (2.53–2.74) ^{b,*}
		2.69 (2.31–3.14) ^d
		5.11 (5.46–6.93) ^{b,**}
	MgCl ₂	0.23 ^a
	Ca(CH ₃ CO ₂) ₂	0.48 ^a
	Mg(CH ₃ CO ₂) ₂	6.61 ^a
	Na ₄ Fe(CN) ₆	2.06 ^a

^a = Dougherty and Smith (2006)

^b = Sanzo and Hecnar (2006)

^c = Brand et al. (2009)

^d = Collins and Russell (2009)

* = Calculated using Spearman-Kärber Analysis

** = Calculated using Probit Analysis

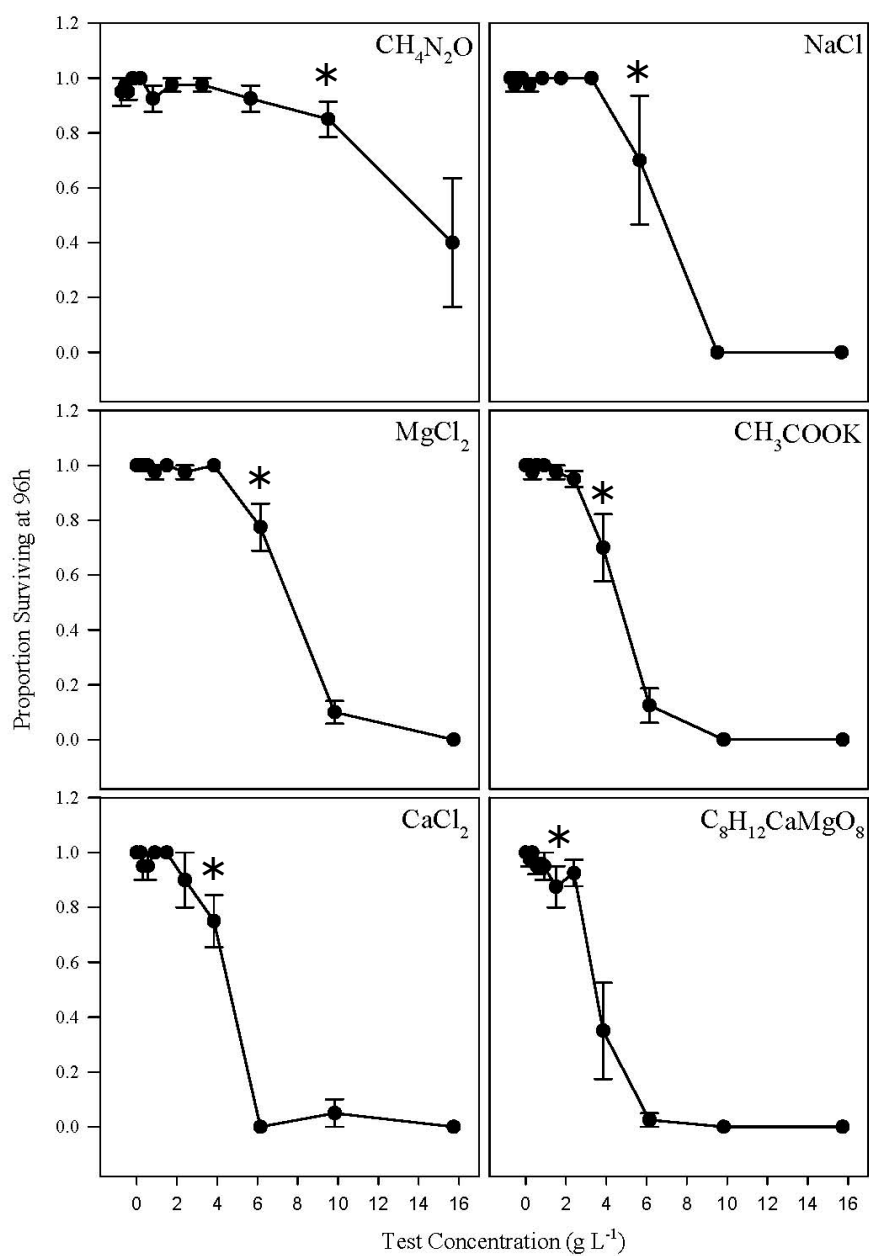


Figure 2.1: Mean proportion of surviving *Rana sylvatica* tadpoles after 96 hours of exposure to 10 nominal test concentrations of six chemical deicers. Error bars represent ± 1 S.E. Asterisks indicate the lowest test concentration at which survival was significantly lower than in the control (n=4 replicates per concentration).

Chapter 3: Sublethal Effects of Road Salt (NaCl) Exposure to Larvae of Two Amphibian Species

Abstract

Road salt (NaCl) is heavily applied in cold climates of North America causing a degradation of quality of surface and groundwater bodies. Amphibians can be considered indicator organisms in freshwater systems and are ideal to address the impacts of road salt use on roadside wetlands and vernal pools. While estimates of the median lethal NaCl concentration (LC₅₀) are useful for effective management, understanding the sublethal or chronic effects of exposure to lower levels of NaCl on these species will provide a better understanding of the potential developmental effects larval amphibians may experience. This is particularly important as species of amphibians using these roadside water bodies employ different life histories strategies. These differences may play a part in the demography of amphibian species in cold climates. I examined relative metrics of fitness in response to chronic NaCl exposure in two amphibian species that employ different life history strategies, wood frogs (*Lithobates sylvatica*) and green frogs (*L. clamitans*). The larvae of these two amphibians responded very differently to exposure to sublethal concentrations of NaCl with significant differences in *L. sylvatica* in terms of survival, size, the proportion of larvae or time to complete metamorphosis, or frequency of physical deformities in 100 days of exposure. However, presence of edema was significantly higher in the highest test concentration (750 mg L⁻¹) suggesting that NaCl exposure is a significant physiologic stressor to these rapidly developing larvae. For *L. clamitans*, NaCl exposure significantly decreased total size of larvae, whereas all other metrics were not significantly affected within 300 days of exposure. Evidence of these potentially harmful responses in these larvae suggests that prolonged exposure to NaCl may compromise fitness in amphibian communities.

Keywords: Road salt, sodium chloride, sublethal, chronic toxicity, edema, *Rana sylvatica*, *Rana clamitans*

Introduction

Sodium chloride (NaCl) is widely used in cold climates to remove or prevent the formation of ice on roads, highways, sidewalks, and airport runways. Each year an estimated 10 million metric tons is spread on these surfaces in the United States (D'Itri 1992). Across the country the application of NaCl varies from 0.3 to 17.6 metric tons per lane mile in 26 states (National Research Council 1991). Sodium chloride applied to a roadway has the potential to reach nearby surface water bodies such as wetlands and vernal pools (Environment Canada 2001; Forman et al. 2003). Not surprisingly, this intensive and widespread application of NaCl on an annual basis over the past few decades has resulted in an increase in the salinity of ground and surface waters in North America (Benbow and Merritt 2004; Ramakrishna and Viraraghavan 2005; Findlay and Kelly 2011) resulting in degradation of ground and surface water quality (Forman and Alexander 1998; Environment Canada 2001; Hoffman et al. 2000; Godwin et al. 2003).

Freshwater organisms vary widely in their tolerance of salinity (e.g., from NaCl) in their immediate environment (Environment Canada 2001). These differences in tolerance may cause a reduction in the diversity and overall abundance of aquatic organisms in roadside environments (Kaushal et al. 2005; Karraker et al. 2008). These differences are likely most notable in communities of less tolerant organisms such as amphibians. Amphibians are sensitive to environmental pollutants because they possess highly permeable skin, aquatic larval stages, and many use roadside wetlands for breeding (Stebbins and Cohen 1995).

Brief exposure to high salinities may decrease survival, decrease development rate, increase malformations (Gosner and Black 1957; Gomez-Mestre et al. 2004; Karraker 2007a,b) and increase the rate of infection of embryonic amphibians by lethal water mold (Karraker and Ruthig 2009). In addition, amphibian species respond differently to road salt exposure, and this tolerance may impact the demography of local species (Sanzo and Hecnar 2006; Karraker et al. 2008). Spring snowmelt could transport NaCl into roadside water bodies (Findlay and Kelly 2011) leading to spring and/or fall peaks in chloride concentrations. However, in some systems high evaporative demand in summer could

cause the chloride levels in these surface water bodies to increase dramatically as salts are concentrated by evaporation of water (Collins and Russell 2009; Kaushal et al. 2005).

Exposure to high salinity during development may cause significant harm to larval amphibians. Thus, it is important to examine the long term or sublethal effects of this exposure on the survival of amphibian larvae. Examining species with different life history strategies will allow for a greater understanding of the potential developmental effects of this stressor. Both wood frogs (*Lithobates sylvatica*) and green frogs (*L. clamitans*) breed in palustrine wetlands in the northern United States; however they tend to display different life history strategies and habitat use. *L. sylvatica* breed primarily in vernal pools and shallow wetlands, whereas *L. clamitans* tend to breed in larger wetlands with longer hydroperiods (Conant and Collins 1998). Further, *L. sylvatica* are one of the first amphibians to breed in the spring while *L. clamitans* breed in early summer. *L. sylvatica* larvae metamorphose quickly (five to nine weeks; Karraker 2007a) with *L. clamitans* larvae remaining as tadpoles for at least one year (Pauley and Lannoo 2005).

Few studies have addressed the impacts of road salt exposure on North American amphibian larvae. Most studies focus on identifying the concentration of NaCl during short-term exposure that leads to survival declines for a specific species (e.g., median lethal concentration or LC₅₀ estimate) (Karraker 2007a; Sanzo and Hecnar 2006; Collins and Russell 2009; Dougherty and Smith 2006; Harless et al. 2011). These resultant estimates are important for effective environmental management, yet many of these estimates exceed the mean environmentally realistic NaCl concentrations measured in roadside water bodies (Collins and Russell 2009; Sanzo and Hecnar 2006). Little is known about the effects of NaCl exposure on other metrics of amphibian fitness such as time to metamorphosis, duration of metamorphosis, size at metamorphosis, and presence of either physical deformities or behavioral abnormalities. These sublethal effects may have substantial negative impacts on amphibian populations inhabiting roadside water bodies. The goal of this study is to examine the sublethal effects of chronic exposure to

environmentally relevant concentrations of NaCl by two amphibian species, *L. sylvatica* and *L. clamitans*, common to cold climates of North America.

Methods

Study Organisms

I collected nine recently deposited *L. sylvatica* egg masses on May 8, 2008 from a palustrine wetland adjacent to a moderately traveled road in Baraga County, Michigan (latitude 46.796 N, longitude 88.390 W). From this same wetland, I collected six recently deposited *L. clamitans* egg masses on July 8, 2008. This road receives a low amount of salt (4.06 tons per lane mile for the winter of 2007-2008) during winter maintenance activities (DJ Mills, Baraga County Road Commission, personal communication). I transported the eggs to the lab and randomly assigned each egg mass to one of four aerated 78 L glass aquaria containing approximately 50 L of filtered water from Portage Lake, Houghton County, Michigan. The *L. sylvatica* eggs began hatching five days after collection with the *L. clamitans* eggs hatching two days after collection.

Chronic Toxicity Tests

Larvae were transferred to experimental test chambers when they reabsorbed their gills and reached Gosner stage 25 (Gosner 1960). I pooled larvae from different egg mass and randomly assigned 30 larvae of similar size to each test chamber. Test chambers consisted of 38 L glass aquaria filled with 20 L of filtered Portage Lake water and the appropriate amount of NaCl to obtain the following five nominal test concentrations: 0 (negative control), 48, 120, 300, and 750 mg L⁻¹. I chose the range of nominal test concentrations of NaCl in this experiment to include the lower environmental concentration of Cl⁻ in wetlands and vernal pools (2.1 – 10300 mg L⁻¹; Turtle 2000; Environment Canada 2001; Dougherty and Smith 2006; Sanzo and Hecnar 2006). I replicated the five nominal test concentrations four times for a total of 20 experimental units. Each test chamber was outfitted with a glass lid and one aerator to provide oxygen to the developing tadpoles.

Larvae were fed a 3:1 mixture of TetraFin Flake Goldfish Food (Tetra Werke, Melle, Germany) and pulverized Purina Rabbit Chow (Purina Mills, St. Louis, MO) every two days throughout the experiment. Test chambers were maintained in the laboratory on a 12:12h light to dark cycle using full spectrum lights. Air temperature remained stable (range: 18.7 – 22.1°C) during each experiment. I performed water changes every 7-10 days or as needed. Water changes consisted of siphoning 18 L of solution and excess food from each tank. Larvae were removed from the test chamber during cleaning and replaced into a cleaned tank with 18 L of fresh water with the appropriate amount of NaCl. The larvae in each test chamber were fed following each water change.

Larvae were checked every 24 hours and mortality was recorded at each interval. Significant developmental events such as the appearance of hind or forelimbs were recorded for each test chamber. Presence of any physical or behavioral deformities and/or abdominal edema was recorded in these daily observations. Larvae that were dead or unresponsive to probing with a small net or those that completed metamorphosis were removed and preserved in a solution of 10% formalin. The *L. sylvatica* experiment was terminated after 100 days when all tadpoles began metamorphosis (emergence of at least one front limb). I terminated the *L. clamitans* experiment after 300 days and preserved all tadpoles remaining in the test chambers. I dry blotted each specimen to obtain its wet mass. Because of a high incidence of edema in *L. sylvatica*, I did not use wet mass data as a metric of fitness in either experiment. All tadpoles and metamorphs were photographed to obtain total length (TL, from snout to tip of tail) and snout-vent length (SVL) measurements using Sigma Scan Pro (Systat Software Inc., San Jose, CA). For *L. sylvatica*, I limited length analyses to those larvae that were removed from the experiment following the completion of metamorphosis. For *L. clamitans*, all larvae surviving to the end of the test were included in the analysis of TL and SVL measurements. Individuals with tail damage (e.g., bitten by conspecifics) or significant deformities resulting in alteration of the tail were removed from this portion of the analysis.

Environmental Concentrations

To determine the environmentally realistic concentrations of Cl^- resulting from road salt application in the local area, I collected water samples from 16 wetlands averaging 2957 m (2 - 19082) from a salt-treated highway (US 41) in Baraga, Houghton, and Keweenaw Counties in the Upper Peninsula of Michigan once every two weeks throughout the amphibian breeding season (May through September). This area receives on average 141 inches of snow annually (NOAA 2011). Road salt application on this highway is through the use of a mixture of sand and rock salt (NaCl). Application rates for the winters of 2009–2010 range from 26.16 to 32.44 tons per lane mile in Baraga and Houghton counties (J. Lake MDOT pers. comm.). These values are much higher than the average application rate in Michigan (12.9 tons per lane mile) as well as average rates across the Great Lakes States (9.36 tons per lane mile; National Research Council 1991). Water samples were returned to the laboratory and stored at 4°C. I filtered the samples through a 0.45 μm cellulose acetate filter and quantified chloride concentration using ion chromatography (Dionex ICS-900, AS22 4 mm column, Fisher Scientific Inc., Sunnyvale CA).

Statistical analysis

In the *L. sylvatica* experiment I removed two tanks (one each of the 300 mg L^{-1} and 750 mg L^{-1} NaCl treatments) due to significant mortality as a result of an overgrowth of bacteria. I used a Shapiro-Wilk test for normality for the distribution of residuals. I used a one-way analysis of variance (ANOVA) to determine if proportion of surviving larvae, TL, SVL, proportion of deformed larvae, proportion of larvae with edema, proportion of larvae reaching metamorphosis or the number of days to complete metamorphosis were significantly affected by NaCl concentration. I then used Tukey multiple comparisons to identify significant differences between test concentrations for these variables.

Chloride concentration failed the Shapiro-Wilk test for normality test even after multiple transformation attempts in both years. I used the nonparametric Kruskal-Wallis one way analysis of variance test to examine differences in chloride concentration across sample

months with multiple post hoc comparisons to test for differences between months. At the end of each experiment, I calculated the proportion of larvae in each test chamber that survived through metamorphosis. I define the start of metamorphosis as the emergence of at least one forelimb (Gosner stage 42), the end of metamorphosis as the complete reabsorption of the tail (tail length <2 mm; Gosner stage 46, Gosner 1960), and the time to complete metamorphosis as the number of days between these developmental achievements. I considered each tank as an experimental unit in both tests. I used “R: A Language and Environment for Statistical Computing” to perform all statistical analyses with an alpha level of 0.05 (R Development Core Team 2010).

Results

Chronic Toxicity Tests

For *L. sylvatica*, the majority of the tadpoles survived to the end of the test (mean = 27.7). Survival remained above 80% across test concentrations (Figure 3.1). I observed the largest larvae in the highest NaCl exposure (750 mgL⁻¹), but size was not significantly different across test concentrations (TL: $F_{4,13} = 1.522$, $P = 0.235$; SVL: $F_{4,13} = 0.372$, $P = 0.824$; Table 3.1). The majority of surviving *L. sylvatica* larvae completed metamorphosis during the experiment (Figure 3.2). Neither the proportion of larvae surviving to the end of test ($F_{4,13} = 1.052$, $P = 0.419$), nor proportion of larvae completing metamorphosis, varied significantly by test concentration ($F_{4,13} = 1.446$, $P = 0.274$). The number of days to complete metamorphosis was highest in the 300 mgL⁻¹ treatment but was not significantly across test concentrations ($F_{4,13} = 0.763$, $P = 0.567$, Figure 3.3).

I observed 27 juvenile *L. sylvatica* (5% of all tadpoles) with physical deformities during the experiment and 40% of these died before the end of the test. The majority of tadpoles had a singular deformity including rear legs that were elongated, stretched out, and thin (70%). Other deformities included bent tails (11%), missing a portion of the bottom jaw (7%), scoliosis (4%), and one missing forelimb (4%) with one tadpole exhibiting both deformed rear legs and missing a portion of the jaw (4%). The proportion of deformed

larvae varied across test concentration but was not significantly different among or between the five treatments ($F_{4,13} = 0.3269$, $P = 0.855$; Figure 3.4).

Swelling as a result of abdominal edema occurred in 119 *L. sylvatica* larvae with 72% of these larvae surviving to the end of the test. The proportion of juvenile *L. sylvatica* exhibiting abdominal edema was significantly different across test concentrations ($F_{4,13} = 7.882$, $P = 0.002$). In addition, larvae in the 750 mgL⁻¹ treatment had a significantly higher proportion of edema than those in the control ($P = 0.001$), 48 mgL⁻¹ ($P = 0.008$), or 120 mgL⁻¹ treatments ($P = 0.017$, Figure 3.5).

For *L. clamitans*, survival was highest in the 48 mgL⁻¹ treatment and lowest in the control ($F_{4,15} = 1.308$, $P = 0.312$; Figure 3.1). There were no significant differences in survival between test concentrations ($P > 0.05$). At the end of the exposure period, tadpoles were significantly different in TL ($F_{4,15} = 4.047$, $P = 0.020$), with the largest tadpoles in the control (Table 3.1). Total tadpole length decreased in higher NaCl concentrations with significantly larger tadpoles in the control versus the 48 mgL⁻¹ ($P = 0.066$), 300 mgL⁻¹ ($P = 0.040$), and 750 mgL⁻¹ treatments ($P = 0.019$). Measurements of SVL did not significantly vary between test concentrations ($F_{4,15} = 0.373$, $P = 0.824$). All other comparisons were not significant ($P > 0.05$). No *L. clamitans* larvae exhibited signs of edema. Nine tadpoles started, but only two completed metamorphosis (one each in the control and 120 mgL⁻¹ concentrations) within 300 days. Thus, I did not examine the number of days to complete metamorphosis in the *L. clamitans* test.

The proportion of *L. clamitans* larvae exhibiting a physical deformity was highest in the control and decreased as test concentration increased (Figure 3.4). I observed 36 larval and juvenile *L. clamitans* with at least one physical deformity (6% of all tadpoles). The majority of these deformities included bent tails (94%) or a double lobed tail (3%) with one larva exhibiting both deformities. Differences in the proportion of deformities were not significant among ($F_{4,15} = 0.250$, $P = 0.905$) or between the test concentrations ($P > 0.05$).

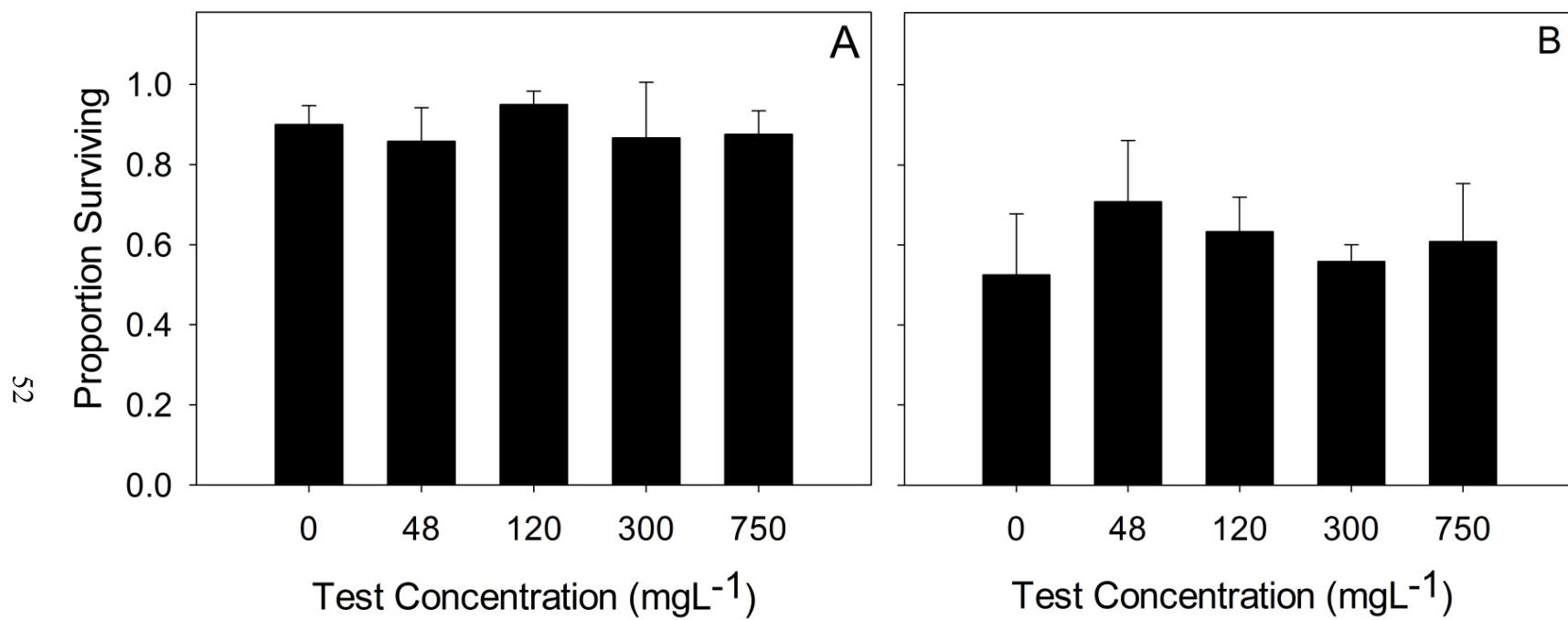


Figure 3.1: Mean proportion of *Lithobates sylvatica* (A) and *L. clamitans* (B) larvae surviving to the end of the test in each test concentration. Error bars represent ± 2 S.E.

Table 3.1

Mean (range) total length (TL) and snout-vent length (SVL) of *L. sylvatica* that completed metamorphosis and *L. clamitans* that survived to the end of the test exposure.

Test Concentration (mgL ⁻¹)	<i>Lithobates sylvatica</i>		<i>Lithobates clamitans</i>	
	TL (mm)	SVL (mm)	TL (mm)	SVL (mm)
0	19.06 (17.8-20.9)	16.29 (15.3-17.3)	57.90 (51.2-65.3)	21.70 (19.9-24.1)
48	19.23 (17.6-21.4)	16.13 (15.3-16.9)	51.15 (49.9-52.5)	19.40 (18.7-20.2)
120	19.63 (19.2-20.6)	16.14 (15.3-16.9)	52.44 (48.3-55.6)	19.06 (17.5-19.9)
300	19.18 (18.6-19.9)	16.14 (15.9-16.3)	50.18 (47.9-51.4)	18.86 (17.7-19.4)
750	19.97 (19.3-20.9)	17.11 (16.9-17.4)	49.66 (47.6-51.9)	18.32 (17.4-19.3)
N	458	488	297	311

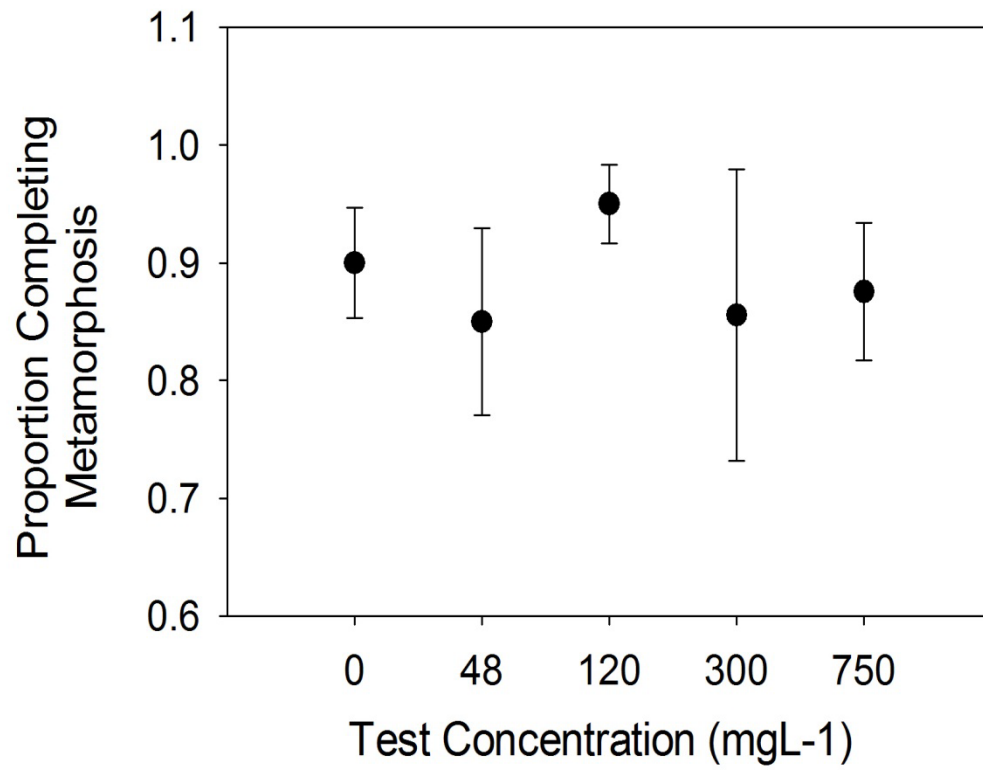


Figure 3.2: Mean proportion of *L. sylvatica* larvae completing metamorphosis in each test concentration. Error bars represent ± 2 S.E.

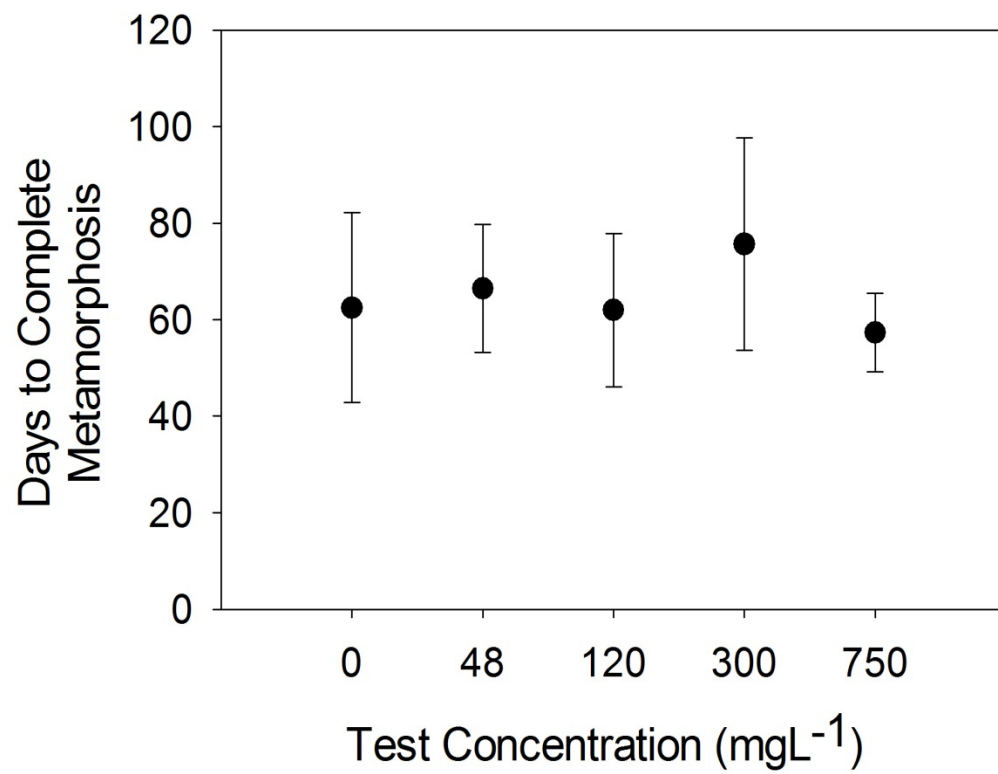


Figure 3.3: Number of days for *L. sylvatica* larvae to complete metamorphosis in each test concentration. Error bars represent ± 2 S.E.

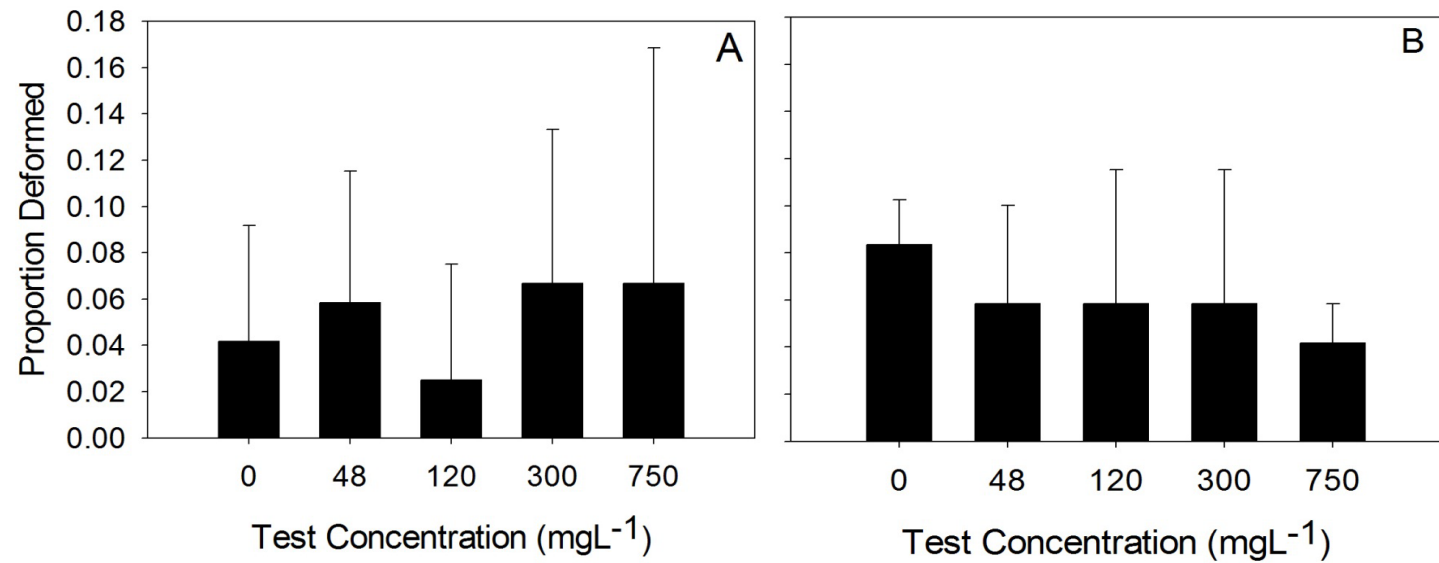


Figure 3.4: Mean proportion of *Lithobates sylvatica* (A) and *L. clamitans* (B) larvae exhibiting a physical deformity in each test concentration. Error bars represent ± 2 S.E.

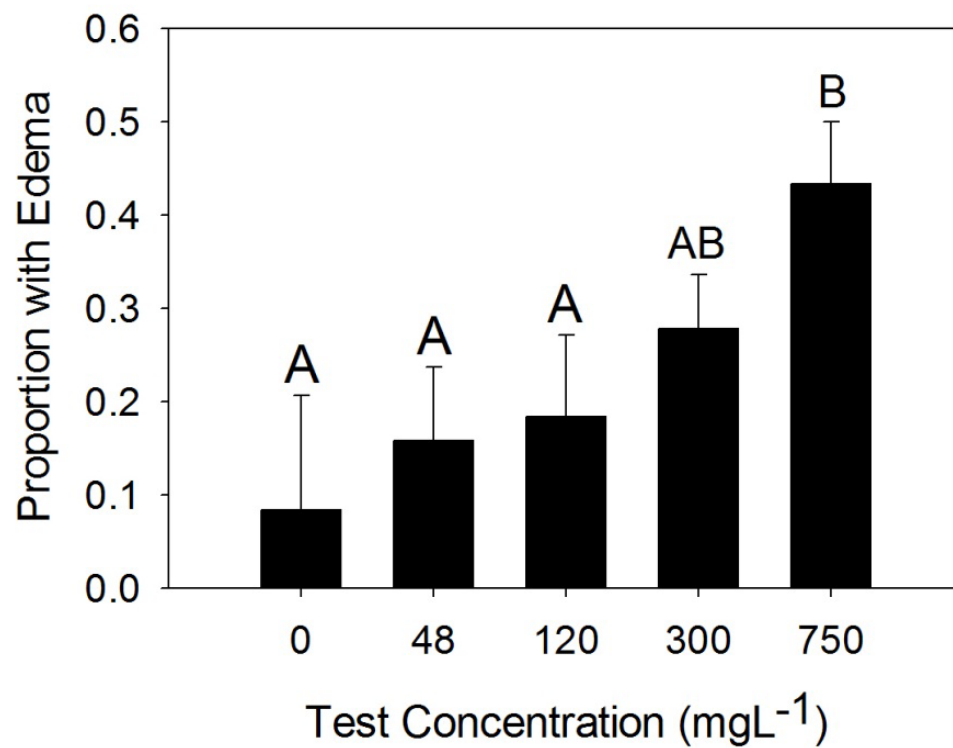


Figure 3.5: Mean proportion of *L. sylvatica* larvae exhibiting abdominal edema in each test concentration with letter indicating significantly different groups. Error bars represent ± 2 S.E.

Environmental Concentrations

Analysis of chloride concentrations in roadside wetlands and vernal pools revealed variation in concentrations in the local environment. Chloride concentrations in local wetlands and vernal pools ranged from 0.0015 to 18.94 mgL⁻¹ (mean = 2.115, max 128.5). Chloride concentrations were highest in late summer (August, Figure 3.6).

However, chloride concentrations were not significantly different across ($H = 4.9432$, $df = 5$, $P = 0.422$) or between sample months ($P > 0.05$ for all tests).

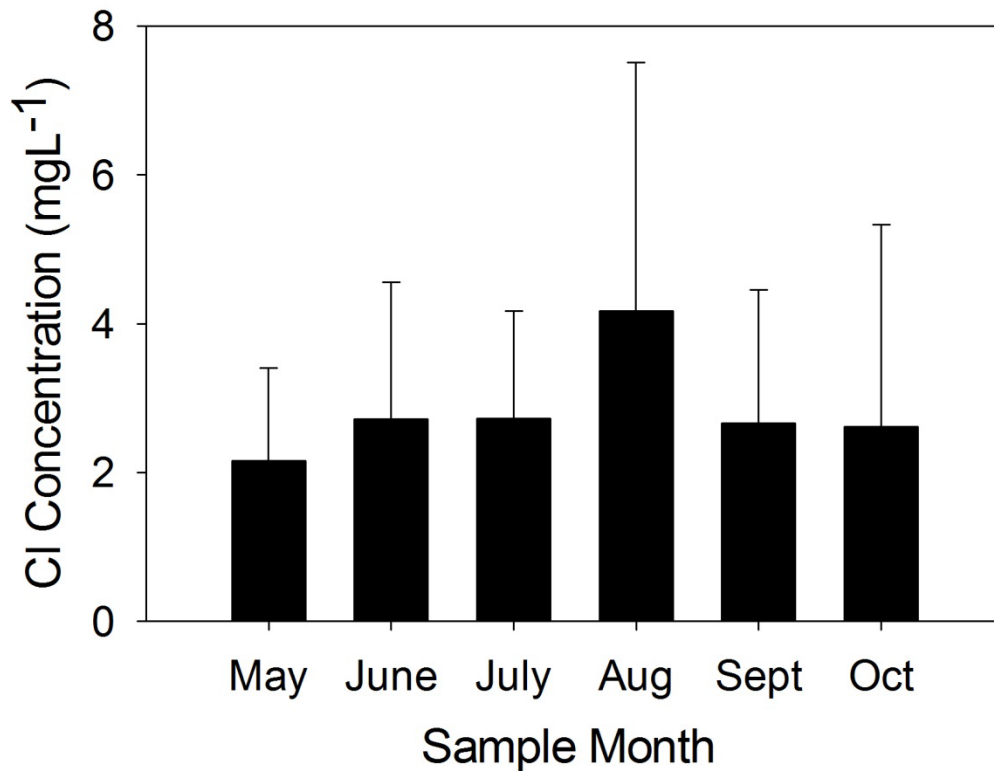


Figure 3.6: Mean environmental concentration of Cl⁻ in wetlands and vernal pools during the amphibian breeding season. Error bars represent ± 2 S.E.

Discussion

Chronic exposure to low concentrations of road salt (NaCl) did not affect survival of either *Lithobates sylvatica* or *L. clamitans* in this study. Survival for both species remained high (>80% for *L. sylvatica* and >50% for *L. clamitans*) across the exposure concentrations, slightly below the natural survival estimates for *L. sylvatica* (95-98%, Siegal 1983). Two studies have examined the effects of chronic exposure to low concentrations of NaCl with native amphibian species (Karraker 2007a,b; Sanzo and Hecnar 2006). Karraker (2007a) observed a significant decrease in survival of both *L. sylvatica* (<20%) and *Ambystoma maculatum* (<11%) larvae in long-term exposure to NaCl concentrations above 945 mgL⁻¹, respectively, yet survival in *L. clamitans* remained above 90% in embryos and larvae exposed to NaCl concentrations from 0 to 465 mgL⁻¹ decreasing to 87% at 945 mgL⁻¹. Additionally, Sanzo and Hecnar (2006) found decreased survival (<5%) in *L. sylvatica* exposed to NaCl concentrations above 1030 mgL⁻¹ whereas survival was <20% in the control, 0.39, and 77.50 mgL⁻¹ treatments. The variability in these studies may be attributed to differences in the life stage and length of exposure periods. Karraker (2007b) used both embryonic and larval amphibians in a 70 day exposure, whereas Sanzo and Hecnar (2006) used larvae in a 90 day exposure. The use of only larval *L. sylvatica* and *L. clamitans* for longer time periods (100 and 300 days) in this study suggests that larvae in this area may be tolerant to long term NaCl exposure than other populations. Furthermore, exposure to high salinity may slow or halt development, as well as cause deformities (Gosner and Black 1957; Gomez-Mestre et al. 2004). Exposure to NaCl did not affect size of larval *L. sylvatica* nor the number days to complete metamorphosis in this study. We would expect *L. sylvatica* to decrease time to metamorphosis resulting in smaller size at metamorphosis when exposed to high NaCl concentrations, as observed by Sanzo and Hecnar (2006).

Wood frog larvae in this study completed metamorphosis in a shorter amount of time (mean = 65 days, range: 37-90) than observed in other studies on chronic effects of salt exposure (77 days, range 65-81; Sanzo and Hecnar 2006). In terms of size at metamorphosis, our results are similar to that of Karraker (2007a) in which neither larvae

of *L. sylvatica* nor *A. maculatum* differed in size during long-term exposure to NaCl. However, our results for *L. clamitans* support the hypothesis that NaCl exposure may accelerate development leading to smaller adults in that we observed the largest individuals (TL) in the control at the end of 300 days. This discrepancy is most likely due to the developmental differences between these species. Wood frogs larvae developing in vernal pools and shallow wetlands must metamorphose quickly in order to survive the variable hydroperiods in these water bodies. Hence these larvae will limit their time of exposure to potentially harmful salt concentrations. In contrast, anurans with larval periods of over one year will potentially be exposed to higher salt concentrations for a longer period. This would result in late and significant effects on adult fitness such as decreased size.

The developmental plasticity of *L. clamitans* may help to explain its tolerance for acute exposure to high concentrations of NaCl (Karraker 2007b). Evidence of significant differences in total length (TL) but not in body size (SVL) suggests that the tail size in *L. clamitans* may be affected by NaCl exposure. Predation risk may induce a number of morphological, behavioral and developmental changes in larval amphibians. *L. clamitans* tadpoles are known to exhibit plasticity of developmental traits (e.g., tail size or shape, behavior, growth, and time to complete metamorphosis; Relyea 2001). Smaller tail size may decrease the ability of these larvae to escape from predators. In addition to physiological stress, these effects could cause serious lethal ramifications for overwintering tadpoles exposed to NaCl.

Sanzo and Hecnar (2006) estimated the median lethal concentration (LC_{50}) from 96 hours of exposure between 2636 and 5109 mgL^{-1} . The low NaCl concentrations used in this experiment are well below the NaCl LC_{50} estimates for this population of *L. sylvatica* (7560 mgL^{-1} ; Harless et al. 2011) suggesting that test concentrations may be too low to observe significant effects on survival during long term exposure in this population. Furthermore, test concentrations used in this study were greater than environmental

concentrations in the local area suggesting that local amphibian larvae are not at risk of exposure to Cl^- levels beyond their tolerance level.

The presence of both edema and physical abnormalities also differed between these two species. Edema in larval anurans indicates a failure of the renal system and is a physiological response to a variety of stressors, including environmental factors, diseases (Pessier 2009), pesticide exposure (Howe et al. 1998), and exposure to NaCl (Karraker 2007b). I observed a high incidence of edema in the wood frog larvae exposed to NaCl above 750 mgL^{-1} whereas no green frog larvae exhibiting edema. In contrast, Karraker (2007b) observed a high percentage (92%) of edema in newly hatched *L. clamitans*. Larvae with edema were sluggish and buoyant, and thus more likely to be consumed by a predator. This increased predatory risk in exposure to low NaCl concentrations may have severe implications for juvenile recruitment in this population. The majority of deformities occurred at the highest salt concentration for *L. sylvatica* but in the control treatment in the *L. clamitans* experiment supporting the hypothesis that green frog larvae are more tolerant of NaCl exposure than wood frogs. Karraker and Ruthig (2009) observed higher incidence of malformations in *A. maculatum* but not *L. clamitans* larvae at high NaCl exposure. In addition, the low rate of overall deformities in this study is similar to rates found in previous studies of long-term NaCl exposure to larval amphibians (reviewed in Karraker 2007a).

Analysis of chloride levels in roadside water bodies indicates a low level of persistent Cl^- as a result of road salt application in the local area. The maximum chloride concentration observed in this study (128.5 mgL^{-1}) is much lower than observed in other studies in which maximum concentrations of 586 (Collins and Russell 2009), 945 (Karraker 2007a) 1,030 (Sanzo and Hecnar 2006), 4,629 (Kaushal et al. 2005), and 18,000 mgL^{-1} (Environment Canada 2001) were observed. I did however observe a similar temporal trend in chloride concentration with highest chloride concentrations in both early spring and again in late summer as noted in previous studies (Godwin et al. 2003; Kaushal et al. 2005; Karraker 2007b; Collins and Russell 2009). These peaks in chloride occur at

critical periods of amphibian development. Increased temperatures and snowmelt in spring triggers breeding activity in wood frogs (*L. sylvatica*; Stebbins and Cohen 1995). Embryonic wood frogs and overwintering *L. clamitans* larvae are at risk of this exposure in early spring. Furthermore, chloride peaks in late summer may have negative impacts on larvae of both species, particularly for *L. sylvatica* that are likely to be completing metamorphosis during this time (Karraker 2007a).

One study has examined the chloride concentration of streams in winter and found a significant increase in chloride during heavy road salt application periods. Kaushal et al. (2005) noted the highest maximum chloride concentrations in urban streams in winter compared to all other seasons. This indicates the potential for high chloride outside of the time periods examined in this study. These high levels may present a significant stressor to amphibian larvae that overwinter, such as those of *L. clamitans*.

In summary, the larvae of these two amphibians responded very differently to exposure to sublethal concentrations of NaCl. *Lithobates clamitans* appears to be more tolerant of high concentrations of NaCl yet prolonged exposure through a lengthy larval period may have significant fitness costs. The high occurrence of edema in *L. sylvatica* larvae suggests that even brief NaCl exposure is a significant physiologic stressor to these rapidly developing larvae. While persistent Cl⁻ in the local area remains low, I cannot assume a lack of effect of prolonged exposure to native amphibians. Future work should address this relationship in an effort to shed more light onto the species differences in sensitivity to NaCl exposure.

Acknowledgements

I am indebted to R. Alger, P. Nankervis, M. Mitchell, H. List, E. Gorsalitz, E. Rogers, N. DelPiccolo, K. Griffith, and R. Datta for assistance in both field and laboratory portions of the experiment. Funding for this research was provided by grants from the DeVlieg Foundation, the Chicago Herpetological Society, the Western New York Herpetological Society, the Amphibian Specialist Group of the International Union for Conservation of

Nature and Natural Resources, the Ecosystem Science Center at Michigan Technological University, with support from the MTU Global Watershed GK-12 program. This research has also been supported by funding and infrastructure provided by Michigan Technological University. Embryos and larvae were collected under permissions granted by the Keweenaw Bay Indian Community Department of Natural Resources. I conducted laboratory research under permit by Michigan Technological University (IUCAC permit L01401).

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Chapter 4: An Analysis of Chloride Concentrations in Surface Water Bodies in Michigan's Upper Peninsula

Abstract

The impacts of roads on surrounding terrestrial and freshwater ecosystems may be quantified through the identification of a road effect zone. This represents the area adjacent to the roadway in which significant impacts on the survival or health of wildlife species may occur. Amphibians experience negative effects on fitness when using these water bodies while exposed to elevated chloride levels. I use the road effect zone concept to identify water bodies near roads that receive road salt (NaCl) that contain elevated levels of Cl that may impact breeding amphibians. I measured residual chloride concentrations in 137 surface water bodies close to and far from a salt treated highway in the three northernmost counties of Michigan's Upper Peninsula from May to September of 2009. Chloride concentrations were significantly higher in waters within 40 m of this highway and varied throughout the amphibian breeding season with the highest levels measured in late summer (range: 0-18.3 mgL⁻¹). While these levels were lower than in previous studies, larval amphibians in these wetlands may experience substantial sublethal impacts on fitness (Chapter 3). The acreage of palustrine wetlands impacted by road salt application in these three counties was highest in Houghton County when using a buffer of 40m as well as a conservative estimate of 250 m. Management efforts should focus on the protection of surface waters near salt-treated highways through reduced salt zones or other non-chemical methods of reducing NaCl application.

Keywords

road salt, de-icing salt, water quality, chloride, road-effect zone, amphibians

Introduction

Substantial negative impacts of roads on wildlife populations has been documented in a variety of species ranging from ground beetles and grizzly bears to amphibians (*reviewed in* Eigenbrod et al. 2009). Road density in the United States is increasing yearly, leaving fewer and fewer tracts of road-less area in states such as Michigan (Figures 4.1 and 4.2). A high density of roads may have substantial negative impacts on local wildlife species. The primary ecological effects of roads on surrounding habitats are from habitat loss or transformation, pollution, avoidance, mortality (from traffic and predation), and the corridor effect (*reviewed in* Jaeger et al. 2005). Of these effects, road-based pollutants are considered a serious threat to terrestrial and aquatic ecosystems (Trombulak and Frissell 2000). Chemicals used in road deicing activities are recognized as a major contributor to habitat transformation and pollution as a growing threat to ecosystem health (Environment Canada 2001; Ramakrishna and Viraraghavan 2005).

Increasing and widespread use of sodium chloride (NaCl) as a deicing agent in cold climates of North America over the past 60 years has resulted in elevated chloride levels in surface water bodies (Kaushal et al. 2005). NaCl is the most common de-icing agent in the United States due to its low cost and availability at a number of salt mines across the country (Forman et al. 2003). NaCl use nationwide is estimated at 10 million tons (D'Itri 1992), ranging from 0.3 to 17.6 tons per lane mile across 26 states (National Research Council 1991). The Great Lakes region receives the highest amount of salt, with Michigan receiving the most NaCl with an average of 12.9 tons per lane-mile (National Research Council 1990). The three northernmost counties of Michigan's Upper Peninsula (Figure 4.2) use a 19:1 mixture of sand to salt. The annual salt load estimates on primary roads in Baraga and Keweenaw counties, as well as on all roads in Houghton County are much lower than the state average (Table 4.1).



Figure 4.1: Density of roadways in the upper and lower peninsulas of Michigan, U.S.A.

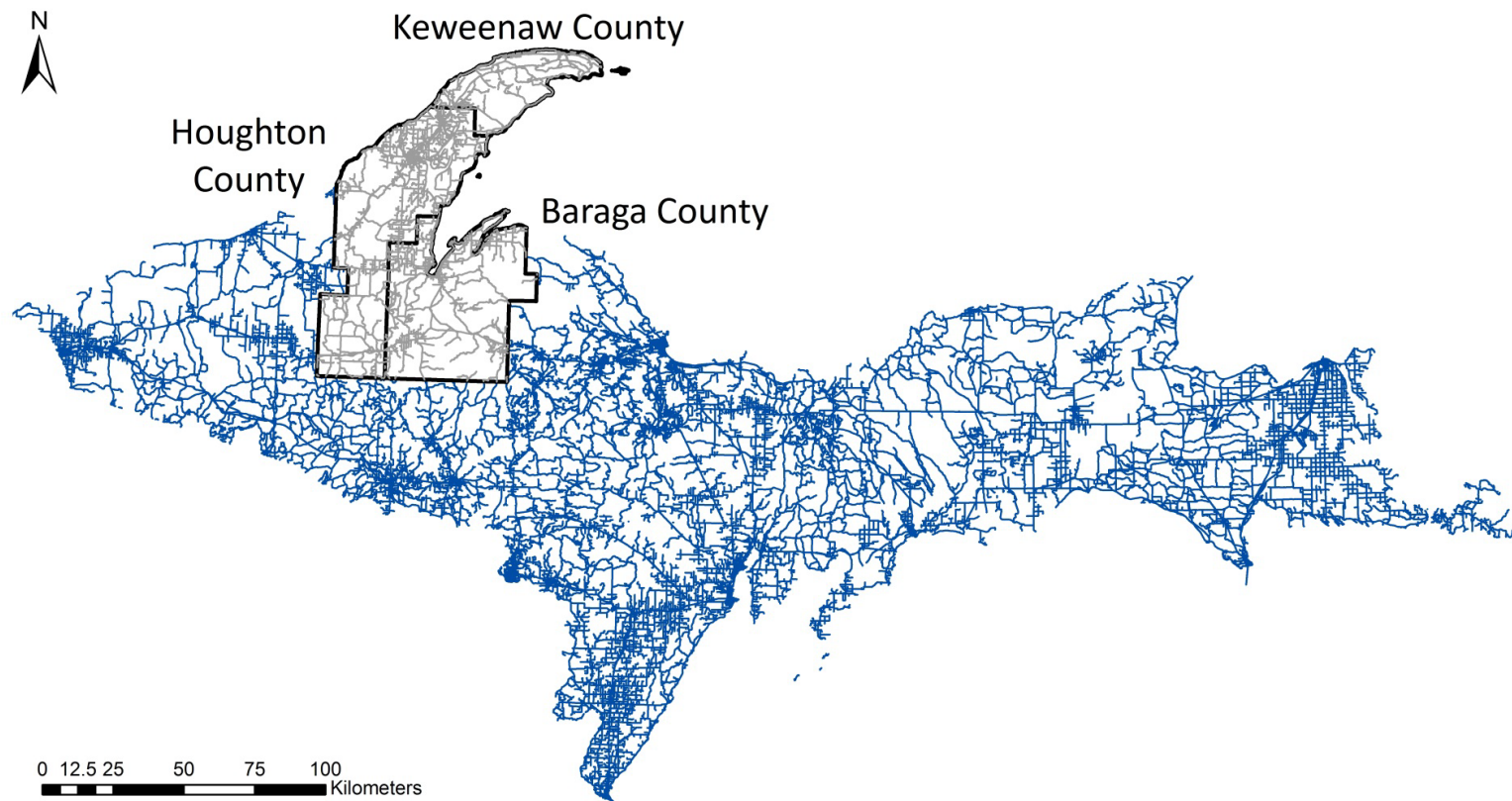


Figure 4.2: Density of roadways in Michigan's Upper Peninsula highlighting the roads in the focal region in this study (grey lines): Baraga, Houghton, and Keweenaw Counties (black outline).

Table 4.1

Average salt (NaCl) and sand use on county and state roads in Baraga, Houghton, and Keweenaw counties of Michigan.

County	Roads	Primary Roads (lane miles)	Sand Used (tons)	NaCl Used (tons)	NaCl Per Lane Mile (tons)
Baraga ^{a,b}	County	156	15,842	634	2.56
	State	245	-	6,410	26.16
Houghton ^{b,c}	County	247	18,000	637	1.61
	State	160	-	5,191	32.44
Keweenaw ^{d,e}	County	112	8,316	504	5.10
	State	91	-	-	-

^a D.J. Mils, Baraga County Road Commission, *pers comm.*

^b J. Lake, Michigan Department of Transportation (MDOT), *pers comm.*

^c Houghton County Road Commission Factsheet.

^d G.M. Patrick, Keweenaw County Road Commission, *pers comm.*

^e Total salt use by county including state highways.

The environmental damage from NaCl application arises from the accumulation of chloride in roadside soils and water bodies. Once applied, NaCl is readily transported into the roadside environment and temporarily held within the soil. Sodium ions are taken up in the soil whereas chloride ions are not subject to natural removal mechanisms (Lunmark and Olofsson 2007). The resultant Cl^- is transported to nearby surface waters where it may eventually accumulate in the groundwater (Environment Canada 2001). Chloride contamination of surface water bodies in the United States is well documented (Godwin et al. 2003; Kaushal et al. 2005). The US Environmental Protection Agency considers an average chloride concentration of 230 mgL^{-1} over a four day period to be harmful to aquatic life (USEPA 1988). Environment Canada considers prolonged exposure to chloride concentrations above 220 mgL^{-1} to be harmful to aquatic organisms. However, chloride levels in excess of 4000 mgL^{-1} in roadside ponds and 18000 mgL^{-1} in urban areas have been observed (Environment Canada 2001). The range of minimum chloride concentrations that produce negative impacts on freshwater organisms range from 0.5 to $1,500 \text{ mgL}^{-1}$ (*reviewed in* Findlay and Kelly 2011). These ranges suggest that freshwater organisms vary greatly in their tolerance of Cl^- concentrations. This highlights the importance of monitoring Cl^- concentrations in surface water bodies in order to characterize the potential negative impacts of NaCl application.

The ecological effects of a road on the surrounding habitat may be quantified using a road-effect zone. Forman and Alexander (1998) introduced the concept of a road-effect zone as a defined distance from a roadway in which substantial negative ecological impacts on a species are observed. Thus far, six studies have attempted to quantify this zone to define the direct and indirect effects of roads on the surrounding ecosystem (Table 4.2). Estimates produced in these studies suggest the road-effect zone ranges from $35 - 1000 \text{ m}$ from the road, depending on the species and metric of analysis.

Table 4.2

Results of studies quantifying the road-effect zone for a variety of organisms.

Focal Species	Metric	Road-effect Estimate (m)	Reference
Forest Birds	Habitat quality	< 200	Reijnen et al. 1995
Forest and Grassland Birds	Abundance	930	Forman and Deblinger 2000
Invasive plants	Survival	100	
Moose	Home range	300	
Deer	Abundance	300	
Desert Tortoises	Abundance	400	Boarman and Sazaki 2006
Salamanders	Abundance	35	Semlitsch et al. 2007
Frogs and Salamanders	Abundance	50	Karraker 2007
Frogs	Species richness	250-1000	Eigenbrod et al. 2009

The road-effect concept may be applied to studying the indirect effects of road salt contamination by examining chloride concentrations in roadside water bodies. The goal of this study is to quantify the relationship between chloride concentration and distance to the nearest salt treated road in the northern most three counties of Michigan's Upper Peninsula. This area has a high density of perennial wetlands with few heavily traveled roads, making it an ideal location to investigate this question (Figure 4.3). I expect to see a negative relationship between distance from salt-treated roadway and chloride concentrations. In addition, I anticipate that chloride concentrations in roadside pools to meet or exceed threshold levels for lethal and sublethal effects of NaCl on local amphibian species (Chapters 2 and 3).

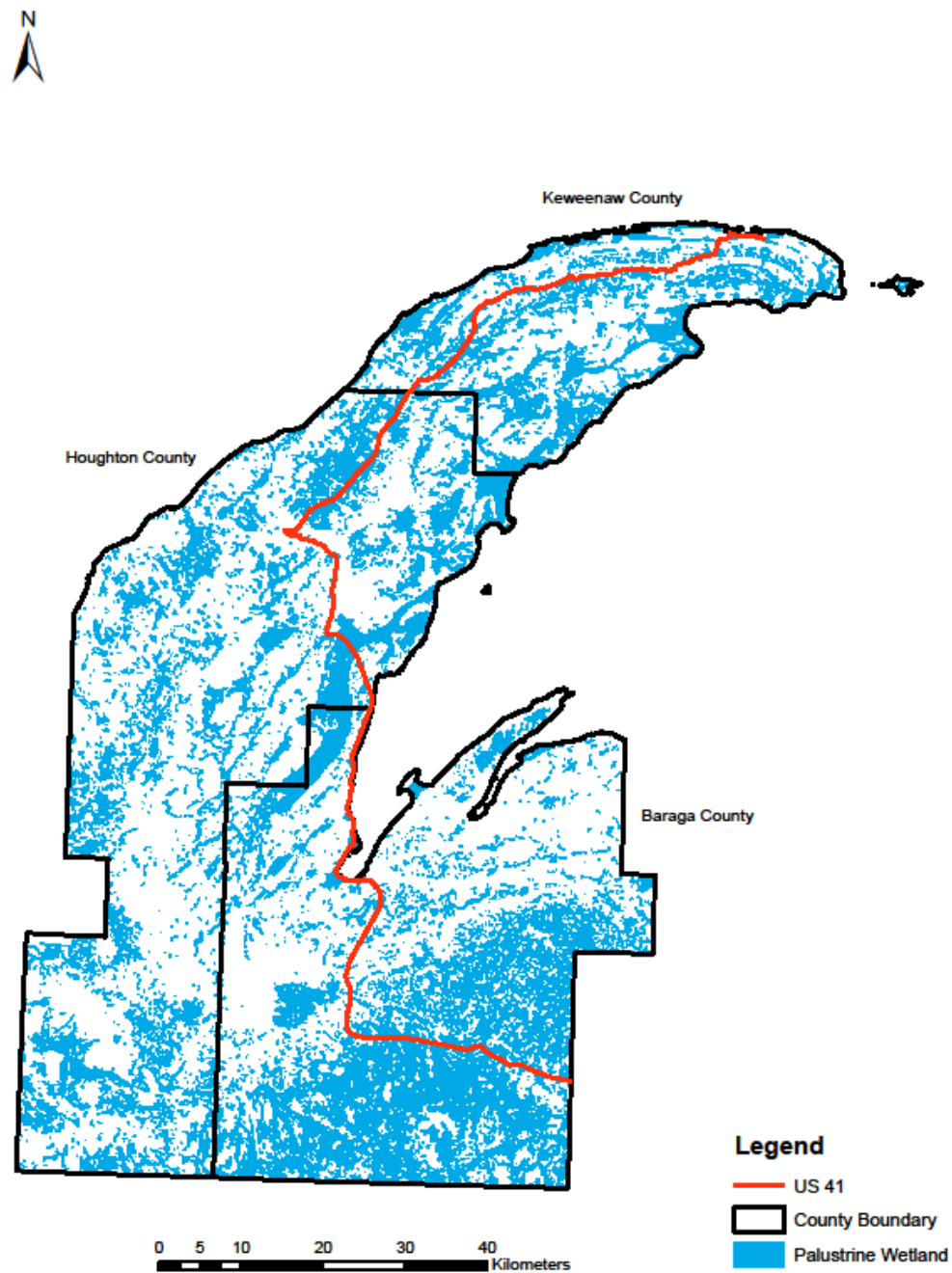


Figure 4.3: Density of palustrine wetlands in Baraga, Houghton, and Keweenaw Counties of Michigan's Upper Peninsula.

Methods

To determine the environmentally realistic concentrations of Cl^- resulting from road salt application in the local area, I collected water samples from 137 perennial wetlands at varying distances from US 41 across Baraga, Houghton, and Keweenaw Counties in the Upper Peninsula of Michigan throughout the 2009 amphibian breeding season (May through September). These water bodies were on average 3.1 km (.005 – 19.08) from a salt-treated highway. I chose to focus our sampling along US 41 it traverses all three northern counties. Water samples were returned to the laboratory and stored at 4°C. I filtered the samples through a 0.45 μm cellulose acetate filter and quantified chloride concentration using ion chromatography (Dionex ICS-900, AS22 4 mm column, Fisher Scientific Inc., Sunnyvale CA). I used ArcGIS to measure the distance from the water sample collection location to the nearest edge of a salt-treated roadway and for the following geoprocessing analysis (ESRI 2011).

Chloride concentration remained non-normally distributed even after multiple transformations in both years using the Shapiro-Wilk test for normality for the distribution of variables. I used the nonparametric Kruskal-Wallis one way analysis of variance test to examine differences in chloride concentration across sample months with multiple post hoc comparisons to test for differences between months (R package ‘pgirmess’). To characterize the relationship between Cl^- concentration and distance to the nearest salt-treated road in local water bodies, I used a linear regression model after a log transformation of these variables. Analysis of the residuals in normal probability plots of this model did not indicate skewness or a heavy-tailed distribution of the residuals that would affect the fit of our regression model (Montgomery et al. 2006). I removed seven data points from the statistical analysis that were identified as exhibiting severe influence on the model fit by analysis of leverage plots (R package ‘car’, maximum concentration = 90.2 mgL^{-1}). These points were collected in the summer (June and July) within 55 m of US 41. I divided the chloride concentration data into four quartiles using R (25, 50, 75, and 100%) based on the distance from the sample location to the nearest salt-treated highway (US 41). I used the nonparametric Kruskal-Wallis one way analysis of variance test to examine differences in chloride concentration across these distance categories with

multiple post hoc comparisons to test for differences between different distance categories (R package ‘pgirmess’).

I obtained spatial data on the infrastructure and hydrology of our study area from the Michigan Center for Geographic Information (CGI; <http://www.michigan.gov/cgi/>). I chose to use the Department of Natural Resources Wetlands data because it discerned between the lacustrine (formed at the bottom or along the shore of a lake) and palustrine wetlands (inland wetlands that are not affected by the lake). All but the palustrine wetlands were removed from these layers prior to analysis. I obtained data for county boundaries using the CGI framework for political-senate boundaries. Isle Royale was removed from the analysis by subtracting 535.4 km² (132.3 acres) from the data for Keweenaw County.

The Michigan Framework (version 11) data layer was used to obtain information on the amount and types of roadways in these three counties. To obtain county totals of specific road types I divided the roads as follows according to the Framework Classification Code (FCC) in Appendix D of the metadata for the Michigan All Roads layer: primary roads are coded as FCC A21 or A23, secondary roads as A31-A33, and tertiary or unmanaged roads as A41-A90. For our analysis I used a conservative road-effect zone of 250 m based on estimates from previous studies on amphibians (Table 4.2). I placed a buffer zone of 40 m and 250 m from US 41 across Baraga, Houghton, and Keweenaw Counties. To determine the area of wetlands that are located within this buffer zone, I used the intersect function on the US 41 buffer layer and the palustrine wetlands layer. I used “R: A Language and Environment for Statistical Computing” to perform all statistical analyses with an alpha level of 0.05 (R Development Core Team 2010).

Results

Water bodies closest to the nearest salt-treated highway contained elevated chloride levels as a result of road salt application (Figures 4.4 – 4.7). However, the concentration of chloride steadily decreased as distance from the highway increased. Distance from the nearest salt-treated highway significantly predicted chloride concentration in these water

bodies ($b = 2.39$, $t_{253} = 10.07$, $P < 0.001$) with chloride levels declining as distance from the nearest salt treated road increased (Adjusted $R^2 = 0.081$, $F_{1,251} = 23.19$, $P < 0.001$). Chloride concentrations ranged from 0.0 to 18.3 mgL⁻¹ (mean = 1.84) and were highest within 250 m of US 41. Chloride concentrations were highest in late summer (August – September) and were significantly different across sample months ($H = 10.98$, $df = 4$, $p\text{-value} = 0.027$). Chloride levels increased significantly from May to June ($P < 0.05$) whereas concentrations were similar in all other months ($P > 0.05$ for all tests). Quantile analysis divided the sampling data into four groups as follows: A: 0-0.40, B: 0.05-0.950, C: 0.960-4.03, and D: 4.04-19.08 km from US 41. Distance classes had similar sample sizes with 63 samples in classes A and D, while classes B and C contained 62 samples. Chloride concentrations in samples within 0.40 km of the highway were wide ranging and were significantly different across distance categories ($H = 83.50$, $df = 4$, $p\text{-value} = <0.001$). Water bodies within 0.40 km of US 41 had significantly greater chloride concentrations compared to each of those in the remaining distance classes (all $P < 0.05$).

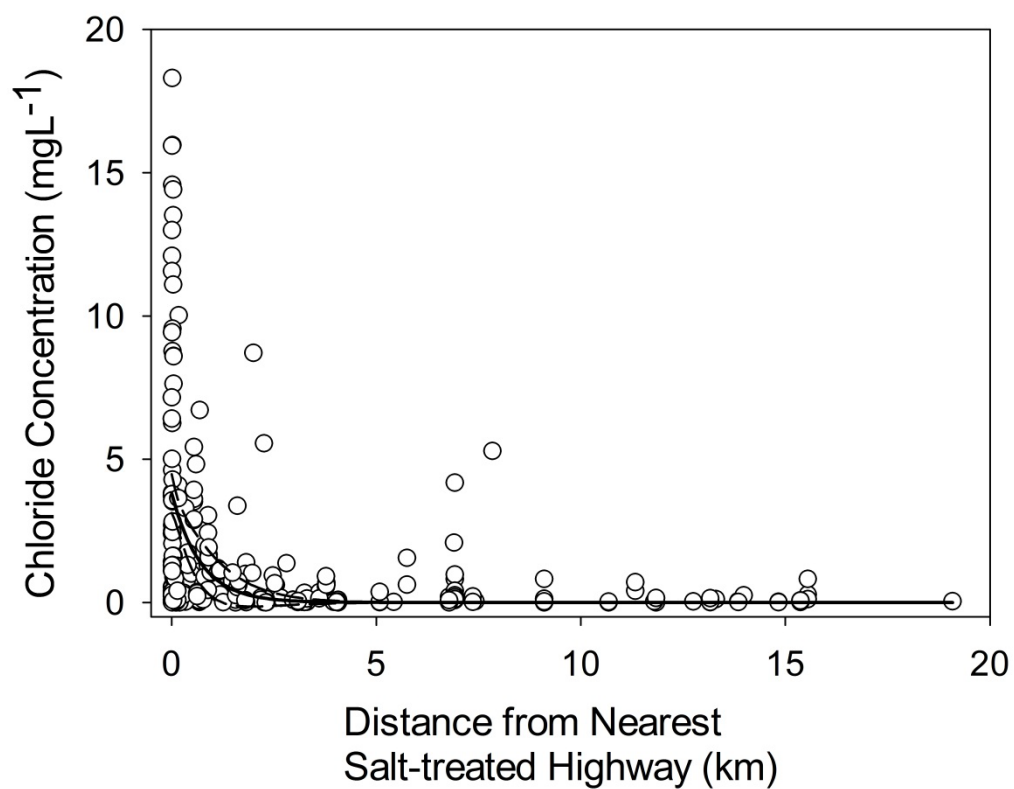


Figure 4.4: Relationship between chloride concentrations in perennial wetlands and distance from the nearest salt treated highway (N = 253) including simple linear regression ($y = 2.39 - 0.208x$) with the 95% confidence bands.

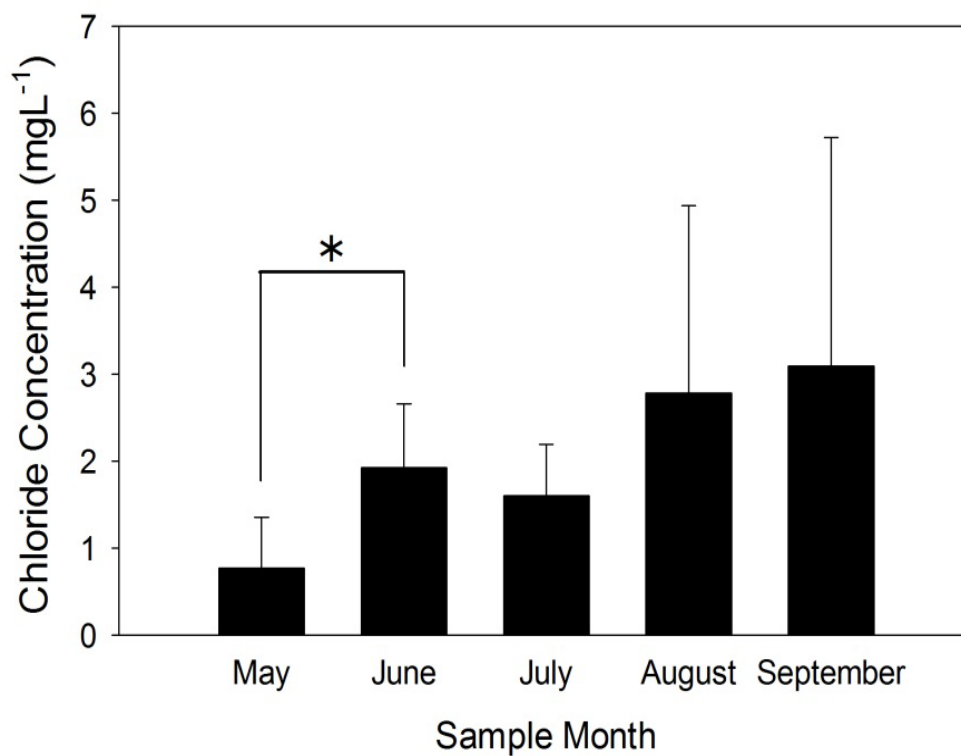


Figure 4.5: Mean environmental concentrations of chloride in surface water bodies during the amphibian breeding season (N = 253). Error bars represent ± 2 S.E. An asterisk indicates a significant increase of chloride from May to June.

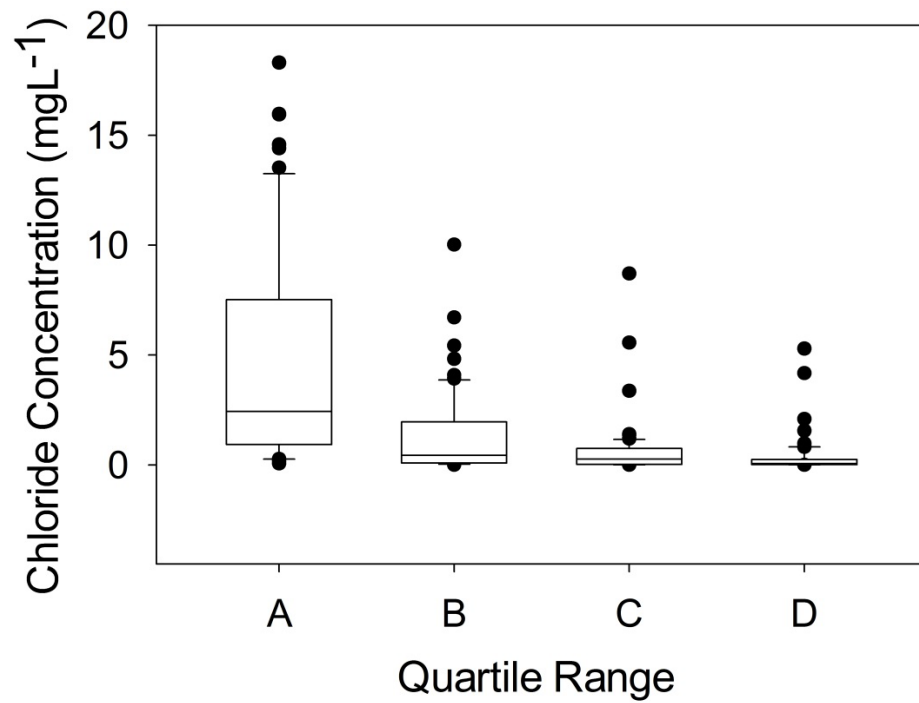


Figure 4.6: Distribution of chloride concentrations (mgL⁻¹) in surface water bodies divided into four quartiles based on sample locations (A: 0-0.40, B: 0.05-0.950, C: 0.960-4.03, D: 4.04-19.08 km) from US 41 across Baraga, Houghton, and Keweenaw Counties.

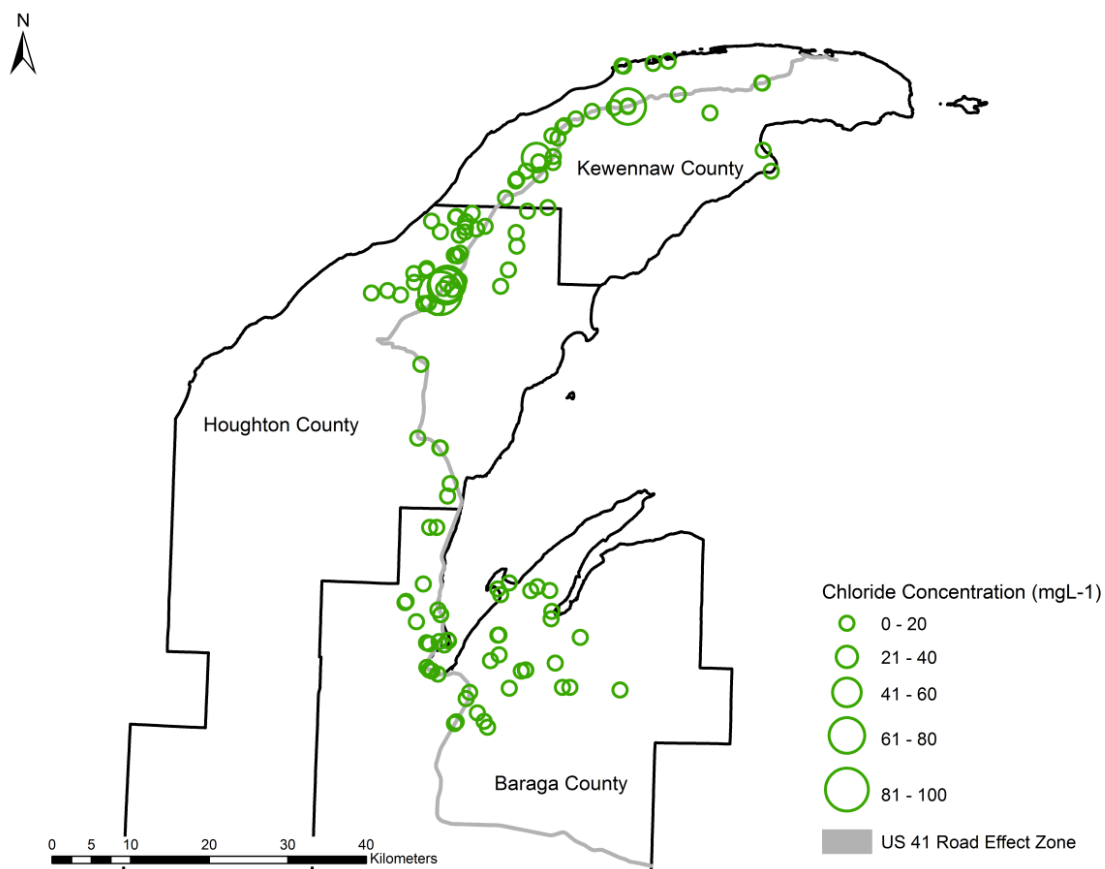


Figure 4.7: Chloride concentration (mgL^{-1}) in sample locations in relation to the US 41 road-effect zone ($N = 259$).

Houghton County contained the largest amount of roadways including 74 km of US 41 (Figure 4.8). Baraga and Keweenaw Counties contain a moderate amount of primary roads with each encompassing 53 km of US 41. Palustrine wetlands covered the largest area in Baraga county (19%) followed closely by Keweenaw (18%) and Houghton (13%) counties (Table 4.3). The 40 m road-effect zone contained a similar proportion of palustrine wetlands across the three counties (0.1%). The 250 m road-effect zone included the largest proportion of Keweenaw County (3.2%) compared to Baraga and Houghton counties (2.5 and 2.4%, respectively). The proportion of palustrine wetlands within the road-effect zone varied by county and was the highest in Houghton County (2.5%, Keweenaw = 2.2%, Baraga = 1.9%).

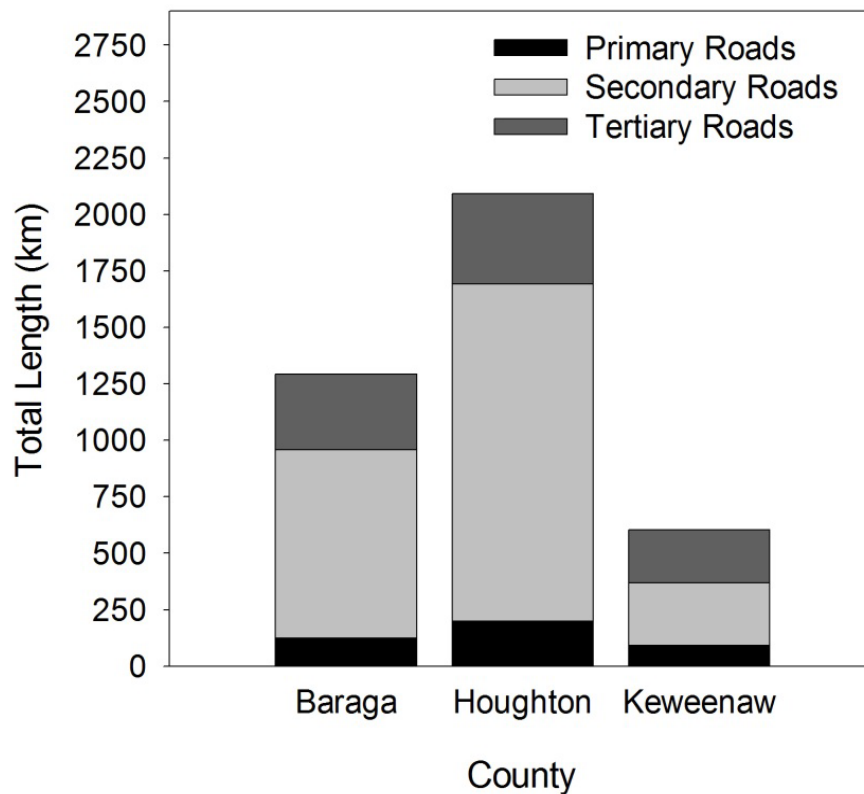


Figure 4.8: Length of primary, secondary, and tertiary roadways in Baraga, Houghton, and Keweenaw Counties of Michigan.

Table 4.3
Acreage of palustrine wetlands within a 40 meter and 250 m road-effect zone adjacent to
US 41 in Baraga, Houghton, and Keweenaw Counties of Michigan.

County	Area of palustrine wetlands	US 41 road-effect zone		Palustrine wetlands	
		40 m	250 m	40 m	250 m
Baraga	111,271	1,620	14,890	154	2,152
Houghton	87,196	1,319	16,755	117	2,210
Keweenaw	64,439	1,185	11,515	69	1,447

Discussion

These results suggest a low level of persistent chloride in surface water bodies as a result of moderate road salt use in upper Michigan. The maximum chloride concentrations measured in this study are much lower than from other studies where estimates ranged from 586 (Collins and Russell 2009) to 4,629 (Kaushal et al. 2005) to 18,000 mgL⁻¹ (Environment Canada 2001). The conservative salting strategies employed by the transportation management agencies in these three counties appear to be effective in minimizing road salt runoff in nearby surface water bodies. However, even at these low chloride concentrations we have observed significant effects on fitness of larval amphibians (29 mgL⁻¹; Chapter 3). This suggests that amphibians using roadside pools in this area are at a greater risk of negative sublethal effects. Chloride levels were also much lower than the estimated LC₅₀ for NaCl of 4585 mgL⁻¹ (Chapter 2; Harless et al. 2011), suggesting these populations are not at risk of lethal effects from local road salt application.

In considering the conservative road-effect zone estimate (250 m) from previous studies, this analysis suggests that Houghton County contains the most acreage of wetlands that may be impacted by road salt use. Houghton County also contains the most mileage of primary (state and federal highways) but the least overall acreage of palustrine wetlands

in these three counties. However, when using a road-effect zone of 40 m the distribution across the three counties is similar. Management efforts focused on reducing the use of road salt would most benefit wetlands in this county, particularly in portions of the northern and southern edge of the county that run through a dense area of these wetlands. Choosing wetlands to protect within a 40 m road buffer will allow for the protection of the most at risk surface water bodies in these three counties. Monitoring and protecting these areas should be a priority to ensure the continued health of these beneficial perennial wetlands in the Upper Peninsula.

The temporal variations I observed in chloride levels are similar to previous studies. Peaks in chloride occur after the melting of the winter snowpack and in summer where evaporative demand is high. While this study only examined chloride concentrations during the local amphibian breeding season (May – September), I would expect to see elevated chloride levels in March or April during snowmelt with the potential for negative impacts on the survival of embryonic, larval, and adult amphibians. Monitoring roadside water bodies during winter is also warranted to obtain a greater understanding of temporal variations in chloride in roadside surface water bodies.

This spatial analysis indicates that surface water bodies within 40 m of highway may contain elevated chloride levels as a result of moderate NaCl application during the winter months. Forman and Deblinger (2000) suggest that the road effect zone is not a constant distance and varies by the ecological factor in question. Heterogeneity in habitat, topography, and resultant hydrology may play a large role in the transport of road salts into the local environment. Groundwater may be recharging these perennial and permanent wetlands decreasing the overall chloride concentrations. In addition, this area is surrounded by large, freshwater lakes that may be contributing to the dilution of these water bodies. An in-depth analysis of the hydrology in these systems could provide valuable insight into this relationship.

Acknowledgements

I would like to thank the following individuals for assistance with the field and laboratory portions of this project: P. Nankervis, E. Gorsalitz, H. List, K. Li, R. Reddy, and R. Datta. Funding for this research was provided by grants from the DeVlieg Foundation, the Ecosystem Science Center at Michigan Technological University, with support from the MTU Global Watershed GK-12 program. This research has also been supported by funding and infrastructure provided by Michigan Technological University.

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Chapter 5: Avoiding the Use of Specific Conductance to Estimate Chloride Concentrations in Natural Water Bodies

Abstract

The health of a freshwater system is often measured using water chemistry variables such as pH, specific conductance or specific conductance, and salinity. Specific conductance is preferred over specific conductance as it adjusts for the temperature of the water sample. Analysis of these systems often requires quantification of specific ions. These data are often expensive and difficult to obtain. Numerous studies have attempted to use linear regression models to predict chloride ion concentrations from measures of specific conductance in natural water bodies. I investigated this relationship using both a large number of water samples collected from natural surface water bodies in the Upper Peninsula of Michigan as well as sodium chloride (NaCl) additions to filtered lake water. This analysis suggests the relationship between chloride concentration and specific conductance may not be sufficient for predicting chloride ion concentrations. While the regression models were significant, residuals were sufficient to warrant caution regarding the use of this method. In addition, many factors other than chloride concentrations affect specific conductance. Researchers should invest in the use of ion chromatography or a similar analytical method to obtain specific ion concentrations.

Key Words: chloride, conductivity, specific conductance, water chemistry, ion chromatography

Introduction

Water chemistry variables are often used to determine the health of surface water bodies. Commonly used metrics include pH, conductivity or specific conductance, salinity, and determination of ionic concentrations. Conductivity is widely employed as an estimate of the amount of disturbance in a freshwater system. It is defined as a measure of the ability of an aqueous solution to carry an electrical current. Conductivity tends to range widely depending on water temperature. Hence, many researchers prefer to use specific conductance in its place as it represents Conductivity normalized to a temperature of 25 °C. Specific conductance may be influenced by the presence of specific ions or the concentration, mobility, and valence of these ions (Eaton et al. 2005). These influential ions may include anions (e.g., chloride, nitrate, sulfate, and phosphate) or cations (e.g., sodium, magnesium, calcium, iron, and aluminum). In general, solutions with a high concentration of ions have high specific conductance. Specific conductance is calculated by taking the reciprocal of the electrical resistance. Portable water meters measure this by passing current between two spatially fixed and chemically inert electrodes (Eaton et al. 2005).

Hamilton (1978) developed a procedure for determining the specific conductance of naturally occurring waters. Using known concentrations of the major ions (Ca^{2+} , Mg^{2+} , Na^+ , K^+ , HCO_3^- , SO_4^{2-} , and Cl^-), specific conductance can be calculated using the conductances of these ions in water with total dissolved solids (TDS) less than 2500 mgL⁻¹. Here the contribution of each ion is calculated by multiplying equivalent conductances by the ionic strength resulting in the infinite dilution specific conductance value. Using these values of ionic concentration, the specific conductance was estimated at 47.88 mS/m. This method retained an error of less than 2% when used on an analysis of 39 natural water bodies (*reviewed in* Hamilton 1978). Because this method requires a number of known values, the reverse calculation of ionic concentrations from specific conductance is not warranted.

Chloride concentrations are often measured to evaluate the health of natural water bodies. Chloride (Cl^-), may originate from a number of natural and anthropogenic sources.

Sodium chloride (NaCl) is widely used as a food additive and passes through the digestive system unchanged. In addition, sodium chloride and other inorganic salts may leach into freshwater systems near oceans hereby elevating the concentration of Cl^- . Industrial and transportation agencies may also cause an increase in Cl^- concentration through the use of NaCl in manufacturing and winter maintenance activities (*reviewed in* Eaton et al. 2005).

While chloride ion concentration estimates are included in Hamilton's algorithm, the concentration of this ion in natural water bodies is not likely to be directly correlated to specific conductance values. The preferred method of determining the concentration of Cl^- in natural water bodies is through the use of ion chromatography. Other methods include the argentometric method, the mercuric nitrate method, the potentiometric method, the automated ferricyanide method, mercuric thiocyanate flow injection analysis, or the capillary ion electrophoresis method (Eaton et al. 2005). Ion chromatography is preferred over many of these methods because it does not require the use of hazardous reagents such as mercury.

For field based studies, the use of ion chromatography presents a logistical challenge. Water samples are sensitive to time and temperature. Ion chromatography methods are not appropriate for long-term monitoring as it requires a significant amount of time to obtain results. In addition, it may be quite expensive to purchase an ion chromatography system or send a large number of samples to a laboratory for analytical ionic analysis.

A number of studies have used evidence of a significant regression fit between specific conductance and chloride concentration in their field samples to estimate Cl^- concentration from other water samples within their study. For example, Zinabu et al. (2002) examined the use of specific conductance as a predictor of both cation concentration and salinity in rivers and lakes in Ethiopia. They determined a significant linear relationship between specific conductance with both total cation concentration and salinity. Curiously, these relationships were not significantly different when comparing values from saline versus freshwater bodies. Paine (2003) used specific conductance to

estimate chloride in Texas water bodies where a significant relationship between chloride, conductivity, and total dissolved solids was demonstrated in this system. Similarly, El-Korashy et al. (2009) identified the use of regression analysis to successfully estimate Cl^- concentration from specific conductance values in a specific drainage system. Brand et al. (2010) used ion chromatography to determine Cl^- concentration in experimental bins then used the regression between chloride and specific conductance in these samples to estimate Cl^- concentration in other experimental samples.

Authors have also used the relationship between conductivity and chloride concentration in natural surface waters to determine sodium chloride (NaCl) tolerance. Karraker (2007a,b) investigated the sensitivity of two larval amphibians to Cl^- exposure through manipulating the conductivity of test solutions by the addition of NaCl. The test solutions were made using laboratory grade water with the appropriate amount of NaCl added to obtain the four test conductivity levels. Cl^- concentration at each conductivity level was determined using Inductively Coupled Plasma - Optical Emission Spectrometry (Karraker 2007b). No information was given as to the relationship between these variables used in this experiment.

While these authors warned against the use of their regression values in systems outside of their study area, the use of this regression raises questions about the validity of the Cl^- estimates derived from this type of analysis. The purpose of this note is to examine the use of regression models to accurately predict chloride concentration from specific conductance in natural water bodies.

Methods

I evaluated this method on water samples collected as a part of a larger study on the impacts of winter de-icing activities on surface water quality in Michigan's Upper Peninsula. I collected field measurements of specific conductance using a YSI 63 probe (Yellow Springs International, Yellow Springs, OH) that was calibrated daily. I collected water samples from 105 wetlands and vernal pools across Baraga, Houghton, and

Keweenaw Counties from May through September in 2009. Water samples were returned to the laboratory and stored at 4°C. I filtered the samples through a 0.45 µm cellulose acetate filter and quantified chloride concentration using ion chromatography (Dionex ICS-900, AS22 4 mm column, Fisher Scientific Inc., Sunnyvale CA). To determine the relationship between sodium chloride (NaCl) additions to specific conductance in natural surface waters, I mixed known concentrations of NaCl (0, 190, 320, 540, 900, 1500, 2400, and 3800 mgL⁻¹) into 2 L of filtered water from Portage Lake, Michigan. I allowed the NaCl to dissolve and took measurements of specific conductance using a YSI 63 probe. I repeated this procedure using deionized water to determine the relationship between specific conductance and NaCl concentration in laboratory water samples. I developed a simple linear regression model from specific conductance values to predict either chloride or NaCl concentrations. I used “R: A Language and Environment for Statistical Computing” to perform all statistical analyses with an alpha level of 0.05 (R Development Core Team 2010).

Results and Discussion

Chloride concentrations in local perennial wetlands ranged from 0.0 to 18.3 mgL⁻¹ (mean = 1.84). A significant linear relationship between specific conductance and chloride in these samples was detected (Adj. R² = 0.391, F_{1,228} = 148.2, P < 0.001; parameter estimates: slope 0.015, intercept -0.783; Figure 5.1). While the correlation between specific conductance and chloride values measured in this study fit a general linear model, examination of the residuals suggest that chloride estimates derived from specific conductance values are wide ranging. Some highly diverged Cl⁻ was detected at specific conductance levels below 300 µS, encompassing 88% of samples in this study. These chloride estimates using this formula on natural water samples were on average 9.4 (0.18-18.3) mgL⁻¹ from the fitted model. Similarly, the linear model underestimate NaCl concentrations in samples using Portage Lake water (Adj. R² = 0.981, F_{1,31} = 1642.56, P < 0.001; parameter estimates: slope 0.72, intercept -272.72) as evidenced by the regression intercept and analysis of the residuals (Figure 5.2). In deionized water, the model tended to overestimate NaCl concentration (Adj. R² = 0.993, F_{1,31} = 4657.76, P <

0.001; parameter estimates: slope 0.58, intercept -42.08; Figure 5.3) yet provided a better fit to NaCl concentration than in the model using natural water samples.

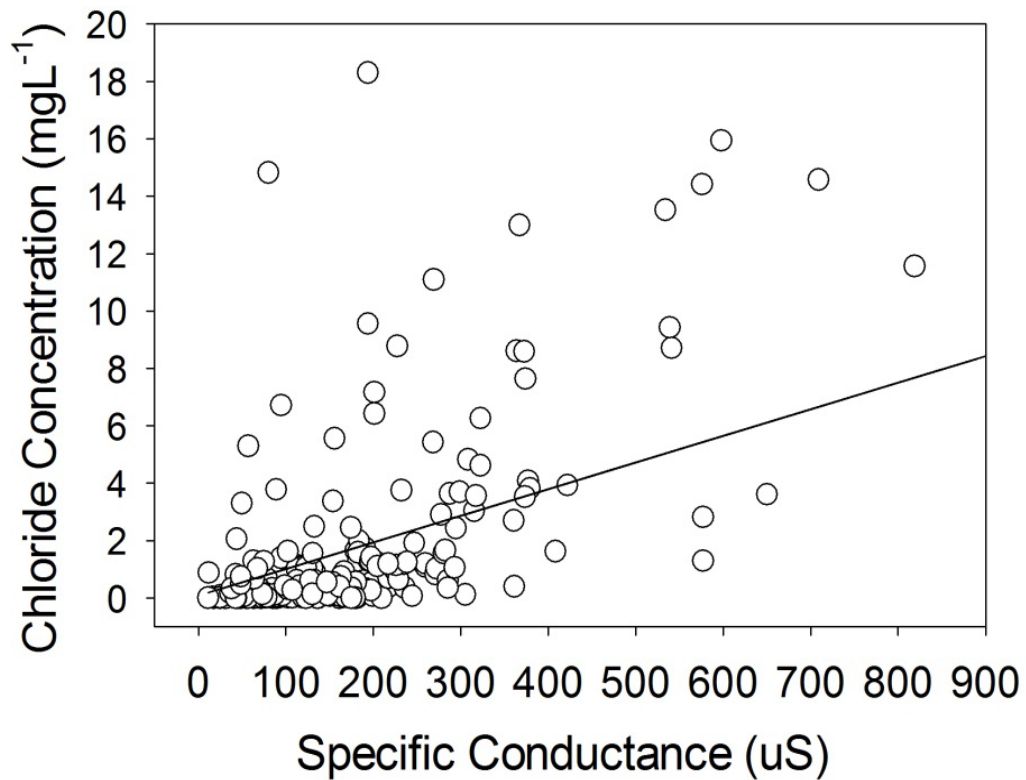


Figure 5.1: The relationship between chloride and specific conductance in natural water samples ($N = 230$). The fit line represents a linear regression model ($y = 0.015x - 0.783$; Adj. $R^2 = 0.391$).

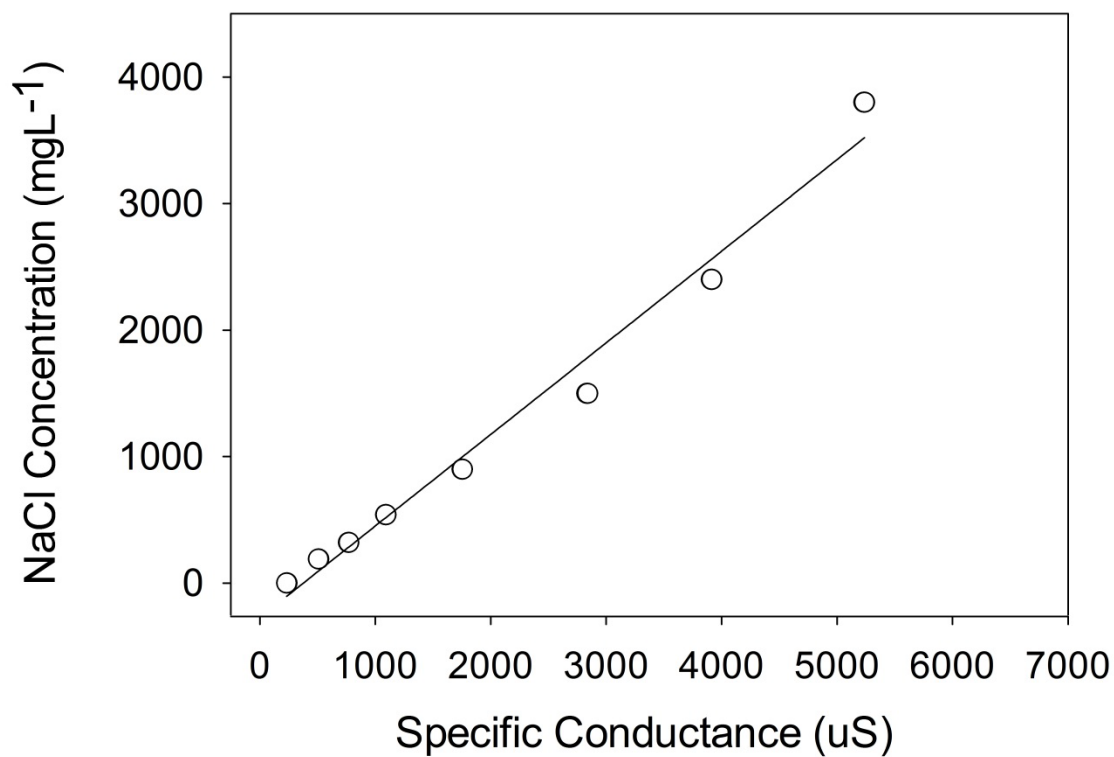


Figure 5.2: The relationship between specific conductance and sodium chloride (NaCl) additions in natural water samples from Portage Lake, Michigan (N = 32). The fit line represents a linear regression model ($y = 0.72x - 272.72$; Adj. $R^2 = 0.981$).

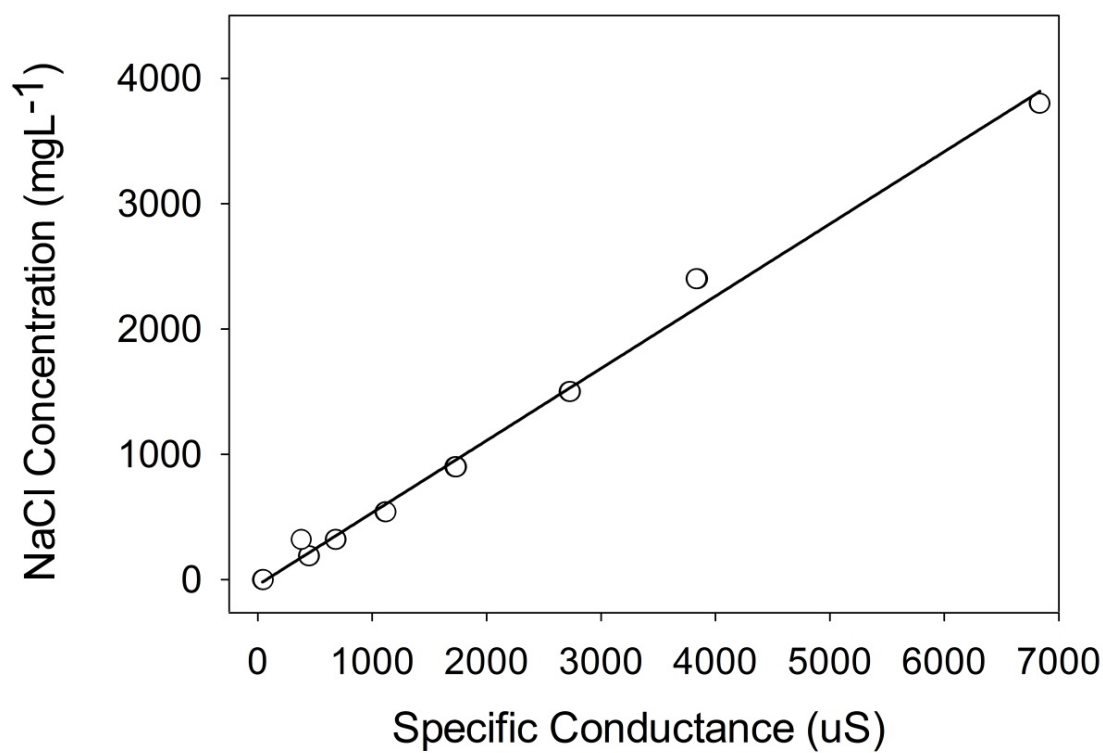


Figure 5.2: The relationship between specific conductance and sodium chloride (NaCl) additions in deionized water ($N = 32$). The fit line represents a linear regression model ($y = 0.58x - 42.08$; Adj. $R^2 = 0.993$).

It is likely that the presence of ions in addition to Cl^- in surface water bodies contributed to the variance in predicted chloride concentration. Hence, it is not recommended to use a simple model like this for estimation of a single ion in natural water bodies without knowledge of the concentration of additional ions (e.g., Hamilton 1978). In addition, we caution against the use of these regression equations between water sources as Cl^- estimates are likely to vary widely when calculated from specific conductance. While this method may be cheap and relatively quick, its use will not take the place of analytical methods such as ion chromatography.

In addition, the use of a linear model derived from one water system should not be used to predict ion concentrations in different water systems. Ion concentrations in freshwater systems may vary greatly in both space and time, making comparisons between analyses using separate water samples difficult. This variance is particularly true for chloride, as it is affected by land use activities (Findlay and Kelly 2011) suggesting caution is advised when attributing an increase in specific conductance to a singular ion.

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Conclusions

The overall goal of this research project was to better understand the impacts of chemical deicers on freshwater ecosystems through a mixture of laboratory studies with larval amphibians and water chemistry analyses. The widespread use of these chemicals to remove or prevent ice from bonding to pavements surfaces represents a significant source of contamination from roads and highways. Freshwater organisms using waters receiving this runoff may be exposed to salts and other chemical beyond their tolerance level. This research adds to the body of literature regarding the environmental impacts of chemical deicers in that it provides greater detail as to the population level response of larval amphibians and the environmental contamination resulting from NaCl application.

Through a series of acute toxicity tests, I determined that not all chemical deicers are created equally. Larval wood frogs (*Lithobates sylvatica*) were least sensitive to urea ($\text{CH}_4\text{N}_2\text{O}$), road salt (NaCl), and magnesium chloride (MgCl_2). However, these larvae were very sensitive when exposed to acetate products ($\text{C}_8\text{H}_{12}\text{CaMgO}_8$, CH_3COOK) and calcium chloride (CaCl_2). The differences in the response of these sensitive organisms suggests that switching to the use of alternative products (e.g., acetates) to avoid chloride contamination of drinking water source may cause greater harm to freshwater ecosystems than previously thought. In addition, the higher tolerance of this population of *L. sylvatica* to high NaCl levels than those in previous studies suggests that population level differences and/or local adaptation of NaCl exposure need further examination.

In Chapter 3, I demonstrated that amphibians employing different life history strategies respond differently to long-term exposure to NaCl during development. Both *L. sylvatica* and green frogs (*L. clamitans*) demonstrated potentially harmful effects of NaCl exposure on individual fitness. The high occurrence of edema in *L. sylvatica* suggests this exposure represents a significant physiological stressor to these rapidly developing larvae. In addition, *L. clamitans* demonstrated a reduction in tail size when exposed to higher levels of NaCl which may decrease adult fitness of these individuals. The phenotypic plasticity

of *L. clamitans* larvae may play a part in this observation and would be worthwhile to examine in more detail.

Analysis of the spatial distribution of chloride in the three northernmost counties of Michigan suggests that amphibians may encounter harmful levels of chloride when using water bodies in this area. The relatively low rates of NaCl application in this region compared to urban areas are reflected in the lowest maximum chloride levels observed in this study. However, these levels are not beyond those in which significant sublethal impacts have been observed. While the road effect zone of NaCl exposure in this area may be large (40 m), the temporal variability in chloride concentrations throughout the amphibian breeding season (e.g., variable hydroperiods) may cause water bodies far from salt-treated roads to experience harmful levels of chloride.

Finally, an in depth analysis of the use of specific conductance to predict chloride concentrations in surface water bodies suggests that this correlation is too variable to accurately predict specific ion content. Researchers interested in this measurement should seek alternative methods of analytical analysis.

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