NOTE TO USERS

WATER CHEMISTRY: ITS EFFECTS ON AMPHIBIANS IN NORTHWESTERN ONTARIO, CANADA

by:

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Abstract

Understanding the role that abiotic environmental factors play in determining the distribution of organisms is of fundamental importance to ecologists. Most amphibians are inherently dependent on aquatic habitats and differences in water may influence community structure. However, few comprehensive studies of amphibian distribution in relation to water chemistry exist, but they suggest that the importance of chemistry may differ among regions. The boreal forest contains nearly half of the world's freshwater wetlands, but its amphibian ecology is poorly understood relative to other biomes. I sampled 73 wetlands in northwestern Ontario for presence/absence of amphibians using repeated day and night visits between April and August 2003. Water samples (late April-early May and late July-August) from each wetland were examined for 37 chemical variables including pH, total dissolved solids, total suspended solids, conductivity, metals, anions, cations and nutrients. I observed eight species, with *Rana sylvatica* being most common (65.8% of wetlands), followed by *Pseudacris crucifer* (32.9%), *Bufo americanus* (28.8%), *P. maculata* (24.7%), *Hyla versicolor* (20.5%), *R. septentrionalis* (16.4%), while *R. clamitans* (5.5%), *R. pipiens* (2.7%) were least common. Local amphibian species richness for the year was 2.4 ± 0.19 SE. Species richness was significantly affected by water chemistry, with positive associations existing with pH and K, and negative associations found with TDS, conductivity, alkalinity, dissolved carbon, Ca, Na, Mg, Cl, various metals, and nutrients such as phosphorus and nitrogen. No differences among species were detected based on water chemistry. Classification of species present at a site and individual species' presence/absence using water chemistry as a predictor was only moderately

successful. Despite the latter, an apparent community level response appears to occur in the region in relation to pollutants, especially those associated with road runoff. In toxicological experiments I exposed wood frog (*R. sylvatica*) tadpoles to NaCl, a major component of road runoff in northern countries used as a winter de-icing agent. Tests revealed a 96-h LC50 of 2636.5 mg/L and tadpoles experienced reduced activity, weight, and displayed physical abnormalities. A 90 d chronic experiment using environmentally realistic concentrations (0.00, 0.39, 77.50, 1030.00 mg/L), revealed significantly lower survivorship, decreased time to metamorphosis, reduced weight and activity, and increased physical abnormalities, especially at the highest salt concentration.

This study indicates that general water chemistry is not a major factor influencing amphibian distribution in northwestern Ontario, however its role in structuring amphibian communities is not entirely dismissed as variables related to acidity and pollution appear to affect species richness. As well, road salts had toxic effects on larvae at environmentally realistic concentrations with potentially far-ranging ecological impacts. More studies examining the role of water chemistry relative to other local and/or landscape levels variables in the region are required. And toxicological studies examining the effects of road salts to amphibians are also urgently needed.

General Introduction

Determining and explaining the patterns of abundance and distribution of organisms are central goals of ecology. These patterns are the products of both biotic and abiotic factors. In recent decades many ecological studies have focused on biotic interactions, despite the fundamental importance of abiotic factors in habitat selection and population persistence (as suggested by early ecologists such as Liebig, Blackman and Shelford). Dunson and Travis (1991) revisited the importance of considering both biotic and abiotic factors in community ecology, and stressed the requirement for abiotic analysis.

The impact of human activity on natural ecosystems has also been a focus for scientific research in recent decades (Myers, 1996; Vitousek et al., 1997; McDaniel and Borton, 2002). There is now consensus among scientists that humans are affecting countless numbers of organisms. Over the last 15 years there has been growing concern about declining populations of amphibians on a global scale (Phillips, 1990; Griffiths and Beebee, 1992; Hedges, 1993; Busby and Parmelee, 1996; Stallard, 2001; Biek et al., 2002; and many others). Concerns for this taxa may be well-warranted because amphibians are important components of many ecosystems, acting as important organisms in energy flow of ecosystems (Seale, 1980), as major components of biomass (Burton and Likens, 1975), in some cases acting as keystone species (Morin, 1981), and as indicators of ecosystem health (Vitt et al., 1990).

Over 5700 species of amphibians inhabit the planet from the tropics to cold climates above the Arctic Circle (AmphibiaWeb, 2004). Amphibians have complex life histories (Wassersug, 1975; Wilbur, 1980, 1984, 1987) using both terrestrial and aquatic

habitats (Stebbins and Cohen, 1995; Beebee, 1996; Pope et al., 2000), and are highly dependent on water to complete their life cycle. All amphibians (anurans, caecilians and salamanders) require water for reproduction and development and most species also have gilled aquatic larvae. Wetlands are also used by adults for foraging and hibernation, as well moisture is important for dispersal and migration. Water is also essential for body maintenance because most amphibians have permeable skin. Their dependence and restriction by water likely makes amphibians perceptive to changes in water chemistry, vulnerable to aquatic pollution (Boyer and Grue, 1995) and sensitive to landscape level changes (Kolozsvary and Swihart, 1999, Pearman, 1997), especially if amphibians exhibit metapopulation dynamics (Gibbs, 1993; Marsh and Trenham, 2001). Therefore declines may be an indication of larger environmental problems.

If amphibian declines are indicative of environmental problems it is important to understand the causal factors. Many possible reasons for declines have been suggested such as increased exposure to ultraviolet radiation (UV), (Blaustein et al., 1995; Tietge et al., 2001; Crump et al., 1999a; Crump et al., 1999b), acidification of habitat (Glooschenko et al., 1992; Rowe et al., 1992), habitat loss and/or habitat degradation (Hedges, 1993; Kolozsvary and Swihart, 1999), introduced predators (Gascon, 1992; Bradford et al., 1993; Fisher and Shaffer, 1996), disease (Cunningham et al., 1993; Beebee, 1996), increased exposure to chemicals (Hecnar, 1995; Oldham et al., 1997; Hall and Henry, 1992; Sparling et al., 2000; Davidson et al., 2002; Hayes et al., 2002) and effects from roads (Fahrig et al., 1995; Trombulak and Frissell, 2000; Hels and Buchwald, 2001), but recent work implicates habitat loss/degradation and

pollution as major contributors (Pechmann et al., 2001; IUCN Conservation International and Nature Serve, 2004).

Studies on amphibians have been conducted in many places around the world but there is limited work in boreal regions such as in northwestern Ontario has occurred (Allin, 1950; Allin, 1961; Elmberg, 1993; Robinson, 2004; Abbott, 2004). The lack of studies in the boreal forest is surprising considering that this biome contains the world's largest expanse of freshwater wetlands (Schindler, 1998), and water covers more than 25% of the landscape in western boreal regions of Ontario (Perera et al., 2000). Previous studies in and near Thunder Bay, Ontario, a region bordering the boreal forests, have examined the effects of local and landscape level factors such as hydroperiod, predatory fish, vegetation and land use on amphibian distribution (Robinson, 2003, Abbott, 2004), but no work has examined the effects of water chemistry. Thus it is important to begin to understand the piece of the puzzle that water chemistry represents in amphibian distribution in the region at its affects on the status of boreal species.

My general objective was to examine the effects of water chemistry on amphibians. I examined the effects of naturally occurring chemical variables on amphibian distribution in northwestern Ontario as well as the effects of a road salts, a potential chemical contaminant being added to the environment in great volumes as a result of human activity.

Chapter 1:

Amphibian distribution in relation to water chemistry in northwestern Ontario, Canada

Introduction

Ecologists have had a long-standing interest in determining the relative importance of abiotic and biotic factors in structuring populations and communities (Dunson and Travis, 1991; Blaustein et al., 2001; Palik et al., 2001). Arguably, studying the role of biotic interactions (i.e. competition, predation) has overshadowed that of abiotic factors in recent decades (Dunson and Travis, 1991; Ricklefs and Schluter, 1993). This apparently unbalanced approach prevailed despite the fundamental importance of abiotic factors in determining the distribution of organisms. Individual taxa are differentially affected by abiotic factors because of their physiological constraints and natural history requirements, and this is true of amphibians. Amphibians have complex life histories (Wassersug, 1975; Wilbur, 1980, 1984, 1987), with aquatic larvae and typically terrestrial adults. They also depend on water for virtually all aspects of their lives making amphibians responsive to changes in water quality (Boyer and Grue, 1995).

The effect of water chemistry and water quality on amphibians has been studied in many regions of the world (Gascon and Planas, 1986; Beebee et al., 1990; Kanamadi and Srinivas, 1991; Bellemakers and van Dam, 1992; Wainscott, 1997). Several

studies have examined large-scale distribution of amphibians based on water chemistry in North America (Dale et al., 1985; Clark, 1986; Freda and Dunson, 1986; Glooschenko et al., 1992; Hecnar and M'Closkey, 1996; Mazerolle, 1999), however varying results have been found. Water chemistry varies greatly among locations and depends on many factors such as type of bedrock, inflows and outflows at the site, and vegetation (Wetzel, 2001), these differences in study regions may explain some of the variation in findings. For example, two notable studies from Ontario, Canada, examining the effects of water chemistry on amphibian distribution, found somewhat different results with similar species assemblages. Glooschenko et al. (1992), working near Sudbury, Ontario, found species presence to be positively related chemical factors related to buffering status of water, while atmospheric deposition (e.g. metals and sulphates) negatively affected species distributions. However, Hecnar and M'Closkey (1996) found that water chemistry played only a minor role in distributions in southwestern Ontario but chemical variables may be helpful in distinguishing between used and unused sites. These studies show that discrepancies among studies, even within a province, make extrapolation of findings from one study to another region problematic.

My goal was to determine if the occurrence and distribution of amphibians in northwestern Ontario was related to chemical composition of a wetland site. To explore for potential relationships, I collected water from wetlands that were previously surveyed for amphibians and analyzed it for a variety of chemical variables. I also surveyed the same network to determine which species were present during the year of sample collection. I address four main questions; first, is amphibian species richness (SR)

related to the water chemistry of a particular site; second, do species present at a site differ based on water chemistry; third, does the presence/absence of individual species depend on water chemistry; and fourth, can one predict which species are present at a site based on water chemistry? Because of the geology and location of northwestern Ontario I expect to find a strong association of amphibian species richness and distribution with water chemistry.

Methods and materials

Study area and study sites

I conducted the study in northwestern Ontario, Canada, in and around the city of Thunder Bay (48° 27' N, 89° 12' W). The region is located in the ecotone of the Boreal and Great Lakes-St. Lawrence forest regions. The dominant tree vegetation for this area is balsam fir (*Abies balsamea* (L.) Miller), white birch (*Betula papyrifera* (Marshall)), trembling aspen (*Populus tremuloides* (Michaux)), white spruce (*Picea glauca* (Moench) Voss) and black spruce (*P. mariana* (Miller)) (Rowe, 1972). Northwestern Ontario is located on the Canadian Shield, which is dominated by Precambrian granitic bedrock, typically under a thin layer of acidic soils (Botts and Krushelnicki, 1988). The region has cool summers and cold winters with an average daily temperature of approximately 2.6 ± 1.9°C SD and precipitation averages 705 mm (700-800 mm) annually (Botts and Krushelnicki, 1988; Environment Canada, 2004).

I selected 73 wetlands within a 100 km radius of the city. They consisted of 44 semi-permanent farm and forest ponds and 29 ephemeral pools such as roadside ditches (Table 1.1). Sites were located on both private and crown land and formed a

subset of a pre-existing network of sites being used for long-term studies of amphibian communities (Robinson, 2004; Abbott, 2004). The wetlands vary from undisturbed forest ponds to highly disturbed ditches and farm ponds, as well as in physical characteristics (Table 1.1).

Amphibian surveys

I conducted amphibian surveys between April and August 2003. Surveys at each site consisted of a minimum of four day and five night visits to ensure accurate species lists (Corn, 1994). Day visits entailed visual searching by walking and/or wading around the perimeter of the wetland looking for various amphibian life stages. Dip-nets were also used to search for larvae in submerged vegetation. Any larvae that were unidentifiable in the field were returned to the lab for identification. I also listened for anuran calls during day visits. Day visits were restricted to favourable weather. Night visits were conducted from May to July (typical breeding time for amphibians found in northwestern Ontario, MacCulloch, 2002). Night surveys involved visiting each site, between dusk and 2:00 AM, to listen for anuran-breeding calls (Shirose et al., 1997; Crouch and Paton, 2002; USGS North American Amphibian Monitoring Program (NAAMP), 2004). I monitored each site for three to five minutes per visit, since most species can be identified within the first three minutes of listening (Shirose et al. 1997).

Based on published range maps and ongoing research in the area, the regional species pool was expected to consist of 13 species of amphibians (Table 1.2) (Conant and Collins, 1998; MacCulloch, 2002; Ontario Ministry of Natural Resources, 2002; AmphibiaWeb, 2004). Surveying was conducted throughout the spring and summer

Table 1.1. Measurements of physical parameters (mean ± SE (range)) of sites including depth, area, perimeter, volume, distance to nearest road.

Variable	Semi-permanent ponds	Temporary sites
Depth (m)	1.9 ± 0.17 (0.30-4.56)	0.5 ± 0.03 (0.25-1.00)
Area (m^2)	3045 ± 772.3 (268-22369)	1790 ± 1236.7 (22-31300)
Perimeter (m)	198 ± 19.3 (62-604)	151 ± 27.1 (25-490)
Volume (m^3)	3261.8 ± 1060.46 (152.15-35836.05)	430.2 ± 300.99 (3.00-7308.00)
Distance to nearest road (m)	147 ± 35.0 (4-1000)	9 ± 3.3 (1-84)

because of species phenology. Some of the species typically breed in the early spring, often times when ice is still present on the wetlands, while others reproduce and are more active at later times of the season and during the summer.

Water sampling

I collected water samples from 59 of the 73 sites in the spring (28 April-7 May) and 53 of the 73 sites in the summer (28 July-14 August) of 2003, the number of sites sampled during collection varies but due to restricted access to property as well as sites being drying during the year. Sampling was conducted in both the spring and summer to examine if water chemistry at different times of the year was influencing species distribution, as well as to incorporate potential differences in phenology.

Two water samples were collected from each site in 1000 ml Nalge^{TM} HDPE wide-mouth bottles. Samples were taken from random locations at opposite ends of the site to capture as much chemical variation as possible. Water samples were collected using a 1 mm handheld strainer covering the mouth of a bottle to reduce the amount of debris in the samples. All samples were transported to Lakehead University in a cooler for further analysis.

I recorded pH (Oakton Waterproof pHTestr 2) and total dissolved solids (TDS) (Oakton TDSTestr Low+) at four locations in each site when collecting water samples. I also recorded water temperature and dissolved oxygen (DO); however I eliminated them from analysis because they were highly variable throughout the day (Hecnar and M'Closkey, 1996; Wetzel, 2001).

Water analysis

Water samples were analyzed at Lakehead University Environmental Laboratories (certified by the Canadian Association for Environmental Analytical Laboratories). All samples were processed within 24 h of collection for a variety of chemical variables (Table 1.3). Analyses were conducted on spring and summer water samples separately, and common sites (45) from both sampling periods were then averaged to produce general water chemistry results for the year.

Statistical analysis

I analyzed 37 water chemistry variables for each site. I reduced the data by eliminating variables that were below detection limits of the instruments, or those that appeared at only a few sites, leaving 28, 25 and 28 variables for spring, summer and general analyses respectively. Because many water chemistry variables can be highly correlated (Wetzel, 2001), I examined correlations in the data. To simplify the data set and produce independent variables, Principal Components Analysis (PCA) was conducted. I used Varimax rotation to maximize the variance of component loadings (Tabachnick and Fidell, 1996) and to aid in interpretation. An Eigenvalue equal to 1 was selected as a cut-off for extraction of components. All principal component (PC) scores were retained for further analysis. I used multiple regression to determine the relationships between the PCs and species richness (the dependent variable). To determine if it was possible to distinguish between presence/absence at sites for individual species, and if there were any differences among species presence at a site

Table 1.2. Amphibian species occurring in northwestern Ontario (based on Conant and Collins, 1998; MacCulloch, 2002; Ontario Ministry of Natural Resources, 2002; AmphibiaWeb, 2004)

Species	Common Name
Ambystoma laterale	Blue-spotted salamander
A. maculatum	Spotted salamander
Bufo americanus	American toad
Hyla versicolor	Gray treefrog
Necturus maculosus	Mudpuppy
Notophthalmus viridescens	Eastern newt
Plethodon cinereus	Eastern red-back salamander
Pseudacris crucifer	Spring peeper
P. maculata	Boreal chorus frog
Rana clamitans	Green frog
R. pipiens	Northern leopard frog
R. septentrionalis	Mink frog
R. sylvatica	Wood frog

Table 1.3. Device and chemical variables analyzed in water samples.

Device	Variables Analyzed	Detection Limits[‡]
Oakton Waterproof pHTestr 2	pH	± 0.1 pH accuracy
Oakton TDSTestr Low+	Total Dissolved Solids (TDS)	0-1999 ± 1% full scale accuracy
Metler DL53 Autotitrator (using 0.02 N H_2SO_4)	Total Alkalinity (as $CaCO_3$)	1.0
VWR Digital Conductivity Meter	Conductivity	0.2 µS/cm
Skalar Autoanalyzer	Dissolved Inorganic Carbon (DIC)	0.2
	Dissolved Organic Carbon (DOC)	0.5
	Total Nitrogen (TotN)	0.015
	Total Phosphorus (TotP)	0.005
	Ammonia (NH_3)	0.03
Varian VistaPro (Cations)	Calcium (Ca)	0.01
	Potassium (K)	0.1
	Magnesium (Mg)	0.005
	Sodium (Na)	0.1
Dionex 120 (Anions)	Chloride (Cl)	0.05
	Sulphate (SO_4)	0.05
	Nitrate (NO_3)	0.009
Varian VistaPro (metals)	Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, S, Sr, Ti, V, Zn	0.001-0.05
Ohaus AP250	Total Suspended Solids (TSS)	2.0

[‡]Units are mg/L unless stated otherwise, pH is unitless.

based on water chemistry, I performed multivariate analysis of variance (MANOVA). I then used discriminant functions analysis (DFA) to explore if it was possible to classify species presence/absence, and to classify which species were present at sites based on differences in water chemistry. Statistical analyses were conducted using SPSS version 12.0 and SYSTAT version 9. Analyses were conducted on both raw and transformed data, but since transformed data did not perform better than raw data, I present the results for raw data.

Results

Amphibian surveys and species richness

I observed 8 of 13 amphibian species that occur in the region (Figure 1.1). Of the 73 wetlands surveyed, 13 sites lacked any amphibians (two ponds, eleven temporary pools). Of the 13 sites, one pond drained where a beaver dam broke; five temporary sites were dry at the start of the season and three other temporary sites dried out during the summer. Sites that dried out were excluded from analysis.

Wood frogs (*R. sylvatica*) were the most common species encountered (65.8% of wetlands surveyed, 58.6% temporary sites and 70.5% ponds), while leopard frogs (*R. pipiens*) were the rarest, occurring at only 2.7% of the sites (0% temporary sites, 4.5% ponds). Mean species richness was 2.4 ± 0.19 SE, (range 0-6 species/pond).

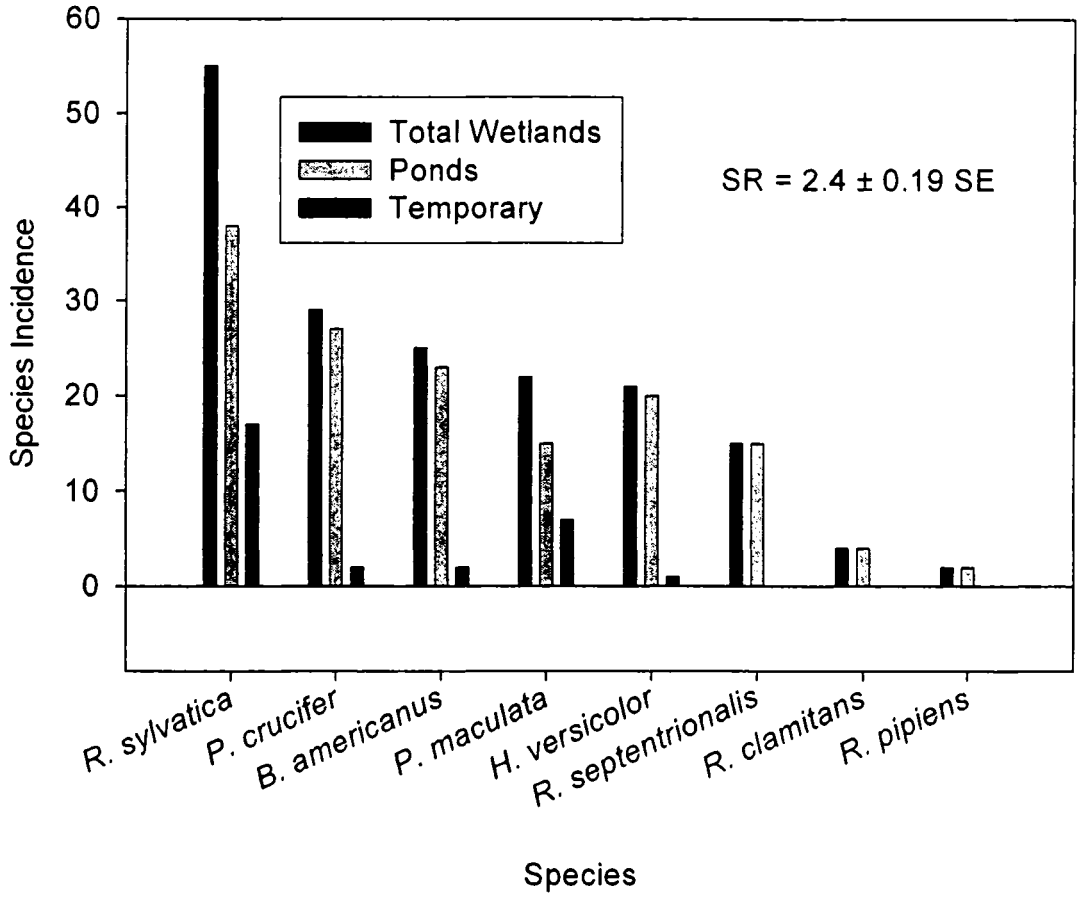

Figure 1.1. Species incidence for 73 wetlands (44 semi-permanent to permanent and 29 temporary sites) in northwestern Ontario during 2003.

Water sampling and analysis

Water chemistry for the region was variable between seasons (Tables 1.4, 1.5, 1.6), and throughout the year (Appendix 1) with many highly correlated chemical variables, as expected.

PCA reduced the spring data set to seven independent components explaining 84.1% of the total variance. PC axes were named based on the weight of component loadings (Table 1.7) for ease of discussion; PC1 was named "spring conductivity", PC2 "metals", PC3 "metal-sulphur", PC4 "pH-alkalinity", PC5 "copper-ammonia", PC6 "organic-phosphorus", and PC7 "nitrate-TSS".

Species richness was significantly related to spring water chemistry (multiple regression analysis, $F = 4.76$, $p < 0.001$, $R^2 = 0.31$). Stepwise regression indicated that all components except the "metals" were significantly influencing amphibian species richness ($F = 5.66$, $p < 0.001$, $R^2 = 0.33$, Table 1.8). No differences, both, between presence/absence at a sites for individual species (Table 1.9), and among species present, based on spring water chemistry were detected (MANOVA, Wilks' $\lambda = 0.558$, $F_{91, 781} = 0.84$, $p = 0.847$). Classification of individual species' presence/absence was only moderately successful using spring water chemistry as a predictor in DFA (Table 1.10). Attempts to classify species present at a site, with spring water chemistry as a predictor in DFA, was only moderately better than that expected by random chance at 26.4% (16.7% correct classification expected by random chance with 6 species).

The summer data set was reduced to six independent components explaining 82.3% of the total variance using PCA. For ease of discussion, PC axes were named based on the weight of component loadings (Table 1.11); PC1 was named "summer

Table 1.4. Descriptive statistics (mean ± SE (range)) for spring water chemistry of the 59-wetland sites.

Variable‡	Value
pH	7.7 ± 0.10 (5.2-9.7)
Total Dissolved Solids	189.9 ± 31.28 (21.0-1127.0)
Total Alkalinity (as $CaCO_3$)	53.6 ± 4.08 (12.0-169.0)
Conductivity (μS/cm)	360.8 ± 72.46 (31.0-3070.0)
Dissolved Inorganic Carbon	11.86 ± 1.026 (2.19-40.90)
Dissolved Organic Carbon	23.50 ± 1.210 (5.96-49.03)
Calcium	23.78 ± 3.196 (2.78-116.45)
Chloride	77.50 ± 24.508 (0.39-1030.00)
Potassium	5.93 ± 0.530 (1.38-18.66)
Magnesium	8.89 ± 1.256 (1.05-55.43)
Sodium	30.54 ± 10.465 (0.23-451.80)
Nitrate	0.24 ± 0.072 (0.01-2.76)
Aluminium	1.14 ± 1.028 (0.01-60.74)
Barium	0.56 ± 0.526 (0.01-31.09)
Copper	0.003 ± 0.0003 (0.001-0.011)
Iron	1.20 ± 0.476 (0.05-28.25)
Manganese	0.18 ± 0.050 (0.0001-2.65)
Nickel	0.002 ± 0.0002 (0.001-0.009)
Sulphur	5.06 ± 1.131 (0.61-64.58)
Strontium	0.12 ± 0.060 (0.04-3.57)
Titanium	0.006 ± 0.0028 (0.002-0.164)
Vanadium	0.40 ± 0.006 (0.01-0.25)
Zinc	0.006 ± 0.0010 (0.001-0.032)
Sulphate	13.40 ± 3.293 (0.04-186.08)

Ammonia	0.11 ± 0.023 (0.02-0.99)
Total Nitrogen	1.25 ± 0.069 (0.014-2.47)
Total Phosphorus	0.1 ± 0.01 (0.004-0.7)
TSS	2.67 ± 0.239 (1.90-9.40)

‡Units are mg/L unless stated otherwise, pH is unitless

Table 1.5. Descriptive statistics (mean ± SE (range)) for summer water chemistry of the 53-wetland sites.

Variable‡	Value
pH	7.9 ± 0.08 (6.3-9.2)
Total Dissolved Solids	220.0 ± 27.07 (41.3-1180.8)
Total Alkalinity (as $CaCO_3$)	113.9 ± 7.73 (18.7-264.5)
Conductivity (µS/cm)	399.9 ± 51.61 (60.5-2180.0)
Dissolved Inorganic Carbon	22.78 ± 1.709 (4.12-54.09)
Dissolved Organic Carbon	21.57 ± 2.053 (3.13-81.05)
Calcium	35.32 ± 3.248 (3.72-149.70)
Chloride	64.99 ± 24.436 (0.23-1136.00)
Potassium	4.99 ± 1.576 (0.20-79.51)
Magnesium	14.06 ± 1.35 (2.23-53.11)
Sodium	20.13 ± 5.473 (1.36-200.29)
Aluminium	0.08 ± 0.028 (0.004-1.23)
Barium	0.044 ± 0.0132 (0.007-0.713)
Copper	0.003 ± 0.0023 (0.001-0.010)
Iron	2.26 ± 0.648 (0.03-28.90)
Manganese	0.35 ± 0.087 (0.01-3.08)
Nickel	0.002 ± 0.0002 (0.001-0.007)
Sulphur	2.34 ± 0.266 (0.49-8.87)
Strontium	0.07 ± 0.008 (0.04-0.40)
Zinc	0.007 ± 0.0036 (0.001-0.193)
Sulphate	14.44 ± 8.356 (0.04-443.71)
Ammonia	0.19 ± 0.053 (0.02-2.67)
Total Nitrogen	1.40 ± 0.169 (0.05-6.83)
Total Phosphorus	0.1 ± 0.01 (0.004-0.3)

TSS	5.32 ± 0.957 (1.90-32.50)

‡Units are mg/L unless stated otherwise, pH is unitless

Table 1.6. Descriptive statistics (mean ± SE (range)) for general water chemistry of the 45-wetland sites.

Variable‡	Value
pH	7.9 ± 0.75 (6.3-9.0)
Total Dissolved Solids	218.0 ± 31.59 (40.0-1002.0)
Total Alkalinity (as $CaCO_3$)	82.9 ± 6.07 (17.9-207.8)
Conductivity (µS/cm)	353.2 ± 56.08 (60.0-1760.0)
Dissolved Inorganic Carbon	17.27 ± 1.411 (3.35-45.74)
Dissolved Organic Carbon	22.29 ± 1.605 (6.41-65.04)
Calcium	28.76 ± 3.153 (3.56-93.99)
Chloride	63.47 ± 23.893 (0.74-975.84)
Potassium	4.84 ± 0.683 (0.88-17.74)
Magnesium	11.42 ± 1.398 (2.10-49.25
Sodium	21.28 ± 6.665 (1.29-226.97)
Nitrate	0.30 ± 0.150 (0.01-6.50)
Aluminium	0.77 ± 0.687 (0.01-30.98)
Barium	0.38 ± 0.345 (0.01-15.56)
Copper	0.003 ± 0.0003 (0.001-0.008)
Iron	1.78 ± 0.655 (0.08-28.57)
Manganese	0.27 ± 0.067 (0.01-2.16)
Nickel	0.002 ± 0.0002 (0.001-0.007)
Sulphur	3.28 ± 0.380 (0.79-13.57)
Strontium	0.10 ± 0.039 (0.04-1.81)
Titanium	0.005 ± 0.0020 (0.002-0.091)
Vanadium	0.034 ± 0.0047 (0.009-0.160)
Zinc	0.006 ± 0.0025 (0.0009-0.113)
Sulphate	13.58 ± 5.13 (0.17-231.92)

Ammonia	0.14 ± 0.017 (0.02-0.52)
Total Nitrogen	1.25 ± 0.094 (0.26-3.39)
Total Phosphorus	0.1 ± 0.01 (0.02-0.4)
TSS	3.91 ± 0.511 (1.90-17.2)

‡Units are mg/L unless stated otherwise, pH is unitless

Table 1.7. PCA component loadings for spring water chemistry (most influential variables for each axis are bolded).

Chemical Variable	Component Loadings						
	1	2	3	4	5	6	7
Conductivity	**0.973**	-0.012	0.099	0.039	0.056	-0.058	0.017
Cl	**0.961**	-0.014	-0.027	-0.060	0.073	-0.021	-0.023
Na	**0.936**	-0.016	-0.017	-0.114	0.111	-0.035	-0.061
Ca	**0.909**	0.013	0.261	0.250	-0.041	-0.073	0.105
TDS	**0.891**	-0.028	0.174	0.254	-0.065	-0.070	0.115
Mg	**0.780**	0.005	0.286	0.397	-0.121	-0.105	0.209
V	**0.768**	-0.021	0.308	0.427	-0.120	-0.136	0.167
Mn	**0.624**	-0.052	-0.076	-0.031	-0.183	0.334	-0.243
Ba	-0.036	**0.994**	-0.041	-0.033	-0.014	-0.015	-0.032
Al	-0.046	**0.994**	-0.040	-0.041	-0.006	-0.013	-0.026
Sr	0.050	**0.994**	-0.006	-0.021	-0.022	-0.047	-0.008
Ti	-0.069	**0.983**	-0.021	-0.068	-0.041	-0.026	0.030
Fe	-0.011	**0.981**	-0.051	-0.058	0.018	0.059	-0.052
S	0.109	-0.031	**0.922**	0.139	-0.144	-0.052	-0.033
SO_4	0.111	-0.034	**0.919**	0.138	-0.152	-0.060	-0.035
Ni	0.120	-0.016	**0.797**	0.040	0.345	-0.015	0.185
Zn	0.217	-0.056	**0.641**	-0.175	0.190	0.165	-0.007
Alkalinity	0.400	0.027	0.119	**0.804**	-0.047	0.000	0.114
DIC	0.426	-0.091	0.145	**0.751**	-0.059	0.015	0.167
K	-0.068	-0.106	0.018	**0.674**	0.464	0.190	-0.175
pH	-0.177	-0.460	-0.082	**0.654**	0.252	-0.217	0.047

TotP	0.100	0.091	-0.130	**0.508**	0.440	0.467	-0.026
NH_3	-0.007	-0.053	0.014	-0.115	**-0.810**	0.165	-0.053
Cu	-0.189	-0.104	0.351	0.005	**0.661**	0.005	0.426
TotN	-0.069	-0.298	0.119	0.188	-0.033	**0.784**	0.253
DOC	-0.276	0.353	-0.072	-0.237	-0.228	**0.731**	-0.072
NO_3	0.022	-0.038	0.035	0.020	-0.062	-0.046	**0.886**
TSS	0.128	-0.012	-0.021	0.094	0.249	0.184	**0.617**
% Variance Explained	23.47	19.81	11.58	10.83	6.84	6.11	6.05

Table 1.8. Regression of species richness vs. spring water chemistry components (PCs noted with asterisks were retained in stepwise analyses).

PC	Standardized coefficient	*t*	*p*	Tolerance
Spring conductivity*	-0.297	-2.728	0.009	1.000
Metals	0.017	0.159	0.874	1.000
Metal-sulphur*	-0.300	-2.757	0.008	1.000
pH-alkalinity*	0.199	1.831	0.073	1.000
Copper-ammonia*	-0.190	-1.745	0.087	1.000
Organic-phosphorus*	-0.324	-2.976	0.004	1.000
Nitrate-TSS*	-0.189	-1.734	0.089	1.000

Table 1.9. Individual species comparisons for differences in presence/absence of sites based on spring water chemistry components using MANOVA, *df* (7,26).

Species	Wilk's λ	*F*	*p*
B. americanus	0.984	0.061	1.000
H. versicolor	0.846	0.677	0.689
P. crucifer	0.954	0.180	0.987
P. maculata	0.958	0.164	0.990
R. clamitans	0.930	0.278	0.957
R. pipiens	0.929	0.285	0.954
R. septentrionalis	0.826	0.784	0.607
R. sylvatica	0.736	1.152	0.363

Table 1.10. Individual species presence/absence classification statistics using spring water chemistry as a predictor in DFA (Due to binary nature of presence/absence data, random chance correct classification is 50%).

Species‡	No. of Sites Present	No. of Sites Absent	Wilk's λ	*F*	*p*	Group Classification (%)
B. americanus	21	38	0.799	1.838	0.100	66.1
H. versicolor	15	44	0.711	2.957	0.011	78.0
P. crucifer	24	35	0.840	1.390	0.230	61.0
P. maculata	18	41	0.875	1.040	0.416	62.7
R. septentrionalis	12	47	0.675	3.351	0.005	86.4
R. sylvatica	48	11	0.702	3.088	0.009	78.0

‡*R. pipiens* and *R. clamitans* were not included as they were present at only two and four sites respectively

conductivity", PC2 "pH-metal", PC3 "nutrients", PC4 "inorganic-phosphorus", PC5 "anions", and PC6 "sulphur-zinc".

Species richness was significantly related to summer water chemistry (multiple regression analysis, $F = 3.57$, $p = 0.006$, $R^2 = 0.23$), while a stepwise regression determined that the "inorganic-phosphorus" and "summer conductivity" PCs were most influential on amphibian species richness ($F = 7.73$, $p = 0.001$, $R^2 = 0.21$, Table 1.12).

Similar to the spring analysis, no differences between presence/absent for individual species (Table 1.13), or among species present based on summer water chemistry were detected (MANOVA, Wilks' $\lambda = 0.784$ $F_{42, 684} = 0.87$, $p = 0.714$). Again classification of individual species' presence/absence was only moderately successful using summer water chemistry as a predictor in DFA (Table 1.14). Similar findings for classification of species present at a site occurred in the summer analysis as compared to the spring analysis. Again only marginal classification success, using summer water chemistry as a predictor in DFA, occurred at 24.1% (with 16.7% correct classification expected by random chance with 6 species). With general water chemistry for the year, PCA reduced the averaged data set to seven independent components explaining 86.9% of the total variance. PC axes were again named based on weight of component loadings (Table 1.15); PC1 was named "metals", PC2 "hardness", PC3 "salt", PC4 "nutrients", PC5 "copper-nickel", PC6 "pH-potassium", PC7 "sulphur-ammonia".

Species richness was significantly related to general water chemistry (multiple regression analysis, $F = 3.03$, $p = 0.013$, $R^2 = 0.24$). Stepwise regression found the "hardness", "pH-potassium" and "salt" components to be most influential on amphibian

Table 1.11. PCA component loadings for summer water chemistry (most influential variables for each axis are bolded).

Chemical Variable	Component Loadings					
	1	2	3	4	5	6
Sr	**0.956**	-0.002	0.016	0.009	0.055	0.163
TDS	**0.947**	0.005	0.066	0.154	0.220	0.104
Conductivity	**0.945**	0.036	0.053	0.183	0.201	0.073
Ba	**0.911**	-0.014	-0.003	-0.217	-0.120	-0.096
Ca	**0.902**	0.010	0.009	0.249	0.057	0.218
Mg	**0.866**	-0.050	0.067	0.289	-0.007	0.197
Na	**0.793**	0.070	-0.120	0.167	0.342	0.000
Fe	-0.050	**0.880**	0.085	0.018	-0.019	-0.047
DOC	-0.136	**0.815**	0.128	0.107	-0.072	0.033
TSS	0.028	**0.798**	0.427	0.189	-0.095	-0.036
Al	-0.044	**0.757**	0.472	-0.241	-0.048	-0.005
pH	-0.122	**-0.704**	0.132	-0.076	-0.117	0.093
Ni	0.269	**0.599**	0.392	0.106	-0.120	0.305
Mn	0.110	**0.537**	-0.013	0.487	0.369	-0.042
K	0.103	0.042	**0.946**	0.054	-0.027	-0.044
NH_3	0.001	0.116	**0.880**	0.158	0.068	-0.018
TotN	-0.024	0.432	0.681	0.366	-0.192	-0.214
Cu	-0.100	0.413	0.557	-0.195	-0.029	0.126
DIC	0.328	-0.003	0.166	**0.830**	0.046	0.241
Alkalinity	0.340	0.001	0.294	**0.795**	0.168	0.238
TotP	-0.008	0.332	-0.121	**0.690**	-0.192	-0.365

SO_4	0.156	-0.064	-0.032	0.039	**0.941**	0.022
Cl	0.618	0.001	-0.033	0.002	**0.763**	-0.055
Zn	0.180	0.218	-0.066	0.071	0.015	**0.815**
S	0.157	-0.246	-0.022	0.049	-0.030	**0.758**
% Variance Explained	26.23	17.58	12.82	10.58	7.94	7.20

Table 1.12. Regression of species richness vs. summer water chemistry components (PCs noted with asterisks were retained in stepwise analyses).

PC Axis	Standardized coefficient	*t*	*p*	Tolerance
Summer conductivity*	-0.278	-2.281	0.027	1.000
pH-metal	-0.189	-1.551	0.128	1.000
Nutrients	0.010	0.083	0.934	1.000
Inorganic-phosphorus*	-0.399	-3.273	0.002	1.000
Anions	-0.185	-1.523	0.135	1.000
Sulphur	-0.106	-0.872	0.388	1.000

Table 1.13. Individual species comparisons for differences in presence/absence of sites based on summer water chemistry components using MANOVA, *df* (6,19).

Species	Wilk's λ	*F*	*p*
B. americanus	0.797	0.807	0.577
H. versicolor	0.707	1.313	0.299
P. crucifer	0.593	2.170	0.092
P. maculata	0.679	1.498	0.232
R. clamitans	0.995	0.015	1.000
R. pipiens	0.935	0.215	0.966
R. septentrionalis	0.878	0.440	0.843
R. sylvatica	0.693	0.095	0.264

Table 1.14. Individual species presence/absence classification statistics using summer water chemistry as a predictor in DFA (Due to binary nature of presence/absence data, random chance correct classification is 50%).

Species[‡]	No. of Sites Present	No. of Sites Absent	Wilk's λ	*F*	*p*	Group Classification (%)
B. americanus	25	28	0.857	1.284	0.234	58.5
H. versicolor	20	33	0.853	1.316	0.269	64.2
P. crucifer	27	26	0.605	4.998	< 0.001	79.2
P. maculata	18	35	0.910	0.755	0.608	60.4
R. septentrionalis	15	38	0.788	2.065	0.076	64.2
R. sylvatica	47	6	0.964	0.289	0.939	83.0

[‡]*R. pipiens* and *R. clamitans* were not included as they were present at only two and four sites respectively

Table 1.15. PCA factor loadings for general water chemistry (most influential variables for each axis are bolded).

Chemical Variable	Component Loadings						
	1	2	3	4	5	6	7
Sr	**0.990**	0.059	0.039	-0.065	0.017	-0.008	-0.070
Ba	**0.989**	-0.073	-0.010	-0.029	0.015	-0.030	-0.054
Al	**0.986**	-0.091	-0.019	-0.021	0.035	-0.036	-0.048
Ti	**0.971**	-0.116	-0.025	-0.005	0.129	-0.004	-0.035
Fe	**0.938**	-0.039	-0.040	0.213	-0.034	-0.190	-0.012
TSS	**0.646**	0.135	-0.075	0.612	0.184	-0.032	0.022
DOC	**0.614**	-0.185	-0.068	0.465	0.097	-0.316	0.200
DIC	-0.177	**0.841**	0.052	0.281	-0.009	-0.025	-0.027
Alkalinity	-0.132	**0.839**	0.117	0.300	-0.108	-0.021	-0.009
Ca	-0.010	**0.819**	0.490	-0.044	-0.025	0.052	-0.093
Mg	-0.020	**0.818**	0.411	-0.015	-0.063	0.176	-0.079
V	-0.053	**0.815**	0.282	-0.071	0.197	0.021	0.107
TDS	0.008	**0.697**	0.586	-0.054	-0.081	0.112	-0.173
Zn	0.105	**0.608**	-0.019	-0.229	0.432	-0.250	0.090
Cl	0.001	0.315	**0.939**	-0.028	-0.083	-0.007	-0.056
NO_3	-0.040	0.054	**0.916**	0.007	-0.005	-0.007	0.138
SO_4	-0.041	0.149	**0.907**	-0.039	-0.034	-0.008	0.214
Na	-0.014	0.460	**0.808**	-0.001	-0.033	-0.141	-0.085
Conductivity	-0.006	-0.673	**0.700**	-0.030	-0.033	0.008	-0.100
Mn	0.053	0.197	**0.606**	0.429	-0.197	-0.496	0.109
TotN	0.011	-0.004	-0.104	**0.871**	0.198	0.065	0.075

TotP	0.076	0.145	0.122	**0.788**	-0.042	0.300	-0.159
Cu	0.013	-0.163	-0.130	0.133	**0.908**	0.059	-0.035
Ni	0.285	0.396	-0.050	0.196	**0.714**	-0.094	-0.067
K	-0.070	0.067	0.017	0.429	0.080	**0.768**	-0.130
pH	-0.464	0.059	-0.183	0.051	-0.218	**0.697**	0.217
NH_3	-0.080	-0.203	0.126	0.028	-0.143	-0.073	**0.793**
S	-0.080	0.554	0.058	-0.121	0.215	0.125	**0.644**
% Variance Explained	21.20	20.51	17.91	9.64	6.63	6.14	4.85

species richness ($F = 5.81$, $p = 0.002$, $R^2 = 0.25$, Table 1.16). Consistent with spring and summer analyses, no differences between presence/absent for individual species (Table 1.17), or among species present based on general water chemistry were detected (MANOVA, Wilks' $\lambda = 0.718$ $F_{49, 588} = 0.81$, $p = 0.819$). Individual species' presence/absence classification was only moderately successful using general water chemistry as a predictor in DFA (Table 1.18). Only marginal classification success for species present at a site occurred at 20.9% (16.7% correct classification expected by random chance with 6 species) using general water chemistry as a predictor in DFA.

Discussion

Amphibian surveys

I observed lower amphibian species richness in 2003 (2.4 ± 0.19 SE) compared to 2002 (3.8 ± 0.18 SE) and 2001 (3.5 ± 0.2 SE) (Robinson, 2004; Abbott, 2004). The lower species richness may have been caused by the harsh winter conditions (December 2002-April 2003) in the region over the season. The regional average winter temperature (based on data between 1971-2000, Environment Canada, 2004) is –8.0°C (ranging from a low of –14.5°C in January to a high of 2.6°C in April). As well average monthly snowfall is 25.9 cm (ranging from a low of 6.9 cm in April to a high of 38.5 cm in December). However during the winter of 2002-2003 the average temperature was -8.8°C (ranging from a low of -16.6°C in February to a high of 1.4°C in April) with the coldest days occurring in early February (19 days in February below –15°C) to mid-March (13 days below –10°C). The average monthly snowfall for

Table 1.16. Regression of species richness vs. general water chemistry components (PCs noted with asterisks retained in stepwise analyses).

PC Axis	Standardized coefficient	*t*	*p*	Tolerance
Metals	0.010	0.073	0.942	1.000
Dissolved components*	-0.358	-2.734	0.010	1.000
Salt*	-0.240	-1.830	0.075	1.000
Nutrients	-0.162	-1.234	0.225	1.000
Copper-nickel	-0.159	-1.217	0.231	1.000
pH-potassium*	0.335	2.557	0.015	1.000
Sulphur-ammonia	0.121	0.924	0.361	1.000

Table 1.17. Individual species comparisons for differences in presence/absence of sites based on general water chemistry components using MANOVA, *df* (7,13).

Species	Wilk's λ	*F*	*p*
B. americanus	0.938	0.123	0.995
H. versicolor	0.816	0.420	0.873
P. crucifer	0.897	0.214	0.976
P. maculata	0.881	0.252	0.962
R. clamitans	0.871	0.276	0.953
R. pipiens	0.878	0.257	0.960
R. septentrionalis	0.853	0.321	0.931
R. sylvatica	0.895	0.217	0.975

Table 1.18. Individual species presence/absence classification statistics using general water chemistry as a predictor in DFA (Due to binary nature of presence/absence data, random chance correct classification is 50%).

Species[‡]	No. of Sites Present	No. of Sites Absent	Wilk's λ	*F*	*p*	Group Classification (%)
B. americanus	21	24	0.808	1.258	0.297	60.0
H. versicolor	14	31	0.685	2.436	0.037	71.1
P. crucifer	22	23	0.680	2.489	0.034	73.3
P. maculata	14	31	0.869	0.798	0.594	53.3
R. septentrionalis	12	33	0.687	2.413	0.039	77.8
R. sylvatica	40	5	0.961	0.215	0.980	84.4

[‡]*R. pipiens* and *R. clamitans* were not included as they were present at only two and four sites respectively

the same period is was only 6.7 cm, with only 5 cm being recorded on the ground at the end of January. The cold temperatures in conjunction with the below average snowfall (snow often acts as insulation and regulates temperatures), may have led to high mortality. Many wetlands froze completely, and limited insulation caused frost lines to reach depths of 2-3 m. Since local amphibians hibernate either in wetlands or in shallow burrows in the ground, mortality due to freezing was likely high. The wood frog, which produces cryoprotectants (Schmid, 1982; Duellman and Trueb, 1994), was the only species that appeared to be relatively unaffected.

Water Chemistry and species richness

Water chemistry varied throughout the year in the network of sites. These findings were not unexpected because sites ranged from highly disturbed farm ponds and roadside ditches, to undisturbed forest ponds. Local wetland characteristics, such as rock type, depth, dominant vegetation and inflows to a site (if present), may also be contributing to variance.

In general I found amphibian species richness in northwestern Ontario to be positively correlated to pH, K, NH_3, Ba, and Ti, and negatively correlated to TDS, conductivity, alkalinity, DIC, Ca, Na, Mg, Cl, a variety of metals and various nutrients such as P and N. The relationships between species richness and water chemistry found in my study appears to be consistent with findings from previous studies examining the effects of chemicals on amphibians.

Differing effects of dissolved carbon on amphibians have been reported. Gascon and Planas (1986) found that increases in total organic carbon and decreases in pH

negatively influenced egg mass abundance and embryonic survival of *R. sylvatica*, while Freda et al. (1990) linked high DOC with reduced survivorship. Other studies suggest that DOC may help protect amphibian eggs from harmful ultraviolet radiation (Nagl and Hofer, 1997; Crump et al., 1999a, 1999b; Licht, 2003). Locally dissolved carbon effects may be related to the dominant vegetation and soils surrounding the wetlands leading to the presence of tannins, which may be a potential explanation for the effects of carbon (Wetzel, 2001).

Conductivity, TDS, Ca, Na, Mg, K, and Cl are all closely related chemical constituents. Conductivity is a measure of the electrical activity and Ca, Na, Mg, and Cl are all ions that readily dissociate in water and are highly conductive. TDS is a measure of the dissolved solids, ionic solids dissolved easiest in water, therefore making TDS related to conductivity. Elevated levels of these chemicals may be present in wetlands due to runoff of road salts. The major salts used for winter road maintenance in northwestern Ontario are NaCl, CaCl and MgCl (Environment Canada, 2001). The proximity of some wetlands to roads combined with the persistence of salts in wetlands, suggests that these chemicals may be having an overall negative effect on amphibians. Studies have found negative effects from salts, related to road runoff, on amphibians (Romspert, 1976; Padhye and Ghate, 1992; Turtle, 2001), fish and invertebrates (Evans and Frick, 2001) and chronic effects have been also been detected for amphibians (Sanzo and Hecnar, in review). Hecnar and M'Closkey (1996) also found that Cl, Mg, conductivity, hardness and turbidity were negatively correlated with amphibian species richness in southwestern Ontario.

Metals can also have negative effects on amphibians (see review in Sparling et al., 2000) so their influence in the region is not a major surprise. With the vast amount of local granitic bedrock containing a variety of metals, it is possible that metals are entering wetlands through watercourses and ground water inputs (Wetzel, 2001). In previous studies, Glooschenko et al. (1992) suggested that metal levels and low pH may be a factor limiting successful breeding for some amphibians in northeastern Ontario, while Freda et al. (1990) found toxic effects from aluminium in soft waters. Reduced amphibian density and reproductive activity were also associated with Al, Cu, Zn and silicon concentrations for *Ambystoma maculatum* in eastern Virginia (Blem and Blem, 1991), further suggesting that the presence of metals in wetlands in northwestern Ontario may be leading to a negative effect on species richness.

Many studies have examined the effects of pH, and its interactions with other chemicals, on amphibians (Clarke, 1986; Freda and Dunson, 1986; Freda et al., 1991; Rowe et al., 1992; Sadinski and Dunson, 1992; Horne and Dunson, 1995). These studies indicate a general negative effect of acidification of wetlands on amphibians. Locally species richness was positively related to the pH-related axes, which is consistent with other studies. Hecnar and M'Closkey (1996) suggested that pH and alkalinity were not important factors in southwestern Ontario, a region with typically hard, alkaline and well-buffered waters, but that other regions such as the Canadian Shield (the location of our study), may find different results because waters are typically soft and poorly buffered, this appears to be the case locally. Among our sites pH ranged from 6.3-9.0, which is relatively high for boreal-type landscapes. It is possible that due to the location of the study area, in the transition zone between the Boreal

forests to the north and the Great Lakes-St. Lawrence forests to the south, pH is exhibiting properties of both biomes. Amphibian species richness decreased as pH decreased, and despite the fact that pH was not significant in the regression analysis (both spring and general water chemistry) it was retained as a component in the stepwise model.

Nutrients, such as nitrogen, may be important factors influencing species richness directly and indirectly. The presence of nitrogen may be related to the agricultural practices and cattle farming in the immediate landscapes surrounding sites. Fertilizers have been shown to have direct detrimental effects on amphibians (Hecnar, 1995; Oldham et al., 1997; Marco et al., 1999; Rouse et al., 1999). Indirect effects of these nutrients may occur from their effects on algae and aquatic vegetation. Wetlands receiving greater concentrations of nutrients may also experience increased productivity, providing more food for larval amphibians; however if there are increased concentrations of nutrients in wetlands, larvae may also experience adverse effects of eutrophication.

My study and others indicate that various chemicals can negatively affect species richness of amphibian communities. The overall negative relationship I observed between species richness and water chemistry may be related to either the adverse effect experienced by amphibians, or by species choosing to avoid the sites. Regression analysis explained only a low percentage of the variance in species richness yet was found to be highly significant, suggesting that the true affects of water chemistry need further clarification. The discrepancy may also be related to the fact that the study area is located in the transition zone between the Great Lakes-St.

Lawrence forests to the south and the Boreal regions to the north, therefore the study area may display characteristics of both regions in wetland chemistry. For example, boreal forests are typically characterized by more acidic soils, which would ultimately affect wetland pH; this in turn would affect the availability of chemicals as acidity changes (Wetzel, 2001). Similarly, the amount and nature of metals present in the bedrock would also change in more northerly locations. Therefore, my findings may be an indication that amphibian species richness may be more greatly affected by water chemistry in "pure" boreal settings.

Water chemistry, species presence/absence and species assemblages

No differences among species present at a site or between occupied and unoccupied sites for individual species was detected by MANOVA. Species classification using water chemistry as a predictor in DFA had only moderate success. Dale et al. (1985) found that water chemistry was unable to predict species' presence, but that acidity negatively affected species composition. Glooschenko et al. (1992) had success at classification of species with DFA, and found that metals and sulphates (most likely the result of atmospheric deposition) negatively affected species' presence, while "buffering status" (a PC in their analysis containing similar chemical variables as my "conductivity" related variables) was positively related to the presence of species. These studies were conducted in regions with similar geology but found slightly different results perhaps related to different land uses. The dominant industry in northwestern Ontario is forestry, potentially explaining why my study found different results.

Beebee (1985) and Pavignano et al. (1990) found that other non-chemical habitat variables were more useful in classification of amphibians in Europe. Hecnar and M'Closkey (1998) found that the area of woodlands within a region and the amount of emergent vegetation in wetlands were the most influential factors associated with amphibian species richness in southwestern Ontario; while Kolozsvary and Swihart (1999) suggested that forest area and permanency of wetland patch, as well as landscape levels variables such as land use patterns and proximity to other wetlands were good predictors of species richness. Wetland hydroperiod (degree of water permanancy) has been suggested as an important factor influencing amphibian distribution (Kolozsvary and Swihart, 1999; Babbit et al., 2003). In a large-scale study in northwestern Ontario area, hydroperiod was also found to positively affect amphibian species richness (Robinson, 2004). Robinson (2004) also found the total amount of roads (km) on the landscape had negative effects on amphibian species richness, while Abbott (2004) suggested that species richness in northwestern Ontario was positively affected by area of agriculture and the percentage of shrubs around a wetland, and distance to the nearby streams. Pope et al. (2000) also suggest that habitat complementation (i.e. amount of summer habitat such as fields or grassy meadows) significantly affected Leopard frog (*R. pipiens*) density. In a study in southern Illinois, examining the potential of riparian zones as dispersal corridors for reptiles and amphibians, found that proximity to core areas and local habitat heterogeneity best explained species richness (Burbrink et al., 1998). Biotic interactions such as predation from fish can also negatively influence amphibian species richness and distribution

(Hayes and Jennings, 1986; Kats et al., 1988; Hecnar and M'Closkey, 1997; Smith et al., 1999).

Conclusions

Species richness was significantly associated with water chemistry based on regression analysis, despite the low percentage of the variance explained by the models. The lack of differences among species present at a site and the moderate classification success using water chemistry as a predictor with DFA suggest that water chemistry does not appear to be a major factor influencing amphibian distribution in northwestern Ontario. This concurs with studies conducted in most other regions. However the significant effect of water chemistry on species richness combined with the lack of differences among species suggests that a community level response may be occurring, whereby all species are responding similarly to general water chemistry, potentially explaining the lack of differences observed at the individual species level.

My results also provide further evidence that pollutants in water can affect amphibians and that the effects of chemicals cannot be dismissed at an important habitat characteristic, in boreal type settings, in explaining amphibian distribution. Different findings from studies conducted in similar geological regions (my study as compared to Dale et al. (1985) and Glooschenko et al. (1992)) indicated further research examining the importance of water chemistry relative to other regional landscape and/or local habitat variables in the northwestern Ontario, and within the Boreal forests is needed. As well studies examining the effects of pollutants on regional species is also required.

Chapter 2:

Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*)

Introduction

A consequence of human population growth is the increasing modification of natural landscapes and ecosystems (Myers, 1996; Dobson et al., 1997; Vitousek et al., 1997; McDaniel and Borton, 2002). One of the most apparent artefacts of human enterprise is the vast network of roads that covers the land surface of many regions (Miller et al., 1996; Forman et al., 2003). There are approximately 8 million km of roads in North America alone (Forman et al., 2003) and nearly 80% of the land in the continental United States is within 1 km of a road (Riitters and Wickham, 2003).

Many negative environmental effects are associated with roads and their fragmentation of natural habitats. These effects include loss of habitat, isolation of populations, increased edge effects, and barriers to movements and gene flow (Reh and Seitz, 1990; deMaynadier and Hunter, 1995; Forman and Alexander, 1998). Roads also increase mortality to wildlife from collisions with vehicles, and they increase pollution from vehicles and road maintenance (Ashley and Robinson, 1996; Forman and Alexander, 1998; Trombulak and Frissell, 2000). Of these effects, the environmental impact of road chemical runoff has generated great concern but limited research (Transportation Research Board, 1991; Wainscott, 1997; Mayer et al., 1999; Environment Canada, 2001).

Road runoff includes many chemicals such as metals, hydrocarbons (e.g. rubber residues and petroleum products) and de-icing agents (Norrstrom and Jacks, 1998). A major component of road runoff that has received increased attention in recent years is salt (Transportation Association of Canada, 1999; Environment Canada, 2001). Salts are commonly used in road maintenance for winter de-icing and summer dust suppression (Environment Canada, 2001). The most commonly used salts include sodium chloride (NaCl), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$), with NaCl accounting for 98% of all usage (Transportation Research Board, 1991; Environment Canada, 2001). In North America alone, about 14.0 million tonnes of road salt are used annually (Transportation Research Board, 1991; Environment Canada, 2001). Environment Canada (2001) estimated that 4.9 million tonnes of road salts were applied to Canadian roads in 1998, which accounted for an input of 3.0 million tonnes of chloride. With such a large addition of a chemical to the environment there is a need for understanding its effects on ecosystems. Terrestrial effects of salts include damage to roadside vegetation and visible changes in animal behaviour, but relatively little is known of the effects in aquatic ecosystems where salts are easily transported and can be highly persistent. Taxa that have greater dependence on wetlands or other aquatic habitats may be more vulnerable to toxicity from road runoff chemicals. Amphibians may be a vulnerable group, however limited literature on salt toxicity exists (Romspert, 1976; Mahajan et al., 1979; Padhye and Ghate, 1992; Turtle, 2001).

Chemical contamination has long been suggested as a possible factor leading to global amphibian declines because amphibians are sensitive to environmental pollution (Phillips, 1990; Blaustein et al., 1994; Blaustein and Wake, 1995; Sparling et al., 2000;

Hayes et al., 2002). A recent global assessment of the status of amphibians now implicates pollution as a factor threatening 21% of the world's species (IUCN, Conservation International and Nature Serve, 2004).

Susceptibility of amphibians to aquatic pollutants is a result of their dependence on water to complete their life cycles (Stebbins and Cohen, 1995). Most amphibians have unprotected (unshelled) aquatic eggs, highly permeable skin, aquatic larval stages, and adults use wetlands for breeding, foraging, and hibernation. Because of these characteristics and their limited movements, amphibians are considered excellent indicators of ecosystem health (Vitt et al., 1990). The wetlands amphibians inhabit range from ephemeral sites such as roadside ditches to permanent lakes (Robinson, 2004). As a result of the vast road network that exists in the world, wetlands may experience increased salt concentrations due to their potential proximity to roads. This in turn may be affecting amphibian populations.

Background chloride concentrations are typically only a few milligrams per litre, with some variability resulting from topography, geology, and geographic location (Wetzel, 2001). However, human inputs significantly raise concentrations. Environment Canada (2001) reports road runoff concentrations to exceed 18 000 mg/L. Salt concentrations can range from 150 mg/L in rural lakes to 5000 mg/L in urban impoundment lakes and snow cleared from streets. Concentrations in ponds and wetlands can reach as high as 4000 mg/L, while watercourses can reach 4300 mg/L (Environment Canada, 2001). Seasonal inputs and environmental persistence of salts result in elevated concentrations that may be present during critical amphibian development times in the early spring and through the summer.

My goal was to determine if road salts had an adverse effect on amphibians at environmentally relevant field concentrations. I report experimental results from acute and chronic exposure of wood frogs (*Rana sylvatica*), to solutions of NaCl and water. The null hypothesis that was under test was that road salts had no effect on the survivorship, or growth and development, of amphibians. I expected to find negative effects of road salts on amphibians.

Materials and methods

Study organism

The wood frog (*Rana sylvatica*) is one of the most widely distributed amphibians in North America, ranging from above the Arctic Circle to the southern United States (Conant and Collins, 1998). Wood frogs use a variety of wetlands that range from shallow temporary ponds to permanent water bodies, but prefer woodland ponds (Conant and Collins, 1998; MacCulloch, 2002). Larvae (tadpoles) are aquatic while adults are largely terrestrial but they return to wetlands for breeding. Wood frogs are early spring breeders across their range and often occur in water bodies in close proximity to roads (personal observations). Development time varies with ambient temperature but tadpoles are potentially exposed to road salts for about 6 to 15 weeks from oviposition to metamorphosis (Harding, 1997).

With the help of a field assistant, I collected recently oviposited wood frog egg masses from numerous sites near Thunder Bay, Ontario, Canada (48° 27' N, 89° 12' W), in May 2004. Eggs were collected only when abundant at sites to minimize impacts

on local populations. I raised eggs in aquaria until they developed into feeding stage tadpoles (Stage 25; Gosner, 1960). Tadpoles were used in all experiments.

Field concentrations

To determine the range of salt concentrations potentially encountered by wood frogs in the region I conducted water analyses on 59 wetlands located in and around Thunder Bay, in the spring of 2003 (Appendix 2). All sites were part of a pre-existing network being used for long-term amphibian studies. Sites sampled varied from temporary pools such as roadside ditches to semi-permanent farm and forest ponds. I collected two water samples from opposite ends of each wetland and transported sample bottles in a cooler to Lakehead University Environmental Laboratories (certified by Canadian Association for Environmental and Analytical Laboratories) for analysis. Chloride content was determined by ion chromatography (Dionex™ DX-120, AG14, AS14 4mm columns) (Table 1.4).

Acute Toxicity

To determine acute effects, I conducted a pilot study to determine an approximate LC50 range for wood frogs to NaCl. A dilution series, of NaCl and dechlorinated water, ranging from 0.00 mg/L to 13 000 mg/L was used. I then exposed tadpoles to NaCl in solution in a 96-h static test. Static non-aerated water was used because wood frogs typically develop in lentic (non-flowing) habitats. I made a dilution series ranging from 0.00 to 9750.00 mg/L. I used commercially available non-iodized coarse pickling salt (Sifto® Canada Inc. ≈99.9% NaCl). Food grade salt rather than

commercial coarse road salt the latter has lower purity and often contains other toxic contaminants (e.g. anti-caking agents and abrasives) (Environment Canada, 2001). As well pickling salt was selected over reagent grade salt because its high purity rivals that of the reagent grade, and the cost effectiveness of pickling salt made it more ideal.

I used 4 L glass jars for containers with each containing 2 L of solution and there were 4 replicates per treatment. I added ten pre-sorted tadpoles to each jar. I used the relatively low number in each jar to avoid density-dependent effects (Cooke, 1979; Hecnar, 1995). Pre-sorted tadpoles of approximately average length were used to avoid individuals that were either too small or large or those that appeared to have any physical abnormalities. Jars were randomly arranged in four rows on a table, with some rearrangement to avoid same treatment level neighbours. Tadpoles were fed so that starvation mortality would not confound mortality resulting from toxicity (Hecnar, 1995). I fed tadpoles boiled lettuce (4 g/jar), which was checked at 48 h and another 4 g was added if needed. Tadpoles were counted and dead individuals were removed every 24 h. I terminated the experiment at 96 h. Daily checks also involved qualitative observations for behavioural and physical abnormalities. Death was defined as no response to continued prodding with a glass rod. Dead tadpoles were removed and weighed (OHAUS^{TM} Model AR3130 scale, readability 0.001 g). At 96 h all surviving larvae from each replicate were removed, counted, excess water was drained, and then tadpoles were weighed. I used mean wet body weight of all tadpoles in a jar for analysis.

To determine if tadpole growth decreased with increasing salt concentration I used linear regression (body weight at 96 h vs. salt concentration). Median lethal

concentrations and their corresponding 95% confidence intervals were calculated for the trimmed Spearman-Karber LC50, using LC50 Calculation Software (Harrass, 1986).

Chronic toxicity

To determine chronic effects of low-level exposure of road salt to tadpoles I used a 4 x 4 replicated randomized design. I used 40 L aquaria, each containing 20 L of solution, and four tanks/treatment. The four treatment levels were 0.00, 0.39, 77.50, 1030.00 mg/L of NaCl mixed in dechlorinated water. To ensure that concentration levels were ecologically relevant, I based my treatment concentrations on my spring water chemistry analysis from my field study (Chapter 1). The low concentration (0.39 mg/L) corresponded with the lowest field concentration found in the region, while the high concentration (1030.00 mg/L) equalled the highest field concentration of chloride in the spring. The medium concentration (77.50 mg/L) was equal to the regional average.

Thirty tadpoles of approximately average length were added to each aquarium. Aquaria were randomly placed in four rows on tables, and some aquaria were rearranged to avoid same treatment level neighbours between adjacent rows. I initially fed tadpoles 5 g of boiled lettuce and then ad libitum for the remainder of the experiment. Aquaria were checked daily, when qualitative observations of behavioural and physical abnormalities were made, dead tadpoles. Water and food levels were also checked daily. I counted the number of surviving tadpoles in each aquarium at 10 d intervals and terminated the experiment at 90 d.

I cleaned tanks every 5 d. This involved siphoning off 15 L of solution, excess lettuce and waste products from each tank; then refilling with the same volume of solution for the given treatment and adding fresh food.

I calculated mean body weight and mean time to metamorphosis for each aquarium at the end of the experiment. To test for differences in survivorship among treatments, I used univariate repeated-measures analysis of variance (ANOVA), with number of tadpoles alive as the dependent variable; salt concentration as the grouping factor, and days (10 d intervals) was the repeated measure. I also used ANOVA to test for differences in time to metamorphosis, the number of metamorphosed frogs, and to explore if there were any differences in mean body weight among treatments. Student-Newman-Keuls and paired t-tests were used for post hoc analysis when ANOVA results were significant. All statistical analyses were conducted using SPSS version 12.0.

Experimental conditions

Laboratory temperature remained at 19 ± 0.3°C SE, and indoor lighting approximated the natural photoperiod. All water used was municipally treated and then dechlorinated on site (residual chlorine < 3.00 ppb, checked approximately weekly). My animal care protocol was approved at the university level (Lakehead University Animal Care Protocol 2003-03) following federal and provincial guidelines.

Results

Acute toxicity

Mortality among salt treatments ranged from 5 to 40 individuals, but no mortality occurred in any control jars (Table 2.1). Median lethal concentration (96-h LC50) was 2636.5 mg/L (95% C.L. 2532.82 mg/L, 2744.4 mg/L) by the trimmed Spearman-Karber method ($\alpha = 0.05$). Significant decreases in weight of tadpoles at 96 h as NaCl concentration increased were found using linear regression (Figure 2.1).

I observed physical and behavioural effects in all salt exposure levels, but they were more pronounced at higher concentrations. Feeding and swimming activity decreased as concentration increased. Tadpoles in exposure jars responded slowly to prodding, but control animals typically reacted to the first prod. I also found many individuals lying on their sides at the bottom of higher concentrations jars. Tadpoles appeared emaciated with increased salt concentration. Bent tails were the only physical abnormalities observed, and typically occurred only in higher treatment levels.

Chronic toxicity

Of 960 tadpoles used in the experiment, 239 metamorphosed and 128 remained as tadpoles at 90 d. Survivorship decreased significantly over time for all treatments (Figure 2.2). Initial mortality occurred from 2 to 40 d into the chronic study. Significant differences in survivorship were found (repeated measures ANOVA, $F_{1.55,\ 43.51} = 170.03$, $p < 0.001$), with post hoc tests indicating significant differences among all time intervals except between 30 and 40 d for all treatment levels. Survivorship between the high

Table 2.1. Number of surviving *Rana sylvatica* tadpoles 96 h acute tests.

Concentration (mg/L)	Exposed	No. of Surviving Individuals
0	40	40
3000	40	35
3750	40	29
4500	40	28
5250	40	16
6000	40	6
6750	40	0
7500	40	16
8250	40	22
9000	40	9
9750	40	0

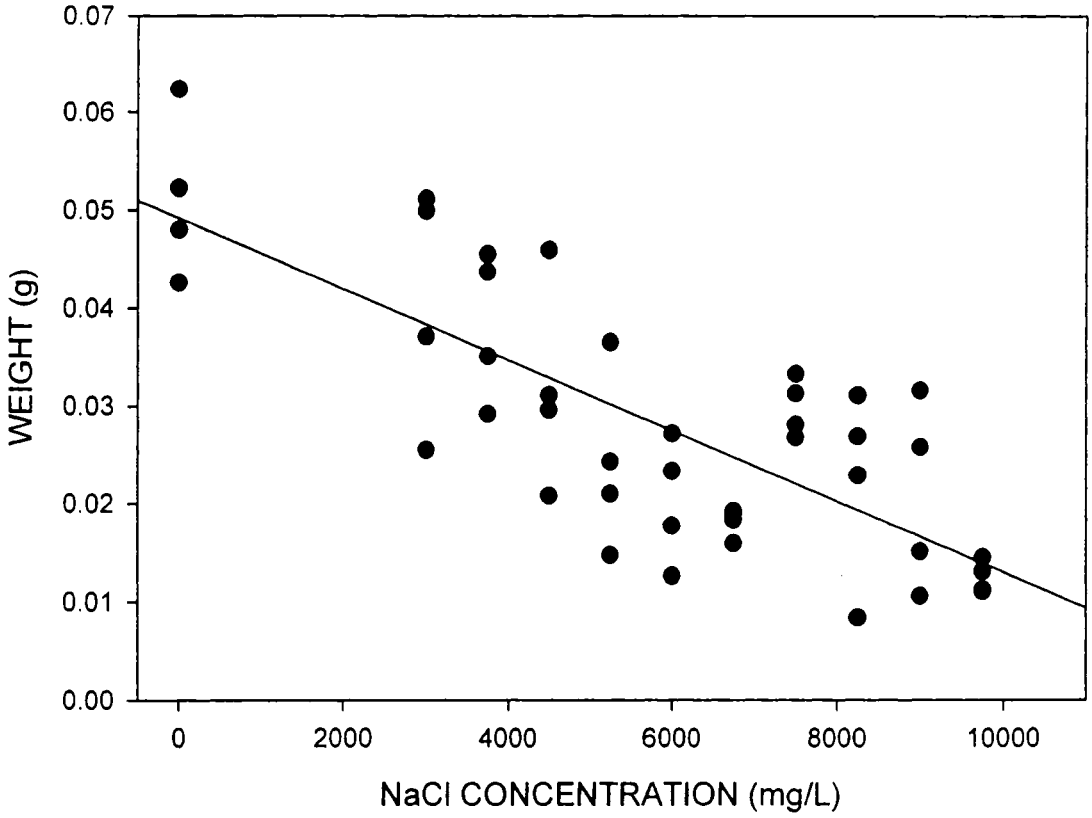

Figure 2.1. Mean body weight of *Rana sylvatica* tadpoles after 96 h exposure to road salt (NaCl), (y = -0.73x + 0.055, $F_{(1, 42)}$ = 49.09, p < 0.001).

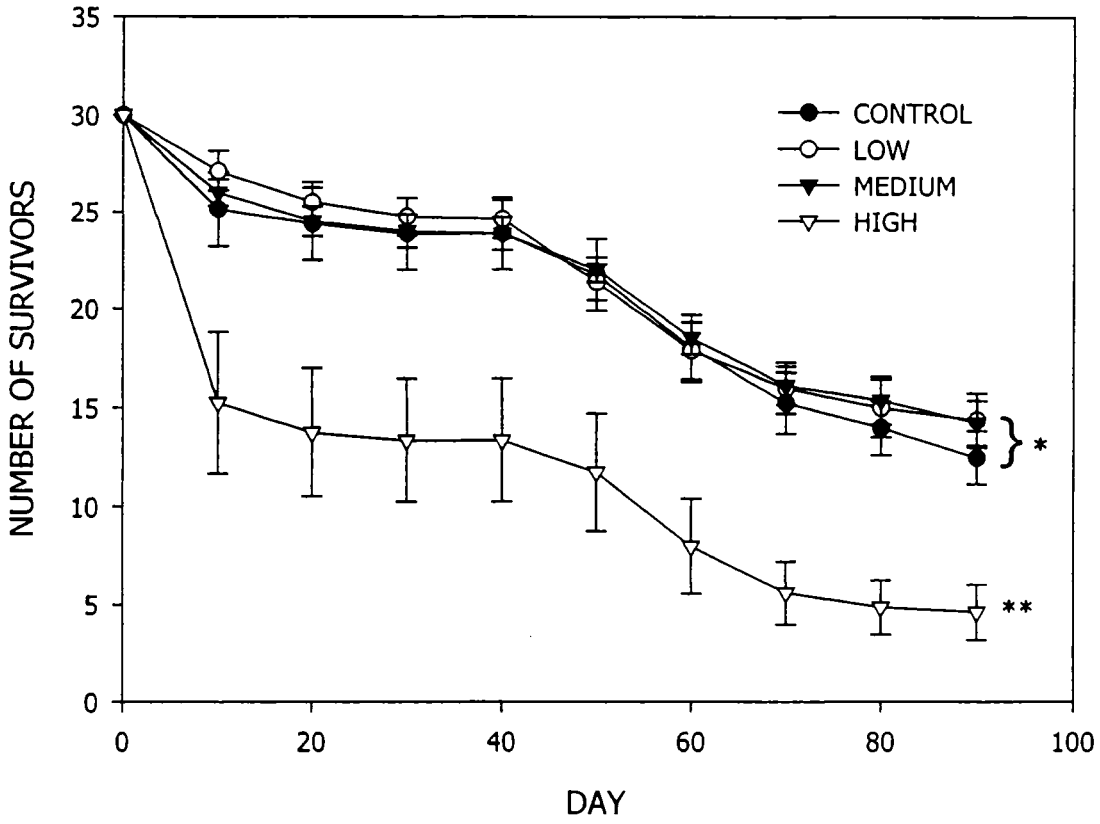

Figure 2.2. Mean survivorship of *Rana sylvatica* tadpoles exposed to road salts (NaCl) for 90 d chronic tests (Control = 0.00 mg/L, Low = 0.39 mg/L, Medium = 77.50 mg/L, and High = 1030/00 mg/L). The asterisks indicate significant differences.

concentration and the other three treatments were found to be significantly different (ANOVA, $F_{3, 28} = 10.25$, $p < 0.001$, Figure 2.2).

The mean time to metamorphosis was 77 d and it differed significantly among treatments (ANOVA, $F_{3, 27} = 3.65$, $p = 0.025$, Figure 2.3). The number of metamorphosing frogs also differed significantly (ANOVA, $F_{3, 28} = 4.56$, $p = 0.01$, Figure 2.3). The fewest individuals metamorphosed from the high treatment, relative to the control, low, or medium treatments as indicated by S-N-K post hoc tests. The weight of both newly metamorphosed frogs and surviving tadpoles did not differ significantly among treatments (ANOVA, $F_{3, 232} = 1.95$, $p = 0.122$, $F_{3, 124} = 1.06$, $p = 0.368$ respectively).

Qualitative observations indicated that exposed tadpoles developed behavioural and physical abnormalities similar to those in the acute experiment. Tadpoles in higher treatments developed bent tails within 5 d. Many exhibited delayed response to prodding as compared to control animals that responded immediately after the first prod. Individuals were also observed struggling and swimming in circles. Tadpoles in higher concentrations developed disintegrating tails.

Discussion

Road salt (NaCl) had a toxic effect on wood frog tadpoles in both acute and chronic tests at environmentally realistic concentrations. In general, I found that tadpoles had decreased survivorship, weight, and activity; they metamorphosed earlier and had increased developmental abnormalities as salt concentration increased. The ecological implications of decreased survivorship for population size are clear.

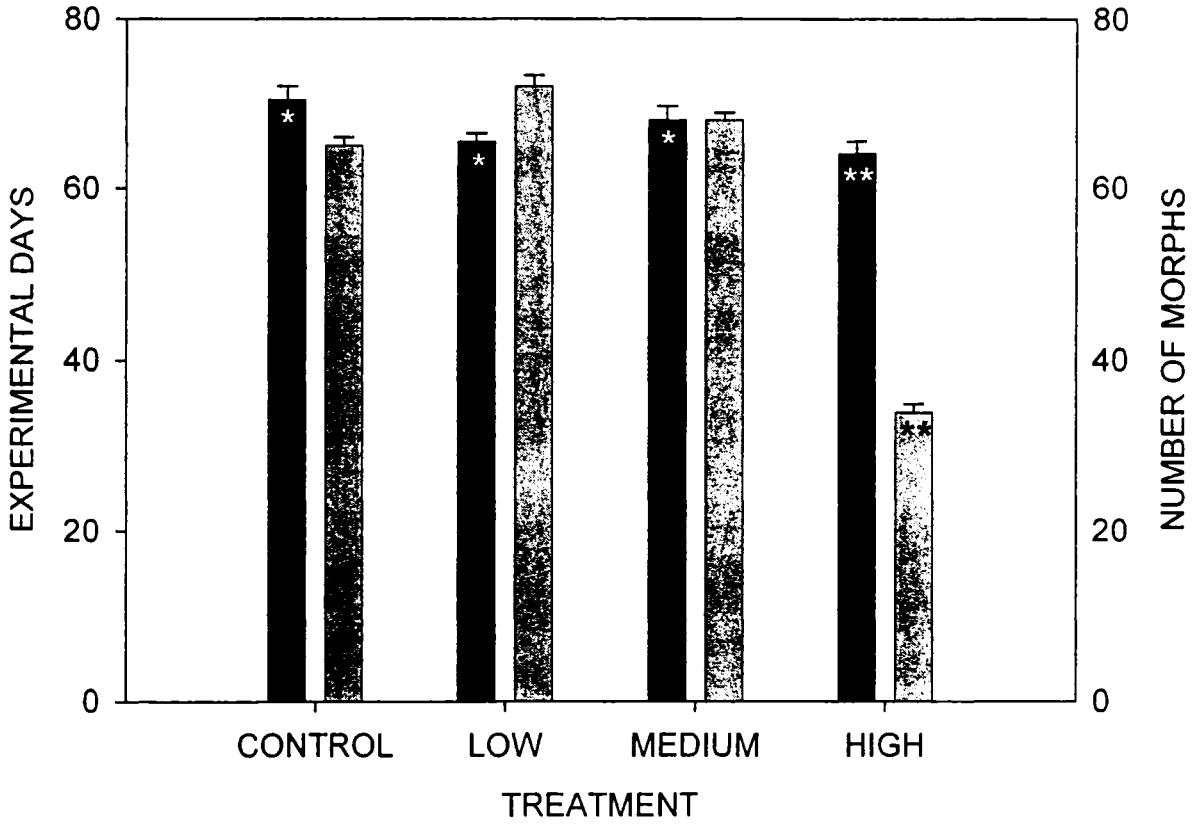

Figure 2.3. Mean time to metamorphosis for *R. sylvatica* (black bars) and number of metamorphosed frogs (grey bars) exposed to road salt (NaCl). Mean time for all metamorphosed frogs was 77 days. Asterisks indicate significant differences among groups. Error bars represent standard error.

However, it is also widely understood that factors affecting body size, time to metamorphosis, and activity in tadpoles can influence the outcome of competitive and predatory encounters and thus affect the structure of populations and communities (for review see Alford, 1999). Because amphibians occupy a pivotal position in food webs and constitute a major component of biomass in forest ecosystems (Burton and Likens, 1975; Stebbins and Cohen, 1995), these effects potentially have far ranging impacts on ecosystems.

Acute toxicity

My acute tests established a median lethal concentration for wood frog tadpoles of 2636.5 mg/L NaCl. This concentration range can be exceeded in the field especially close to roads (Environment Canada, 2001). Most mortality and abnormalities occurred quickly (< 24 h). NaCl-exposed tadpoles exhibited reduced activity and feeding, delayed responses to prodding with a glass rod, were emaciated and also developed bent and disintegrated tails. If similar effects are occurring in nature many ecological implications may exist. Tadpoles with reduced activity and delayed reactions may be more susceptible to predation (see Alford, 1999). With reduced feeding, tadpoles require more time to metamorphose, are less likely to attain sufficient resources to survive to metamorphosis, or metamorphose at a smaller size. Extended time to metamorphosis can reduce survival in wood frogs (Berven, 1990). Wood frog tadpoles experience nearly constant mortality in the field from predaceous aquatic beetles (Herreid and Kinney, 1966) and remain vulnerable until reaching metamorphosis (Formanowicz and Brodie, 1982). Vulnerability to predation also decreases as body

size increases in wood frog tadpoles (Brodie and Formanowicz, 1983). In general, tadpoles that metamorphose at a smaller size also have reduced success and fitness as adults (Wells, 1978; Smith, 1987; Wells and Bevier, 1997; Alford, 1999)

Several other studies have reported NaCl LC50s for amphibians. Padhye and Ghate (1992) found toxic effects between 1646.0 and 4206.0 mg/L for *Microhyla ornata*, an Asian anuran species typically found in lowlands to mountainous areas. Romspert (1976) found LC50 values between 8869.0 and 9926.0 mg/L for *Xenopus laevis*, the African clawed frog, a species known to be almost entirely aquatic. African clawed frogs inhabit a variety of wetlands including shallow ephemeral ponds that are subject to drying and increased concentration of salts, suggesting this species may be adapted to higher salt concentrations. In comparison, my LC50 values were relatively low and closer to those found by Padhye and Ghate (1992). This may suggest that "terrestrial" amphibians may be less tolerant of salts vs. highly aquatic amphibians.

Previous studies suggested that salts may affect amphibians physiologically in a variety of ways such as interfering with osmotic regulation (Romspert, 1976) and maintenance of urea concentration (Bentley and Schmidt-Nielsen, 1971), causing abnormal development (Ruibal, 1959; Padhye and Ghate, 1992), as well as having possible circulatory effects (Parsons et al., 1990; Romspert, 1976). If similar stresses as above were influencing wood frog larvae similarly, they may account for the slow responses and reduced activity levels in my experiment. My observations of reduced swimming activity differ from those found by Wainscott (1997). He reported increased swimming activity in salt-treated water by *R. catesbeiana* tadpoles and also observed that larvae did not actively avoid salty water in a choice experiment. Bullfrog tadpoles

however exhibit a higher tolerance for road salts (NaCl and $CaCl_2$) with 10 000 mg/L being lethal (Wainscott, 1997). His findings may be related to the large size of the larvae and the lengthy larval period of the highly aquatic species.

Several other possibilities may explain reduced feeding by *R. sylvatica*. Sodium and chloride ions are known to affect muscle activity (Hill and Wyse, 1988) and salt may have interfered with locomotion or sensory processes making it difficult for tadpoles to feed. Many tadpoles struggled while swimming as a result of bent tails, making it harder for those individuals to acquire food and orientate themselves while feeding. Other studies have suggested that contaminants can affect the digestive system of larval amphibians (e.g. nitrates, Hecnar, 1995). Lettuce may have also absorbed salt making it less palatable.

As previously mentioned, limited literature of the toxic effects of salts exists. Most focus on four-day toxicity tests for fish and aquatic invertebrates (see Evans and Frick, 2001). For invertebrates, LC50 values range from 3939.0 to 10 254.0 mg/L while values for fish range from 7341.0 to 21 571.0 mg/L. The relatively higher tolerance of fish to NaCl compared to terrestrial amphibians is concerning because fish are well-known predators of amphibians (Kats *et al.*, 1988; Hecnar and M'Closkey, 1997). Differential tolerance between predator and prey coupled with reduced tadpole size and activity, and slower response times to attacks, would exacerbate the impact of predation on tadpoles.

Chronic toxicity

Chronic exposure to road salts decreased wood frog survivorship during the experiment, decreased the number of frogs that metamorphosed, and decreased time to metamorphosis. The ecological implications of these results are the same as discussed for the acute experiment. I did not find differences in weight between surviving tadpoles and metamorphosed frogs. However, most surviving tadpoles were in stages of advanced development approaching metamorphosis (stages 36 to 43, Gosner, 1960). Similarities in behavioural and developmental abnormalities to those in the acute experiment suggest that general toxic effects exist.

Survivorship differed between the high treatment and the three remaining treatments. Survivorship in the control, low, and medium treatments was about 50%, which is similar to survivorship in the field (37%, Seigel, 1983). The significantly lower survivorship in the high treatment (≈17%) may be a result of salt interfering with physiological processes or food avoidance discussed above. A field study on the spotted salamander (*A. maculatum*) in New Hampshire indicated that there was reduced embryonic survivorship in roadside pools exposed to de-icing salts (Turtle, 2001). This also supports the idea that exposure to salts at critical times of development may have detrimental effects on populations.

Decreased time to metamorphosis in the high treatment level relative to the control may be attributed to a drying response. Tadpoles of some species may be able to sense increases in chemical concentration and respond by accelerating development (Alford, 1999). Hydroperiod (length of time a site contains standing water) also influences the number and size of metamorphosing frogs, the time of metamorphosis

and the effects of diseases (Semlitsch, 1987; Semlitsch et al., 1988; Pechmann et al., 1989; Kiesecker and Skelly, 2001). When salt concentrations are higher, tadpoles may associate this with a change in water level and develop faster. However, the trade-off with accelerated development yields smaller adults with reduced fitness and delayed maturity (Smith, 1987; Semlitsch et al., 1988).

My results indicated that fewer tadpoles metamorphosed at the highest salt concentration. Similar physiological and developmental effects, as noted for the acute study, might be producing these results. These results suggest that even relatively low level exposure to chloride salts (below LC50 values) over a prolonged period of time may have negative impacts on populations. It should also be noted that more tadpoles metamorphosed at low and medium concentration as compared to the control treatment (where individuals were "salt starved") indicating the importance of salt for physiological processes.

Body weight did not differ between metamorphosed frogs and surviving tadpoles. The lack of a difference may be confounded by density of survivors among treatments. Density-dependent effects on tadpole growth are well documented in tadpoles (Alford, 1999) including wood frogs (Wilbur and Collins, 1973; Wilbur, 1977; Berven, 1990). I can discount competition for food as the cause because I fed all tanks ad libitum. Density-dependent growth responses or 'crowding effects' independent of food are documented for wood frogs (Adolph, 1931; Lynn and Edelman, 1936; but see Berven and Chadra, 1988).

Other chemicals commonly found in road runoff (petroleum/oil residues and metals) have also been examined for their potential effects on amphibians and have

produced mixed results. Pyastolova and Danilova (1986) found that low concentrations of oil contamination had negative effects on development and survival of *R. arvalis*. In a study of mole salamanders (Genus *Ambystoma*), Lefcort et al. (1997) suggested that used motor oil may have negative effects at the community level and may be a factor leading to global amphibian declines.

The toxicological effects of metals and other contaminants on amphibians have been examined in some detail (see Sparling et al., 2000). The most common metals examined include As, Cd, Cr, Cu, Pb, Hg and Zn (Sparling et al., 2000), but other metals have also been studied. Most work tends to focus on median lethal concentrations and physiological effects, however recent studies have indicated that ecologically relevant non-lethal concentrations may still have adverse population level effects (Rowe et al., 1996; Rowe et al., 1998; Raimondo et al., 1998). These findings on non-lethal effects are similar to the work present here. It is also possible that synergistic effects, similar to those that exist with metals and other physical or chemical factors (Beattie and Tyler-Jones, 1992; Horne and Dunson, 1994; Horne and Dunson, 1995), are affecting amphibian populations exposed to a 'soup' of road runoff chemicals.

Conclusion

My study indicates that wood frog larvae experience stress, increased mortality, and altered development resulting from acute and chronic exposure to salts used on roads. To my knowledge no other studies have demonstrated chronic effects of road salts on amphibians. If my findings are indicative of a general detrimental effect of

environmentally realistic concentrations of road salts, then other species may be experiencing similar fates. The potential impact of road salts on amphibians may be underestimated by my study because northwestern Ontario has one of the lowest average recommended application rates for road salts in Canada (Environment Canada, 2001). Concentrations used in this study are often exceeded in more heavily populated areas of northern countries where salts are used during road maintenance. Globally, the application of salts during road maintenance may be devastating populations of amphibians. Surprisingly, road salts have rarely been considered as a factor in amphibian declines. Further investigations examining the ecological effects of road salts on other amphibian species and other aquatic taxa are urgently needed.

General Discussion

General water chemistry does not appear to be a major factor affecting amphibian distribution in the Boreal/Great Lakes-St. Lawrence forest regions of northwestern Ontario. Amphibian species richness was significantly associated with water chemistry, but I was unable distinguish among species or among occupied and unoccupied sites for individual species. In addition, classification attempts using water chemistry as a predictor in DFA was only moderately successful. My findings seem somewhat counterintuitive considering the dependence of amphibians on water, however my findings are consistent with other studies from regions within Ontario, within Canada and other countries, which suggest that water chemistry in conjunction with other factors may be important in structuring amphibian communities. Studies have suggested local habitat and/or regional landscape factors as being important in explaining patterns of amphibian communities. It is also possible that the vast number of wetlands available in the region allow recolonisation of less favourable sites. The significant community response (species richness) but lack of differences among species or between sites (presence/absence) may be interpreted as a common, albeit weak, collective response of amphibians to chemistry (i.e. good ponds vs. poor ponds).

The limited studies on the effects of road salts focus on acute effects only and are taxonomically biased towards fish and aquatic invertebrates. The lack of research raises many ecological concerns. In my experimental study road salts (NaCl) had an adverse effect on *R. sylvatica* tadpole growth and development at environmentally realistic concentrations. I found a decrease in larval survivorship, number of metamorphosing frogs, and development time, as well as physical and behavioural

abnormalities. The concentrations used in my study were based on spring water samples taken from my study network in northwestern Ontario. The large variation in road salt application volumes across Canada suggests that the regional concentrations in this study are much lower than those found closer to larger urban centres at northern latitudes. In fact, wetlands and watercourses have been documented as having concentrations four times greater than the highest treatment used in my experiment (Environment Canada, 2001). Considering the importance of amphibians as indicator and keystone species, and the observed negative chronic effects at concentrations lower than LC50 values, many more organisms might be suffering similar or worse fates, highlighting the urgent need further studies examining the effects of previously ignored chemicals that commonly enter the environment.

In conclusion, despite the limited use of general water chemistry in describing patterns of amphibian distribution it is important not to ignore pollutants. Some commonly used chemicals such as road salts do appear to be negatively affecting individuals and populations and may ultimately have consequences for communities and ecosystems. My work demonstrates the value of studies examining the influence of abiotic factors on amphibians and the importance of ecologically relevant toxicological studies. Further research is needed to determine all the factors affecting amphibian distribution in northwestern Ontario as well as the effects of road salts on the environment. Future analyses should combine important water chemistry variables from my study with other local and landscape variables known to influence amphibian distribution in northwestern Ontario. There is also an urgent need to examine the

sensitivity and ecological effects of road salts on other amphibian species that occur in northern regions.

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Appendix 1: Water chemistry for select sites showing "yearly" variation (sites were sampled on approximately 2-3 week intervals)

Table A1 Water chemistry for site 4T

Chemical	4T-Spring	4T-1	4T-2	4T-3	4T-Summer
pH	7.2	7.3	7.8	8.0	7.1
TDS	339	417	470	461	653
Total Alk	55.250	144.900	139.800	207.200	240.550
Conductivity	741.000	1014.500	1083.000	863.500	1428.000
DIC	5.475	47.860	31.235	54.515	54.090
DOC	19.185	21.671	21.642	27.672	20.889
Calcium	20.610	40.935	51.575	31.390	53.370
Chloride	228.010	275.930	34.895	159.765	262.355
Potassium	6.520	4.088	4.170	1.357	0.530
Magnesium	7.071	16.211	20.240	11.345	20.230
Sodium	113.005	139.570	132.560	128.916	200.290
Nitrate	0.008	0.008	0.008	0.008	0.008
Aluminum	0.069	0.046	0.064	1.274	0.053
Barium	0.032	0.045	0.060	0.041	0.059
Copper	0.001	0.001	0.003	0.011	0.004
Iron	1.824	4.699	12.050	3.358	3.269
Manganese	0.417	1.097	1.758	0.372	1.372
Nickel	0.001	0.001	0.001	0.005	0.003
Sulphur	3.296	0.830	0.562	2.029	0.767
Strontium	0.040	0.040	0.127	0.083	0.135
Titanium	0.002	0.002	0.002	0.002	0.002
Vanadium	0.031	0.068	0.101	0.033	0.103
Zinc	0.007	0.004	0.005	0.011	0.005
Sulphate	8.225	1.340	0.450	4.420	0.040
Ammonia	0.020	0.310	0.694	0.078	0.283
Total N	1.592	2.021	2.035	1.315	2.831
Total P	0.183	0.164	0.197	0.138	0.329
TSS	1.900	18.300	34.350	9.050	7.950

Table A2. Water chemistry for 18T (site dried by early July 2003)

Chemical	18T-Spring	18T-1	18T-2	18T-3	18T-Summer
pH	8.4	7.7	7.9	0.0	0.0
TDS	151	163	131 --	--	
Total Alk	64.900	76.600	43.150	0.000	0.000
Conductivity	253.000	247.500	198.000	0.000	0.000
DIC	11.420	16.765	8.640	0.000	0.000
DOC	25.035	25.386	35.873	0.000	0.000
Calcium	18.160	17.675	9.770	0.000	0.000
Chloride	31.120	27.245	35.305	0.000	0.000
Potassium	10.858	12.985	10.485	0.000	0.000
Magnesium	7.242	89.205	6.169	0.000	0.000
Sodium	12.870	14.530	14.490	0.000	0.000
Nitrate	0.008	0.008	0.008	0.000	0.000
Aluminum	0.107	0.061	0.127	0.000	0.000
Barium	0.034	0.039	0.033	0.000	0.000
Copper	0.004	0.004	0.004	0.000	0.000
Iron	0.826	1.593	4.379	0.000	0.000
Manganese	0.121	0.044	0.137	0.000	0.000
Nickel	0.003	0.003	0.004	0.000	0.000
Sulphur	2.833	1.683	1.351	0.000	0.000
Strontium	0.040	0.040	0.040	0.000	0.000
Titanium	0.002	0.002	0.006	0.000	0.000
Vanadium	0.034	0.040	0.027	0.000	0.000
Zinc	0.001	0.002	0.002	0.000	0.000
Sulphate	6.175	2.510	1.215	0.000	0.000
Ammonia	0.020	0.253	0.255	0.000	0.000
Total N	2.115	2.861	0.448	0.000	0.000
Total P	0.172	0.191	0.024	0.000	0.000
TSS	1.900	4.850	1.900	0.000	0.000

Table A3. Water chemistry for site 21T

Chemical	21T-Spring	21T-1	21T-2	21T-3	21T-Summer
pH	8.0	7.8	7.8	8.0	8.1
TDS	182	252	270	226	196
Total Alk	92.100	179.850	193.650	188.500	175.850
Conductivity	246.500	384.500	378.000	368.500	349.500
DIC	22.665	41.900	68.820	47.095	38.615
DOC	19.255	16.740	19.963	16.245	13.015
Calcium	24.805	43.925	45.135	41.680	39.415
Chloride	19.650	17.795	16.970	8.980	2.935
Potassium	6.755	5.544	4.533	1.656	1.139
Magnesium	11.112	20.180	19.055	18.120	18.040
Sodium	5.214	6.661	6.810	5.481	5.579
Nitrate	0.008	0.008	0.008	0.008	0.008
Aluminum	0.059	0.011	0.013	0.009	0.004
Barium	0.025	0.029	0.027	0.024	0.019
Copper	0.004	0.002	0.001	0.001	0.001
Iron	0.249	0.325	0.830	1.810	0.912
Manganese	0.017	0.051	0.121	0.137	0.054
Nickel	0.002	0.002	0.001	0.001	0.001
Sulphur	5.667	3.674	1.025	0.687	0.614
Strontium	0.040	0.072	0.068	0.065	0.061
Titanium	0.002	0.002	0.002	0.002	0.002
Vanadium	0.056	0.099	0.098	0.094	0.009
Zinc	0.001	0.001	0.001	0.001	0.001
Sulphate	15.640	9.970	2.720	1.075	0.865
Ammonia	0.020	0.096	0.217	0.072	0.150
Total N	1.006	0.980	1.509	0.918	1.206
Total P	0.012	0.065	0.136	0.055	0.056
TSS	1.900	2.850	7.000	5.100	1.900

Table A4. Water chemistry for site P16

Chemical	P16-Spring	P16-1	P16-2	P16-3	P16-Summer
pH	8.4	8.2	7.8	7.8	8.1
TDS	120	149	147	122	121
Total Alk	48.000	80.800	83.750	79.400	89.200
Conductivity	159.500	230.000	217.000	200.000	216.000
DIC	9.495	19.420	19.895	20.070	19.915
DOC	20.365	20.160	28.293	19.080	16.931
Calcium	13.865	19.875	20.910	17.515	20.790
Chloride	18.305	27.580	12.045	13.775	8.645
Potassium	11.614	15.555	12.400	8.089	9.082
Magnesium	5.565	7.740	7.848	7.105	7.629
Sodium	3.457	4.775	4.911	4.397	4.651
Nitrate	0.008	0.008	0.008	0.008	0.008
Aluminum	0.043	0.004	0.035	0.018	0.393
Barium	0.033	0.047	0.055	0.058	0.068
Copper	0.005	0.001	0.001	0.004	0.001
Iron	2.843	4.225	3.811	3.701	6.765
Manganese	0.093	0.296	0.064	0.205	0.368
Nickel	0.003	0.001	0.002	0.001	0.001
Sulphur	2.038	0.653	0.491	0.617	0.584
Strontium	0.040	0.040	0.040	0.040	0.040
Titanium	0.002	0.002	0.002	0.002	0.015
Vanadium	0.026	0.036	0.037	0.038	0.009
Zinc	0.003	0.001	0.002	0.002	0.003
Sulphate	4.520	0.790	0.525	0.375	0.040
Ammonia	0.020	0.020	0.433	0.284	0.960
Total N	1.254	1.141	0.480	1.509	2.469
Total P	0.082	0.149	0.045	0.105	0.163
TSS	1.900	3.650	7.300	5.800	9.000

Table A4. Water chemistry for site TB3

Chemical	TB3-Spring	TB3-1	TB3-2	TB3-3	TB3-Summer
pH	7.7	8.4	8.4	8.4	7.9
TDS	75	125	136	141	127
Total Alk	44.050	89.000	96.050	100.000	101.950
Conductivity	113.500	188.500	194.000	211.000	220.000
DIC	10.035	21.845	22.620	25.005	17.490
DOC	21.034	9.372	8.886	8.302	8.486
Calcium	14.000	25.050	28.310	27.825	28.060
Chloride	3.600	5.730	2.610	4.365	4.110
Potassium	1.955	1.143	0.911	0.697	1.069
Magnesium	3.810	6.549	7.458	7.517	8.301
Sodium	1.645	2.521	2.507	2.435	2.523
Nitrate	0.223	0.008	0.008	0.008	0.008
Aluminum	0.093	0.017	0.012	0.011	0.010
Barium	0.012	0.022	0.019	0.021	0.021
Copper	0.001	0.004	0.001	0.001	0.004
Iron	0.378	0.233	0.151	0.210	0.178
Manganese	0.036	0.067	0.043	0.036	0.028
Nickel	0.001	0.001	0.001	0.001	0.002
Sulphur	2.213	3.485	2.420	2.262	1.727
Strontium	0.040	0.040	0.040	0.040	0.040
Titanium	0.003	0.002	0.002	0.002	0.002
Vanadium	0.013	0.038	0.037	0.043	0.009
Zinc	0.002	0.001	0.001	0.001	0.001
Sulphate	5.545	9.430	7.260	5.280	4.560
Ammonia	0.157	0.020	0.088	0.068	0.051
Total N	0.519	0.092	1.261	0.469	0.537
Total P	0.004	0.035	0.127	0.033	0.037
TSS	1.900	1.900	1.900	1.900	1.900

Table A4. Water chemistry for site TB6

Chemical	TB6-Spring	TB6-1	TB6-2	TB6-3	TB6-Summer
pH	8.1	8.0	8.1	8.4	8.4
TDS	1037	742	693	637	550
Total Alk	121.500	136.100	128.250	111.400	88.500
Conductivity	1422.500	1106.500	1007.000	999.500	1058.000
DIC	28.310	26.940	25.060	28.810	14.635
DOC	6.544	5.353	4.665	5.130	6.267
Calcium	103.750	81.395	78.545	67.435	59.370
Chloride	371.550	306.480	28.785	254.900	260.035
Potassium	3.950	3.842	3.282	2.840	3.481
Magnesium	55.430	45.930	35.640	33.545	43.060
Sodium	80.495	62.260	55.830	52.148	57.820
Nitrate	2.128	0.017	0.034	0.013	0.485
Aluminum	0.009	0.005	0.012	0.007	0.006
Barium	0.233	0.211	0.173	0.145	0.122
Copper	0.001	0.001	0.001	0.003	0.001
Iron	0.191	0.905	0.804	0.369	0.349
Manganese	0.057	0.135	0.099	0.029	0.036
Nickel	0.001	0.002	0.001	0.001	0.003
Sulphur	7.544	5.613	2.819	4.718	2.997
Strontium	0.312	0.317	0.284	0.268	0.255
Titanium	0.002	0.002	0.002	0.002	0.002
Vanadium	0.251	0.221	0.203	0.194	0.009
Zinc	0.003	0.002	0.001	0.001	0.001
Sulphate	20.720	16.425	8.430	12.450	59.335
Ammonia	0.092	0.020	0.221	0.133	0.050
Total N	1.213	0.251	0.875	0.444	0.052
Total P	0.243	0.042	0.064	0.013	0.040
TSS	5.350	2.550	2.750	1.900	1.900

Appendix 2: Information regarding distribution of sites relative to spring chloride concentrations

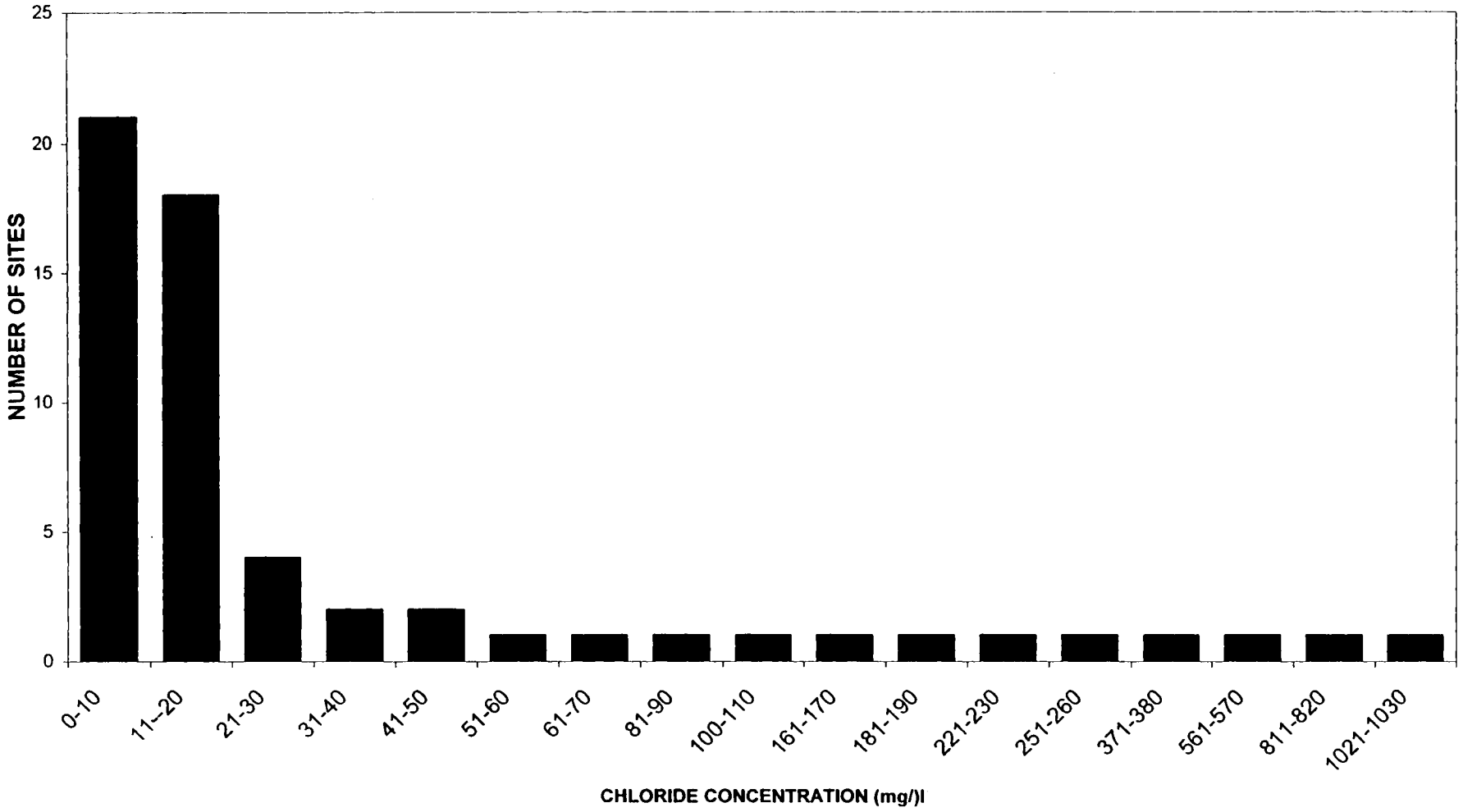

Figure A1. Distribution of sites in various chloride concentrations (Based on spring water chemistry analysis, 2003, Chapter 1)