Winter 2009

Spatial and temporal trends of dissolved nitrous oxide in the Lamprey River Watershed and controls on the end-products of denitrification

Emily DiFranco
University of New Hampshire, Durham

Recommended Citation

DiFranco, Emily, "Spatial and temporal trends of dissolved nitrous oxide in the Lamprey River Watershed and controls on the end-products of denitrification" (2009). *Master's Theses and Capstones*. 535.
https://scholars.unh.edu/thesis/535

SPATIAL AND TEMPORAL TRENDS OF DISSOLVED NITROUS OXIDE IN THE LAMPREY RIVER WATERSHED AND CONTROLS ON THE END-PRODUCTS OF DENITRIFICATION

BY

EMILY DIFRANCO

Baccalaureate Degree B.S. Geography, McGill University, 2001

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science
In
Natural Resources

December, 2009

This thesis has been examined and approved.

William H. McDowell

Thesis Director, Dr. William H. McDowell, Professor
Natural Resources and the Environment

Ruth K. Varner

Dr. Ruth K. Varner, Research Assistant Professor
Institute for the Study of Earth, Oceans and Space and
Earth Sciences

Gretchen M. Gettel

Dr. Gretchen M. Gettel, Postdoctoral Research/Teaching
Natural Resources and the Environment

6/21/09

Date

DEDICATION

This thesis and all the work that went into it is dedicated to my wonderful and loving husband, Brett Fletcher, and our growing family. I couldn't have done any of this without you!

ACKNOWLEDGMENTS

First, I would like to thank my committee members, Ruth Varner and Gretchen Gettel for their support, encouragement, and insights throughout this project. A special thanks goes to my advisor, Bill McDowell for providing a solid foundation of knowledge, reviewing early drafts of my thesis, and for finding the funding to support this project. For field and lab support, as well as always being around and willing to answer my endless questions, thanks to Jeff Merriam, Jody Potter, and Michelle Daley. For extensive background knowledge about L1, thank you to Danna Truslow.

I must also thank my husband, Brett Fletcher, for always offering to assist in field work (even at two in the morning!), and for his unwavering support in all aspects of my life. Lastly, a thanks to my parents, Steve and Debbie DiFranco, for always encouraging me to push myself academically.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

SPATIAL AND TEMPORAL TRENDS OF DISSOLVED NITROUS OXIDE IN THE LAMPREY RIVER WATERSHED AND CONTROLS ON THE END-PRODUCTS OF DENITRIFICATION

by

Emily DiFranco

University of New Hampshire, December, 2009

Thesis Advisor: William H. McDowell

An understanding of the controls on the production of the greenhouse gas N_2O is important to assess the atmospheric contribution of N_2O from freshwater streams. Concentration and percent saturation of dissolved N_2O were measured from March 2008 to February 2009 and were used as to measure the potential for streams in the Lamprey River Watershed to lose N_2O to the atmosphere. Almost all streams were oversaturated most of the year, and though field concentrations of dissolved N_2O did show trends with stream chemistry, it is possible that N_2O is not only being produced within the streams themselves, but is entering the streams via groundwater and surface runoff. A controlled laboratory experiment was also conducted to determine controls on N_2O and N_2O production from denitrification. These controls were much more obvious than in the field study, suggesting that laboratory experiments may misrepresent field conditions.

INTRODUCTION

Background

The Effects of Greenhouse Gases on Global Temperatures

Over the past century, global temperatures have changed in response to the growing presence of greenhouse gases in the atmosphere (IPCC, 2007; Crowley, 2000; Mitchell et al., 1995). The increasing concentration of various greenhouse gases in the atmosphere has resulted in the largest change in global temperatures over the past century and is the focus of current environmental policy (IPCC, 2007). Greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and chloroflourocarbons (CFCs) have the ability to effectively "trap" solar radiation in the atmosphere, which, over time, has been shown to increase temperatures globally (EPA, 2007; Crowley, 2000; Mann et al., 1998; Mitchell, 1995). A recent report by the Intergovernmental Panel on Climate Change (IPCC) noted a warming trend of approximately 1.0 to 1.7 degrees Fahrenheit from 1906-2005 that they attribute to a rise in atmospheric greenhouse gases (IPCC, 2007). Though many of these gases, such as CO_2 and N_2O, occur naturally in the atmosphere, direct and indirect anthropogenic production of these gases has increased dramatically over the past few decades (Crowley, 2000; Mann et al., 1998; Vitousek et al., 1997)

<u>Carbon Dioxide:</u> CO_2 is the most prevalent greenhouse gas in the atmosphere, arising naturally as part of the carbon cycle and from human sources such as the burning of fossil fuels (EPA, 2007; Vitousek, 1994). Over the past few decades, much attention has been aimed at reducing the anthropogenic input of CO_2 to the atmosphere. The

publication of the Keeling Curve in the 1980s clearly showed an increase in the atmospheric concentration of CO_2 since 1958 over Mauna Loa, Hawaii (Vitousek, 1994). This rising concentration of CO_2 has since been linked to increasing global temperatures through various climate models, and has become the focus of much scientific study (Bauer, 2007; IPCC, 2007; Crowley, 2000).

Nitrous Oxide: The concentration of N_2O in the atmosphere has also increased in the past century, and is currently increasing at a rate of 0.2 - 0.3% per year (Granier et al., 2006; McSwiney et al., 2001; Bouwman et al., 1995). Global concentrations of N_2O have increased from approximately 270 ppbv in 1750 to 311 ppbv in 2000 (IPCC, 2007; EPA, 2007; Garnier et al, 2005; Bange, 2000). N_2O is of growing concern to air quality as it is not only a greenhouse gas (with a warming potential of 310 times that of CO_2), but also contributes to the depletion of stratospheric ozone (Granier et al., 2006; Vitousek et al., 1997). Identifying the sources of N_2O has become the focus of much scientific study, though one potential source, freshwater streams, has received little attention (Ullah and Zinati, 2006; Groffman, 1998).

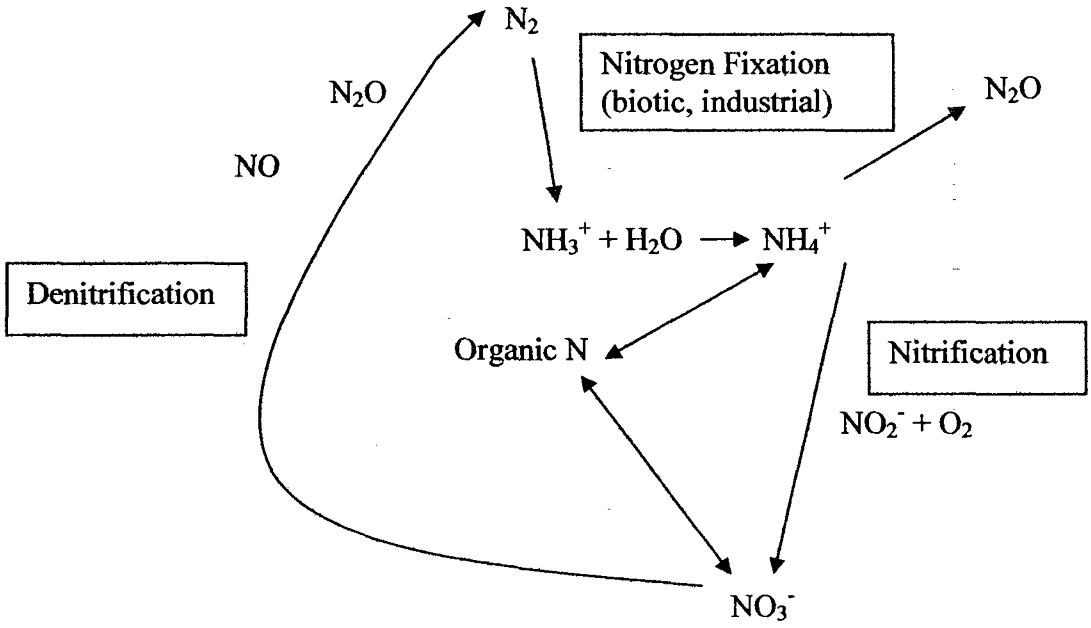
The Nitrogen Cycle

Nitrogen makes up approximately 78 percent of Earth's atmosphere and is an essential element for all life on Earth. It is part of the DNA, RNA, and enzymatic proteins that make up living cells. It is an essential nutrient in plant growth. Despite its necessity, most nitrogen is unavailable for use by plants and animals, as it exists as double-bonded atoms in the form of dintrogen gas (N_2) in the atmosphere (Galloway and Cowling, 2002).

For nitrogen to be changed into a reactive form that can be used by plants and animals, the strong bond between N_2 molecules must be broken (Figure 1-1). This occurs naturally by lightning and by the process of biologic nitrogen fixation, which is carried out by nitrogen-fixing bacteria. This type of bacteria may exist on the roots of some plants, such as leguminous crops, or as free-floating species, such as cyanobacteria (Vitousek et al., 1997). Nitrogen fixation produces ammonia (NH_3^+), a useable, reactive form of nitrogen. When combined with water, NH_3^+ reacts to form ammonium (NH_4^+). NH_4^+ can be taken up by plants or microbes, or, under oxic conditions, undergo the process of nitrification. During nitrification, ammonium (NH_4^+) is first oxidized to nitrite (NO_2^-) and then to (nitrate) NO_3^- by microbes. This process converts nitrogen into a form available for uptake by plants (Barnard et al., 2005, Hefting et al., 2003). Some N_2O may be produced and released to the atmosphere during the first process of nitrification (Figure 1-1). However, this contribution is generally less than 1% of the total oxidized NH_4^+ (Hefting et al., 2003).

Once nitrogen is in the form of NO_3^- it may be taken up by plants, or it may be returned to the atmosphere through a process called denitrification. During denitrification, NO_3^- is first reduced to NO_2^- and then to the gases nitric oxide (NO), N_2O, and N_2 through a series of reductions by microbial activity. Denitrification requires a source of labile carbon and anaerobic conditions (Figure 1-1) (Barnard et al., 2005). Although N_2 represents the completion of denitrification, in sub-optimal conditions such as low oxygen, increased NO_3^- availability, and low pH and temperature, the N_2O: N_2 produced during denitrification is often greater than in more idealized conditions (Barnard et al., 2005; Hefting et al., 2003).

Figure 1-1: Basic Nitrogen Cycle



Human Alteration of the Natural Nitrogen Cycle

Over the past century, humans have altered the natural nitrogen cycle by increasing the amount of available nitrogen for use in agriculture and by releasing various forms of nitrogen into the atmosphere as a by-product of fossil fuel combustion. In the early 1900s, a new way to produce large amounts of reactive nitrogen was invented through a process that is now known as the Haber-Bosch process. This process involves the large-scale synthesis of ammonia from nitrogen and hydrogen, and led to mass production of nitrogen fertilizers throughout the world. The ability of humans to "fix" nitrogen into available forms has led to dramatic changes in the global nitrogen budget. It is predicted that the contribution of anthropogenically-fixed nitrogen is approximately equal to the amount produced through natural nitrogen fixation by microorganisms (Galloway and Cowling, 2002; Vitousek et al., 1997).

Natural processes such as denitrification and plant uptake are able to remove or store excess nitrogen within a system. However, when nitrogen inputs exceed the amount of nitrogen that can be processed within a system, nitrogen saturation may occur. The excess nitrogen, most often in its most mobile form (NO_3^-), will then be exported from the system (Aber, 2004).

This input of excess reactive nitrogen has led to a new suite of environmental problems. Nitrate-enriched freshwater has increased as nitrogen fertilizers applied to agricultural fields "runoff" into nearby streams or leach into underlying groundwater. High levels of nitrate in freshwater systems can lead to human health problems such as methemoglobinemia, also known as blue baby syndrome, and environmental effects such as eutrophication in receiving coastal waters (Peters et al., 2005; Vitousek et al., 1997).

It has been shown that riparian buffer zones are effective in removing nitrate from groundwater and overland flow before it reaches rivers and streams (Groffman et al., 1998). These streams, however, still act as receiving waters for anthropogenic inputs of various forms of available nitrogen. The ability of a stream to remove this excess nitrogen through processes such as denitrification has been the focus of recent studies (Clough et al., 2006). However, the actual end product of this denitrification (N_2O or N_2) and the contribution of these streams to global N_2O production are not fully understood.

Project Goals, Objectives, and Hypotheses

The overall goal of this project is to understand spatial and temporal patterns in dissolved N_2O dynamics in the Lamprey River Watershed. Secondary goals include developing an understanding of controls on the end products of denitrification and relating these controls to field conditions. These goals were accomplished through the following objectives:

Objective 1: Quantify the concentration of dissolved N_2O in 16 streams throughout the Lamprey River Watershed and relate the concentration to in-stream characteristics.

Hypothesis 1: The concentration of dissolved N_2O will be highest in streams with the highest concentration of NO_3^- and the lowest pH as the ratio of N_2O: N_2 produced during denitrification has been shown to increase under these conditions.

Objective 2: Identify small-scale spatial patterns of the concentration of dissolved N_2O in one stream in the Lamprey River Watershed and relate them to in-stream characteristics.

Hypothesis 2: The concentration of dissolved N_2O will vary spatially throughout the 1-kilometer reach due to variations in the concentration of NO_3^-.

Objective 3: Identify diel variation in the concentration of dissolved N_2O in one stream in the Lamprey River Watershed and relate this to in-stream characteristics.

Hypothesis 3: The concentration of dissolved N_2O will vary throughout a 24-hour period.

Objective 4: Understand controls on N_2O production in a controlled laboratory experiment using streambed sediment from one stream in the Lamprey River Watershed.

Hypothesis 4: Streambed sediment amended with high concentrations of NO_3^- and exposed to low pH conditions will have a higher N_2O: N_2 than other sediments as soils exposed to these conditions have been shown to have a higher N_2O: N_2 than other soils.

CHAPTER 1

SPATIAL AND TEMPORAL TRENDS IN DISSOLVED N_2O IN THE LAMPREY RIVER WATERSHED

ABSTRACT

Increasing concentrations of available nitrogen in freshwater streams have the potential to increase the contribution of nitrous oxide (N_2O) from streams to the atmospheric pool. The concentration and percent saturation of dissolved N_2O were measured from March 2008 to February 2009 and were used to determine the potential for freshwater streams in the Lamprey River Watershed to lose N_2O to the atmosphere. Concentrations of dissolved N_2O ranged from 0.19 -2.71 μg N_2O-N/L and percent saturation ranged from 45-705%. Overall, dissolved N_2O was highest in April 2008 with snowmelt, and lowest in July 2008. Almost all streams were oversaturated through most of the year, indicating that streams in the Lamprey River Watershed are net sources of N_2O to the atmosphere. Although concentrations of dissolved N_2O did vary with stream chemistry and land use, these relationships were inconsistent throughout the year. Both in-stream and watershed production of N_2O are possible significant sources of the N_2O found in the stream water of the Lamprey River Watershed.

INTRODUCTION

Background

Over the last century, anthropogenic activities such as fossil fuel combustion and fertilizer application have increased the amount of available nitrogen that reaches freshwater bodies (DiFranco, Introduction). Although riparian buffer zones are effective in removing excess nitrate from groundwater and overland flow before it reaches rivers and streams,, freshwater streams still act as receiving waters for anthropogenic inputs of various forms of nitrogen, including nitrate and N_2O from the entire watershed. As such, these streams have the potential to have high concentrations of dissolved N_2O through both in-stream production and watershed inputs (Clough et al., 2006; Hefting et al., 2003; Groffman et al., 1998). Few studies have evaluated the potential flux of N_2O from rivers and streams, particularly small streams, to the atmosphere (but see Beaulieu et al., In Prep; Clough et al., 2006; Granier, 2006; Laursen and Seitzinger, 2004; Cole and Caraco, 2001; McMahon and Dennehy, 1998).

Dissolved N_2O may enter rivers directly through runoff or groundwater inputs, or it may be produced in the system by denitrification and nitrification (McMahon and Dennehy, 1998). Denitrification generally occurs in conditions of low oxygen or at the interface between the anaerobic streambed and the river, while nitrification may occur within the water column (Clough et al., 2006). Seitzinger and Kroeze (1998) estimate that the contribution of N_2O from rivers may be as much as 1.8 Tg N y^{-1} globally. Models such as these have been shown to overestimate the contribution of N_2O to the atmosphere from rivers and streams. For instance, Cole and Caraco (2001) measured emissions of N_2O from the Hudson River in New York over a two-year cycle. Although they found their study reach of the Hudson River to be a net source of N_2O to the atmosphere, the amount measured was lower than modeled values (Cole and Caraco, 2001). A study by Clough et al. (2006) also showed that N_2O emissions from a spring-fed river in New Zealand were a net source to the atmosphere, though the actual amount produced was less than that predicted using models developed by the IPCC.

Overall, the amount of dissolved N_2O and the flux of N_2O from the few rivers studied thus far have been highly variable, ranging between 0.2 and 8.0 μmol N m^{-2} h^{-1} (Cole and Caraco, 2001), although there is a consistent trend that rivers are a net source of gaseous N_2O to the atmosphere. The predominant source of N_2O in these rivers is also unknown. A study by McMahon and Dennehy (1998) on N_2O in the South Platte River in Colorado showed that emission rates of N_2O increased with percent N_2O saturation, total inorganic nitrogen, and stream temperature. In other studies, N_2O flux was related to the concentration of NO_3^- within the water column (Cole and Caraco, 2001). However, Beaulieu et al. (In Prep) found that N_2O emission rates were not related to stream NO_3^- concentrations when these concentrations were below 95 μg L^{-1}.

Denitrification, and thus the amount of N_2O produced in a stream, may also be limited by carbon availability, as denitrifying bacteria require a carbon source for denitrification (Hefting et al., 2003; Groffman et al., 1998). Some studies have shown that higher levels of DOC yield higher rates of denitrification (Groffman et al., 1998), though other studies have found the effect of DOC to be negligible (Garcia-Ruiz et al., 1998a).

Soil pH has also been shown to affect the relative amounts of N_2O and N_2 produced during denitrification (Firestone et al., 1980). Microbial activity (and thus denitrification) is inhibited in very acidic soils ($pH < 5.0$) and is only carried out by microbes that have been able to adapt to harsh conditions (Parkin et al., 1985). Despite this potential for microbial inhibition, N_2O has been shown to be the favored end-product of denitrification in soils with a pH below 7.0, while basic to neutral soils (pH 7.0-8.0) favor the production of N_2 (Yamulki et al., 1997).

Project Goals, Objectives, and Hypotheses

The overall goal of this project is to understand spatial and temporal patterns in dissolved N_2O dynamics in the Lamprey River Watershed. A secondary goal is to use these trends to develop an understanding of the relationships between N_2O concentration and a suite of in-stream characteristics as well as to determine the presence of internal production and external sources of N_2O. These goals were accomplished through the following objectives.

Objective 1: Quantify the concentration of dissolved N_2O in 16 streams throughout the Lamprey River watershed and relate the concentration to in-stream characteristics.

Hypothesis 1: The concentration of dissolved N_2O will be highest in streams with the highest concentration of NO_3^- and the lowest pH as the ratio of N_2O: N_2 produced during denitrification has been shown to increase under these conditions.

Objective 2: Identify small-scale spatial patterns of the concentration of dissolved N_2O in one stream in the Lamprey River watershed and relate to in-stream characteristics.

Hypothesis 2: The concentration of dissolved N_2O will vary spatially throughout the 1-kilometer reach due to variations in the concentration of NO_3^-.

Objective 3: Identify diel variation in the concentration of dissolved N_2O in one stream in the Lamprey River watershed and relate to in-stream characteristics.

Hypothesis 3: The concentration of dissolved N_2O will vary throughout a 24-hour period.

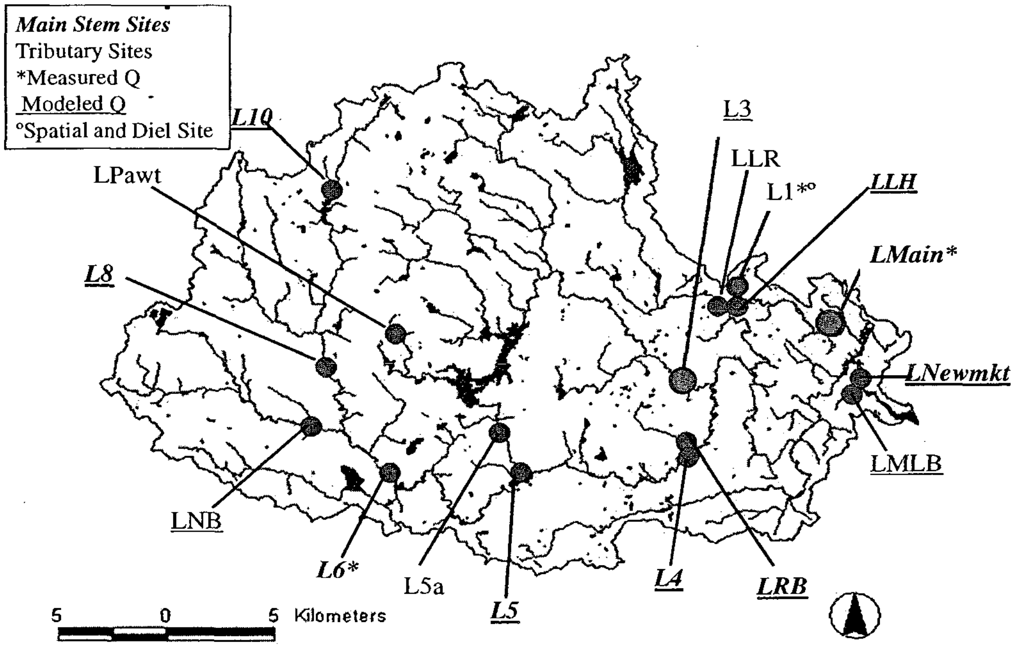
MATERIALS AND METHODS

Study Sites

Site Description

Sites were chosen in the Lamprey River Watershed that included a range of land use characteristics, stream chemistry, and field parameters (discharge, DO, EC, and pH) (Figure 1-2). The Lamprey River Watershed covers approximately 479 km^2 in southeastern New Hampshire, and includes the towns of Durham, Candia, Raymond, Fremont, Brentwood, Epping, Exeter, Newfields, Newmarket, Lee, Nottingham, Barrington, Northwood, and Deerfield (43.998° to 43.223° Latitude and -71.905° to -71.364° Longitude). Although much of the area is still rural with small farms, it is becoming increasingly suburbanized. The watershed has a total population density of 53 people km^{-2}. The relative location of each monthly sampling site within the Lamprey River Watershed is shown in Figure 1-2, and land use characteristics for the watershed and the 16 sub-basins used in this study are listed in Table 1-1.

Figure 1-2: Map of the Lamprey River Watershed with the location of the 16 sampling locations (LRHO, 2009)

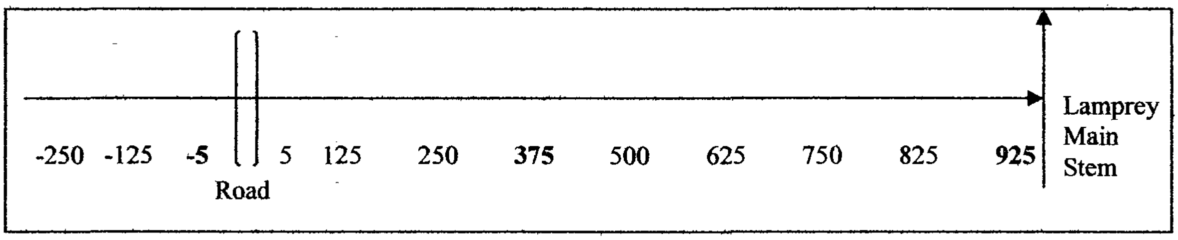


Wednesday Hill Brook (L1): To study spatial differences of N_2O concentration in one stream, twelve sites along L1, a tributary to the main stem of the Lamprey River, were identified. Sites were located approximately 125 meters apart and measured relative to an existing culvert (approximately 1 km from the mouth). Sites -5, 375, and 925 were selected for diel sampling due to accessibility (Figure 1-3).

Table 1-1: Watershed characteristics for Lamprey River Watershed and 16 sub-basins (Main stem sites*) (as classified by NH GRANIT, the statewide GIS warehouse)

Site	Watershed Area (km^2)	Agriculture (%)	Forested (%)	Urban (%)	Wetlands (%)	Population Density ($people/km^2$)
L10*	14.7	1.2	87.2	0.4	6.7	19.9
L8*	80.5	3.0	78.0	2.6	6.8	30.6
L6*	144.7	2.3	75.6	3.0	7.5	40.7
L5*	197.6	2.1	70.5	4.6	8.6	60.7
L4*	251.4	2.5	69.3	4.6	8.8	63.7
L-LH*	468.6	2.9	69.6	4.0	10.3	52.9
L-Main*	478.8	3.0	69.5	4.1	10.2	53.4
LNwmkt*	548.9	3.3	68.1	4.4	11.0	59.1
L-LR	51.8	0.8	73.2	3.7	11.6	44.5
L-1	1.3	11.3	54.0	15.6	5.7	158.4
L-MLB	1.4	2.7	37.2	30.6	1.6	853.7
L-NB	41.2	1.6	75.6	3.3	7.0	42.2
L-5a	25.9	1.0	74.4	1.4	7.9	29.9
L-RB	2.9	6.7	61.8	6.3	11.7	103.3
L-3	128.7	2.9	73.2	2.6	11.0	31.0
L-Pawt	2.6	0.0	81.4	0.0	14.2	4.3

Figure 1-3: Relative location of 12 spatial sampling points along L1 (meters from road). Diel sampling locations in bold.



Field Procedures

Water and Gas Sample Collection

Water samples were collected monthly from the 16 sites in the Lamprey River Watershed from March 11, 2008 through February 2009 (with the exception of January 2009), every three weeks at 12 sites in L1 from February 14, 2008 through August 21, 2008, and twice (at four-hour intervals for 24 hours) at three sites in L1 in March and

August 2008. Field parameters (dissolved oxygen concentration, pH, water temperature, and specific conductivity) were recorded at each site using a YSI meter. Water samples for analysis of NO_3^-, NH_4^+, Cl^-, TDN, and DOC (measured as non-purgeable organic carbon) were collected using a syringe and passed through a pre-combusted glass fiber filter (Whatman GF/F) into a 60-mL bottle. The syringe, filter holder, and bottle were acid-washed in the lab and rinsed with stream water three times in the field. These samples were transported in a cooler and frozen in the lab prior to analysis.

Water samples for N_2O were collected in triplicate using a 60-mL syringe with three-way stopcock. Syringes were submerged in the stream to prevent atmospheric contamination, flushed once with stream water, filled to 40-mL, and closed under water. These samples were unfiltered and taken to minimize the introduction of any bubbles.

Discharge Measurements

Discharge was continuously measured at LMain along the main stem of the Lamprey River by a USGS gage throughout the entire study period (USGS station number 01073500). From this information, as well as local precipitation (NCDC, 2009), discharge at the nearby Oyster River (USGS station number 0107300), and actual measured discharge from many of the study tributaries, a regression model was created using SPSS 17.0 to predict discharge for each study date at L1 ($R^2 = 0.897$, $p < 0.001$), L3 ($R^2 = 0.969$, $p < 0.001$), L6 ($R^2 = 0.967$, $p < 0.001$), LRB ($R^2 = 0.896$, $p < 0.001$), LNB ($R^2 = 0.838$, $p < 0.001$). For the main stem sites L10 and L8, discharge was estimated using the area-weighted discharge from discharge modeled at L6. For the main stem sites below L6, (L5, L4, LLH, and LNewmkt), discharge was calculated using the area-weighted discharge from L-Main. Discharge at LMLB was estimated using area-weighted discharge at L1, as there were not enough measured discharge values to create a model. From the few data points available, area-weighted discharge was found to be similar to actual measured discharge for this site. Discharge could not be estimated for L5a, LLR, or LPawt as models were not statistically significant.

Lab Procedures

Water and Gas Sample Processing and Analysis

All water samples were analyzed in the Ecosystems Analysis Lab at the University of New Hampshire under the direction of Lab Manager, Jeff Merriam. Samples were analyzed for NH_4^+ using a WestCo Scientific SmartChem 200 discrete automated colorimetric analyzer (detection limit 5 µg N/L). Samples were analyzed for Cl^- (detection limit 0.1 mg Cl/L) and NO_3^- (detection limit ~3 µg N/L) through ion chromatography using an Anions/Cations Dionex ICS-1000 with AS40 Autosampler. Samples were analyzed for DOC (detection limit 0.1 mg C/L) and TDN (detection limit 0.07 mg N/L) using a Shimadzu TOCV with TNM-1 Nitrogen Detector.

N_2O processing and analysis

Samples collected for N_2O analysis were brought back to the lab and placed in a water bath. Under water, each sample was injected with 20-mL of high purity helium directly into the syringe. The equilibration temperature of the water bath was recorded. Syringes were agitated for five minutes to equilibrate the headspace and to allow for total extraction of gases from the water sample. The 20-mL headspace was then injected into over-pressurized, evacuated 20-mL glass vials sealed with butyl septa stoppers. Silicon grease was applied to the stoppers after injection to prevent leakage or contamination from atmospheric gases. Vials were stored at room temperature and were analyzed between 6 hours and 48 hours of collection.

Analysis of N_2O was performed on a Shimadzu gas chromatograph (GC) equipped with an electron capture detector in the Complex Systems Research Center at the University of New Hampshire under the direction of Dr. Ruth K. Varner. The carrier gas was ultra-high purity CH_4-Argon. The column and injector/detector temperatures were 75 °C and 330 °C, respectively. Three standards ranging from 0.1 N_2O ppmv to 10 N_2O ppmv were run in triplicate at the beginning of each analysis to allow a standard calibration curve to be developed.

Calculations

The area under each curve determined from the GC provided N_2O in units of parts per million by volume (ppmv) at equilibrium for each sample. To calculate the concentration of N_2O in the field from these areas, the following set of equations were used (from Flint, 2006 following Mulholland et al., 2004 and LINX II calculations):

Equation 1: $\mathbf{Bsc} = 2.7182818 \wedge \{-165.8806 + [222.8743 * (100/\mathbf{T})] + [92.0792 * \ln(\mathbf{T}/100)] + [-1.48425 * (\mathbf{T}/100)\wedge 2]\} * 0.0821 * 293.15$

Equation 2: $\mathbf{C_1 = AREA}/(0.0821 * 273.15)$

Equation 3: $\mathbf{C_2 = AREA * BP * Bsc_L} * \mathbf{(Bsc_F/Bsc_L)} * 1/(0.0821 * 293.15)$

Equation 4: $\mathbf{G = (C_1 * V_1) + (C_2 * V_2)}$

Equation 5: $\mathbf{C^* = G/V_2}$

Bsc is the Bunsen solubility coefficient for headspace equilibrium in a container (L/L-atm). As samples were not equilibrated in the field, it was necessary to calculate a Bsc for laboratory conditions (Bsc_L) and for field conditions (Bsc_F). For Bsc_L, T is the temperature of the water bath during equilibration (°K), and for Bsc_F, T is the stream temperature (°K). C_1 and C_2 are the N_2O headspace mixing ratio and liquid concentration, respectively (µmol/L). AREA is the area under the curve calculated for each sample (ppmv) determined from the area under the curve calculated by the GC software. To convert the AREA from ppmv to µmol/L, the term "1/(0.0821*293.15)" was used, to account for room temperature conditions. BP is the barometric pressure (atm) in the field at the time of sample collection. V_1 and V_2 are the volumes of the headspace and the liquid in the collection syringe during equilibration (L). G is the total dissolved gas in the water sample (µmol). C* is the concentration of dissolved N_2O in each water sample (µmol/L). C* was converted to µg/L by multiplying by 28 g N/mol N_2O.

To calculate percent saturation, the following formula was used:

$$\textbf{Percent Saturation } (\%) = (C^*/ C^*_{sat})*100$$

where C^*_{sat} was calculated as described above to represent the concentration of dissolved N_2O at atmospheric conditions (with AREA equal to 0.311 ppmv) (EPA, 2007).

Statistical Analysis

To estimate discharge, a forward multiple regression model was created using measured discharge at LMain (USGS station number 01073500), local precipitation (NCDC, 2009), discharge at the nearby Oyster River (USGS station number 01073000), and actual measured discharge from many of the study tributaries.

Three replicates were taken at each sample location on each sample date. From the average of these replicates, median N_2O for each site throughout the entire study period was then determined as a measure of central tendency as mean values were not normally distributed.

Significant relationships between the overall median concentration of dissolved N_2O and the overall median concentrations of NO_3^-, NH_4^+, TDN, DOC, and Cl^- as well as the SC and pH of the stream water were determined using linear regressions. Linear regressions were then run to determine significant relationships between the concentration of all dissolved N_2O samples collected and the concentration of all NO_3^-, NH_4^+, TDN, DOC, and Cl^- as well as the SC and pH of the stream water. Each variable was analyzed for normality, and those that displayed skewness and kurtosis were log transformed ($\log_{10}$) before entering into the model.

Significant differences between the 12 study sites along L1 for each study date were determined using a one-way ANOVA with post-hoc Tukey. Significant relationships between the overall median concentration of dissolved N_2O and the overall median concentrations of NO_3^-, NH_4^+, TDN, and DOC as well as the SC and pH of the stream water were determined using linear regressions. Regressions were then run to determine significant relationships using all dissolved N_2O and stream chemistry data. Each variable was analyzed for normality, and those that displayed skewness and kurtosis were log transformed ($\log_{10}$) before entering into the model.

Significant differences between the sample times for a specific site for the diel sampling on L1 for each study date were determined using a one-way ANOVA with post-hoc Tukey. SPSS 17.0 was used for all statistical analyses.

RESULTS

Lamprey River Watershed Survey

In general, the magnitude of dissolved N_2O was similar in all streams for a specific study date (Figures 1-5 and 1-6). With the exception of four streams (L6, L3, LMain, and LNewmkt), all of the streams followed a similar temporal pattern throughout the year, with highest concentrations of dissolved N_2O occurring in April 2008 (1.99 – 2.46 μg N_2O-N/L) and lowest concentrations occurring in July 2008 (0.19-0.56 μg N_2O-N/L) (Figure 1-4, Tables 1-2 and 1-3). Over the study period, dissolved N_2O was highest in L6 (2.71 μg N_2O-N/L) and lowest in LRB (0.19 μg N_2O-N/L) both on July 10, 2008 (Figures 1-7 and 1-8). Concentrations at L1, and LMLB were consistently highest throughout the study period (Figure 1-5), while LMain and LNewmkt were often the lowest (Figure 1-6). Average concentrations and standard deviations for dissolved N_2O for each stream on each sample date can be found in Appendix A.

Figure 1-4: Concentration of average dissolved N_2O (μg N_2O-N/L) for all streams over the study period (March 2008 – February 2009)

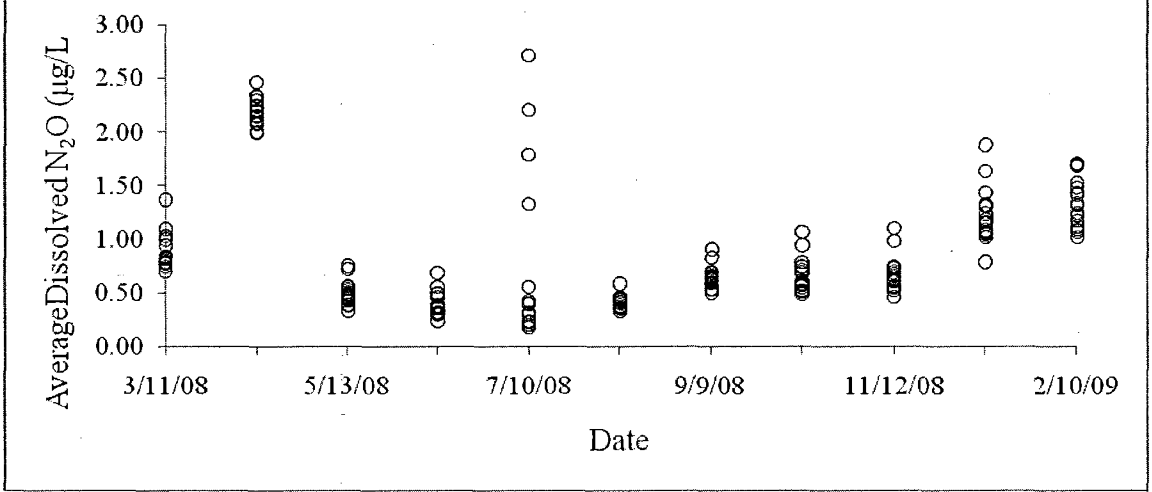


Figure 1-5: Average concentration of dissolved N_2O (µg N_2O -N/L) and standard deviation of the three replicate samples per site per sample date for all sites along the main stem of the Lamprey River for all study dates

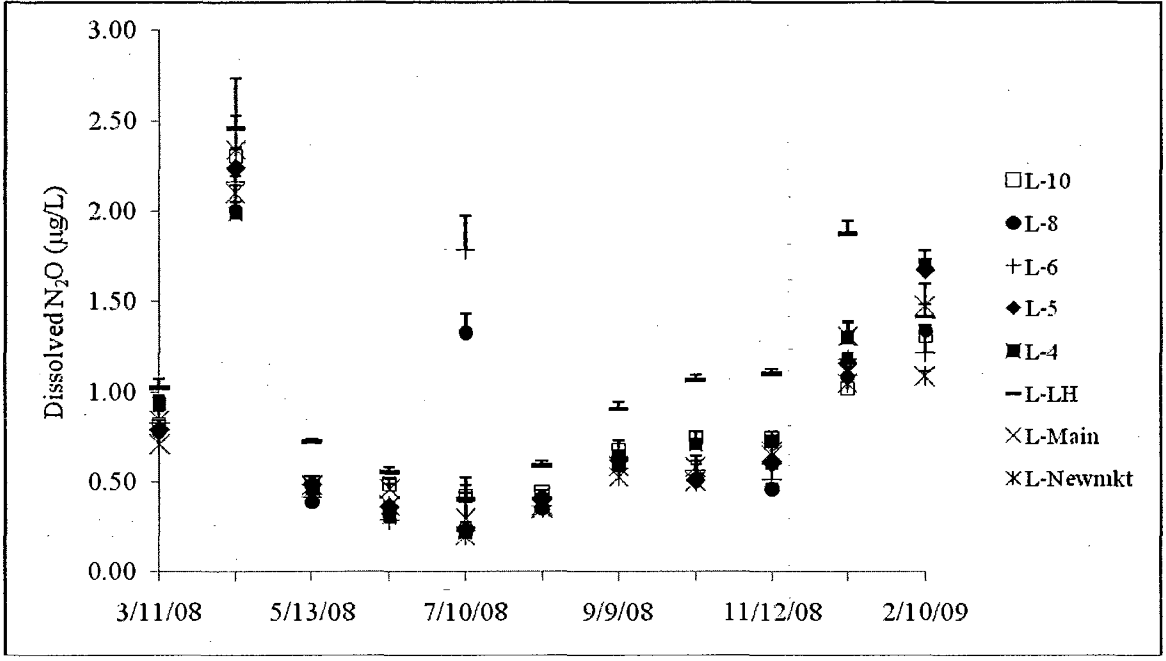


Figure 1-6: Average concentration of dissolved N_2O (µg N_2O -N/L) with standard deviation of the three replicate samples per site per sample date for all sampled tributaries of the main stem of the Lamprey River for all study dates

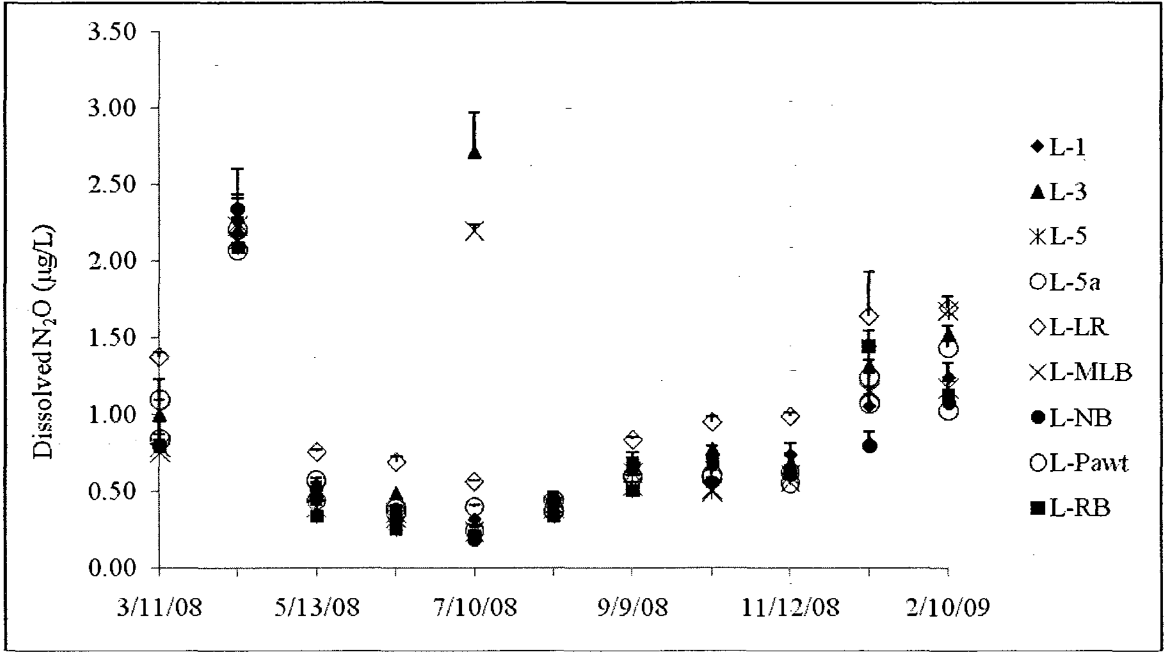


Table 1-2: Maximum and minimum average concentrations of dissolved N_2O (μg N_2O-N/L) for all study dates

Date	**Maximum Concentration of Dissolved N_2O (μg N_2O-N/L)**	**Minimum Concentration of Dissolved N_2O (μg N_2O-N/L)**
3/11/08	1.37 ± 0.19 (L1)	0.71 ± 0.13 (L5a)
4/8/08	2.46 ± 0.30 (LMLB)	1.99 ± 0.03 (L5)
5/13/08	0.75 ± 0.03 (L1)	0.34 ± 0.01 (LPawt)
6/10/08	0.69 ± 0.03 (L1)	0.25 ± 0.03 (LPawt)
7/10/08	2.71 ± 0.19 (L6)	0.19 ± 0.06 (LRB)
8/12/08	0.59 ± 0.11 (LMLB)	0.34 ± 0.03 (LPawt)
9/9/08	0.91 ± 0.03 (LMLB)	0.50 ± 0.04 (LPawt)
10/14/08	1.07 ± 0.03 (LMLB)	0.49 ± 0.02 (L3)
11/12/08	1.10 ± 0.10 (LMLB)	0.46 ± 0.04(LNewt)
12/16/08	1.88 ± 0.12 (LMLB)	0.80 ± 0.11 (LRB)
2/10/08	1.70 ± 0.04 (L5)	1.03 ± 0.01 (LNB)

Table 1-3: Range of average concentrations and standard deviations of dissolved N_2O (μg N_2O-N/L) by stream (italics indicates main stem sites)

Stream	**Range of concentrations of dissolved N_2O (μg N_2O-N/L)**
L10	0.32 ± 0.06 – 2.26 ± 0.06
L8	0.43 ± 0.11 – 2.30 ±0.14
L6	0.46 ± 0.02 – 2.71 ± 0.19
L5	0.22 ± 0.01 - 1.99 ± 0.03
L4	0.24 ± .06 – 2.24 ± 0.07
LLH	0.24 ± 0.03 – 2.08 ± 0.28
LMain	0.29 ± 0.02 – 2.16 ± 0.09
LNewmkt	0.32 ± 0.02 – 2.01 ±0.19
LLR	0.30 ± 0.01 – 2.11 ± 0.07
L1	0.38 ± 0.02 – 2.19 ± 0.35
LMLB	0.41 ± 0.04 – 2.46 ± 0.30
LNB	0.36 ± 0.01 – 2.21 ± 0.07
L5a	0.20 ± 0.04 – 2.34 ± 0.11
LRB	0.19 ± 0.06 – 2.34 ± 0.08
L3	0.33 ± 0.00 – 2.20 ± 0.26
LPawt	0.21 ± 0.02 – 2.09 ± 0.08

Figure 1-7: : Average concentration of dissolved N_2O (µg N_2O -N/L) of the three replicate samples per site per sample date for all main stem sites (in order from headwaters to mouth) of the Lamprey River for all study dates

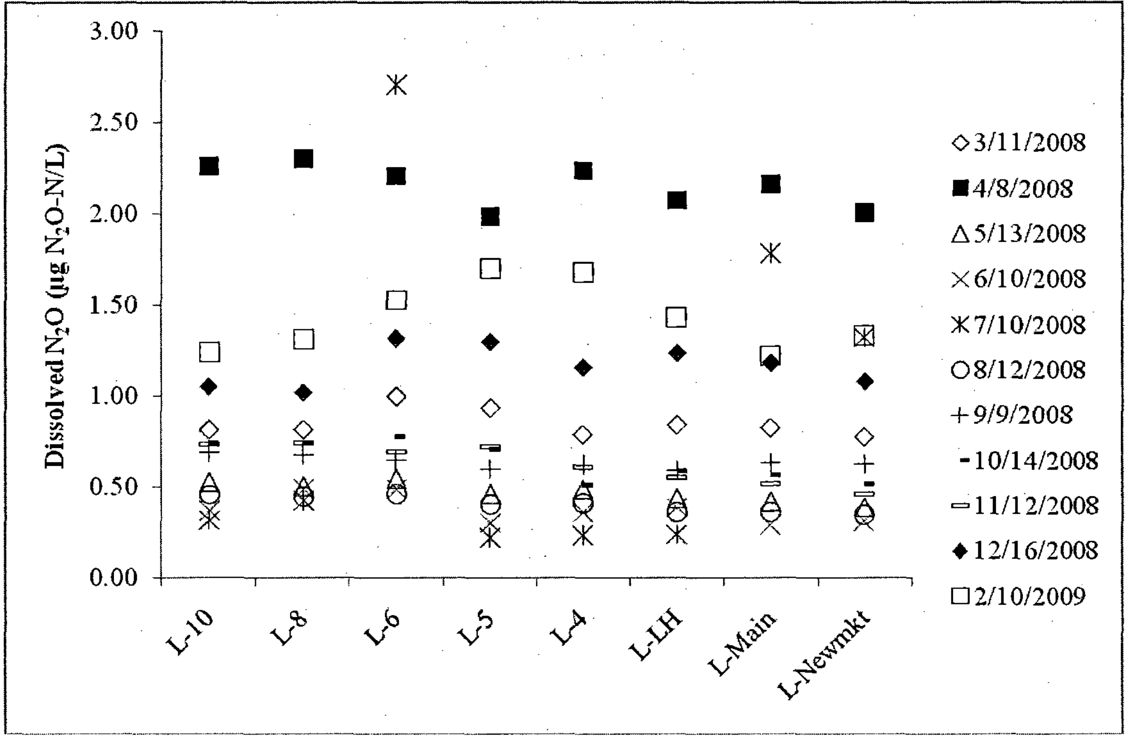
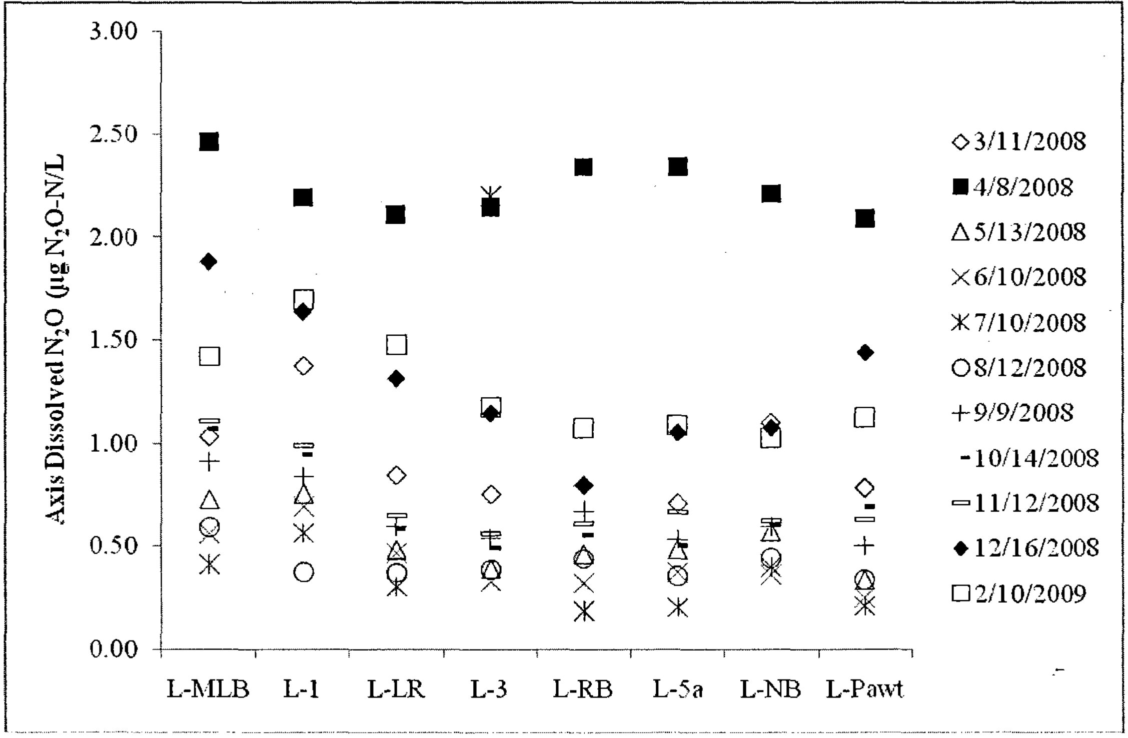
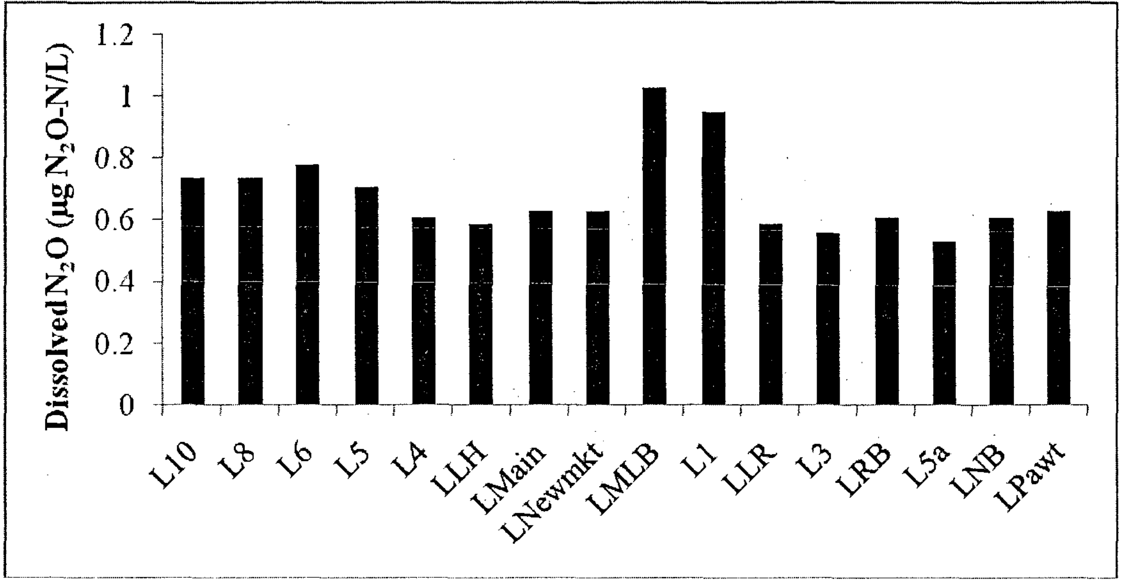


Figure 1-8: Average concentration of dissolved N_2O (µg N_2O -N/L) of the three replicate samples per site per sample date for all tributaries of the main stem of the Lamprey River for all study dates



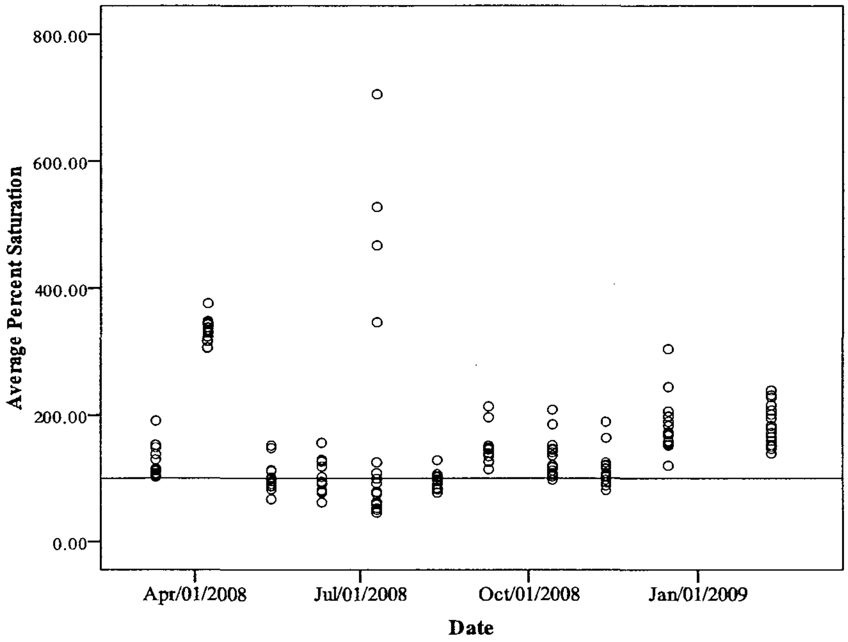
Median concentrations of dissolved N_2O for each stream for the entire study period was highest in MLB (1.03 µg N_2O-N/L) and lowest at L5a (0.53 µg N_2O-N/L) (Figure 1-9).

Figure 1-9: Median concentrations of dissolved N_2O (µg N_2O-N/L) by stream



Average percent saturation exceeded 100% in most streams in the fall, spring, and winter months, while the summer months often yielded percent saturation values lower than 100%. For the study period, average percent saturation for dissolved N_2O was highest in L6 (705.2%) and lowest in LRB (45.77%) in July 2008 (Figure 1-10, Appendix A). All streams had a percent saturation above 300% in April 2008.

Figure 1-10: Average percent saturation for dissolved N_2O all streams for each sample date (line indicates 100% saturation)



Dissolved N_2O and Discharge

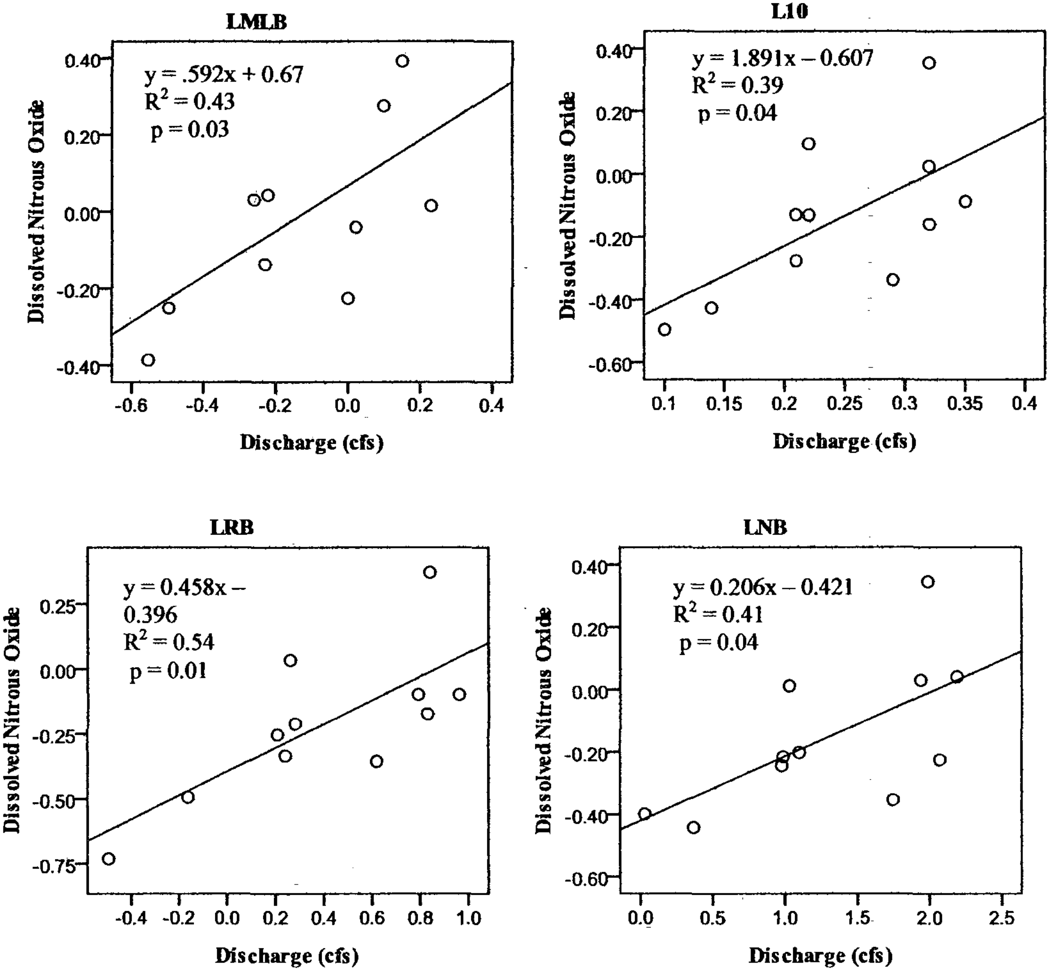
Discharge was highest in April and September 2008 and lowest in July 2008 for all streams in which discharge was determined (Appendix B). Overall, the highest discharge for the study streams was 2040.59 cfs at LNewmkt in September 2008 and the lowest discharge was 0.27 cfs at L1 in July 2008. Runoff depth (discharge normalized for watershed size) was lowest in L10 and increased in the following order: L10 < L8 < LRB < L3 < LMLB < L1 < L6 < L5, L4, LLH, LMain, LNewmkt (Table 1-4, Appendix B). (Note no discharge data available for L5a, LLR, LPawt).

Table 1-4: Median discharge (cfs) for each stream throughout the study period (main stem sites in italics), watershed area, and discharge normalized for watershed area (runoff depth)

Stream	**Median Q (cfs)**	**Watershed Area (km^2)**	**Runoff Depth (cfs/km^2)**
L10	1.66	14.70	0.11
L8	10.23	80.50	0.13
L6	66.07	144.70	0.46
L5	98.63	197.60	0.50
L4	125.48	251.40	0.50
LLH	233.91	468.80	0.50
LMain	239.00	478.80	0.50
LNewmkt	273.99	548.90	0.50
LMLB	.60	1.40	0.43
L1	.63	1.30	0.45
L3	51.29	128.7	0.40
LRB	1.91	4.9	0.39
LNB	12.59	41.20	0.31

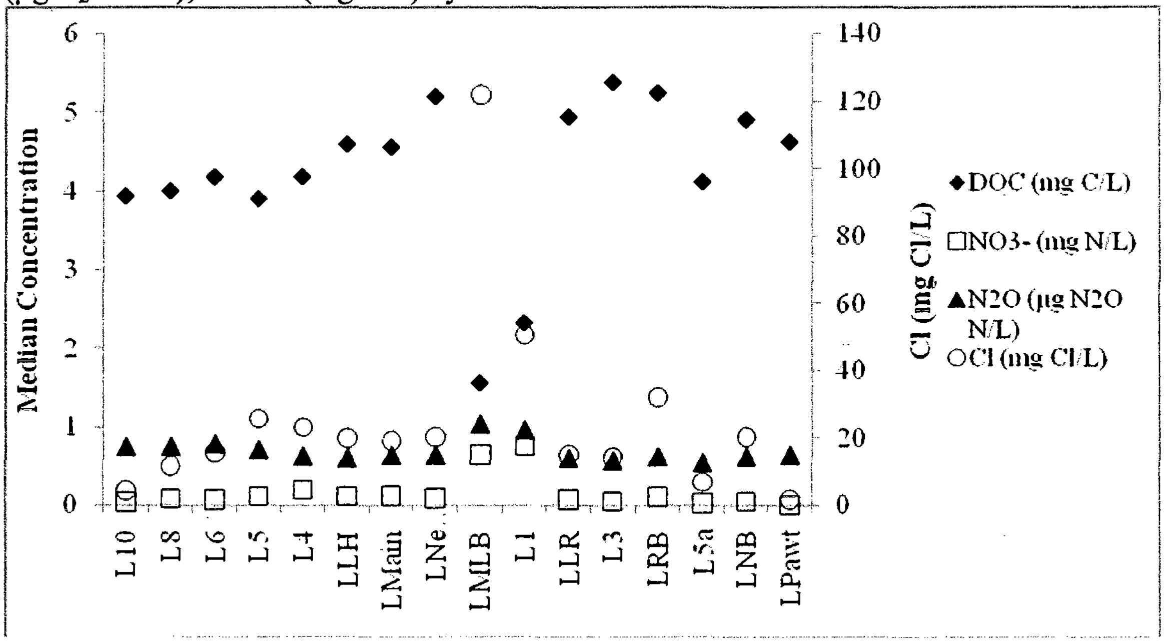
Using regression analysis, no relationships were found between median dissolved N_2O and median discharge ($R^2 = 0.221$, $p = 0.105$) or runoff depth ($R^2 = 0.007$, $p = 0.791$). By analyzing the data file by stream, four streams showed a significant positive relationship between dissolved N_2O ($\log_{10}$) and discharge ($\log_{10}$): LMLB ($R^2 = 0.428$, $p = 0.29$); L10 ($R^2 = 0.628$, $p = 0.039$); LRB ($R^2 = 0.544$, $p = 0.010$); and LNB ($R^2 = 0.405$, $p = 0.035$) (Figure 1-11).

Figure 1-11: Significant positive relationships between dissolved N_2O (µg N_2O-N/L) (log_{10}) and discharge (cfs) (log_{10}) for four streams in the Lamprey River Watershed



Dissolved N_2O and Stream Chemistry

Figure 1-12: Median concentrations of DOC (mg C/L), NO_3^- (mg N/L), dissolved N_2O (µg N_2O-N/L), and Cl^- (mg C/L) by stream



Dissolved N_2O and NO_3^-: Overall, NO_3^- was highest in the winter, spring, and fall months, and lowest in the summer months. NO_3^- ranged from 1.30 mg N/L in L1 in March 2008 to below detection limits (< 3 µg N/L) in LPawt for most of the sample period, and many other streams at one time during the sample period. L1 and LMLB consistently had the highest concentrations of NO_3^- with values typically higher than 0.30 mg N/L. No other sites had concentrations above 0.30 mg N/L, and often had concentrations at or near the detection limit (Appendix C). Median NO_3^- for the entire study period ranged from 0.75 mg N/L in L1 to below detection limits in LPawt (Figure 1-12).

A strong, positive relationship was found between overall median dissolved N_2O (µg N_2O-N/L) and overall median NO_3^- (mg N/L) ($R^2 = 0.696$, $p < 0.001$) (Figure 1-13). A weaker but significant positive relationship between dissolved N_2O (µg N_2O-N/L)

($\log_{10}$) and NO_3^- (mg N/L) ($\log_{10}$) was found using linear regression when all data points were used ($R^2 = 0.056$, $p = 0.002$). However, no significant relationships were found for an individual stream when the entire data set for the year was included. A strong positive relationship was observed between dissolved N_2O (µg N_2O-N/L) ($\log_{10}$) and NO_3^- (mg N/L) ($\log_{10}$) for all dates ($R^2 = 0.262$ -0.746) except April and July ($R^2 = 0.262$ -0.746) (Figure 1-14).

Figure 1-13: Significant positive relationship between median dissolved N_2O (µg N_2O-N/L) and median NO_3^- (mg N/L) for all streams in the Lamprey River Watershed throughout the study period

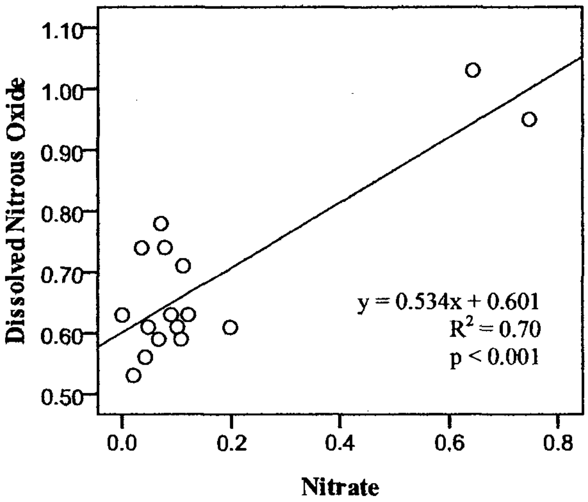
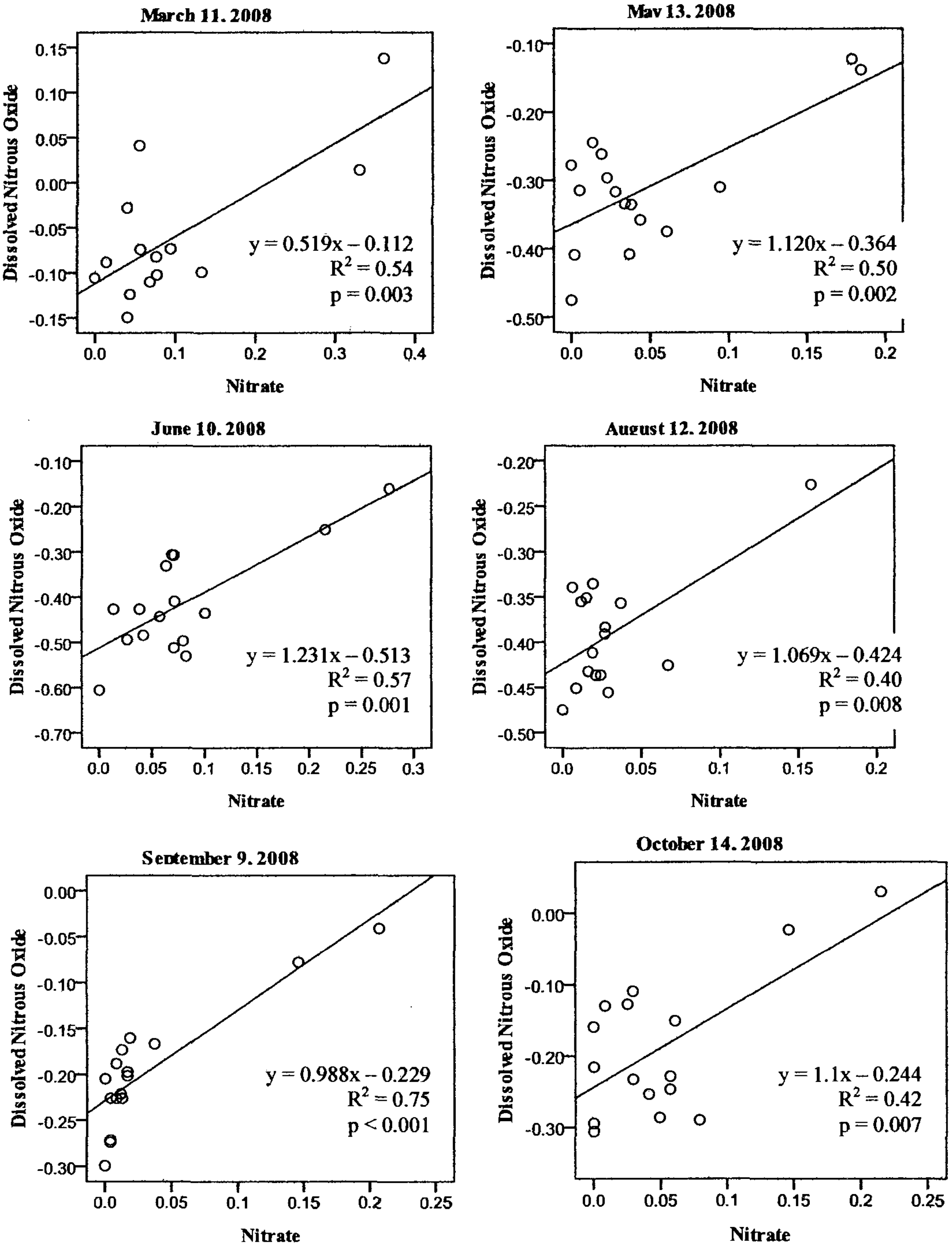
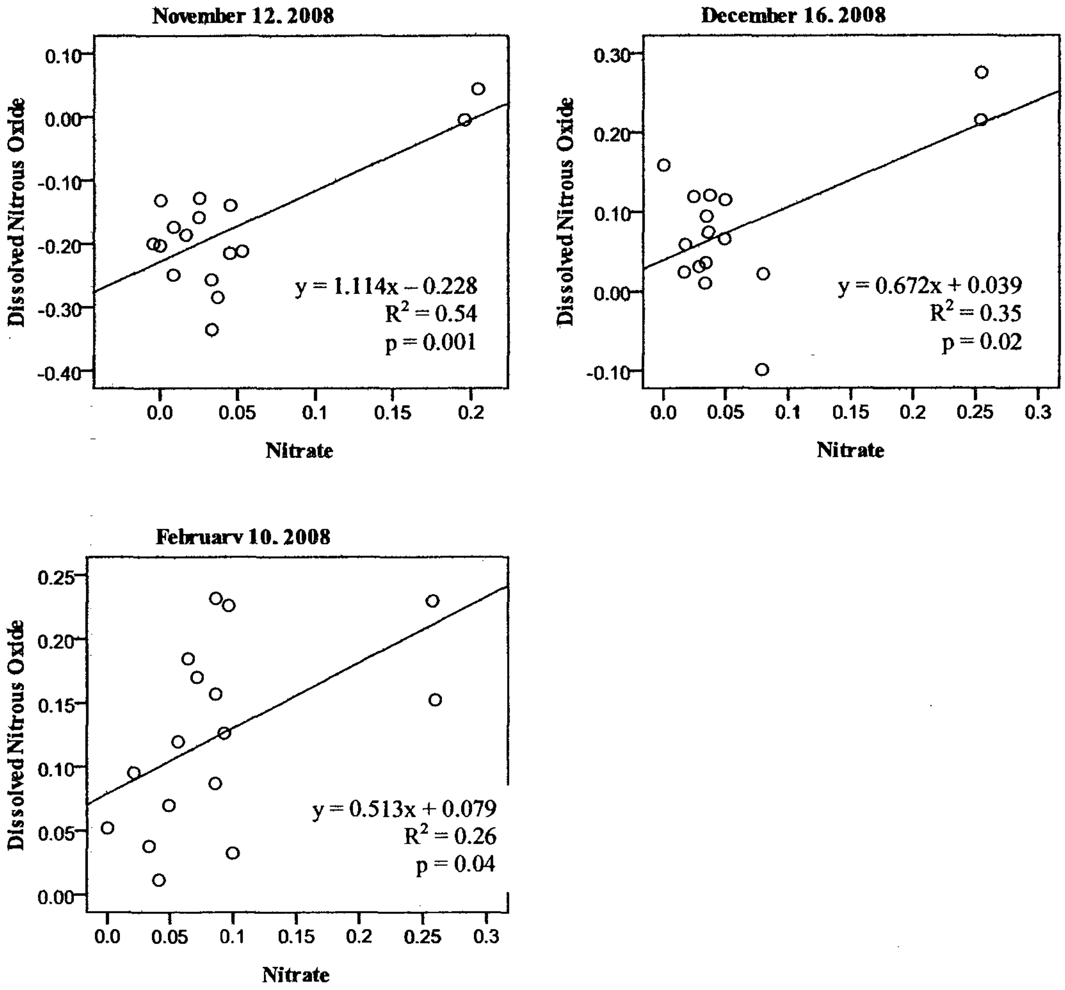


Figure 1-14: Significant positive relationships between dissolved N_2O (µg N_2O-N/L) ($\log_{10}$) and NO_3^- (mg N/L) ($\log_{10}$) for 9 of 11 sample dates for all streams in the Lamprey River Watershed

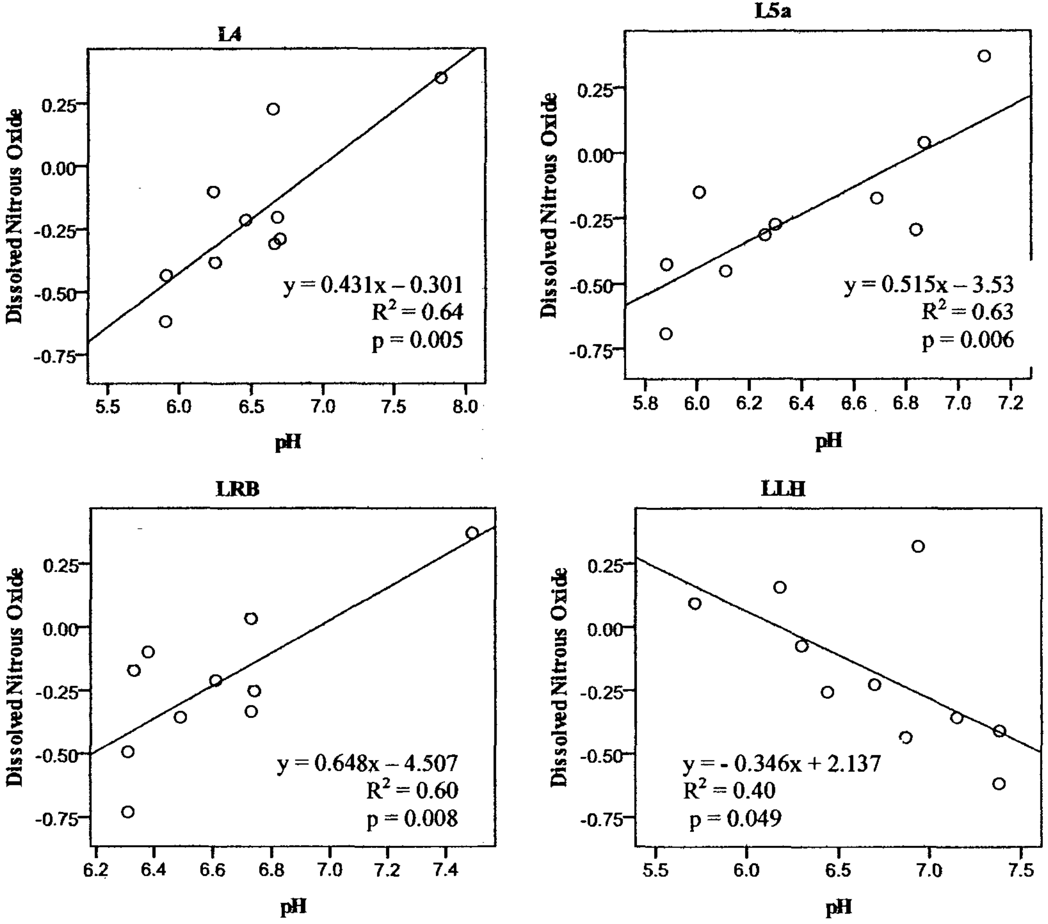




Dissolved N_2O and pH: pH ranged from 3.85 in L-Main (February 2009) to 8.67 in L-MLB (June 2008) and L-Main (July 2008). Most sites ranged between 6 and 7 throughout the study period, while L-Pawt ranged from a pH of 5 to 6 (Appendix C). Median pH for each stream for the entire study period was highest at L1 (7.06) and lowest at L10 (5.80).

No significant relationships were found between median dissolved N_2O and pH ($R^2 = 0.047$, $p = 0.422$), when using all of the streams and all sample dates ($R^2 = 0.001$, $p = 0.766$) or when splitting the data by date. However, significant relationships were found for four of the sixteen sites (L4, L5a, LLH, and LRB) (Figure 1-15).

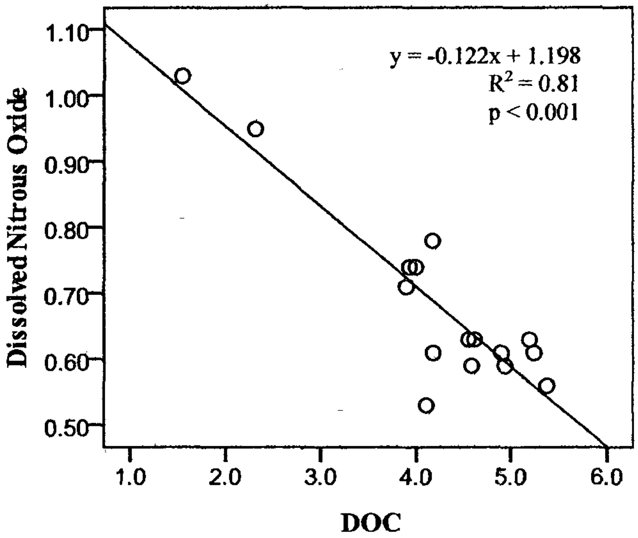
Figure 1-15: Significant relationships between dissolved N_2O (μg N_2O-N/L) ($\log_{10}$) and pH for 4 of 16 streams for all sample dates in the Lamprey River Watershed (note the negative relationship in LLH)



Dissolved N_2O and DOC: DOC ranged from below detection limits (0.1 mg C/L) (L3 in April 2008) to 10.10 mg C/L (LRB in August 2008). Few concentrations of DOC were above 10.00 mg C/L. Higher concentrations were seen in the late summer and early fall (August and September 2008) and in February 2009 (Appendix C). Median DOC for each stream over the entire study period was highest in L3 (5.38 mg C/L) and lowest in LMLB (1.55 mg C/L) (Figure 1-12).

A strong significant negative relationship was found between median dissolved N_2O (μg N_2O-N/L) and DOC (mg C/L) ($R^2 = 0.809$, $p < 0.001$) (Figure 1-16), while a weaker but significant negative relationship was found between dissolved N_2O (μg N_2O-N/L) ($\log_{10}$) and DOC (mg C/L) when all data points were used ($R^2 = 0.092$, $p = 0.001$).

Figure 1-16: Significant negative relationship between median dissolved N_2O (μg N_2O-N/L) and median DOC (mg C/L) for all streams in the Lamprey River Watershed for all sample dates



Only one significant relationship was found for the individual streams using all of the sampling dates (L-Pawt) (Figure 1-17). However, six of the eleven sampling dates showed significant negative relationships between dissolved N_2O (μg N_2O-N/L) ($\log_{10}$) and DOC (mg C/L) for all streams (Figure 1-18).

Figure 1-17: Significant negative relationship between dissolved N_2O (μg N_2O-N/L) ($\log_{10}$) and DOC (mg C/L) for L-Pawt in the Lamprey River Watershed for all sample dates

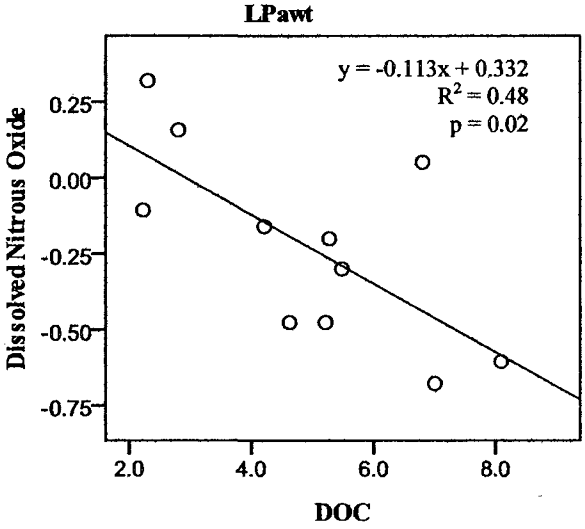
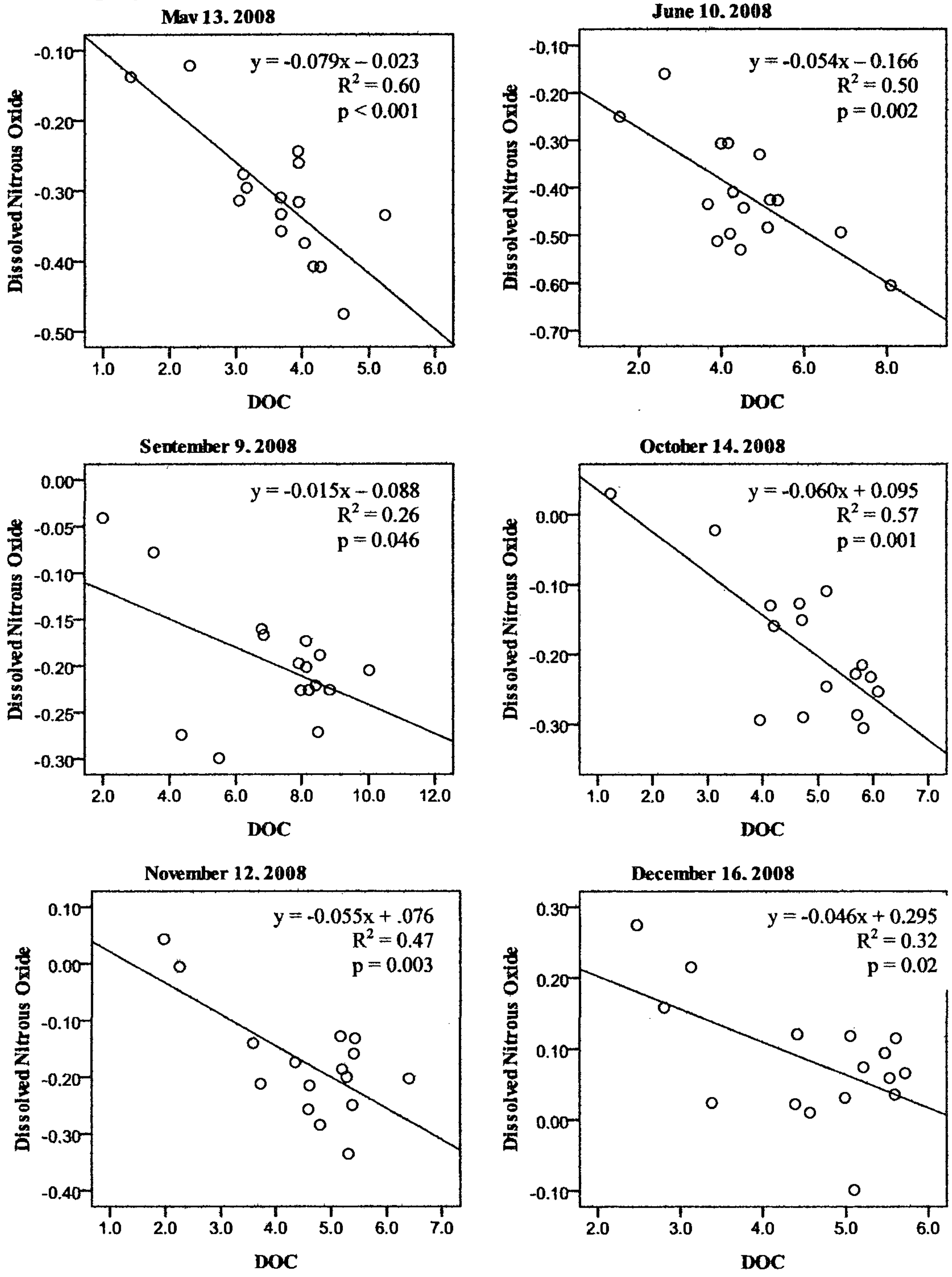


Figure 1-18: Significant negative relationships between dissolved N_2O (μg N_2O-N/L) ($\log_{10}$) and DOC (mg C/L) for six of the eleven sampling dates using all streams sampled in the Lamprey River Watershed



Dissolved N_2O and TDN : TDN ranged from 0.08 mg N/L (L-Pawt, December 2008) to 1.17 mg N/L (L-1, March 2008). Lower concentrations were found in the late summer, fall, and winter, while the highest concentrations occurred in the late spring and early summer (Appendix C). Median concentrations of TDN (mg N/L) for each site over the entire study period were highest at LMLB (0.84 mg N/L) and lowest at L10 (0.16 mg N/L).

A significant positive linear relationship was found between median dissolved N_2O (μg N_2O-N/L) and TDN (mg N/L) ($R^2 = 0.630$, $p < 0.001$) (Figure 1-19), though no significant linear relationship was found between dissolved N_2O (μg N_2O-N/L) ($\log_{10}$) and TDN (mg N/L) when all data were used ($R^2 = 0.011$, $p = 0.169$). Significant relationships were found when analyzed by date (March, May, June, September, November 2008 and Febuary 2009) (Figure 1-20).

Figure 1-19: Significant relationships between median dissolved N_2O (μg N_2O-N/L) and median TDN (mg N/L) for each stream in the Lamprey River Watershed over the entire study period

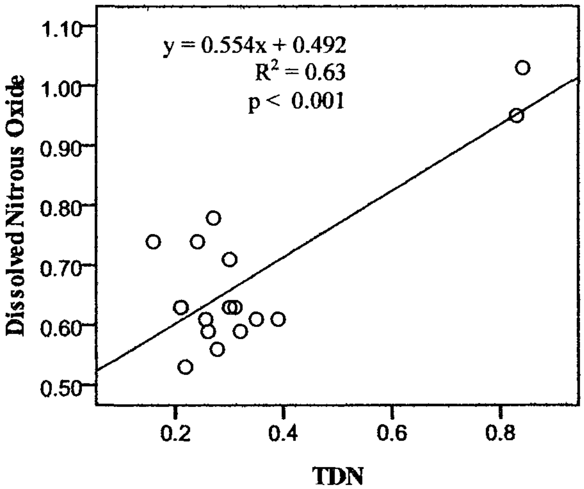
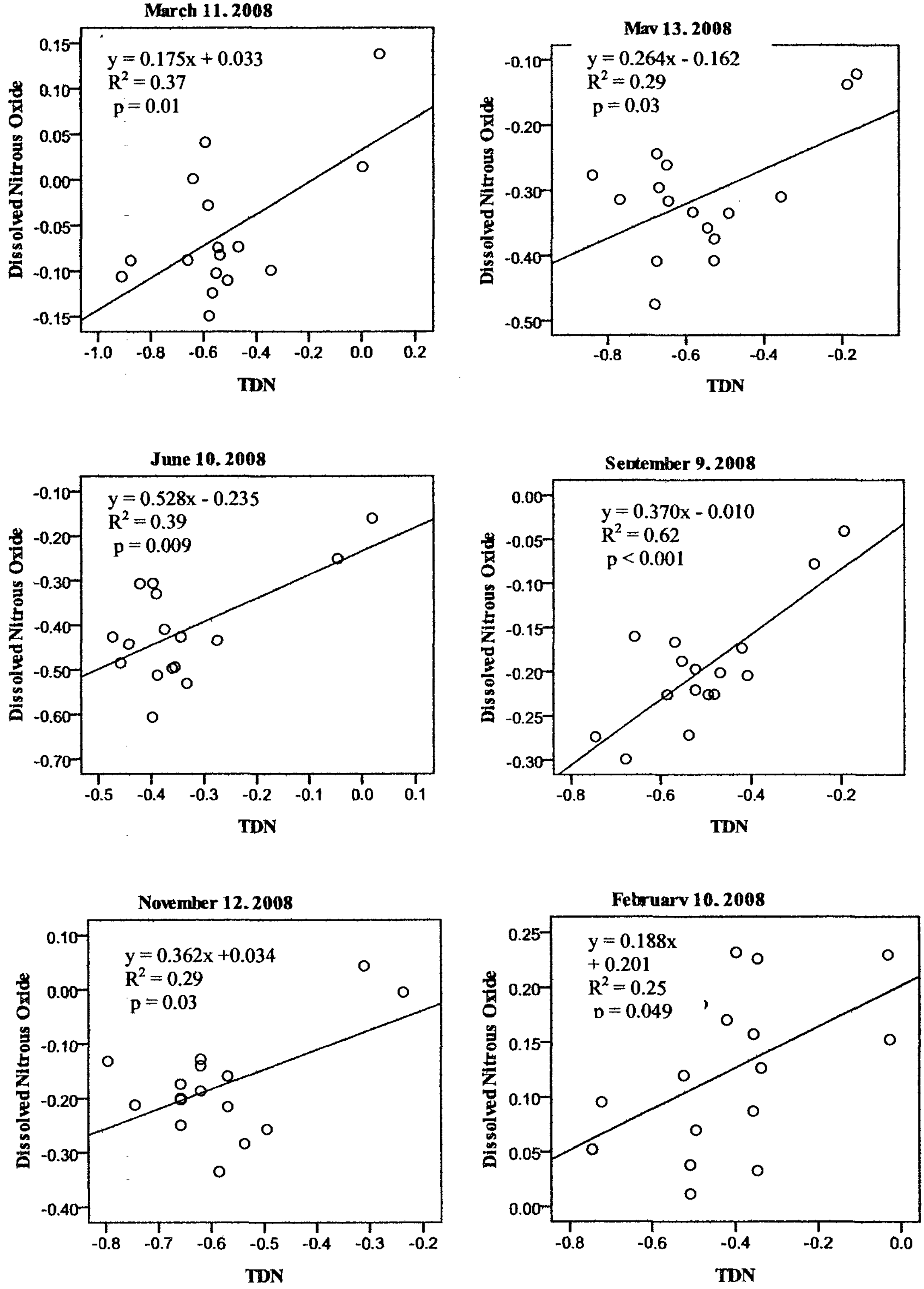
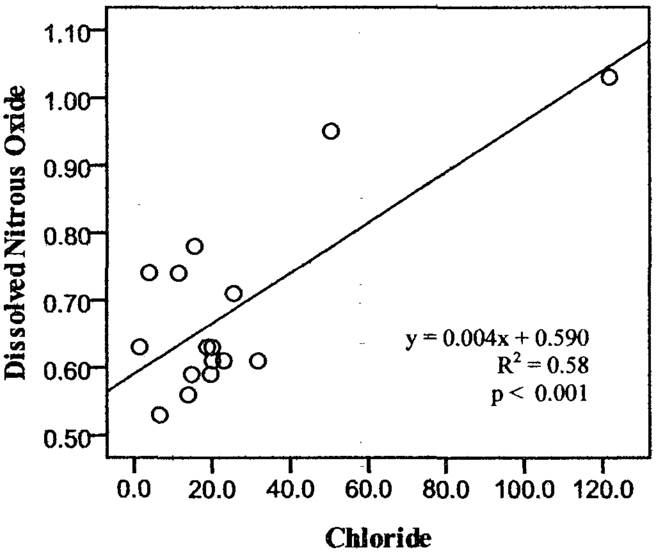


Figure 1-20: Significant relationships between dissolved N_2O (µg N_2O-N/L) ($\log_{10}$) and TDN (mg N/L) ($\log_{10}$) for six of the eleven sampling dates using all streams sampled in the Lamprey River Watershed



Dissolved N_2O and Cl^-: Cl^- ranged from 0.80 mg Cl/L (L-Pawt, August 2008) to 138.84 mg Cl/L (LMLB, July 2008) (Appendix C). Concentrations in LMLB were almost always over 100 mg Cl/L, which is twice as high as L1 (the stream with the second highest concentration of Cl^-) and almost 100 times higher than LPawt (the stream with the lowest concentration of Cl^-). Median concentrations of Cl^- for each site over the entire study period were highest at LMLB (121.79 mg Cl/L) and lowest at LPawt (1.44 mg Cl/L) (Figure 1-12). A significant positive linear relationship was found between median dissolved N_2O (µg N_2O-N/L) and Cl^- (mg Cl/L) ($R^2 = 0.583$, $p < 0.001$) (Figure 1-21).

Figure 1-21: Significant relationships between median dissolved N_2O (µg N_2O-N/L) and median Cl^- (mg Cl/L) for each stream in the Lamprey River Watershed over the entire study period



Small Scale Spatial and Temporal Trends of Dissolved N_2O: L1

Spatial Trends of Dissolved N_2O in L1

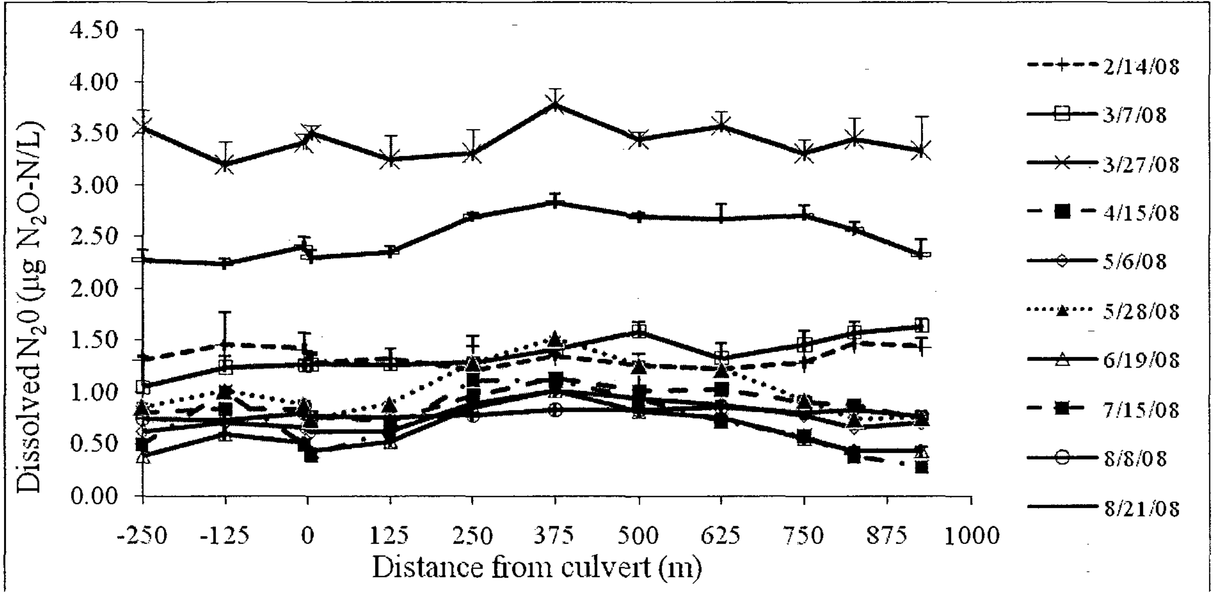
Average dissolved N_2O ranged from 0.28 ± 0.03 µg N_2O-N/L (July 15, 2008) to 3.78 ± 0.16 µg N/L (March 27, 2008) (Table 1-5, Figure 1-22, Appendix D). Dissolved

N_2O was highest at all sites on March 27, 2008 and lowest on June 18, 2008 and July 15, 2008. Significant differences were found among the twelve sites sampled on L1 on eight of the ten study dates (Table 1-5).

Table 1-5: Significant differences in dissolved N_2O between all sites measured in L1 for each study date with the range of average dissolved N_2O and discharge (measured discharge in bold) for that date

Date	**Range of Average Dissolved N_2O (μg N_2O-N/L)**	**Significance**	**Discharge (cfs)**
2/14/2008	1.22 ± 0.02 – 1.47 ± 0.10	--	2.19
3/7/2008	1.05 ± 0.28 – 1.64 ± 0.07	$p = 0.002$	1.20
3/27/2008	3.20 ± 0.23 – 3.78 ± 0.16	--	1.23
4/15/2008	0.75 ± 0.04 – 1.14 ± 0.02	$p < 0.001$	1.10
5/6/2008	0.62 ± 0.01 – 1.02 ± 0.03	$p < 0.001$	0.93
5/28/2008	0.74 ± 0.01 - 1.52 ± 0.02	$p < 0.001$	0.36
6/19/2008	0.39 ± 0.05 – 1.02 ± 0.01	$p < 0.001$	**0.61**
7/15/2008	0.28 ± 0.03 – 1.12 ± 0.06	$p < 0.001$	**0.19**
8/8/2008	0.73 ± 0.02 – 0.86 ± 0.01	$p < 0.001$	**1.07**
8/21/2008	2.24 ± 0.05 – 2.83 ± 0.09	$p < 0.001$	**0.56**

Figure 1-22: Average dissolved N_2O (μg N_2O-N/L) for all sites in L1 for all study



Discharge was highest on February 14, 2008 and lowest on May 28, 2008 (Appendix B). A spatial pattern in dissolved N_2O was more apparent on dates with lower discharge (below 1 cfs) as determined by an increase in post-hoc Tukey significant differences in dissolved N_2O between sites (Appendix E). Overall, on low discharge dates, sites 250-625 were consistently significantly different than the other sites (Figure 1-24 and Appendix E). However, on sample dates where discharge exceeded 1 cfs, significant differences in both these particular sites, as well as between all of the sites were not always found (Figure 1-23). Though significant differences were found between sites, dissolved N_2O did not range more than 1 μg N_2O-N/L on a specific date between the 12 sites.

Figure 1-23: Average dissolved N_2O (μg N_2O-N/L) for all sites in L1 for study dates with discharge above 1 cfs

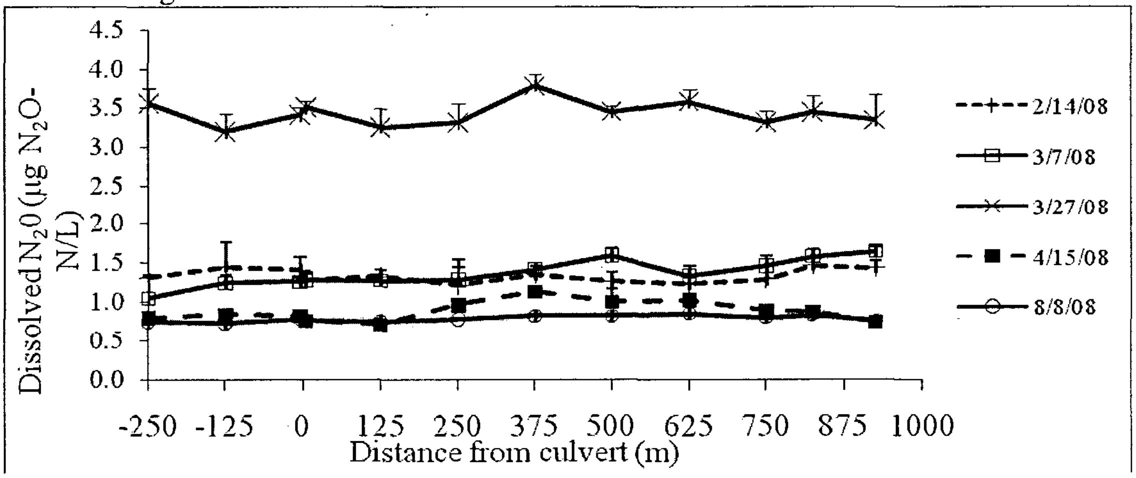
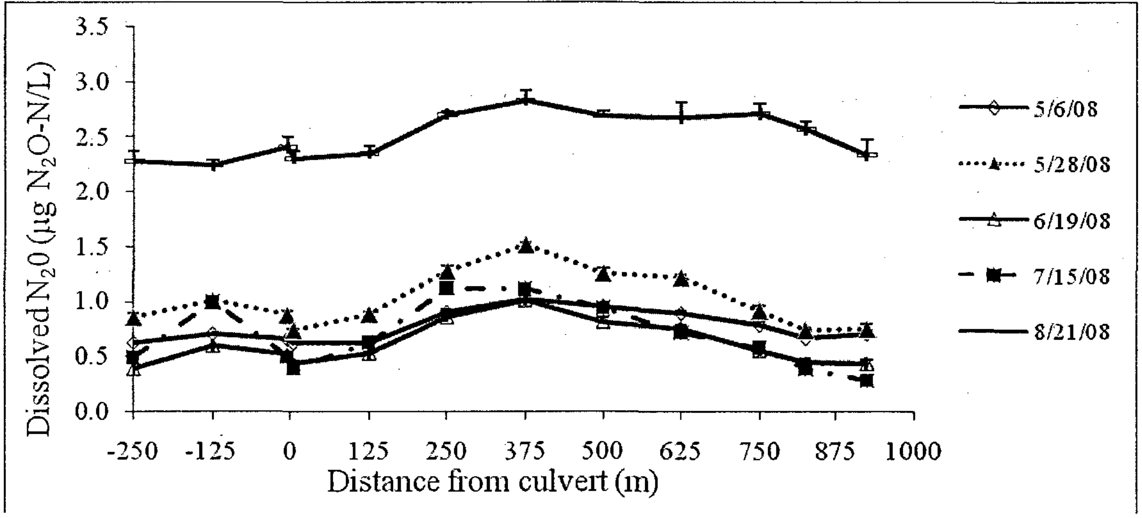
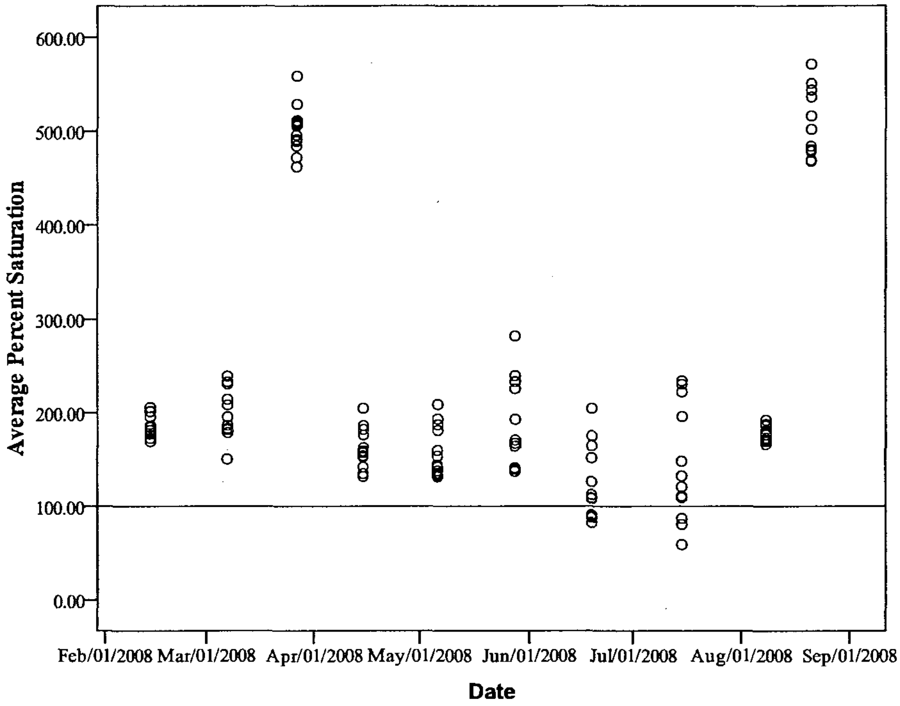


Figure 1-24: Average dissolved N_2O (μg N_2O-N/L) for all sites in L1 for study dates with discharge below 1 cfs



Overall, average percent saturation was above 100% for most of the study period at all of the sites (Figure 1-25, Appendix D). Percent saturation was highest at all sites on 3/27/08 and on 8/21/08, corresponding with the highest concentration of dissolved N_2O, and lowest in the summer, reaching below 100% at some sites. Average percent saturation of dissolved N_2O ranged from 59.23% (Site 925 on 7/15/08) to 571.65% (Site 375 on 8/21/08).

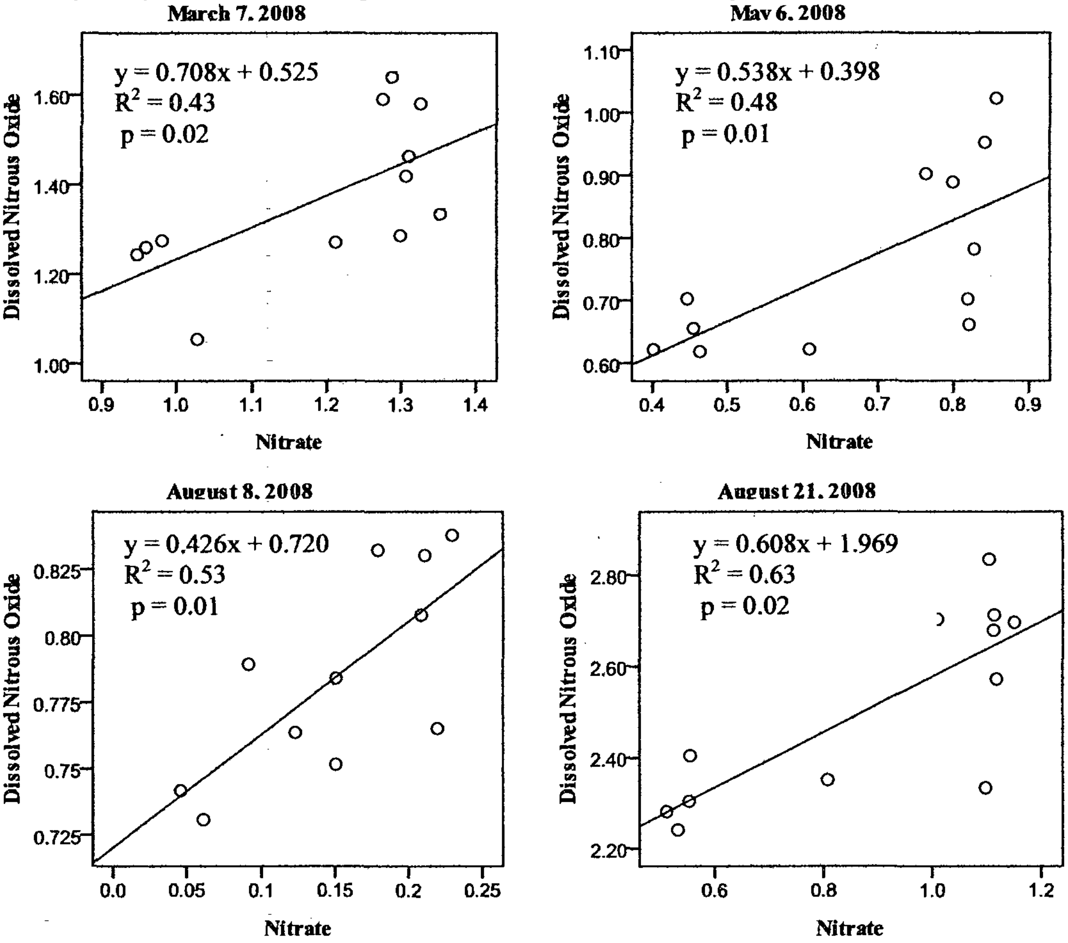
Figure 1-25: Average percent saturation of dissolved N_2O for all sites along L1 throughout the study period (reference line indicates 100% saturation)



Dissolved N_2O and Stream Chemistry

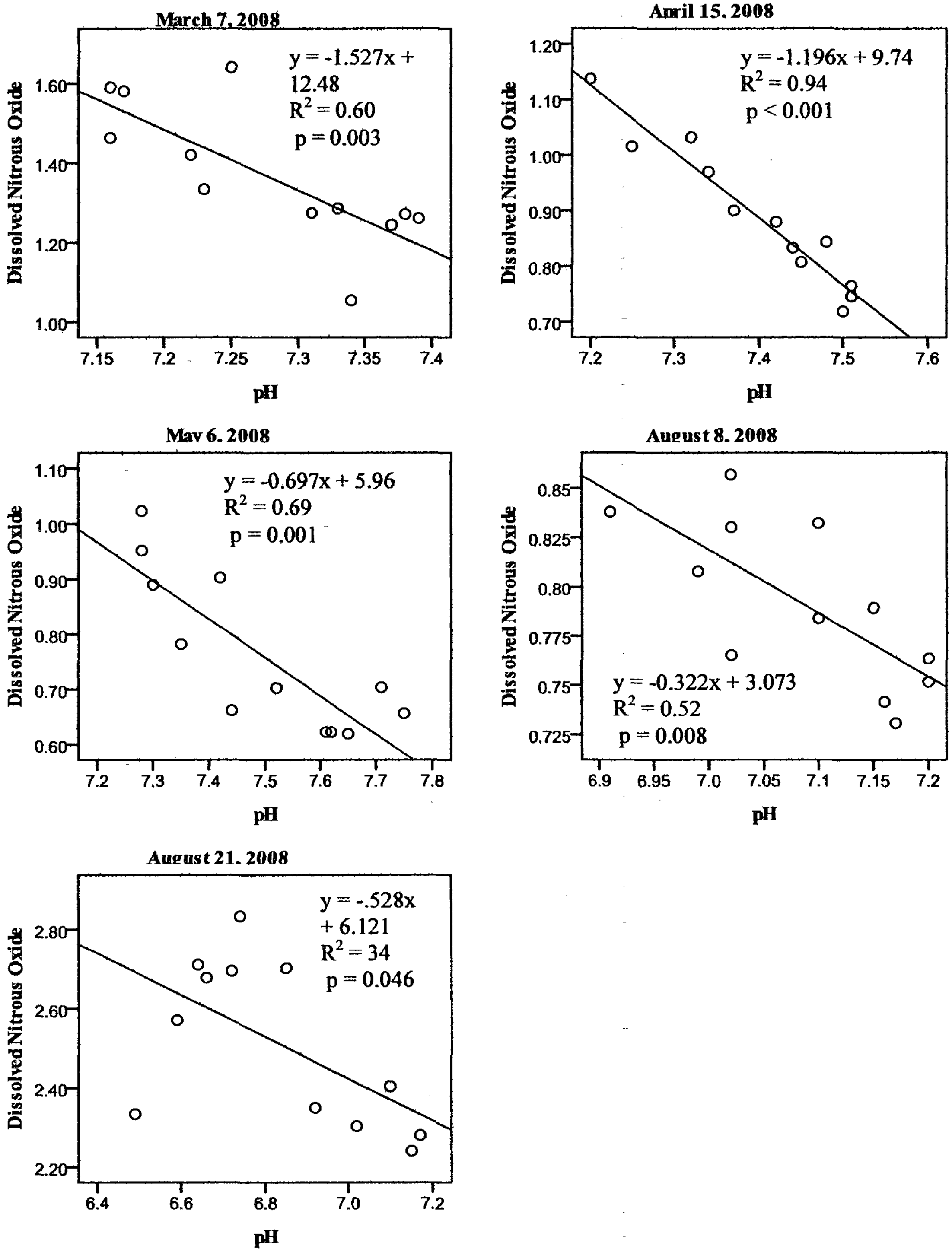
Dissolved N_2O and NO_3^-: NO_3^- ranged from 0.05 mg N/L (site -250 on 8/8/08) to 1.80 mg N/L (site 250 on 7/15/08) (Appendix F). Overall, NO_3^- was lowest at all sites on 8/8/08. NO_3^- was consistently lower at the upstream sites than at the downstream sites beginning at site 250. No significant linear relationship was found between median dissolved N_2O (μg N_2O-N/L) and median NO_3^- (mg N/L) for a specific site for all sample dates ($R^2 = 0.193$, $p = 0.153$), or for all sites on a specific date ($R^2 = 0.002$, $p = 0.909$). However, significant relationships were found when all data was analyzed by date (3/7/08, 5/6/08, 8/8/08, and 8/21/08) (Figure 1-26).

Figure 1-26: Significant positive relationships between dissolved N_2O (µg N_2O-N/L) and NO_3^- (mg N/L) for 4 of 11 sample dates for all 12 sites along L1



Dissolved N_2O and pH: pH ranged from 6.02 (site 925 on 7/15/08) to 7.77 (site -250 on 6/19/08) (Appendix F). No linear relationship was found between median dissolved N_2O and median pH for a specific site for all sample dates (R^2 = 0.192, p = 0.155), or for all sites on a specific date (R^2 = 0.002, p = 0.905). However, significant negative relationships were found for five of the ten study dates (3/7/08, 4/15/08, 5/6/08, 8/8/08, and 8/21/08) when all data points were used (Figure 1-27).

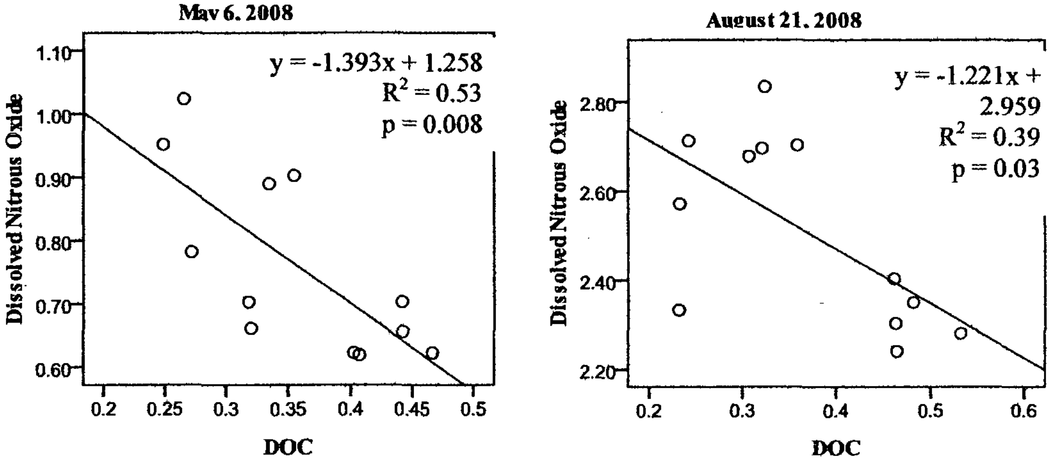
Figure 1-27: Significant negative relationships between dissolved N_2O (µg N_2O-N/L) and pH for 5 of 10 sample dates for all 12 sites along L1



<u>Dissolved N_2O and DOC</u>: DOC ranged from 0.83 mg C/L (site 625 on 7/15/08) to 9.37 mg C/L (site -125 mg C/L) (Appendix F). No significant linear relationship was

found between median dissolved N_2O and median DOC for a specific site for all sample dates ($R^2 = 0.086$, $p = 0.356$), or for all sites on a specific date ($R^2 = 0.022$, $p = 0.685$). However, significant negative relationships were found for two of the ten sample dates when all data was used (Figure 1-28).

Figure 1-28: Significant negative relationships between dissolved N_2O (μg N_2O-N/L) and DOC (mg C/L) ($\log_{10}$) for 2 of 10 sample dates for all 12 sites along L1



<u>Dissolved N_2O and SC</u>: SC ranged from 95 μS/cm (site 375 on 8/8/08) to 465 μS/cm (site 250 on 3/27/08) (Appendix F). Overall, SC was highest on 3/27/08 and lowest on 8/8/08. No significant linear relationship was found between median dissolved N_2O and median SC for a specific site for all sample dates ($R^2 = 0.238$, $p = 0.153$), or for all sites on a specific date ($R^2 = 0.022$, $p = 0.685$).

Diel Trends of Dissolved N_2O in L1

Average dissolved N_2O ranged from 2.37 ± 0.10 μg N/L (Site -5: 18:00) to 2.80 ± 0.06 μg N/L (Site -5: 6:00) for the diel sampling on 3/20-3/21/08 and 2.09 ± 0.14 μg N/L (Site -5: 2:00) to 2.70 ± 0.04 μg N/L (Site 275: 2:00) (Figures 1-29 and 1-30, Appendix G). Average percent saturation of dissolved N_2O ranged from 350-450% in March 2008, and between 400-500% in August 2008 (Appendix G).

Significant differences in dissolved N_2O based on time of day for a specific site were only found for site -5 during the March sampling ($p = 0.043$). A post-hoc Tukey test indicates these differences were found between sampling at 6:00 and 18:00 ($p = 0.023$) only. No other temporal patterns were found on either sample date for any of the other sites.

Figure 1-29: Average dissolved N_2O (μg N_2O- N/L) for three sites on L1 for diel sampling on March 20 and 21, 2008

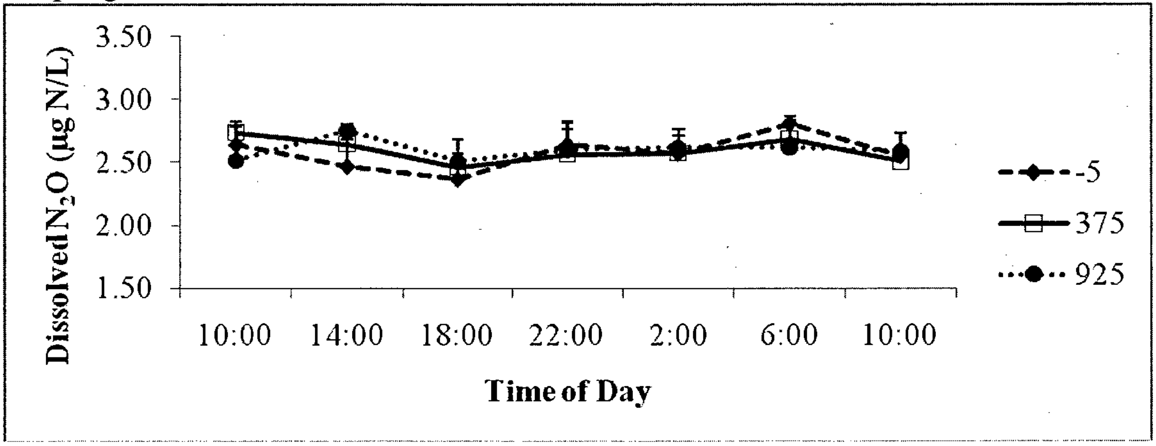
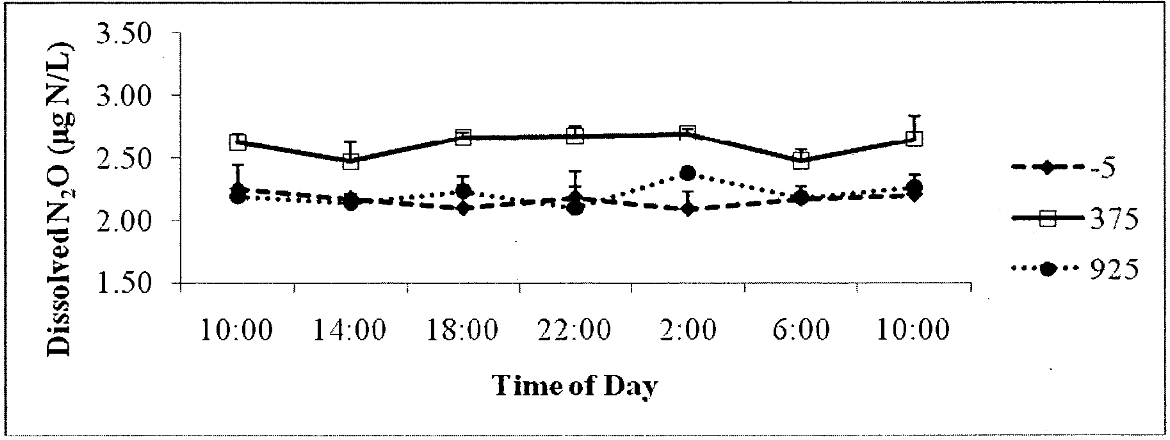


Figure 1-30: Average dissolved N_2O (μg N_2O -N/L) for three sites on L1 for diel sampling on August 21 and 22, 2008



DISCUSSION

Lamprey River Watershed Survey

An understanding of the spatial and temporal trends in dissolved N_2O and the controls on these trends is important to determine the potential of freshwater streams to contribute to the global pool of atmospheric N_2O. The concentrations found in this study (0.19 – 2.71 μg N_2O- N/L) were within the range of values found in the literature (Table 1-6).

Table 1-6: Concentrations and percent saturation of dissolved N_2O for the Lamprey River Watershed compared with literature values

River/Watershed/Stream Location	Concentration Range (μg N_2O -N/L)	Percent Saturation (%)	Discharge (cfs)	Data Source
Lamprey River Watershed	0.19 – 2.71	45 - 705	0.27 - 2040.59	This study
North Island, New Zealand	0.16 – 11.35	--	0.03 – 3.85	Wilcox and Sorrell (2008)
South Island, New Zealand	0.43 – 1.89	--	26.82	Clough et al. (2006)
Indiana/Illinois/New Jersey	0.006 – 0.017	104 - 209	--	Laursen and Seitzinger (2004)
Hudson River, New York	0.196 – 0.606	125-385	2752.87	Cole and Caraco (2001)

The percent saturation of dissolved N_2O exceeded the range (both higher and lower) than values found in the literature possibly due to the range of stream sizes and the varying land use cover of the sub-watersheds for the streams used in this study as well as the lack of published results on the percent saturation on dissolved N_2O in streams, as most publications note flux instead of percent saturation (Table 1-6). In this study, percent saturation was used to understand the potential for degassing of N_2O from freshwater streams in the Lamprey River Watershed (if percent saturation exceeded

100%, there is potential for loss of N_2O to the atmosphere), as the number of streams and their relative sizes made it difficult to measure flux for each stream for each date. For every sample date, at least half of the streams exceeded 100% saturation of N_2. For March, April, September, December 2008 and February 2009, all of the streams exceeded 100% saturation, indicating that during these months, there was a greater potential for the entire watershed to contribute to the atmospheric pool of N_2O.

Concentrations of dissolved N_2O were highest in April (with a few exceptions in July) and lowest overall in July. The peak of dissolved N_2O in April also resulted in a watershed-wide peak in percent saturation (> 300% for all streams), indicating this month had the greatest potential for loss of N_2O from these streams to the atmosphere. Seasonal trends were found to be inconsistent in the literature. Wilcox and Sorrell (2008) noted a seasonal trend of highest concentrations in the summer and lowest in the winter for two of their study streams, while the third stream showed the opposite trend. Cole and Caraco (2001) also found higher concentrations in the summer months for one year of study, and the opposite the following year. On the other hand, Beaulieu et al. (2009) found peaks in in-stream N_2O production rates in the winter months corresponding to precipitation patterns and snowmelt. Overall, many riverine studies of N_2O were conducted on only a few study dates due to the often labor-intensive procedures, and do not lend themselves to seasonal analysis. More studies over longer periods of time are necessary to determine the true seasonal influence on concentrations of dissolved N_2O.

Effect of discharge and stream size on concentrations of dissolved N_2O

Other field studies have shown that smaller, shallower streams generally have higher concentrations of a dissolved gas than larger, deeper rivers, and as such, have a greater potential for gas exchange with the atmosphere (Wilcox and Sorrell, 2008; Allan and Castillo, 2007). In this study, tributaries to the main stem of the Lamprey River generally had higher concentrations of dissolved N_2O throughout the study, while the main stem sites with the highest discharge (LMain and LNewmkt) often had the lowest concentrations, indicating a dilution effect. Though no direct relationship was found between median dissolved N_2O and median discharge or median runoff depth for all streams on all study dates, significant positive relationships between discharge and dissolved N_2O concentration were found for four tributaries for all sample dates. This may have occurred because the increase in discharge in a specific stream has the potential to carry with it NO_3^- and externally produced dissolved N_2O originating in overland flow from adjacent soils. Beaulieu et al. (2009), found a spike in both NO_3^- concentrations and subsequent in-stream dissolved N_2O production as a result of winter rain and snowmelt that flushed the soils of excess NO_3^-. This result is counterintuitive, as it was expected that lower discharges would allow for increased potential for the interaction of stream water and the streambed sediment, which has been shown to increase denitrification rates (Allan and Castillo, 2007). As these results are not universal for all streams studies, more research on the effect of discharge on the concentration of dissolved N_2O is needed.

Effects of stream chemistry on dissolved N_2O

Effects of stream concentrations of NO_3^-: Many field studies have linked the concentration of NO_3^- in the stream water, groundwater, and soil pores to N_2O production (Beaulieu et al., 2009; Beaulieu et al., In Prep; Inwood et al., 2007; Wall et al., 2005; Hefting et al., 2003). This occurs because NO_3^- can be a limiting nutrient in denitrification and N_2O: N_2 has been shown to increase in conditions of high NO_3^-

(Hefting et al., 2003; Firestone et al., 1980). Stream concentrations of N_2O may result from in-stream production through processes such as denitrification. However, streams also receive inputs of N_2O from groundwater and overland flow (McMahon and Dennehy, 1998). This indicates that though factors (such as NO_3^- concentration) that have been shown to influence rates of denitrification may predict concentrations of dissolved N_2O these factors may not have as much influence on the dissolved N_2O in a stream as has been shown in soil field studies or laboratory experiments.

In this study, a significant positive relationship was found between median NO_3^- and N_2O for each stream throughout the study period ($R^2 = 0.696$, $p < 0.001$). By examining NO_3^- concentrations for each stream, it is clear that the range of values for a specific stream does not change much throughout the study period. In other words, streams with low NO_3^- had low NO_3^- throughout the entire study period and streams with high NO_3^- had high NO_3^- values throughout the study period. However, for a specific stream, concentrations of dissolved N_2O had a much larger range throughout the study period. For a specific sample date, the magnitude of the concentration of N_2O was similar throughout the watershed. This indicates that stream NO_3^- concentrations may have influenced dissolved N_2O concentrations in the Lamprey River Watershed for most of the study period.

This relationship was not seen in April and July 2008. April 2008 marked the end of snowmelt and yielded the highest overall concentrations of dissolved N_2O of all study dates (up to three times higher). Inputs of dissolved N_2O from groundwater and overland flow related to spring runoff may have influenced these concentrations, while NO_3^- concentrations did not change in magnitude during this period. Wall et al. (2005) showed a similar peak in N_2O coinciding with spring runoff. July 2008 had unusually high concentrations of N_2O for four streams which did not correspond with a similar peak in stream NO_3^- concentrations. This may have occurred as these streams could have received outside inputs of N_2O from groundwater or overland flow. Flint (2006) also found similar high summer concentrations of dissolved N_2O in streams near the Lamprey River Watershed, indicating that these high concentrations are possibly not unique.

Effects of pH: Soil pH has been shown to affect the relative amounts of N_2O and N_2 produced during denitrification (Firestone et al., 1980) as microbial activity (and thus denitrification) is inhibited in very acidic soils ($pH < 5.0$) and is only carried out by microbes that have been able to adapt to harsh conditions (Parkin et al., 1985). In this study, no relationship was found between overall median pH and the concentration of dissolved N_2O. However, significant relationships were found for four of the sixteen streams though three of these relationships were positive and one was negative. Thus, there appears to be no consistent effect of stream pH on in-stream concentrations of dissolved N_2O in the LRW.

The lack of consistent trends found in this study may be because the range of pH values was not large enough to yield an effect on the end-products of denitrification, or because the inputs of N_2O from other sources such as groundwater and overland flow overshadowed any in-stream effect. Further, controlled laboratory experiments that have shown the effect of pH on the production of N_2O do not take into account these other inputs of N_2O or the fluid nature of streams. It is possible that the trends seen in laboratory experiments cannot be seen in streams as the denitrifying microbes are not in direct contact with the overlying stream water for the same length of time. More field studies on the effect of pH on stream concentrations of dissolved N_2O are needed to fully understand the lack of consistent trends.

Effects of DOC: Denitrification may also be limited by carbon availability, as denitrifying bacteria require a carbon source for denitrification (Hefting et al., 2003; Groffman et al., 1998). In this study, a significant negative relationship was found between median stream concentrations of DOC and dissolved N_2O for all streams for all sample dates. This may have either been a direct effect of stream DOC concentration on denitrification, or may be an indirect result as streams with the highest concentrations of NO_3^-, generally had the lowest concentrations of DOC.

The negative relationship between DOC and dissolved N_2O may be a direct result of denitrification. For instance, the influence of DOC on N_2O production may affect the proportion of N_2O: N_2 produced. Garcia-Ruiz et al. (1998a) found that though stream sediment denitrification did not increase with the addition of DOC, the proportion of N_2O produced relative to N_2 was smaller. If this is the case, it is possible that the negative relationships found in this study are a result of this effect on denitrification end-products. Alternatively, this negative relationship may be a direct result of N_2O production driving down the total amount of DOC in the stream water as microbes use this carbon during the process of denitrification.

It is also possible that the negative relationship between DOC and dissolved N_2O may have occurred as an indirect result of other relationships. For instance, streams with the highest concentrations of NO_3^- in this study, generally had the lowest concentrations of DOC. A significant negative relationship was found between NO_3^- and DOC (R^2 = 0.72, $p < 0.001$), indicating that it is possible that the effect of in-stream concentration of

NO_3^- may be more important in driving the concentration of dissolved N_2O than the concentration of DOC. This is counter-intuitive as it has been shown that microbes require a carbon source for the process of denitrification to occur, indicating that the more carbon in a stream, the more potential for denitrification to occur. However, it has been shown that the influence of the in-stream concentration of DOC on the production and concentration of N_2O may be less important than the sediment organic matter and carbon content. For instance, Beaulieu et al. (2009) did not find a relationship between sediment N_2O production and in-stream DOC concentrations, though positive relationships with sediment organic matter and carbon content were found. This may have occurred because the carbon measured in in-stream DOC concentrations may not be available to denitrifying bacteria, as DOC concentrations lump multiple types of organic molecules and does not differentiate by quality. A study by Inwood et al. (2007) suggests that it may be the quality of the DOC and not the quantity that affects the rates of denitrification in stream sediment. More studies on the effects of both the quantity and quality of DOC on denitrification are needed to understand these relationships.

Effects of Cl^-: No studies found have examined the relationship between the concentration of Cl^- and the concentration of dissolved N_2O in streams. However, studies have linked the concentration of Cl^- to the predominant land use of the watershed particularly in the Northeast and Midwest. Salts used as de-icing compounds in the winter have been shown to lead to an increase in the Cl^- concentration of nearby rivers and streams. Streams in watersheds with suburban to urban land uses (areas with a greater percentage of impervious surfaces) have been shown to have a higher concentration of Cl^- than streams in more rural watersheds (Allan and Castillo, 2007).

In this study, LMLB and L1 have the highest percentage of urban land use within their sub-watersheds (30.6% and 15.6% respectively) of all sampled streams, and as such, also have the highest concentration of Cl^-. The significant positive relationship between Cl^- concentration and dissolved N_2O may result from land use within the sub-watershed, though more studies are needed to fully understand this relationship.

Small scale spatial trends in dissolved N_2O on L1

Most studies on N_2O in rivers have been completed on large rivers, taking measurements of dissolved N_2O and fluxes of N_2O kilometers apart on a quarterly or monthly sampling schedule. McMahon and Dennehy (1998), for instance measured N_2O emissions and dissolved N_2O concentration at nine locations along the South Platte River, Colorado. The study reach was approximately 733-km long and sample sites were located 20-60 km from each other on three sampling dates throughout the year. It is thought that studies such as this may either over or under-estimate the actual flux of N_2O, as it has been shown to vary both spatially and temporally (Laursen and Seitzinger, 2004; Reay, 2002).

In this study, one tributary of the Lamprey River (L1) was sampled intensively to try to understand small-scale spatial differences in the concentration of dissolved N_2O. A spatial pattern was easier to detect both graphically and through the increase in post-hoc Tukey differences between sites on sample dates with discharge below 1 cfs. On these dates, sites 250-625 were consistently significantly different than the other sites, indicating this area may either experience increased denitrification or may receive inputs from groundwater or overland flow that contains dissolved N_2O.

The controls on the rates and end-products of denitrification such as NO_3^-, DOC, and pH may also cause differences in dissolved N_2O in L1. However, significant positive relationships between dissolved N_2O and NO_3^- concentration were only found for four of the eleven sample dates, indicating that although the concentration of NO_3^- may influence the concentration of dissolved N_2O at certain times of the year, it is likely not the only factor. Similarly, the negative relationship between DOC and dissolved N_2O was only found for two of the eleven sample dates. This may have occurred because of the overall low amount of DOC in L1. pH was found to have a significant negative relationship with dissolved N_2O. This may have an influence on the end-products of denitrification as low pH has been shown to produce relatively higher ratios of N_2O: N_2 (DiFranco, Chapter 2; Firestone et al., 1980), and could result in higher concentrations of dissolved N_2O. More studies over a larger range of pH are necessary to fully understand the influence of pH on dissolved N_2O in riverine systems.

Spatial differences in dissolved N_2O may also be attributed to external inputs of N_2O from groundwater or overland flow. A study by Reay et al. (2002) on emissions of N_2O and concentrations of dissolved N_2O from agricultural drainage waters was the only study found focusing on a small spatial scale. This study showed strong spatial variation in both concentrations of dissolved N_2O and in emission of N_2O within a 300-m study reach (sampling every 50-m). This variation was thought to be attributed to external inputs of dissolved N_2O from the surrounding agricultural fields, and not related to in-stream processing of NO_3^- through denitrification. As such, it is possible that spatial differences in L1 may be due to external inputs of dissolved N_2O somewhere near site 250.

Though significant differences between sites along L1 were found for most sample dates, these differences represent a variation of no more than 1 μg N_2O-N/L. Much larger differences in the concentration of dissolved N_2O were found between sample dates in L1. Further, within the same sample month, dissolved N_2O was found to vary up to approximately 3 μg N_2O-N/L, indicating the overall date of sampling may result in a larger variation in dissolved N_2O than small scale spatial differences along the length of the stream. Similar results were obtained for the Lamprey River watershed as a whole, where spatial variability was much less than the consistent temporal variability shown among all the study sites.

Diel trends in dissolved N_2O in L1

Few studies have measured diel differences in dissolved N_2O in rivers and those that have, have yielded different results. Laursen and Setizinger (2004) found N_2O flux to be higher at night than during the day possibly due to changes in pH or dissolved O_2. In contrast, Harrison et al. (2005) noted a decline in the concentration of dissolved N_2O at night over a two-day study in the summer. This decline was thought to be related to decline of both the concentration of dissolved oxygen and the concentration of NO_3^-. In this study, significant differences were only found between two sample times for one site on one sample date (6:00 and 18:00, March 2008), indicating that overall, no true diel variability in dissolved N_2O exists for L1 on the sample dates. Though diel trends have been noted in other streams (Harrison et al., 2005; Laursen and Seitzinger, 2004), a study by Beaulieu et al. (In Prep) of 72 headwater streams as part of the Lotic Intersite Nitrogen eXperiment (LINX II), noted diel patterns in only two streams indicating that diel differences may be the exception rather than the rule.

Conclusion

Based on the percent saturation of dissolved N_2O, it is likely that most of the streams in the Lamprey River Watershed are a net source of N_2O to the atmosphere at most times throughout the year. Though trends between dissolved N_2O and discharge, NO_3^-, DOC, Cl^-, and pH could be found, many relationships were weak or inconsistent throughout the year. As such, it is possible that N_2O is not only being produced within the streams themselves, but is entering the streams via groundwater and surface runoff. More studies are needed to identify potential sources of dissolved N_2O to streams in the Lamprey River Watershed.

Small scale spatial trends along L1 indicate that estimates of N_2O in streams may either be under or over-estimated when sampling at one location along the length of a stream on one sample date. These spatial trends may be due to internal production or external inputs of N_2O or a combination of both. However, variation along the length of the stream was smaller than variation between sample dates indicating that date sampled may be more important than sampling location along a stream. Further, in the Lamprey River Watershed, diel differences in dissolved N_2O were not found overall indicating that time of sampling does not influence the concentration of dissolved N_2O as has been found in other studies.

CHAPTER 2

CONTROLS ON THE RATES AND END-PRODUCTS OF DENITRIFICATION IN STREAMBED SEDIMENT

ABSTRACT

Denitrification in streams is an important process as it represents a permanent loss of NO_3^- from the system in the form of N_2O and N_2 gas. An understanding of the controls on the rates and end-products of denitrification is important as N_2O is a potent greenhouse gas. Denitrification enzyme activity (DEAs) were used to measure denitrification rates and the ratio of N_2O and N_2 in streambed sediment in a controlled laboratory setting under conditions of low, medium and high concentrations of NO_3^- and low or neutral levels of pH. Denitrification rates ranged from 0.017 μg N/g soil/hour in the low NO_3^-, acidic treatment to 0.262 μg N/g soil/hour in the medium NO_3^-, neutral treatment. This variation was explained by both the concentration of NO_3^- and the level of pH. The molar ratio of N_2O:N_2 ranged from 0.111 to 3.826 in the low NO_3^-, neutral treatment and the high NO_3^-, acidic treatment respectively. This ratio is much higher than the ratio measured in whole-stream experiments across North America, suggesting that molar ratios derived from slurry experiments must be used with considerable caution when extrapolating to whole-system rates.

INTRODUCTION

Background

Over the last century, anthropogenic activities such as the burning of fossil fuels and increased fertilizer application have greatly increased the input of nitrogen to both terrestrial and aquatic systems (Peters et al., 2005). High levels of nitrate in freshwater systems can lead to human health effects such as methemoglobinemia, also known as blue baby syndrome, and environmental effects such as eutrophication in receiving coastal waters (Peters et al., 2005; Vitousek et al., 1997). An understanding of the natural processes of NO_3^- removal in streams (such as biological assimilation and denitrification) is therefore important to determine the ability of these streams to handle increasing nitrogen loads (Beaulieu et al., In Prep; Wall et al., 2005).

Denitrification in streambed sediments is particularly important as it represents a permanent removal of NO_3^- from the system in the form of N_2 or N_2O gas. In streambed sediment, denitrification typically occurs in the top 2-5 cm where conditions are generally anoxic, and carbon and NO_3^- are readily available (Wall et al., 2005). Though conditions in which denitrification occurs have been the focus of many studies (Beaulieu et al., In Prep; Ullah and Zinati, 2006; Groffman et al., 1998), the actual controls on which end-product of denitrification (N_2 or N_2O) is favored in an area are not fully understood.

Overall, it is more energy efficient for microbes to reduce nitrate before N_2O, indicating that in areas with high loads of nitrate, more N_2O will be produced than N_2 as there is an ample supply of nitrate (Ullah and Zinati, 2006; Hefting et al., 2003). In fact, it has been shown that denitrifying microbes in riparian soils exposed to long-term loads of nitrate may actually lose the ability (and in fact the enzyme) to completely reduce nitrate to N_2 (Hefting et al., 2003). Though the overall amount of denitrification may not increase in these soils, the relative amount of N_2O produced during denitrification compared to other soils that have not been continuously loaded with nitrate should, and have been shown to, increase (Ullah and Zinati, 2006; Hefting et al., 2003).

Soil pH has been shown to affect the relative amounts of N_2O and N_2 produced during denitrification. Microbial activity (and thus denitrification) is inhibited in very acidic soils (pH < 5.0) and is only carried out by microbes that have been able to adapt to harsh conditions (Parkin et al., 1985). Despite this potential for microbial inhibition, N_2O appears to be the favored end product of denitrification in soils with a pH below 7.0, while basic to neutral soils (pH 7.0-8.0) favor the production of N_2 (Yamulki et al., 1997).

These trends may be synergistic effects of NO_3^- availability and pH. For instance, Firestone et al. (1980) found N_2O to be the favored end-product in more acidic soils that also had high concentrations of nitrate, but did not find a significant effect of pH in lower nitrate soils. More research is needed to tease apart interactions between variables that may control the production of N_2O to determine the true effect of each variable, and to allow a larger scale assessment of areas where excessive N_2O production may be taking place.

Project Goals, Objectives, and Hypotheses

The goal of this study was to examine the controls on the production of N_2O in a controlled laboratory experiment using streambed sediment from one stream in the Lamprey River Watershed. The main objective was to understand the influence of NO_3^-

concentration and pH on the end products of denitrification. It was hypothesized that sediment amended with high concentrations of NO_3^- and exposed to low pH conditions would have a higher $N_2O:N_2$ and higher denitrification rates than sediments amended with other treatments, as these trends have been seen in soils.

MATERIALS AND METHODS

Study Site

For this experiment, streambed sediment for denitrification assays were collected in a predominantly sandy reach of L1 just upstream of the culvert at site -5 on July 24 and 25, 2008 (DiFranco, Chapter 1). Weather and discharge conditions for the two days were similar.

Field Procedures

Sediment Collection for N_2O Experiment

Sediments (approximately 15 ccs) were collected using a 60-mL syringe with the tip removed and immediately placed in small Mason jars for transport back to the lab. To ensure the sediment did not dry out before treatment, a small amount of stream water was added to the jars.

Lab Procedures

Experimental Design

The main objective of this experiment was to understand the influence of NO_3^- concentration and pH on the end products of denitrification. Sixty sediment samples (thirty per consecutive day) from the same location on L1 were amended with a low (0.004 mg N/L), medium (1 mg N/L), or high concentration of NO_3^- (40 mg N/L) (20 reps each, 10 per day), and of those, half were amended with a dilute solution of 2 N HCl to achieve a pH between 4 and 5 (Figure 2-1). A time series experiment was run prior to this experiment (June 2008) to ensure NO_3^- would not be limiting to denitrification.

Figure 2-1: Experimental Set-up for N_2O Experiment (60 total reps)

	Low NO_3^- (0.004 mg N/L)	**Medium NO_3^- (1 mg N/L)**	**High NO_3^- (40 mg N/L)**
Low pH (4-5)	5-Acetylene 5-No Acetylene	5-Acetylene 5-No Acetylene	5-Acetylene 5-No Acetylene
Neutral pH (7)	5-Acetylene 5-No Acetylene	5-Acetylene 5-No Acetylene	5-Acetylene 5-No Acetylene

Experimental Procedures:

Denitrification enzyme activity (DEA) procedures were amended from those described in Groffman et al. (1999). A NO_3^- stock solution (0.6061g/mL) was used to create a low, medium, and high solution of NO_3^- (0.004 mg N/L, 1 mg N/L, and 40 mg N/L). A solution of dextrose (to ensure carbon would not be a limiting factor in denitrification) and chloramphenicol (to inhibit further microbial production), and a solution of media (containing major nutrients, vitamins, and trace metals, made according to Caron, 1993) were made to ensure the bacteria were not limited by these factors. A solution of 2N HCl was made to allow acidic conditions within certain jars to be attained. All equipment, solutions, and sediment samples were put into an anaerobic chamber overnight to become anoxic.

Once anoxic conditions had been reached, the solutions were added to the 30 jars/day. To 1 L of DI water, 1 mL of mixed media solution and 0.4 mL of the dextrose/chloramphenicol solution were added. In turn, 0.4 µL, 10 µL, and 400 µL of the NO_3^- stock solution were added to the DI solution to create solutions with low, medium, and high concentrations of NO_3^-. The jar containing the DI solution was rinsed with DI water between preparations of solutions of the different levels of NO_3^-. 75 mL of the three DI solutions were added to ten jars each, and of those, five were amended with 100 μL of 2N HCl to create conditions between pH 4-5.

The jars were capped and then removed from the anaerobic chamber. Half of the jars for each treatment for each day were amended with 10 mL of H_2SO_4-purified acetylene to prevent the reduction of N_2O to N_2. Gas samples were taken at 30 and 90 minutes via a 5 mL syringe from a rubber stopper. 5 mL of helium was added to replace the gas removed from the headspace. Gas samples were analyzed for N_2O by gas chromatography (DiFranco, Chapter 1) with the addition of an N_2O standard of 100 ppmv.

To determine sediment weight, the pre-weighed jars were dried overnight in an oven and re-weighed when completely dry.

Calculations

It was necessary to first calculate the concentration of N_2O in the headspace. The following equation was used (from Holland et al., 1999):

$$\mathbf{C_m = (C_v \times M \times P)/(R \times T)}$$

where C_m is the mass/volume concentration (μg N_2O –N/L), C_v is the volume/volume concentration (from the GC in ppmv), M is the molecular weight of the species (28 μg N_2O –N/L), P is the barometric pressure of the anaerobic chamber (atm), R is the universal gas constant (0.0821 L atm K mole), and T is the temperature of the anaerobic chamber (K). From this, the rate of denitrification (μg N kg $soil^{-1}$ h^{-1}) can be determined using the following equation (from Groffman et al., 1999):

$$\mathbf{DR = [C_{90} \times H) - (C_{30} \times H)]/(D \times T)}$$

where DR is the denitrification rate (μg N kg $soil^{-1}$ h^{-1}), C_{30} is the N_2O concentration at 30 minutes (μg N_2O-N/L), C_{90} is the N_2O concentration at 90 minutes (μg N_2O-N/L), H is the flask headspace volume (L), D is dry soil weight (g), and T is the total duration of the incubation (hours).

To determine the relative proportion of N_2O and N_2 produced by each treatment, it was first necessary to subtract the average DR of the soil not amended with acetylene from the average DR of the soil amended with acetylene. The average DR for N_2O was then divided by the average DR for N_2 to produce the ratio $N_2O:N_2$ for each treatment.

Statistical Analysis

To determine significant differences in denitrification rates based on treatment, a three-way univariate ANOVA with post-hoc Tukey for NO_3^- level was run using SPSS 17.0. The effect of "day" on denitrification rates was also determined using the same method.

RESULTS

Total rates of denitrification ranged from 0.017 μg N/g soil/hour in the low NO_3^-, acidic treatment to 0.262 μg N/g soil/hour in the medium NO_3^-, neutral treatment (Table 2-1, Figure 2-2).). $N_2O:N_2$ ranged from 0.111 to 3.826 in the low NO_3^-, neutral treatment and the high NO_3^-, acidic treatment respectively (Table 2-1, Figure 2-3). Denitrification rates for individual jars can be found in Appendix H.

Table 2-1: Total Rates of Denitrification, N_2O and N_2 production, and N_2O: N_2 for all treatments

Treatment	**Total Denitrification Rates (μg N/g soil/hour)**	**N_2O (μg N/g soil/hour)**	**N_2 (μg N/g soil/hour)**	**N_2O:N_2**
Low NO_3^- - Acidic	0.017	0.008 ± 0.006	0.009 ±0.015	0.872
Low NO_3^- - Neutral	0.022	0.002 ± 0.003	0.020 ± 0.012	0.111
Medium NO_3^- - Acidic	0.078	0.061 ± 0.060	0.017 ± 0.091	3.673
Medium NO_3^- - Neutral	0.262	0.028 ± 0.036	0.234 ± 0.105	0.119
High NO_3^- - Acidic	0.095	0.075 ± 0.068	0.020 ± 0.078	3.826
High NO_3^- - Neutral	0.229	0.084 ± 0.091	0.145 ± 0.198	0.581

Figure 2-2: Total Denitrification Rates and Relative Proportion of N_2O: N_2 for all treatments

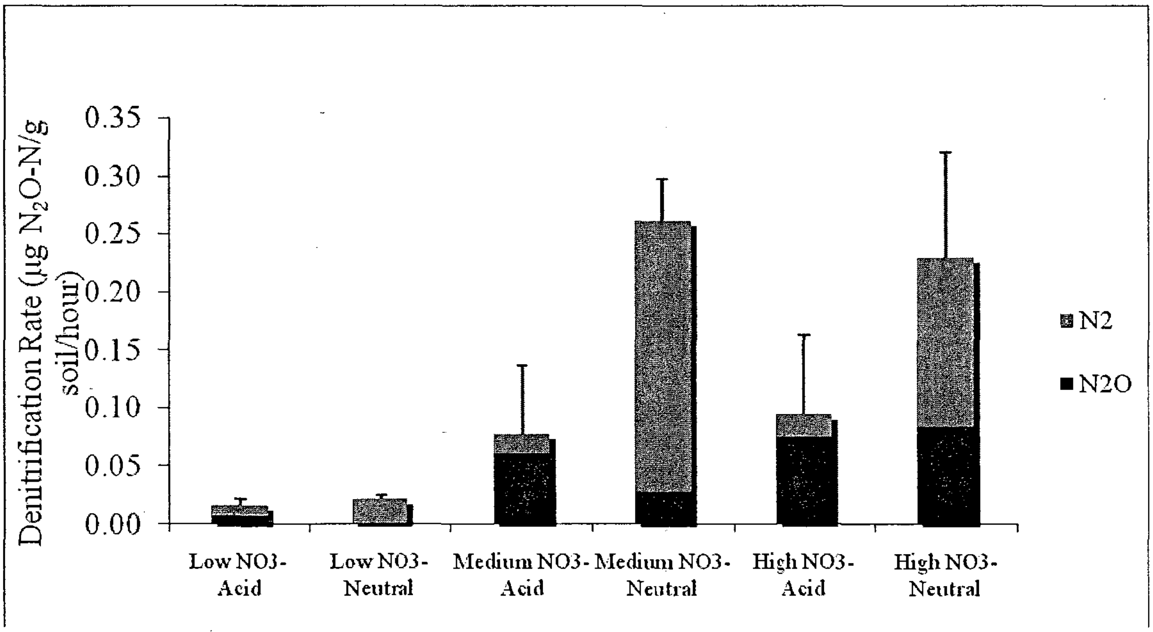
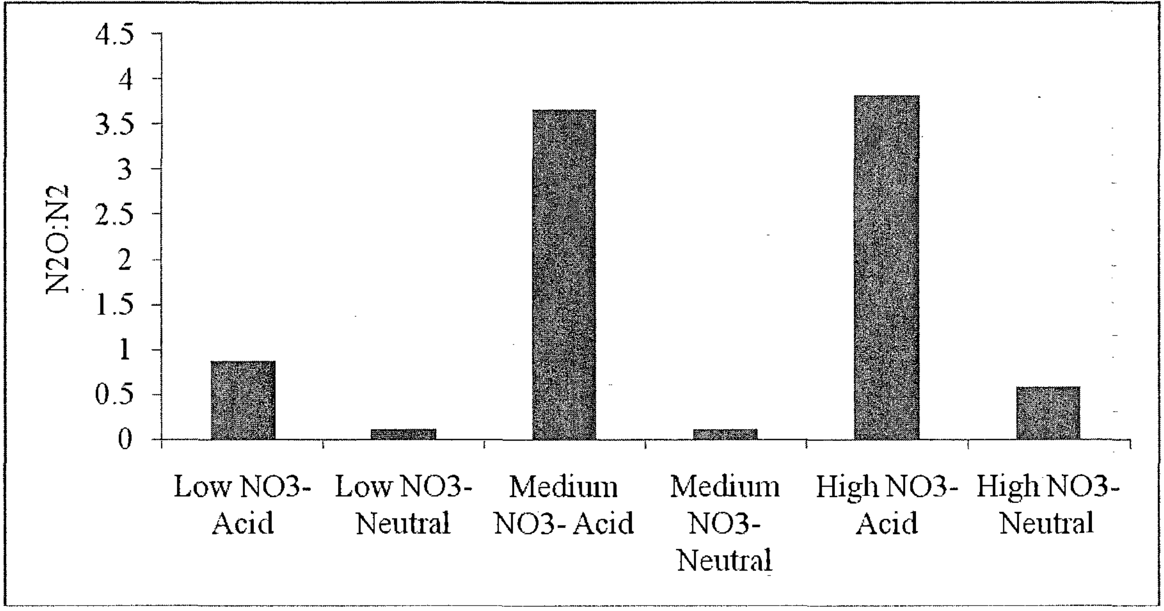


Figure 2-3: Ratio of N_2O:N_2 for all treatments (a ratio > 1 indicates N_2O is the dominant end-product of denitrification)



The percent of N_2O and N_2 of the total denitrification rate ranged from 9 – 79% (N_2O) and 21 – 91% for N_2 for all treatments (Figures 2-4 and 2-5). The percent of N_2O and N_2 of the total denitrification rate for neutral treatments ranged from 9 – 37% (N_2O) and 63 – 91% for N_2 (Figure 2-4). The percent of N_2O and N_2 of the total denitrification rate for the acidic treatments ranged from 47 – 79% (N_2O) and 21 – 53% for N_2 (Figure 2-5).

Figure 2-4: Percent of N_2O and N_2 of the total denitrification rates for all neutral treatments

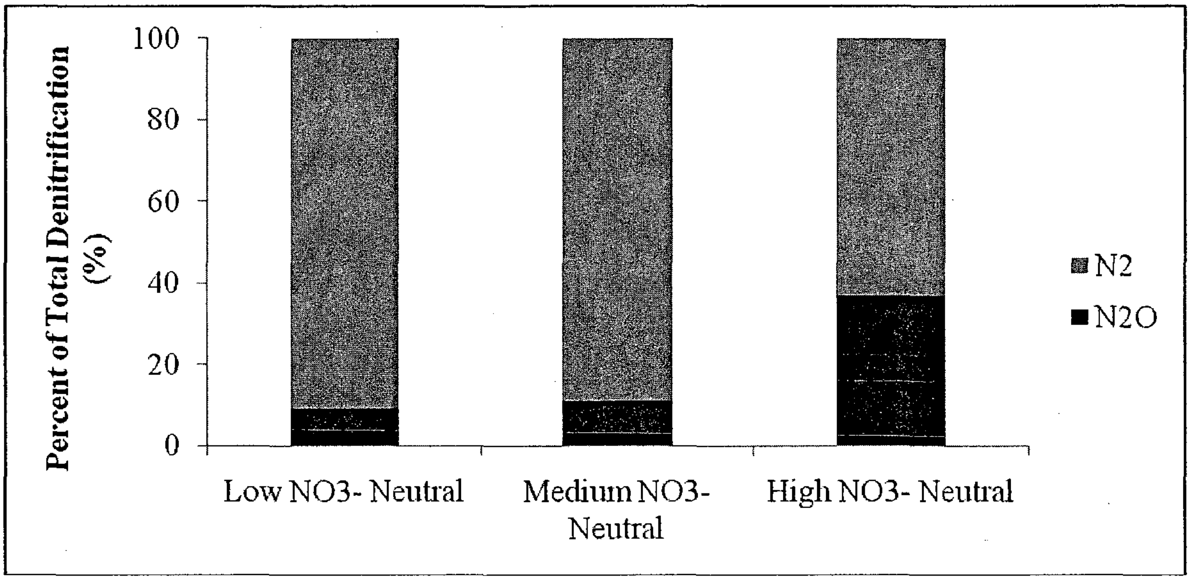
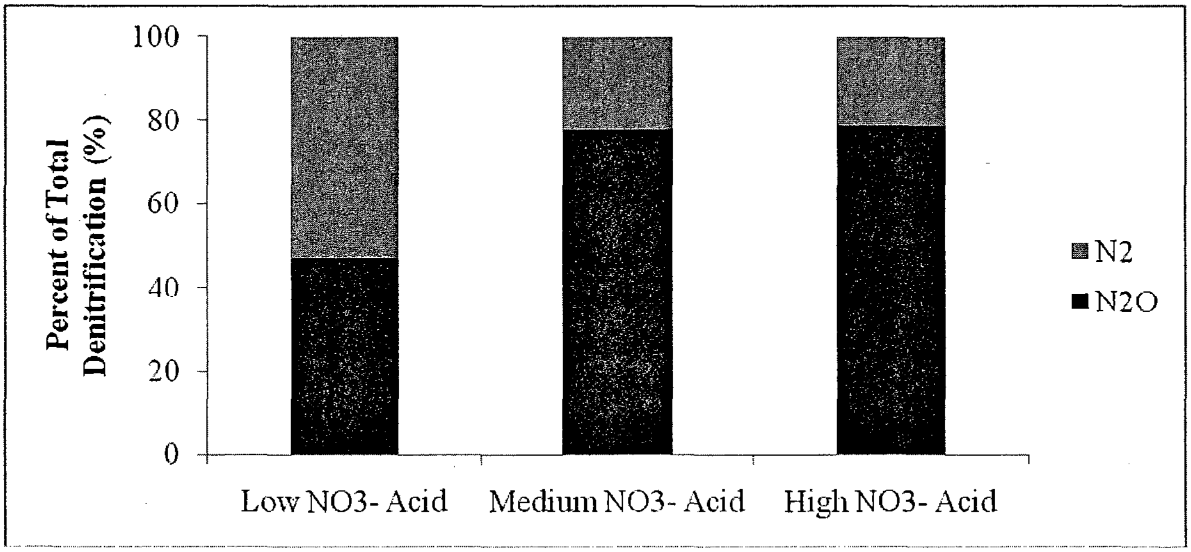


Figure 2-5: Percent of N_2O and N_2 of the total denitrification rates for all acidic treatments



Significant differences were found in denitrification rates based on the presence or absence of acetylene ($p = 0.001$), the level of NO_3^- ($p < 0.001$), and the level of pH ($p = 0.027$) accepting the hypothesis of difference. A post-hoc Tukey test showed significant differences in denitrification rates between the low and medium NO_3^-

treatment ($p = 0.002$) and between the low and high NO_3^- treatment ($p < 0.001$), but not between the medium and high NO_3^- treatments ($p = 0.859$). Significant differences were also found in the interaction between acetylene and pH level ($p = 0.008$), but not between any other interaction terms. No significant differences were found between similar treatments based on day ($p = 0.994$).

DISCUSSION

An understanding of the controls on the rates and the end-products of denitrification is important to determine conditions in which efficient removal of NO_3^- as well as the excessive production of the greenhouse gas N_2O may occur.

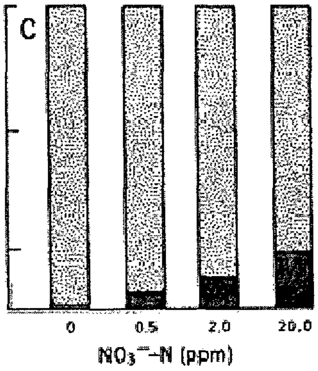
The rates of denitrification in this study (0.017 μg N/g soil/hour - 0.262 μg N/g soil/hour) fall within the range of values reported in a similar study on denitrification in river sediments. Wall et al. (2005) found sediment denitrification rates to range from 0 to 0.270 μg N/g soil/hour in riverine sites in tributaries of the Mississippi River. Garcia-Ruiz (1998) examined denitrification rates and N_2O production at 50 sites throughout northern England using the acetylene-block technique with river sediment and stream water. They found denitrification rates ranging from 0.00007 μg N/g soil/hour - 3.64 μg N/g soil/hour from a variety of different sediment types. In a study by Drury et al. (1992), sandy loam soils (most similar to this study) were shown to have denitrification rates up to 0.280 μg N/g soil/hour.

Effects of NO_3^- level on denitrification rates and end-products

Many field studies have linked the concentration of NO_3^- in the stream water, ground water, and soil pores to rates of denitrification and N_2O emission (Beaulieu et al., In Prep; Inwood et al., 2007; Wall et al., 2005; Hefting et al., 2003). In this study, denitrification rates from the sediments amended with medium and high concentrations of NO_3^- were significantly different from those amended with low concentrations of NO_3, though not significantly different from each other. This may have occurred because of the abundant supply of NO_3^- in those jars, providing the microbes with more medium to denitrify.

Overall, the magnitude of the percent of N_2O of the total denitrification rate for low, medium, and high levels of NO_3^- found in this study was similar to the findings of Firestone et al. (1980), indicating that this trend is not only found in soils, but also in streambed sediment. Firestone et al. (1980) found N_2O to be produced as an end-product of denitrification more often in soils with medium to high levels of NO_3^- than in soils with low NO_3^- under neutral conditions (Figure 2-6). In this study, the percent of N_2O of the total denitrification rate was similar for the low and medium NO_3^- treatments and almost three times as high for the high NO_3^- treatments under neutral conditions (Figure 2-4). If this experiment was conducted over a longer period of time, it is possible that the ratio for the low and medium levels of NO_3^- would decrease farther as the microbes would have exhausted the NO_3^- present in the solution and would then begin to reduce N_2O.

Figure 2-6: Percent of N_2O produced (in black) of total denitrification (100%) under different concentrations of NO_3^- (adapted from Firestone et al., 1980)



Effects of level of pH on denitrification rates and end-products

Previous work in terrestrial soils has shown that denitrification occurs at a slower rate in acidic soils than in neutral soils, and that the optimum range for denitrification is a neutral pH between 6 and 8 (Yamulki et al., 1997; Parkin et al., 1985). Results from this study show that the pH level of the solution had a significant effect on overall denitrification rates of streambed sediments (Table 2-1) with lower denitrification rates in sediments exposed to acidic conditions for each level of NO_3^-.

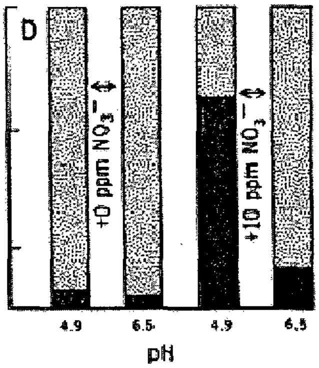
This may have occurred because denitrifying bacteria may be inhibited in the newly introduced acidic environment. Klemedtsson et al. (1977) concluded that raising the pH from 3.5 to 6.5 stimulated denitrification in peat soils. Though it may be possible that some denitrifying bacteria can adapt to long-term exposure to acidic conditions, Parkin et al. (1985) found that soils exposed to years of acidic conditions from fertilizer application had lower rates of denitrification when compared to nearby neutral soils, even though they still represented a significant loss of N from the system.

The relative proportion of N_2O:N_2 produced during denitrification may also be affected by acidity. In this study, the N_2O:N_2 of sediment exposed to acidic conditions was higher than those exposed to neutral conditions for all levels of NO_3^- (Table 2-1, Figure 2-3 – 2-5). This may have occurred because despite the potential for acidic soils to inhibit microbial activity, previous studies from terrestrial soils have shown that the ratio of N_2O:N_2 increases as the pH decreases (Yamulki et al., 1997).

Effect of the interaction of NO_3^- and pH on denitrification rates and end-products

The interaction between the effect of NO_3^- and pH on N_2O may also explain the differences in denitrification rates and N_2O production in stream sediment. For instance, for some treatments in this study, the ratio of N_2O:N_2 exceeded 3, indicating that 3 times more N_2O was produced than N_2 under the same conditions. This may be due to an interaction between pH conditions and the concentration of NO_3^-. Firestone et al. (1980) found N_2O to be the favored end-product in more acidic soils that also had high concentrations of nitrate, but did not find a significant effect of pH in lower nitrate soils (Figure 2-7). However, in this study, the percent of N_2O of the total denitrification rate was higher under acidic conditions than under neutral conditions for all levels of NO_3^- (Figure 2-5). As noted previously, it has been shown that it is more energy efficient for microbes to reduce NO_3^- before N_2O (Ullah and Zinati, 2006). If this is the case, bacteria in streambed sediment may choose to reduce NO_3^- before N_2O to conserve energy in a stressful, acidic environment.

Figure 2-7: Percent of N_2O produced (in black) of total denitrification (100%) under different concentrations of NO_3^- and levels of pH (adapted from Firestone et al., 1980)



Implications and limitations of findings

Overall, it has been shown that approximately 1 – 6% of denitrified NO_3^- is released as N_2O in aquatic systems (Beaulieu et al., 2009; Seitzinger, 1988). In this study, N_2O ranged from 9 – 79% depending on treatment. Similarly, Firestone et al. (1980) found N_2O production rates ranging from approximately 1 – 70% depending on treatment. These discrepancies between the field and lab data indicate that laboratory

DEAs may overestimate N_2O production rates via denitrification. This may have occurred because laboratory experiments allow for longer interaction between the microbes in the sediment and the NO_3^- in the solution. Further, this experiment used high NO_3^- concentrations (40 mg N/L) which may not have been found in the field.

Despite the potential for overestimation, overall trends on the controls of N_2O production may be similar for both laboratory and field conditions. For instance, Seitzinger (1988) showed that N_2O production rates could be as high as 6% of total denitrification in streams under conditions of high NO_3^- concentration and low pH. Though the overall magnitude of the percent of N_2O produced during denitrification is smaller than those found in laboratory experiments, the trend with NO_3^- concentration and pH are similar.

These findings are important to understand the controls on both denitrification and denitrification end-products, particularly in the case of N_2O production. The results from this study suggest a link between NO_3^- concentration, pH level, denitrification, and N_2O production in sandy streambed sediment. This study allowed for the removal of other potentially limiting factors such as carbon availability and oxygen level, permitting the full effect of NO_3^- concentration and pH level to be examined. Comparison with field data of N_2O emissions and denitrification rates in a variety of environments will further our knowledge of the combined effects of NO_3^- concentration and pH level.

The major limitation of this study is that N_2 could not be measured directly, but was found as the difference between the N_2O produced in the acetylene-treated sediments and the non-acetylene-treated sediments. In other words, N_2 and N_2O were not measured directly from the same jar. This may have skewed the results of the ratio of N_2O:N_2 slightly, as standard error could not be produced.

LIST OF REFERENCES

Aber, J. and Magill, A. 2004. Chronic nitrogen additions at the Harvard Forest (USA): the first 15 years of a nitrogen saturation experiment, *Forest Ecology and Management*, 196: 1-5.

Allan, J.D. and Castillo, M. 2007. Stream Ecology: Structure and Function of Running Waters 259-285.

Bange W.H. 2000. It's not a gas. *Nature* 408: 301–302.

Barnard, R. and Leadley, P. 2005. Global change, nitrification, and denitrification: a review, *Global Biogeochemical Cycles* 19: GB1007.

Bauer, E., Petoukhov, V., Ganopolski, A., and Eliseev, A. 2007. Climatic response to anthropogenic sulphate aerosols versus well-mixed greenhouse gases from 1850 to 2000 AD in Climber-2, *Tellus B* (Online Early Articles): 1-15.

Beaulieu, J., Arango, C., and Tank, J. 2009. The effects of season and agriculture on nitrous oxide production in headwater streams, *Journal of Environmental Quality*: 38: 637-646.

Beaulieu, J., Tank, J., Hamilton, S., Mulholland, P., Helton, A., Poole, G., Hall, R., Peterson, B., Ashkenas, L., Cooper, L., Dahm, C., Dodds, W., Findlay, S., Gregory, S., Grimm, N., Johnson, S., McDowell., W., Meyer, J., Valett, H.M., Webster, J., Arango, C., Bernot, M., Burgin, A., Crenshaw, C., Johnson, L., Niederlehner, B.R., O'Brien, J., Potter, J., Sheibley, R., Sobota, D., Thomas, S. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. In Prep.

Bouwman, A.F., Van der Hoek, and Olivier, J.G.J. 1995. Uncertainties in the global source distribution of nitrous oxide, *Journal of Geophysical Research* 100 (D2): 2785-2800.

Clough, T., Bertram, J., Sherlock, R., Leonard, R., and Nowicki, B. 2006. Comparison of measured and EF5-r-derived N_2O fluxes from a spring-fed river, *Global Change Biology* 12: 477-488.

Cole, J., and Caraco, N. 2001. Emissions of nitrous oxide (N_2O) from a tidal, freshwater river, the Hudson River, New York, *Environmental Science and Technology* 35 (6): 991-996.

Crowley, T.J. 2000. Causes of climate change over the past 1000 years, *Science* 289 (5477): 270-277.

Drury, C., McKenney, D., and Findlay, W. 1992. Nitric-oxide and nitrous-oxide production from soil-water and oxygen effects, *Soil Science Society* 56 (3): 766-770.

EPA 2007. Climate Change, < http://www.epa.gov/climatechange >. Accessed 1/3/09.

Firestone, M., Firestone, R., and Tiedje, J. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. *Science* 208 (4445): 749-751.

Flint, S. 2006. Impacts of headwater wetlands on surface water quality in the Lamprey River Watershed, NH. UNH Master's Thesis.

Galloway, J., and Cowling, E. 2002. Reactive nitrogen and the world, *Ambio* 31 (2): 64-71.

Garnier, J., Cebron, A., Tallec, G., Billen, G., Sebilo, M., and Martinez, A. 2006. Nitrogen behaviour and nitrous oxide emission in the tidal Seine River estuary (France) as influenced by human activities in the upstream watershed, *Biogeochemistry* 77: 305–326.

Garcia-Ruiz, R., Pattinson, S., and Whitton, B. 1998. Denitrification in river sediments: relationship between process rate and properties of water and sediment, *Freshwater Biology* 39: 467-476.

Garcia-Ruiz, Pattison, S., and Whitton, B. 1998a. Denitrification and nitrous oxide production in the sediments of the Wiske, a lowland, eutrophic river, *Science of the Total Environment* 210: 307-320.

Groffman, P., Holland, E., Myrold, D., Robertson, G., and Zou, X. 1999. Denitrification, Standard Soil Methods for Long –Term Ecological Research, 272-288.

Groffman, P., Gold, A., and Jacinthe, P. 1998. Nitrous oxide production in riparian zones and groundwater, *Nutrient Cycling in Agroecosystems* 52: 179-186.

Harrision, J., Matson, P., and Fendorf, S. 2005. Effects of a diel oxygen cycle on nitrogen transformations in a eutrophied subtropical stream, *Aquatic Science* 67: 308-315.

Hefting, M., Bobbink, R., and de Caluwe, H. 2003. Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones, *Journal of Environmental Quality* 32: 1194-1203.

Inwood, S., Tank, J., and Bernot, M. 2007. Factors controlling sediment denitrification in Midwestern streams of varying land use, *Microbial Ecology* 53: 247-258.

IPCC, 2007. Climate Change 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning (eds.)].

Klemedtsson, L., Svensson, B.H., Lindberg, T., and Rosswall, T. 1977. The Use of Acetylene Inhibition of Nitrous Oxide Reductase in Quantifying Denitrification of Soils. *Swedish Journal of Agriculture* 7: 179-185.

Lamprey River Hydrologic Observatory 2009. Maps of the LRHO <http://www.wrrc.unh.edu/lrho/maps.htm>. Accessed 4/21/09.

Laursen, A., and Seitzinger, S., 2004. Diurnal patterns of denitrification, oxygen consumption and nitrous oxide production in rivers measured at the whole-reach scale, *Freshwater Biology* 49: 1448-1458.

Mann, M., Bradley, R., and Hughes, M. 1998. Global-scale temperature patterns and climate forcing over the past six centuries, *Nature* 392: 779-787.

McMahon, P., and Dennehy, K. 1998. N_2O emissions from a nitrogen-enriched river, *Environmental Science and Technology* 33: 21-25.

McSwiney, C., McDowell, W., and Keller, M. 2001. Distribution of nitrous oxide and regulators of its production across a tropical rainforest catena in the Luquillo Experimental Forest, Puerto Rico, *Biogeochemistry* 56: 265-286.

Mitchell, J., Johns, T., Gregory, J., and Tett, S. 1995. Climate response to increasing levels of greenhouse gases and sulphate aerosols, *Nature* 376: 501-504..

Mulholland, P., Valett, H., Webster, J., Thomas, S., Cooper, L., Hamilton, S., and Peterson, B. 2004. Stream denitrification and total nitrate uptake rates measured using a field N tracer addition approach. *Limnology and Oceanography* 49 (3): 809-820.

National Climatic Data Center 2009. NH Durham 2 SSW.

Parkin, T.B., Sexstone, A.J., and Tiedje, J.M. 1985. Adaptation of Denitrifying Populations of Low Soil pH. Applied and Environmental Microbiology 49 (5): 1053-1056.

Peters, N., Meybeck, M., and Chapman, D. 2005. Effects of Human Activities on Water Quality, *Encyclopedia of Hydrological Sciences.*

Reay, D., Smith, K., and Edwards, A. 2003. Nitrous oxide emission from agricultural drainage waters, *Global Change Biology* 9: 15-203.

Seitzinger, S., and Kroeze, C. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine environments, *Global Biogeochemical Cycles*, 12: 93-113.

Ullah, S., and Zinati, G. 2006. Denitrification and nitrous oxide emissions from riparian forest soils exposed to prolonged nitrogen runoff, *Biogeochemistry*, 81:253-267.

Vitousek, P. 1994. Beyond global warming: ecology and global change, *Ecology*, 75 (7):1861-1876.

Vitousek, P.M., Aber, J.D., Howarth, R.H., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. and Tilman, D.G. 1997. Human alteration of the global nitrogen cycle: source, and consequences, *Ecological Applications* 7: 737-750.

Wall, L., Tank, J., Royer, T., and Bernot, M. 2005. Spatial and temporal variability in sediment denitrification within an agriculturally influenced reservoir, *Biogeochemistry* 76: 85-111.

Wanninkhof, Mulholland, P., and Elwood, J., 1992. Gas exchange rates for a first-order stream determined with deliberate and natural tracers, *Water Resources Research* 26 (7): 1621-1630.

Wilcock, R., and Sorrell, B. 2008. Emissions of greenhouse gases CH4 and N2O from low-gradient streams in agriculturally developed catchments, *Water Air Soil Pollut* 188: 155-170.

Yamulki, S., Harrison, R., Goulding, K., and Webster, C. 1997. N2O, NO, and NO2 fluxes from a grassland: effect of soil pH, *Soil Biology Biochemistry*, 29 (8): 1199-1208.

APPENDIX A

Sample date, average dissolved N_2O with standard deviation, and average percent saturation with standard deviation for each stream sampled in the Lamprey River Watershed from March 2008 – February 2009

Site	**Date**	**Average Dissolved N_2O (µg N_2O-N/L)**	**Standard Deviation N_2O (µg N_2O-N/L)**	**Average Percent Saturation (%)**	**Standard Deviation Percent Saturation(%)**
L-10	3/11/08	0.82	0.03	113.12	3.52
L-10	4/8/2008	2.26	0.06	334.07	8.50
L-10	5/13/2008	0.53	0.03	101.88	5.04
L-10	6/10/2008	0.37	0.02	90.91	6.03
L-10	7/10/2008	0.32	0.06	78.94	14.70
L-10	8/12/2008	0.46	0.01	103.56	2.95
L-10	9/9/2008	0.69	0.05	151.57	11.11
L-10	10/14/2008	0.74	0.04	144.61	7.11
L-10	11/12/2008	0.74	0.04	119.46	5.73
L-10	12/16/2008	1.06	0.06	153.38	8.27
L-10	2/10/2009	1.25	0.05	171.80	7.54
L-8	3/11/08	0.82	0.03	112.92	4.30
L-8	4/8/2008	2.30	0.14	338.78	20.21
L-8	5/13/2008	0.51	0.02	99.49	4.47
L-8	6/10/2008	0.49	0.07	126.48	19.24
L-8	7/10/2008	0.43	0.11	108.36	27.35
L-8	8/12/2008	0.45	0.01	101.84	1.68
L-8	9/9/2008	0.68	0.05	152.00	10.46
L-8	10/14/2008	0.75	0.08	146.95	16.72
L-8	11/12/2008	0.74	0.03	121.23	5.32
L-8	12/16/2008	1.02	0.10	151.82	15.29
L-8	2/10/2009	1.32	0.02	179.14	2.67
L-6	3/11/08	1.00	0.07	137.90	9.08
L-6	4/8/2008	2.21	0.05	337.47	8.27
L-6	5/13/2008	0.55	0.03	111.69	6.80
L-6	6/10/2008	0.49	0.03	129.69	7.67
L-6	7/10/2008	2.71	0.19	705.20	49.55
L-6	8/12/2008	0.46	0.02	106.64	3.48
L-6	9/9/2008	0.65	0.03	147.51	6.60
L-6	10/14/2008	0.78	0.08	152.25	15.84
L-6	11/12/2008	0.69	0.06	115.96	9.30
L-6	12/16/2008	1.32	0.02	198.21	2.55

L-6	2/10/2009	1.53	0.12	207.51	15.83
L-5	3/11/08	0.94	0.08	129.75	10.76
L-5	4/8/2008	1.99	0.03	306.71	4.47
L-5	5/13/2008	0.46	0.02	95.63	4.32
L-5	6/10/2008	0.31	0.02	81.26	4.59
L-5	7/10/2008	0.22	0.01	58.54	2.68
L-5	8/12/2008	0.41	0.01	95.65	1.25
L-5	9/9/2008	0.60	0.02	139.76	4.54
L-5	10/14/2008	0.71	0.02	141.01	3.17
L-5	11/12/2008	0.72	0.02	124.94	4.01
L-5	12/16/2008	1.30	0.12	189.64	17.32
L-5	2/10/2009	1.70	0.04	231.68	5.56
L-4	3/11/08	0.79	0.05	108.41	6.37
L-4	4/8/2008	2.24	0.07	342.58	10.46
L-4	5/13/2008	0.49	0.03	99.16	5.18
L-4	6/10/2008	0.37	0.01	94.91	2.80
L-4	7/10/2008	0.24	0.06	61.17	14.91
L-4	8/12/2008	0.41	0.00	96.87	1.04
L-4	9/9/2008	0.62	0.03	144.99	7.05
L-4	10/14/2008	0.51	0.03	102.85	6.52
L-4	11/12/2008	0.61	0.04	104.16	6.70
L-4	12/16/2008	1.16	0.09	170.84	13.71
L-4	2/10/2009	1.68	0.08	228.26	10.90
L-LH	3/11/08	0.84	0.05	115.09	6.52
L-LH	4/8/2008	2.08	0.28	316.80	42.16
L-LH	5/13/2008	0.44	0.01	91.71	2.37
L-LH	6/10/2008	0.39	0.02	102.15	6.37
L-LH	7/10/2008	0.24	0.03	62.62	8.39
L-LH	8/12/2008	0.37	0.02	85.06	5.43
L-LH	9/9/2008	0.59	0.04	139.45	8.82
L-LH	10/14/2008	0.59	0.03	117.72	5.94
L-LH	11/12/2008	0.55	0.03	93.37	5.15
L-LH	12/16/2008	1.24	0.07	182.81	9.65
L-LH	2/10/2009	1.44	0.06	194.76	8.65
L-Main	3/11/08	0.83	0.00	112.21	0.46
L-Main	4/8/2008	2.16	0.09	329.95	14.01
L-Main	5/13/2008	0.42	0.02	88.72	3.24
L-Main	6/10/2008	0.29	0.02	77.87	5.88
L-Main	7/10/2008	1.79	0.22	468.23	58.33
L-Main	8/12/2008	0.37	0.01	82.31	3.36
L-Main	9/9/2008	0.63	0.01	148.98	3.06

L-Main	10/14/2008	0.57	0.04	112.52	6.96
L-Main	11/12/2008	0.52	0.05	88.62	8.45
L-Main	12/16/2008	1.19	0.07	173.22	10.56
L-Main	2/10/2009	1.22	0.12	165.57	16.26
L-Newmkt	3/11/08	0.78	0.06	104.92	8.73
L-Newmkt	4/8/2008	2.01	0.19	306.34	29.64
L-Newmkt	5/13/2008	0.39	0.01	82.89	2.45
L-Newmkt	6/10/2008	0.32	0.02	77.24	3.67
L-Newmkt	7/10/2008	1.33	0.08	347.09	21.28
L-Newmkt	8/12/2008	0.35	0.03	82.43	7.60
L-Newmkt	9/9/2008	0.63	0.03	147.58	7.45
L-Newmkt	10/14/2008	0.52	0.01	102.62	1.34
L-Newmkt	11/12/2008	0.46	0.04	81.17	7.06
L-Newmkt	12/16/2008	1.09	0.17	156.93	24.39
L-Newmkt	2/10/2009	1.34	0.03	183.35	3.82
L-MLB	3/11/08	1.03	0.21	148.67	29.61
L-MLB	4/8/2008	2.46	0.30	376.01	45.51
L-MLB	5/13/2008	0.73	0.02	148.05	4.23
L-MLB	6/10/2008	0.56	0.03	127.59	5.71
L-MLB	7/10/2008	0.41	0.04	92.32	8.58
L-MLB	8/12/2008	0.59	0.11	129.20	23.86
L-MLB	9/9/2008	0.91	0.03	213.56	6.32
L-MLB	10/14/2008	1.07	0.03	208.22	6.57
L-MLB	11/12/2008	1.10	0.10	189.00	17.19
L-MLB	12/16/2008	1.88	0.12	304.49	19.81
L-MLB	2/10/2009	1.42	0.05	215.34	7.74
L-1	3/11/08	1.37	0.02	190.83	3.16
L-1	4/8/2008	2.19	0.35	334.38	53.33
L-1	5/13/2008	0.75	0.03	152.45	5.69
L-1	6/10/2008	0.69	0.03	156.14	7.55
L-1	7/10/2008	0.56	0.01	125.55	2.90
L-1	8/12/2008	0.38	0.02	82.40	4.01

L-1	9/9/2008	0.84	0.07	196.12	15.34
L-1	10/14/2008	0.95	0.05	185.03	10.42
L-1	11/12/2008	0.99	0.08	163.53	12.60
L-1	12/16/2008	1.64	0.07	244.68	11.18
L-1	2/10/2009	1.70	0.09	239.38	13.12
L-LR	3/11/08	0.84	0.03	115.61	4.27
L-LR	4/8/2008	2.11	0.07	323.89	11.12
L-LR	5/13/2008	0.48	0.01	96.15	2.47
L-LR	6/10/2008	0.47	0.04	118.89	10.32
L-LR	7/10/2008	0.30	0.01	75.96	1.52
L-LR	8/12/2008	0.37	0.02	84.58	4.50
L-LR	9/9/2008	0.59	0.02	139.42	4.75
L-LR	10/14/2008	0.59	0.04	117.21	7.43
L-LR	11/12/2008	0.65	0.03	108.09	4.29
L-LR	12/16/2008	1.31	0.29	197.42	43.52
L-LR	2/10/2009	1.48	0.08	201.05	10.69
L-3	3/11/08	0.75	0.10	102.42	13.33
L-3	4/8/2008	2.15	0.06	343.33	10.22
L-3	5/13/2008	0.39	0.02	82.88	3.30
L-3	6/10/2008	0.33	0.00	79.48	1.12
L-3	7/10/2008	2.20	0.26	527.81	61.68
L-3	8/12/2008	0.39	0.01	90.60	1.37
L-3	9/9/2008	0.54	0.08	125.63	17.98
L-3	10/14/2008	0.49	0.02	97.77	3.98
L-3	11/12/2008	0.56	0.05	93.80	8.16
L-3	12/16/2008	1.15	0.04	167.59	5.49
L-3	2/10/2009	1.17	0.06	159.34	7.51
L-RB	3/11/08	0.80	0.04	111.57	5.20
L-RB	4/8/2008	2.34	0.08	345.50	12.17
L-RB	5/13/2008	0.46	0.01	87.54	1.12
L-RB	6/10/2008	0.32	0.03	79.83	7.01
L-RB	7/10/2008	0.19	0.06	45.77	14.26
L-RB	8/12/2008	0.44	0.05	98.54	12.27
L-RB	9/9/2008	0.67	0.02	147.54	3.79
L-RB	10/14/2008	0.56	0.02	108.39	4.62
L-RB	11/12/2008	0.61	0.03	97.85	4.82
L-RB	12/16/2008	0.80	0.11	120.54	16.18
L-RB	2/10/2009	1.08	0.03	146.83	3.81
L-5a	3/11/08	0.71	0.02	104.83	3.64
L-5a	4/8/2008	2.34	0.11	348.05	16.37
L-5a	5/13/2008	0.49	0.02	96.26	4.37

L-5a	6/10/2008	0.37	0.01	94.17	2.70
L-5a	7/10/2008	0.20	0.04	50.58	9.42
L-5a	8/12/2008	0.35	0.01	85.04	1.54
L-5a	9/9/2008	0.53	0.04	126.83	10.04
L-5a	10/14/2008	0.51	0.05	105.83	9.62
L-5a	11/12/2008	0.67	0.03	115.09	5.14
L-5a	12/16/2008	1.05	0.20	154.09	29.75
L-5a	2/10/2009	1.09	0.05	150.43	6.32
L-NB	3/11/08	1.10	0.19	152.75	25.80
L-NB	4/8/2008	2.21	0.07	331.76	10.80
L-NB	5/13/2008	0.57	0.01	113.27	1.88
L-NB	6/10/2008	0.36	0.01	92.31	3.20
L-NB	7/10/2008	0.40	0.02	99.74	6.05
L-NB	8/12/2008	0.44	0.02	101.82	4.10
L-NB	9/9/2008	0.59	0.03	134.69	7.92
L-NB	10/14/2008	0.61	0.03	120.81	6.21
L-NB	11/12/2008	0.63	0.03	102.21	4.80
L-NB	12/16/2008	1.07	0.09	159.22	13.65
L-NB	2/10/2009	1.03	0.01	139.39	1.74
L-Pawt	3/11/08	0.78	0.13	107.24	17.92
L-Pawt	4/8/2008	2.09	0.08	317.19	12.57
L-Pawt	5/13/2008	0.34	0.01	67.29	2.97
L-Pawt	6/10/2008	0.25	0.03	61.96	7.24
L-Pawt	7/10/2008	0.21	0.02	53.36	4.09
L-Pawt	8/12/2008	0.34	0.03	77.26	7.40
L-Pawt	9/9/2008	0.50	0.04	114.18	8.25
L-Pawt	10/14/2008	0.69	0.05	135.97	8.96
L-Pawt	11/12/2008	0.63	0.00	102.41	0.79
L-Pawt	12/16/2008	1.44	0.11	205.91	15.32
L-Pawt	2/10/2009	1.13	0.05	153.23	6.30

APPENDIX B

Sample date, modeled and measured discharge, and area-weighted discharge for each stream sampled in the Lamprey River Watershed from March 2008 – February 2009 (italics indicates actual measured discharge)

Site	**Date**	**Discharge (cfs)**	**Area-Weighted Discharge (cfs/km^2)**
L-10	3/11/08	2.24	0.15
L-10	4/8/2008	2.09	0.14
L-10	5/13/2008	1.62	0.11
L-10	6/10/2008	1.38	0.09
L-10	7/10/2008	1.26	0.09
L-10	8/12/2008	1.95	0.13
L-10	9/9/2008	2.09	0.14
L-10	10/14/2008	1.62	0.11
L-10	11/12/2008	1.66	0.11
L-10	12/16/2008	2.09	0.14
L-10	2/10/2009	1.66	0.11
L-8	3/11/08	38.02	0.47
L-8	4/8/2008	30.20	0.38
L-8	5/13/2008	9.55	0.12
L-8	6/10/2008	4.47	0.06
L-8	7/10/2008	2.82	0.04
L-8	8/12/2008	20.42	0.25
L-8	9/9/2008	27.54	0.34
L-8	10/14/2008	9.12	0.11
L-8	11/12/2008	10.23	0.13
L-8	12/16/2008	26.92	0.33
L-8	2/10/2009	10.00	0.12
L-6	3/11/08	676.08	4.67
L-6	4/8/2008	457.09	3.16
L-6	5/13/2008	56.23	0.39
L-6	6/10/2008	15.14	0.10
L-6	7/10/2008	6.31	0.04
L-6	8/12/2008	223.87	1.55
L-6	9/9/2008	398.11	2.75
L-6	10/14/2008	52.48	0.36
L-6	11/12/2008	66.07	0.46
L-6	12/16/2008	380.19	2.63
L-6	2/10/2009	63.10	0.44

L-5	3/11/08	734.60	3.72
L-5	4/8/2008	511.75	2.59
L-5	5/13/2008	78.83	0.40
L-5	6/10/2008	25.59	0.13
L-5	7/10/2008	13.62	0.07
L-5	8/12/2008	327.27	1.66
L-5	9/9/2008	590.16	2.99
L-5	10/14/2008	79.65	0.40
L-5	11/12/2008	98.63	0.50
L-5	12/16/2008	262.22	1.33
L-5	2/10/2009	86.67	0.44
L-4	3/11/08	934.50	3.72
L-4	4/8/2008	651.00	2.59
L-4	5/13/2008	100.28	0.40
L-4	6/10/2008	32.55	0.13
L-4	7/10/2008	17.33	0.07
L-4	8/12/2008	416.33	1.66
L-4	9/9/2008	750.75	2.99
L-4	10/14/2008	101.33	0.40
L-4	11/12/2008	125.48	0.50
L-4	12/16/2008	588.00	2.34
L-4	2/10/2009	110.25	0.44
L-LH	3/11/08	1742.07	3.72
L-LH	4/8/2008	1213.58	2.59
L-LH	5/13/2008	186.93	0.40
L-LH	6/10/2008	60.68	0.13
L-LH	7/10/2008	32.30	0.07
L-LH	8/12/2008	776.10	1.66
L-LH	9/9/2008	1399.53	2.99
L-LH	10/14/2008	188.89	0.40
L-LH	11/12/2008	233.91	0.50
L-LH	12/16/2008	1096.13	2.34
L-LH	2/10/2009	20.55	0.04
L-Main	3/11/08	*1780*	3.72
L-Main	4/8/2008	*1240*	2.59
L-Main	5/13/2008	*191*	0.40
L-Main	6/10/2008	*62*	0.13
L-Main	7/10/2008	*33*	0.07
L-Main	8/12/2008	*793*	1.66
L-Main	9/9/2008	*1430*	2.99
L-Main	10/14/2008	*193*	0.40

L-Main	11/12/2008	*239*	0.50
L-Main	12/16/2008	*1120*	2.34
L-Main	2/10/2009	*210*	0.44
L-Newmkt	3/11/08	2040.59	3.72
L-Newmkt	4/8/2008	1421.54	2.59
L-Newmkt	5/13/2008	218.96	0.40
L-Newmkt	6/10/2008	71.08	0.13
L-Newmkt	7/10/2008	37.83	0.07
L-Newmkt	8/12/2008	909.10	1.66
L-Newmkt	9/9/2008	1639.35	2.99
L-Newmkt	10/14/2008	221.26	0.40
L-Newmkt	11/12/2008	273.99	0.50
L-Newmkt	12/16/2008	1283.97	2.34
L-Newmkt	2/10/2009	240.74	0.44
L-MLB	3/11/08	1.70	1.21
L-MLB	4/8/2008	1.41	1.01
L-MLB	5/13/2008	0.59	0.42
L-MLB	6/10/2008	0.32	0.23
L-MLB	7/10/2008	0.28	0.20
L-MLB	8/12/2008	1.00	0.71
L-MLB	9/9/2008	1.05	0.75
L-MLB	10/14/2008	0.55	0.39
L-MLB	11/12/2008	0.60	0.43
L-MLB	12/16/2008	1.26	0.90
L-MLB	2/10/2009	0.60	0.43
L-1	3/11/08	1.62	1.25
L-1	4/8/2008	1.38	1.06
L-1	5/13/2008	0.60	0.46
L-1	6/10/2008	0.35	0.27
L-1	7/10/2008	0.30	0.23
L-1	8/12/2008	*1.00*	0.77
L-1	9/9/2008	*1.05*	0.81
L-1	10/14/2008	*0.58*	0.45
L-1	11/12/2008	0.63	0.48
L-1	12/16/2008	1.23	0.95
L-1	2/10/2009	0.63	0.48
L-3	3/11/08	281.84	2.19
L-3	4/8/2008	309.80	2.41
L-3	5/13/2008	43.65	0.34
L-3	6/10/2008	10.72	0.08
L-3	7/10/2008	6.03	0.05

L-3	8/12/2008	199.53	1.55
L-3	9/9/2008	281.84	2.19
L-3	10/14/2008	44.67	0.35
L-3	11/12/2008	51.29	0.40
L-3	12/16/2008	263.03	2.04
L-3	2/10/2009	44.67	0.35
L-RB	3/11/08	9.12	1.86
L-RB	4/8/2008	6.92	1.41
L-RB	5/13/2008	1.74	0.36
L-RB	6/10/2008	0.69	0.14
L-RB	7/10/2008	0.32	0.07
L-RB	8/12/2008	4.17	0.85
L-RB	9/9/2008	6.76	1.38
L-RB	10/14/2008	1.62	0.33
L-RB	11/12/2008	1.91	0.39
L-RB	12/16/2008	6.17	1.26
L-RB	2/10/2009	1.82	0.37
L-NB	3/11/08	154.88	3.76
L-NB	4/8/2008	97.72	2.37
L-NB	5/13/2008	9.55	0.23
L-NB	6/10/2008	2.34	0.06
L-NB	7/10/2008	1.07	0.03
L-NB	8/12/2008	56.23	1.36
L-NB	9/9/2008	117.49	2.85
L-NB	10/14/2008	9.77	0.24
L-NB	11/12/2008	12.59	0.31
L-NB	12/16/2008	87.10	2.11
L-NB	2/10/2009	10.72	0.26

APPENDIX C

Sample date and stream chemistry for each stream studied in the Lamprey River Watershed for March 2008 – February 2009 (ND = Below detection limit)

Site	Date	NO_3^- (mg N/L)	DOC (mg C/L)	TDN (mg N/L)	Cl^- (mg Cl/L)	pH
L-10	3/11/08	0.03	2.90	0.13	4.22	4.95
L-10	4/8/2008	0.03	2.55	0.12	4.08	6.02
L-10	5/13/2008	ND	3.11	0.14	3.87	6.09
L-10	6/10/2008	0.09	5.19	0.34	6.82	5.54
L-10	7/10/2008	0.09	4.68	0.29	10.93	5.54
L-10	8/12/2008	0.01			3.36	5.57
L-10	9/9/2008	0.05	6.79	0.22	2.37	5.49
L-10	10/14/2008	0.02	4.15	0.16	3.97	6.44
L-10	11/12/2008	ND	5.43	0.16	4.82	6.29
L-10	12/16/2008	0.04	3.38	0.09	3.53	
L-10	2/10/2009	0.05	3.71	0.19	4.61	6.2
L-8	3/11/08		3.35	0.22	11.02	5.54
L-8	4/8/2008	0.07	3.02	0.18	10.85	6.57
L-8	5/13/2008	0.05	3.16	0.21	14.80	6.49
L-8	6/10/2008	0.18	4.00	0.38	17.42	6.34
L-8	7/10/2008	0.15	3.77	0.40	19.24	6.34
L-8	8/12/2008	0.04	7.11	0.30	10.56	6.22
L-8	9/9/2008	0.09	6.85	0.27	8.29	6.2
L-8	10/14/2008	0.06	4.67	0.22	13.17	6.75
L-8	11/12/2008	0.06	5.17	0.24	13.02	6.78
L-8	12/16/2008	0.08	4.57	0.18	7.57	
L-8	2/10/2009	0.14	3.94	0.30	11.49	6.23
L-6	3/11/08		3.69	0.23	12.91	5.6
L-6	4/8/2008	0.07	3.28	0.20	15.57	6.77
L-6	5/13/2008	0.05	3.95	0.22	19.76	6.24
L-6	6/10/2008	0.17	4.18	0.40	23.53	6.12
L-6	7/10/2008	0.11	4.13	0.31	25.93	6.12
L-6	8/12/2008	0.05	7.94	0.38	13.61	6.05
L-6	9/9/2008	0.02	8.55	0.28	9.95	5.93
L-6	10/14/2008	0.07	5.16	0.24	17.71	6.46
L-6	11/12/2008	0.06	5.41	0.27	16.59	6.54
L-6	12/16/2008	0.09	4.42	0.21	9.62	
L-6	2/10/2009	0.16	3.35	0.33	14.53	6.57
L-5	3/11/08	0.10	3.52	0.26	22.93	6.06

L-5	4/8/2008	0.10	3.38	0.24	20.67	7.24
L-5	5/13/2008	0.08	3.69	0.26	30.03	6.64
L-5	6/10/2008	0.18	3.90	0.41	40.17	6.58
L-5	7/10/2008	0.11	4.11	0.33	38.19	6.58
L-5	8/12/2008	0.06	6.97	0.37	22.63	6.53
L-5	9/9/2008	0.03	8.43	0.30	13.19	6.28
L-5	10/14/2008	0.15	4.71	0.30	26.89	6.89
L-5	11/12/2008	0.11	3.59	0.24	25.64	6.65
L-5	12/16/2008	0.12	5.61	0.24	14.76	
L-5	2/10/2009	0.22	3.50	0.40	25.65	6.6
L-4	3/11/08	0.20	3.76	0.28	20.67	6.24
L-4	4/8/2008	0.11	3.65	0.16	20.18	7.83
L-4	5/13/2008	0.24	3.68	0.44	32.54	6.66
L-4	6/10/2008	0.26	3.67	0.53	43.44	5.91
L-4	7/10/2008	0.22	4.18	0.45	39.75	5.91
L-4	8/12/2008	0.06	6.40	0.35	18.33	6.25
L-4	9/9/2008	ND	10.02	0.39	13.04	6.68
L-4	10/14/2008	0.20	4.73	0.40	23.43	6.70
L-4	11/12/2008	0.11	4.61	0.27	23.15	6.46
L-4	12/16/2008	0.12	5.72	0.26	13.12	
L-4	2/10/2009	0.25	3.13	0.45	23.09	6.65
L-LH	3/11/08	0.14	4.09	0.29	19.72	6.3
L-LH	4/8/2008	0.09	3.43	0.22	16.88	6.94
L-LH	5/13/2008	0.11	3.69	0.28	23.77	7.15
L-LH	6/10/2008	0.18	4.29	0.42	30.37	7.38
L-LH	7/10/2008	0.13	4.66	0.36	30.06	7.38
L-LH	8/12/2008	0.06	7.51	0.37	12.66	6.87
L-LH	9/9/2008	0.03	8.22	0.32	12.68	
L-LH	10/14/2008	0.14	5.68	0.31	20.55	6.70
L-LH	11/12/2008	0.08	4.59	0.32	17.96	6.44
L-LH	12/16/2008	0.08	5.47	0.26	11.87	5.72
L-LH	2/10/2009	0.22	3.96	0.44	22.71	6.18
L-Main	3/11/08	0.19	3.58	0.29	19.32	5.62
L-Main	4/8/2008	0.10	3.50	0.24	16.83	6.46
L-Main	5/13/2008	0.15	4.04	0.30	24.66	6.83
L-Main	6/10/2008	0.21	4.48	0.47	31.36	7.3
L-Main	7/10/2008	0.12	4.56	0.35	29.26	8.67
L-Main	8/12/2008	0.05	8.56	0.51	14.25	5.97
L-Main	9/9/2008	0.04	7.91	0.30	13.74	
L-Main	10/14/2008	0.14	5.15	0.30	21.26	6.79

L-Main	11/12/2008	0.09	4.81	0.29	18.50	6.91
L-Main	12/16/2008	0.09	5.21	0.24	11.87	5.42
L-Main	2/10/2009	0.22	3.84	0.44		3.85
L-Newmkt	3/11/08	0.17	4.04	0.31	20.65	5.62
L-Newmkt	4/8/2008	0.10	3.80	0.26	17.16	6.42
L-Newmkt	5/13/2008	0.09	4.18	0.30	24.65	6.75
L-Newmkt	6/10/2008	0.20	4.22	0.44	31.33	7.19
L-Newmkt	7/10/2008	0.05	5.20	0.32	27.11	7.3
L-Newmkt	8/12/2008	0.07	7.65	0.39	16.97	6.75
L-Newmkt	9/9/2008	0.04	8.14	0.34	15.23	
L-Newmkt	10/14/2008	0.12	5.70	0.30	20.70	6.64
L-Newmkt	11/12/2008	0.08	5.31	0.26	18.36	6.53
L-Newmkt	12/16/2008	0.08	5.59	0.24	12.41	4.34
L-Newmkt	2/10/2009	0.24	3.86	0.46	20.19	5.59
L-MLB	3/11/08	1.14	1.77	1.01	130.17	6.43
L-MLB	4/8/2008	0.77	1.33	0.95	98.68	6.68
L-MLB	5/13/2008	0.53	1.42	0.65	116.32	7.01
L-MLB	6/10/2008	0.64	1.54	0.90	130.10	8.67
L-MLB	7/10/2008	0.73	1.49	0.92	138.84	7.19
L-MLB	8/12/2008	0.44	3.00	0.59	87.87	6.10
L-MLB	9/9/2008	0.61	2.00	0.64	115.58	
L-MLB	10/14/2008	0.64	1.23	0.59	121.79	6.63
L-MLB	11/12/2008	0.60	1.97	0.49	126.28	6.59
L-MLB	12/16/2008	0.80	2.47	0.84	95.16	5.79
L-MLB	2/10/2009	0.82	1.55	0.94	128.75	5.76
L-1	3/11/08	1.30	2.31	1.17	48.83	6.7
L-1	4/8/2008	0.75	2.26	0.83	43.91	7.15
L-1	5/13/2008	0.51	2.30	0.69	53.66	7.59
L-1	6/10/2008	0.89	2.62	1.05	50.59	7.55
L-1	7/10/2008	0.95	1.87	1.06	55.33	7.55
L-1	8/12/2008	0.17	6.75	0.48	35.31	6.57
L-1	9/9/2008	0.40	3.53	0.55	50.36	

L-1	10/14/2008	0.40	3.14	0.47	51.47	7.07
L-1	11/12/2008	0.57	2.25	0.58	50.39	7.04
L-1	12/16/2008	0.80	3.13	0.86	35.17	5.91
L-1	2/10/2009	0.81	2.23	0.93	36.18	5.38
L-LR	3/11/08	0.24	4.23	0.34	58.80	6.17
L-LR	4/8/2008	0.05	3.85	0.18	14.84	6.27
L-LR	5/13/2008	0.07	3.95	0.23	18.25	6.65
L-LR	6/10/2008	0.16	4.94	0.41	20.42	7.1
L-LR	7/10/2008	0.14	4.05	0.33	19.83	7.1
L-LR	8/12/2008	0.04	7.71	0.38	13.68	4.80
L-LR	9/9/2008	0.02	7.98	0.26	10.06	
L-LR	10/14/2008	0.07	5.96	0.24	14.33	6.79
L-LR	11/12/2008	0.04	5.20	0.24	11.81	6.13
L-LR	12/16/2008	0.06	5.05	0.20	14.68	5.55
L-LR	2/10/2009	0.18	3.59	0.38	15.74	6.16
L-3	3/11/08	0.11	4.09	0.27	23.04	5.87
L-3	4/8/2008	0.06	-0.15		13.98	6.22
L-3	5/13/2008	ND	4.28	0.21	15.48	6.9
L-3	6/10/2008	0.10	5.13	0.35	20.39	6.91
L-3	7/10/2008	0.04	5.47	0.28	20.19	6.91
L-3	8/12/2008	0.05	7.65	0.36	12.81	6.67
L-3	9/9/2008	0.01	8.50	0.29	8.82	
L-3	10/14/2008	ND	5.82	0.20	15.84	6.61
L-3	11/12/2008	0.02	5.38	0.22	12.64	
L-3	12/16/2008	0.04	5.53	0.17	13.46	5.23
L-3	2/10/2009	0.12	3.72	0.32	13.28	6.06
L-RB	3/11/08	0.36	3.94	0.45	31.86	6.38
L-RB	4/8/2008	0.17	3.36	0.25	26.55	7.49
L-RB	5/13/2008	0.09	5.24	0.32	36.53	6.73
L-RB	6/10/2008	0.06	6.89	0.44	42.64	6.31
L-RB	7/10/2008	0.07	5.87	0.35	44.70	6.31
L-RB	8/12/2008	0.09	10.10	0.47	23.52	6.49
L-RB	9/9/2008	0.03	8.13	0.38	25.43	6.33
L-RB	10/14/2008	0.10	6.09	0.32	32.60	6.74
L-RB	11/12/2008	0.13	3.73	0.18	32.21	6.61
L-RB	12/16/2008	0.20	5.09	0.33	17.43	
L-RB	2/10/2009	0.26	3.72	0.45	26.88	6.73
L-5a	3/11/08	0.10	3.42	0.26	7.41	6.01
L-5a	4/8/2008	0.07	3.02	0.19	7.52	7.1

L-5a	5/13/2008	0.01	3.05	0.17	9.31	6.26
L-5a	6/10/2008	0.03	5.37	0.45	14.05	5.88
L-5a	7/10/2008	ND	4.24	0.27	14.79	5.88
L-5a	8/12/2008	0.02	4.06	0.22	5.76	6.11
L-5a	9/9/2008	0.01	4.36	0.18	5.54	6.3
L-5a	10/14/2008	ND	3.96	0.20	5.36	6.84
L-5a	11/12/2008	0.02	4.36	0.22	6.59	6.69
L-5a	12/16/2008	0.20	4.39	0.21	5.61	
L-5a	2/10/2009	0.08	4.11	0.31	6.57	6.87
L-NB	3/11/08	0.14	3.78	0.26	23.09	5.8
L-NB	4/8/2008	0.05	3.47	0.19	19.03	6.68
L-NB	5/13/2008	0.03	3.94	0.21	23.82	6.53
L-NB	6/10/2008	0.14	4.55	0.36	27.70	6.09
L-NB	7/10/2008	0.11	4.90	0.37	30.89	6.09
L-NB	8/12/2008	0.03	7.20	0.30	20.40	6.51
L-NB	9/9/2008	ND	8.85	0.33	12.48	6.05
L-NB	10/14/2008	ND	5.80	0.23	19.50	6.82
L-NB	11/12/2008	ND	6.40	0.22	20.22	6.55
L-NB	12/16/2008	0.07	4.99	0.16	11.40	
L-NB	2/10/2009	0.10	3.69	0.31	17.15	6.46
L-Pawt	3/11/08	ND	2.22	0.12	1.44	4.92
L-Pawt	4/8/2008	ND	2.29	0.12	1.54	6.35
L-Pawt	5/13/2008	ND	4.62	0.21	1.53	5.87
L-Pawt	6/10/2008	ND	8.09	0.40	1.03	5.61
L-Pawt	7/10/2008	ND	7.00	0.38	1.26	5.61
L-Pawt	8/12/2008	ND	5.21	0.21	0.80	5.37
L-Pawt	9/9/2008	ND	5.48	0.21	0.86	6.45
L-Pawt	10/14/2008	ND	4.21	0.21	1.22	6.15
L-Pawt	11/12/2008	ND	5.28	0.22	3.06	6.2
L-Pawt	12/16/2008	ND	2.80	0.08	2.09	
L-Pawt	2/10/2009	ND	3.68	0.18	2.53	5.76

APPENDIX D

Sample date, average dissolved N_2O with standard deviation, and average percent saturation with standard deviation for each site along L1 sampled from February 2008 – August 2008

Site	Date	Average Dissolved N_2O (µg N_2O-N/L)	Standard Deviation N_2O (µg N_2O-N/L)	Average Percent Saturation (%)	Stand Deviation Percent Saturation
-250	2/14/2008	1.32	0.04	181.80	5.51
-125	2/14/2008	1.46	0.31	201.77	42.69
-5	2/14/2008	1.42	0.16	195.98	22.47
5	2/14/2008	1.29	0.08	178.57	10.83
125	2/14/2008	1.33	0.09	184.69	13.03
250	2/14/2008	1.22	0.24	169.49	32.77
375	2/14/2008	1.36	0.78	186.34	1.62
500	2/14/2008	1.26	0.12	177.41	16.25
625	2/14/2008	1.23	0.10	172.87	13.99
750	2/14/2008	1.29	0.01	181.18	0.89
825	2/14/2008	1.47	0.10	205.85	13.89
925	2/14/2008	1.44	0.08	201.53	11.73
-250	3/7/2008	1.05	0.28	150.88	40.44
-125	3/7/2008	1.24	0.11	178.88	15.52
-5	3/7/2008	1.26	0.06	181.88	8.51
5	3/7/2008	1.27	0.11	182.54	16.05
125	3/7/2008	1.27	0.15	183.37	21.52
250	3/7/2008	1.29	0.26	186.64	37.47
375	3/7/2008	1.42	0.05	208.41	7.58
500	3/7/2008	1.59	0.10	233.27	14.03
625	3/7/2008	1.33	0.14	195.57	20.04
750	3/7/2008	1.46	0.13	214.49	19.75
825	3/7/2008	1.58	0.10	230.83	15.06
925	3/7/2008	1.64	0.07	239.23	10.73
-250	3/27/2008	3.56	0.18	511.51	26.38
-125	3/27/2008	3.20	0.23	462.30	33.23
-5	3/27/2008	3.41	0.09	495.81	13.47
5	3/27/2008	3.51	0.08	505.66	11.17
125	3/27/2008	3.25	0.24	471.99	34.94
250	3/27/2008	3.31	0.24	484.31	34.40
375	3/27/2008	3.78	0.16	558.51	24.16
500	3/27/2008	3.45	0.08	509.45	11.40

625	3/27/2008	3.58	0.15	528.63	21.88
750	3/27/2008	3.32	0.13	489.21	19.85
825	3/27/2008	3.45	0.21	507.62	30.89
925	3/27/2008	3.34	0.33	491.13	49.20
-250	4/15/2008	0.81	0.04	153.01	6.83
-125	4/15/2008	0.84	0.01	157.40	1.51
-5	4/15/2008	0.83	0.01	154.28	2.21
5	4/15/2008	0.77	0.02	142.11	3.64
125	4/15/2008	0.72	0.00	132.07	0.61
250	4/15/2008	0.97	0.03	176.32	5.36
375	4/15/2008	1.14	0.02	205.01	3.97
500	4/15/2008	1.01	0.16	182.34	29.41
625	4/15/2008	1.03	0.04	186.39	6.42
750	4/15/2008	0.90	0.01	162.77	1.55
825	4/15/2008	0.88	0.03	159.38	5.48
925	4/15/2008	0.75	0.04	135.18	7.60
-250	5/6/2008	0.62	0.01	138.08	1.36
-125	5/6/2008	0.70	0.00	153.85	0.95
-5	5/6/2008	0.66	0.02	142.21	3.42
5	5/6/2008	0.62	0.02	133.79	4.82
125	5/6/2008	0.62	0.01	131.64	2.85
250	5/6/2008	0.90	0.03	187.32	5.99
375	5/6/2008	1.02	0.031	208.845	6.315
500	5/6/2008	0.95	0.041	193.536	8.367
625	5/6/2008	0.89	0.032	181.314	6.510
750	5/6/2008	0.78	0.037	160.134	7.657
825	5/6/2008	0.66	0.060	135.788	12.407
925	5/6/2008	0.70	0.034	144.467	6.902
-250	5/28/2008	0.86	0.033	164.587	6.367
-125	5/28/2008	1.01	0.030	193.125	5.800
-5	5/28/2008	0.88	0.040	167.482	7.624
5	5/28/2008	0.74	0.01	141.39	2.66
125	5/28/2008	0.89	0.02	167.61	3.11
250	5/28/2008	1.28	0.04	239.99	7.43
375	5/28/2008	1.52	0.02	282.32	3.68
500	5/28/2008	1.26	0.05	233.50	9.12
625	5/28/2008	1.22	0.02	225.82	4.25
750	5/28/2008	0.92	0.04	170.90	7.61
825	5/28/2008	0.74	0.02	137.40	3.88
925	5/28/2008	0.76	0.05	139.71	8.85
-250	6/19/2008	0.389	0.054	82.823	11.390

-125	6/19/2008	0.600	0.006	126.670	1.321
-5	6/19/2008	0.515	0.015	108.345	3.120
5	6/19/2008	0.433	0.030	90.895	6.325
125	6/19/2008	0.531	0.015	109.200	3.052
250	6/19/2008	0.862	0.022	175.341	4.469
375	6/19/2008	1.017	0.007	204.650	1.444
500	6/19/2008	0.819	0.036	164.769	7.162
625	6/19/2008	0.757	0.037	151.792	7.418
750	6/19/2008	0.561	0.007	112.482	1.328
825	6/19/2008	0.444	0.035	89.087	7.095
925	6/19/2008	0.438	0.034	87.964	6.786
-250	7/15/2008	0.497	0.006	111.154	1.249
-125	7/15/2008	1.002	0.016	222.507	3.470
-5	7/15/2008	0.494	0.038	109.535	8.457
5	7/15/2008	0.395	0.013	87.124	2.936
125	7/15/2008	0.623	0.054	132.584	11.553
250	7/15/2008	1.116	0.057	234.522	12.076
375	7/15/2008	1.112	0.019	230.829	4.029
500	7/15/2008	0.94	0.02	195.83	4.35
625	7/15/2008	0.72	0.07	148.41	13.99
750	7/15/2008	0.58	0.03	120.97	7.27
825	7/15/2008	0.39	0.09	80.71	18.63
925	7/15/2008	0.28	0.03	59.23	7.00
-250	8/8/2008	0.74	0.04	169.76	8.41
-125	8/8/2008	0.73	0.02	166.64	4.69
-5	8/8/2008	0.79	0.02	179.42	3.84
5	8/8/2008	0.76	0.02	173.38	4.04
125	8/8/2008	0.75	0.00	170.16	1.10
250	8/8/2008	0.78	0.02	176.97	4.87
375	8/8/2008	0.83	0.01	187.10	3.31
500	8/8/2008	0.83	0.01	186.51	1.65
625	8/8/2008	0.86	0.01	192.55	2.88
750	8/8/2008	0.81	0.04	181.44	9.34
825	8/8/2008	0.84	0.05	188.21	11.64
925	8/8/2008	0.77	0.05	171.90	11.46
-250	8/21/2008	2.28	0.09	478.52	18.97
-125	8/21/2008	2.24	0.05	469.32	10.92
-5	8/21/2008	2.40	0.10	502.08	20.58
5	8/21/2008	2.30	0.07	480.63	14.07
125	8/21/2008	2.35	0.06	484.07	13.00
250	8/21/2008	2.70	0.03	550.86	6.46

375	8/21/2008	2.83	0.09	571.65	17.63
500	8/21/2008	2.70	0.04	543.50	7.90
625	8/21/2008	2.68	0.14	536.44	27.83
750	8/21/2008	2.71	0.10	543.76	19.65
825	8/21/2008	2.57	0.07	516.07	14.33
925	8/21/2008	2.33	0.14	467.85	28.63

APPENDIX E

Post-hoc Tukey results for dissolved N_2O for all sites along L1 for dates with discharge > 1 cfs

	-250	-125	-5	5	125	250	375	500	625
250	0.037°			0.004°	< .001°				
375	< .001° 0.034´	< .001° 0.012´	< .001°	< .001°	< .001°	0.029°			
500	0.007* 0.003° 0.041´	0.024° 0.015´	0.014°	< .001°	< .001°				
625	0.001° 0.003´	0.010° 0.001´	0.006°	< .001° 0.027´	< .001° 0.009´				
750					0.014°		0.001°		
825	0.009* 0.020´	0.007´			0.041°		< .001°		
925	0.003*					0.001°	< .001°	< .001°	< .001° 0.031´

3/7/08*, 4/15/08°, 8/8/08´

Post-hoc Tukey results for dissolved N_2O for all sites along L1 for dates with discharge < 1 cfs

	-250	-125	-5	5	125	250	375	500	625	750
-125	$< .001^{2}$ $< .001^{3}$ $< .001^{4}$									
-5	0.001^{3}	0.003^{2} 0.048^{3} $< .001^{4}$								
5	0.015^{2}	$< .001^{2}$	0.002^{2}							

		$< .001^{3}$ $< .001^{4}$								
125	$< .001^{3}$ $.020^{4}$	0.007^{2} $< .001^{4}$	$0\ .016^{4}$	0.001^{2} 0.014^{3} $< .001^{4}$						
250	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ 0.015^{5}	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $.001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $0\ .003^{5}$					
375	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $< .001^{5}$	0.004^{1} $< .001^{2}$ $< .001^{3}$				
500	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $0\ .020^{4}$ $< .001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $0\ .019^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $.001^{5}$	$< .001^{1}$ $< .001^{2}$ $< .001^{3}$ $< .001^{4}$ $0\ .003^{5}$	$< .001^{4}$	$< .001^{2}$ $< .001^{3}$ $< .001^{4}$			

625	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ 0.001^5	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ $< .001^5$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ 0.032^5	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ $.001^5$	$< .001^1$ $< .001^2$ $< .001^3$ 0.006^5	$.007^3$ $< .001^4$	0.001^1 $< .001^2$ $< .001^3$ $< .001^4$	0.020^4		
750	$< .001^1$ $< .001^3$ $< .001^5$	$< .001^4$ $< .001^5$	0.002^1 0.011^5	$< .001^1$ $< .001^2$ 0.001^3 0.012^4 $< .001^5$	$< .001^1$	0.004^1 $< .001^2$ $< .001^3$ $< .001^4$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$	0.012^1 $< .001^2$ $< .001^3$	
825	0.012^2 0.020^5	$< .001^2$ $< .001^3$ $< .001^4$ 0.005^5	0.002^2	$.040^5$	0.001^2 0.038^3 $< .001^4$ 0.002^5	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ 0.047^5	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$	0.004^1 $< .001^2$ 0.002^3 0.008^4
925	0.036^2 0.002^4	$< .001^2$ $< .001^3$ $< .001^4$	0.006^2 0.003^4		0.002^2 0.023^3 $< .001^4$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ $.001^5$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ $< .001^5$	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ 0.002^5	$< .001^1$ $< .001^2$ $< .001^3$ $< .001^4$ 0.003^5	$< .001^2$ $.001^3$ $< .001^4$ $.001^5$

5/6/08[1], 5/28/08[2], 6/19/08[3], 7/15/08[4], 8/21/08[5]

APPENDIX F

Sample date and stream chemistry for each site studied in L1 for February 2008 – August 2008

Site	Date	NO_3^- (mg N/L)	DOC (mg C/L)	TDN (mg N/L)	SC (µS/cm)	pH
-250	2/14/2008	0.62	3.43	0.63	157.00	7.02
-125	2/14/2008	0.59	3.26	0.68	156.00	7.02
-5	2/14/2008	0.63	3.34	0.72	155.00	7.06
5	2/14/2008	0.61	3.35	0.71	157.00	7.08
125	2/14/2008	0.64	3.23	0.77	160.00	7.06
250	2/14/2008	0.69	3.52	0.81	162.00	7.06
375	2/14/2008	0.71	2.69	0.72	167.00	7.03
500	2/14/2008	0.70	3.06	0.8	165.00	7.05
625	2/14/2008	0.71	3.14	0.8	164.00	6.96
750	2/14/2008	0.73	3.03	0.8	160.00	6.99
825	2/14/2008	0.83	2.74	0.84	161.00	7.07
925	2/14/2008	0.79	3.13	0.93	159.00	7.38
-250	3/7/2008	1.03	2.15	0.84	214.00	7.34
-125	3/7/2008	0.95	2.29	1.01	213.00	7.37
-5	3/7/2008	0.96	2.09	0.93	209.00	7.39
5	3/7/2008	0.98	1.69	0.91	214.00	7.31
125	3/7/2008	1.21	1.92	1.11	222.00	7.38
250	3/7/2008	1.30	1.94	1.16	228.00	7.33
375	3/7/2008	1.31	1.73	1.18	233.00	7.22
500	3/7/2008	1.28	1.86	1.16	230.00	7.16
625	3/7/2008	1.35	1.66	1.18	226.00	7.23
750	3/7/2008	1.31	1.93	1.22	227.00	7.16
825	3/7/2008	1.33	2.1	1.26	228.00	7.17
925	3/7/2008	1.29	1.41	0.95	227.00	7.25
-250	3/27/2008	0.80	1.93	0.83	445.00	7.58
-125	3/27/2008	0.78	2.09	0.85	441.00	7.59
-5	3/27/2008	0.82	1.85	0.88	438.00	7.56
5	3/27/2008	0.81	1.76	0.85	443.00	7.54
125	3/27/2008	0.78	1.73	0.88	456.00	7.57
250	3/27/2008	0.96	1.79	1.05	465.00	7.47
375	3/27/2008	1.01	1.80	1.03	405.00	7.45
500	3/27/2008	0.95	1.56	0.95	459.00	7.44
625	3/27/2008	1.00	2.03	1.04	448.00	7.42
750	3/27/2008	1.00	1.68	1.02	459.00	7.41

825	3/27/2008	1.00	1.72	1.08	454.00	7.31
925	3/27/2008	1.02	1.88	1.08	398.00	7.29
-250	4/15/2008	0.64	2.03	0.70	290.00	7.45
-125	4/15/2008	0.67	2.04	0.76	288.00	7.48
-5	4/15/2008	0.69	2.08	0.79	288.00	7.44
5	4/15/2008	0.68	1.99	0.76	302.00	7.51
125	4/15/2008	0.78	1.78	0.87	281.00	7.50
250	4/15/2008	0.89	1.65	0.97	308.00	7.34
375	4/15/2008	0.81	1.53	0.90	306.00	7.20
500	4/15/2008	0.96	1.54	1.04	306.00	7.25
625	4/15/2008	0.90	1.72	0.96	310.00	7.32
750	4/15/2008	0.64	1.31	0.65	304.00	7.37
825	4/15/2008	0.95	1.83	1.03	306.00	7.42
925	4/15/2008	0.97	1.75	1.03	304.00	7.51
-250	5/6/2008	0.40	2.93	0.55	292.00	7.62
-125	5/6/2008	0.45	2.76	0.58	262.00	7.71
-5	5/6/2008	0.46	2.77	0.58	255.00	7.75
5	5/6/2008	0.46	2.55	0.58	291.00	7.65
125	5/6/2008	0.61	2.53	0.71	291.00	7.61
250	5/6/2008	0.76	2.26	0.84	273.00	7.42
375	5/6/2008	0.86	1.84	0.96	293.00	7.28
500	5/6/2008	0.84	1.77	0.89	289.00	7.28
625	5/6/2008	0.80	2.16	0.86	255.00	7.30
750	5/6/2008	0.83	1.87	0.84	256.00	7.35
825	5/6/2008	0.82	2.09	0.87	290.00	7.44
925	5/6/2008	0.82	2.08	0.92	297.00	7.52
-250	5/28/2008	0.74	2.10	0.85	306.00	7.73
-125	5/28/2008	0.79	1.89	0.88	299.00	7.70
-5	5/28/2008	0.80	2.01	0.92	302.00	7.69
5	5/28/2008	0.79	1.86	0.87	299.00	7.67
125	5/28/2008	1.09	1.56	1.17	299.00	7.60
250	5/28/2008	1.32	1.26	1.32	245.00	7.44
375	5/28/2008	1.42	1.06	1.39	223.00	7.32
500	5/28/2008	1.38	1.24	1.37	294	7.32
625	5/28/2008	1.32	1.08	1.32	304.00	7.36
750	5/28/2008	1.29	1.53	1.29	293.00	7.44
825	5/28/2008	1.27	1.12	1.34	303.00	7.37
925	5/28/2008	1.35	1.29	1.32	304.00	7.25
-250	6/19/2008	0.51	3.18	0.79	336.00	7.77

-125	6/19/2008	0.57	3.09	0.82	286.00	7.66
-5	6/19/2008	0.59	2.93	0.84	333.00	7.69
5	6/19/2008	0.24	1.75	0.39	326.00	7.64
125	6/19/2008	0.87	2.37	1.09	332.00	7.42
250	6/19/2008	1.16	1.93	1.43	246.00	7.42
375	6/19/2008	1.16	1.61	1.41	339.00	7.25
500	6/19/2008	0.42	1.07	0.52	343.00	7.28
625	6/19/2008	1.30	1.59	1.36	357.00	7.33
750	6/19/2008	1.40	1.56	1.22	354.00	7.34
825	6/19/2008	1.34	1.45	1.30	354.00	7.27
925	6/19/2008	1.36	1.73	1.38	357.00	7.23
-250	7/15/2008	0.93	1.39	0.97	394.00	7.05
-125	7/15/2008	1.06	1.64	1.13	382.00	6.93
-5	7/15/2008	1.07	1.44	1.06	336.00	6.92
5	7/15/2008	0.95	1.24	0.93	366.00	6.78
125	7/15/2008	1.47	1.15	1.49	374.00	6.73
250	7/15/2008	1.80	0.94	1.89	388.00	6.68
375	7/15/2008	1.71	0.88	1.72	387.00	6.64
500	7/15/2008	1.72	1.07	1.70	304.00	6.64
625	7/15/2008	1.69	0.83	1.66	401.00	6.54
750	7/15/2008	1.67	1.13	1.66	396.00	6.43
825	7/15/2008	1.59	0.93	1.66	396.00	6.37
925	7/15/2008	1.63	1.13	1.65	403.00	6.02
-250	8/8/2008	0.05	9.28	0.37	132.00	7.16
-125	8/8/2008	0.06	9.37	0.38	131.00	7.17
-5	8/8/2008	0.09	9.35	0.42	133.00	7.15
5	8/8/2008	0.12	9.23	0.40	133.00	7.20
125	8/8/2008	0.15	9.16	0.44	135.00	7.20
250	8/8/2008	0.15	8.76	0.45	135.00	7.10
375	8/8/2008	0.18	8.82	0.46	95.00	7.10
500	8/8/2008	0.21			120.00	7.02
625	8/8/2008	0.00	9.06	0.52	134.00	7.02
750	8/8/2008	0.21	9.04	0.51	129.00	6.99
825	8/8/2008	0.23	9.13	0.53	128.00	6.91
925	8/8/2008	0.22	8.65	0.52	127.00	7.02
-250	8/21/2008	0.51	3.41	0.56	423.00	7.17
-125	8/21/2008	0.53	2.91	0.60	317.00	7.15
-5	8/21/2008	0.56	2.89	0.62	411.00	7.10
5	8/21/2008	0.55	2.90	0.63	399.00	7.02

125	8/21/2008	0.81	3.03	0.88	374.00	6.92
250	8/21/2008	1.01	2.28	1.09	388.00	6.85
375	8/21/2008	1.10	2.11	1.17	374.00	6.74
500	8/21/2008	1.15	2.10	1.17	368.00	6.72
625	8/21/2008	1.11	2.03	1.21	349.00	6.66
750	8/21/2008	1.11	1.75	1.17	381.00	6.64
825	8/21/2008	1.12	1.71	1.22	382.00	6.59
925	8/21/2008	1.10	1.70	1.22	384.00	6.49

APPENDIX G

Sample date, time, average dissolved N_2O with standard deviation, and average percent saturation with standard deviation for each site sampled in L1 every four hours on March 20-21, 2008 and August 21-22, 2008

Site	Date	Time	Average Dissolved N_2O (µg N_2O-N/L)	Standard Deviation N_2O (µg N_2O-N/L)	Average Percent Saturation (%)	Standard Deviation Percent Saturation (%)
-5	3/20/2008	10:00	2.64	0.19	413.03	29.34
-5	3/20/2008	14:00	2.47	0.21	390.97	33.32
-5	3/20/2008	18:00	2.37	0.10	373.96	15.85
-5	3/20/2008	22:00	2.64	0.18	407.99	27.31
-5	3/21/2008	2:00	2.57	0.07	387.57	10.70
-5	3/21/2008	6:00	2.80	0.06	411.54	8.81
-5	3/21/2008	10:00	2.56	0.05	374.90	7.37
375	3/20/2008	10:00	2.74	0.06	431.51	9.73
375	3/20/2008	14:00	2.65	0.04	424.01	6.97
375	3/20/2008	18:00	2.46	0.11	394.62	17.68
375	3/20/2008	22:00	2.57	0.25	405.45	39.37
375	3/21/2008	2:00	2.58	0.19	398.31	29.50
375	3/21/2008	6:00	2.69	0.15	404.75	22.75
375	3/21/2008	10:00	2.51	0.08	373.91	11.80
925	3/20/2008	10:00	2.52	0.27	395.51	42.28
925	3/20/2008	14:00	2.75	0.06	437.31	10.10
925	3/20/2008	18:00	2.51	0.17	400.02	27.10
925	3/20/2008	22:00	2.60	0.16	409.52	25.69
925	3/21/2008	2:00	2.62	0.09	402.39	13.82
925	3/21/2008	6:00	2.62	0.03	392.73	5.03
925	3/21/2008	10:00	2.59	0.14	386.57	21.17
-5	8/21/2008	10:00	2.25	0.19	472.47	39.28
-5	8/21/2008	14:00	2.18	0.03	476.71	7.40
-5	8/21/2008	18:00	2.10	0.18	467.13	40.41
-5	8/21/2008	22:00	2.19	0.09	478.53	19.01
-5	8/22/2008	2:00	2.09	0.14	450.23	30.12
-5	8/22/2008	6:00	2.17	0.02	458.14	4.68
-5	8/22/2008	10:00	2.21	0.07	471.92	15.08
375	8/21/2008	10:00	2.63	0.07	535.53	15.18
375	8/21/2008	14:00	2.47	0.15	523.50	32.15
375	8/21/2008	18:00	2.67	0.04	567.82	9.07

375	8/21/2008	22:00	2.68	0.08	557.85	16.41
375	8/22/2008	2:00	2.70	0.04	555.82	8.65
375	8/22/2008	6:00	2.47	0.09	502.27	18.75
375	8/22/2008	10:00	2.65	0.19	548.42	40.02
925	8/21/2008	10:00	2.19	0.07	447.29	14.70
925	8/21/2008	14:00	2.15	0.01	457.71	2.43
925	8/21/2008	18:00	2.24	0.12	477.51	25.06
925	8/21/2008	22:00	2.10	0.29	438.08	60.51
925	8/22/2008	2:00	2.38	0.04	487.39	9.16
925	8/22/2008	6:00	2.19	0.08	442.17	16.73
925	8/22/2008	10:00	2.27	0.10	467.95	20.70

APPENDIX H

Jar number, day, treatment, and denitrification rate for each assay in the controlled denitrification experiment

Jar Number	Day	NO_3^- Treatment	pH Treatment	Acetylene?	Denitrification Rate (μg N_2O-N/g soil/hour
63	1	Low	Acidic	No	0.005
2	1	Low	Acidic	No	0.000
59	2	Low	Acidic	No	0.008
41	2	Low	Acidic	No	0.011
72	2	Low	Acidic	No	0.014
90	1	Low	Acidic	Yes	0.036
37	1	Low	Acidic	Yes	0.004
23	1	Low	Acidic	Yes	0.000
48	2	Low	Acidic	Yes	0.025
3	2	Low	Acidic	Yes	0.015
18	1	Low	Neutral	No	0.000
85	1	Low	Neutral	No	0.000
33	1	Low	Neutral	No	0.000
73	2	Low	Neutral	No	0.005
95	2	Low	Neutral	No	0.007
84	1	Low	Neutral	Yes	0.017
42	1	Low	Neutral	Yes	0.017
25	2	Low	Neutral	Yes	0.042
62	2	Low	Neutral	Yes	0.011
93	2	Low	Neutral	Yes	0.022
76	1	Medium	Acidic	No	0.005
27	1	Medium	Acidic	No	0.002
24	2	Medium	Acidic	No	0.112
9	2	Medium	Acidic	No	0.129
31	2	Medium	Acidic	No	0.057
28	1	Medium	Acidic	Yes	0.021
15	1	Medium	Acidic	Yes	0.037
68	1	Medium	Acidic	Yes	0.000
34	2	Medium	Acidic	Yes	0.114
69	2	Medium	Acidic	Yes	0.220
61	1	Medium	Neutral	No	0.009
78	1	Medium	Neutral	No	0.001
53	1	Medium	Neutral	No	0.000
57	2	Medium	Neutral	No	0.053

B	2	Medium	Neutral	No	0.079
20	1	Medium	Neutral	Yes	0.100
46	1	Medium	Neutral	Yes	0.256
7	2	Medium	Neutral	Yes	0.268
4	2	Medium	Neutral	Yes	0.390
45	2	Medium	Neutral	Yes	0.293
8	1	High	Acidic	No	0.009
29	1	High	Acidic	No	0.011
56	2	High	Acidic	No	0.072
66	2	High	Acidic	No	0.121
17	2	High	Acidic	No	0.163
39	1	High	Acidic	Yes	0.006
42	1	High	Acidic	Yes	0.039
77	1	High	Acidic	Yes	0.077
55	2	High	Acidic	Yes	0.165
26	2	High	Acidic	Yes	0.186
36	1	High	Neutral	No	0.036
3	1	High	Neutral	No	0.023
87	1	High	Neutral	No	0.016
22	2	High	Neutral	No	0.115
52	2	High	Neutral	No	0.231
31	1	High	Neutral	Yes	0.105
30	1	High	Neutral	Yes	0.129
47	2	High	Neutral	Yes	0.213
19	2	High	Neutral	Yes	0.124
44	2	High	Neutral	Yes	0.575