Stormwater Retention Ponds: Hydrogen Sulfide Production, Water Quality and Sulfate-Reducing Bacterial Kinetics

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A thesis submitted under the supervision of Drs. Robert Delatolla and Frances Pick in partial fulfillment of the requirements for the degree of Master of Applied Sciences in Civil Engineering.

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ABSTRACT

Stormwater retention basins are an integral component of municipal stormwater management strategies in North America. The province of Ontario's Ministry of the Environment and Climate Change obligates land developers to implement stormwater management in their land use and development plans to mitigate the effects of urbanization (Bradford and Gharabaghi, 2004). When stormwater retention ponds are improperly designed or maintained, these basins can fail at improving effluent water quality and may exasperate water quality issues.

Intense H_2S production events in stormwater infrastructure is a serious problem which is seldom encountered and documented in stormwater retention ponds. This study monitored two stormwater retention ponds situated in the Riverside South community, Ottawa, Ontario, Canada for a period of 15 consecutive months to thoroughly characterize intense hydrogen sulfide (H_2S) production in a stormwater retention pond under ice covered conditions during winter operation and during periods of drought under non-ice covered conditions during the summer.

Field experiments showed a strong relationship ($p < 0.006$, $R > 0.58$, $n = 20+$) between hypoxic conditions (dissolved oxygen (DO) concentration < 2 mg/L) and the intense production of H_2S gas. Ice-capping of the stormwater ponds during winter severely hindered reaeration of the pond and led to significant production of total sulfides in the Riverside South Pond #2 (RSP2), which subsequently resulted in the accumulation of total sulfides in the water column (20.7 mg/L) during winter in this pond. There was a perceived lag phase between the drop in DO and the increase in total sulfides near the surface, which was potentially indicative of slow movement of total sulfides from the benthic sediment into the water column. These high-sulfide conditions persisted in RSP2 from early January 2015 until the spring thaw, in mid-April, 2015. Riverside South Pond #1 (RSP1), the reference pond studied in this work, showed significantly less production of total sulfides across a significantly shorter period of time.

Analysis of the microbial communities showed that there was little change in the dominant bacterial populations present in the benthic sediment of the pond demonstrating significant total sulfide production (RSP2) and the pond that did not demonstrate significant total sulfide production (RSP1). Additionally, it was found that locations with the most accumulated sediment had the highest propensity for the production of H_2S gas. Furthermore, there was no perceivable community shift in the two ponds throughout the seasons, indicating that the sulfate-reducing bacteria (SRB) in stormwater benthic sediment are ubiquitous, exist in an acclimatized microbial population and are robust. Study of the microbial abundances revealed that SRB represented approximately 5.01 ± 0.79 % of the microbes present in the benthic sediment of RSP2. Likewise, in the stormwater pond which did not experience intense H_2S gas production, RSP1, 6.22 ± 2.11 % of microbes were of the SRB type, demonstrating that H_2S gas production does not correspond to higher concentrations of SRB or the proliferation of dominant species, but rather is a symptom of increased bacterial activity due to favourable environmental conditions.

In addition, this work also covers the kinetics of sediment oxygen demand (SOD), ammonification and sulfate-reduction, and attempts to understand the processes leading to H_2S gas production events.

In doing so, it was observed that kinetics obtained full-scale field studies were greater than in laboratory kinetic experiments. Laboratory experiments at 4°C identified total SOD, ammonification and sulfate-reduction kinetics to be 0.023 g/m^2/day, 0.027 g N/m^2/day and 0.004 g S/m^2/day, respectively. Meanwhile, kinetics calculated from the field study of stormwater retention ponds for total SOD, ammonification and sulfate-reduction were of 0.491 g/m^2/day, 0.120 g N/m^2/day and 0.147 g S/m^2/day, respectively. It is expected that this difference is due to the depth of active sediment influencing the total rates of production/consumption, making area-normalized daily rates of production/consumption

($g/m^2/day$) unsuitable for the comparison of field and laboratory studies, without some scaling factor. This study also measured supplementary kinetic parameters such as the Arrhenius coefficients and the half-saturation coefficient, to add to existing knowledge of sulfate-reduction.

ACKNOWLEDGEMENTS

This whole research project and subsequent thesis would have been impossible to complete with the incessant support from several persons and organizations. I would like to outline a few of the people and organizations which helped me. This is no way an exhaustive list and I am sure I will forget some elements, but here goes:

First and foremost, I would like to express my infinite gratitude to my supervisors (Dr. Delatolla & Dr. Pick), the City of Ottawa, the University of Ottawa, all the professors who helped me throughout my journey in this school (Drs. Narbaitz, Rennie, Sartaj, Infante, to name a few). I would also take the time to thank the academic staff (Luc & Meghan) at the department, and last but not least, my friends at the University of Ottawa (Brad, Ru, Liyu, Alex, Rochelle, Warsama, Xin, Bing, Juan-Pablo, Anis, Bahman, Pablo, Carl, Allie and all the others I will have forgotten in this list which have influenced my experience from up-close or from afar).

I would also like to thank my parents Carole & Jérôme and my fiancée Baisha, for their never-ending patience and support. I would also like to thank my friends at the City of Gatineau and will also take the time to thank the Civil and Environmental Engineering students, my friends at the Office of Risk Management and individuals at CUPE local 2626.

Last but not least, I would like to thank the City of Ottawa and all the staff which helped on during our sampling and experiments, braving the snow, ice, rain, wind, thunder and other generally unpleasant meteorological conditions without complaint. Special thanks to Chris, Alain, Marlene, Paul, Kristina and the rest of team, both on the field and in the laboratory or at ROPEC.

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LIST OF ABBREVIATIONS

aDcp	Acoustic Doppler current profiler
ADV	Acoustic Doppler velocimetry
CLSM	Confocal laser scanning microscope
CRD	Collaborative Research and Development
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
ECA	Environmental Compliance Approval
EDTA	Ethylenediaminetetraacetic acid
HDPE	High density polyethylene
LDPE	Low density polyethylene
M.A.Sc	Masters of applied sciences
MOE	Ministry of the Environment and Climate Change
NSERC	Natural Sciences and Engineering Research Council of Canada
PCR	Polymerase chain reaction
PETE	Polyethylene
PI	Principal investigator
PI	Propidium iodide
RPM	Revolutions per minute
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RSP1	Riverside South Pond #1
RSP2	Riverside South Pond #2

CHAPTER 1 : INTRODUCTION

1.1. BACKGROUND

Stormwater, according to the Oxford Dictionaries is defined as "*surface water in abnormal quantity resulting from heavy falls of rain or snow*" (Oxford Dictionaries, 2016). In the context of civil engineering and more specifically municipal and stormwater engineering, stormwater is generally referred to as any water which has been generated due to urbanization or the creation of impervious surfaces, impeding rainfall from re-entering the water cycle naturally. As a result of increased urbanization and the use of impervious construction materials, the volume of water which needs to be captured, stored and conveyed back to receiving waters is increasing rapidly. In addition to capture and conveyance, because stormwater can lift up and transport pollutants and other undesirable substances from the ground, (Lee and Bang, 2000), stormwater must typically undergo some degree of treatment or quality improvement process otherwise it can severely impact receiving waters. In fact, stormwater is widely regarded as one of the main sources of surface water pollution in urbanized regions (Eriksson et al., 2007; National Research Council, 2008). A popular option to manage this issue in North America is the use of stormwater ponds to mitigate these issues.

Stormwater retention ponds maintain a permanent portion of the pond filled with water, termed the permanent pool of the pond. They are an economical (Wossink and Hunt, 2003) and effective option to manage stormwater. They improve water quality, mitigate flooding risks in flood-prone zones (Grigg, 2005) and can also bring interesting aesthetic and/or recreational elements to urban environments (CSQA, 2003; Lawrence and Breen, 1998). Improved water quality during cold temperature operation in northern climates such as in Ottawa, is, however, not guaranteed. In fact, some studies report that ponds may become sources of pollutants under

cold conditions (Semadeni-Davies, 2006). As a result of climate change, governments around the world are now striving to meet increasing demands on their stormwater infrastructure, which typically translates to updating urban stormwater management policies and constructing bigger stormwater ponds, and more of them. Herein lies the problem as these new, larger facilities, might increase the occurrence of hydrogen sulfide (H_2S) gas generation in these facilities.

Although literature is currently lacking on the topic of H_2S production in stormwater ponds, at least one other instance of intense H_2S gas production has been recorded in the last two years in Canada (Ku et al., 2016). The problem is not novel, however, as in 1995, Makepeace (1995) already recognized that stormwater retention ponds had the potential to produce H_2S gas. Microbial sulfate-reduction is a process which can occur in aquatic environments, in the presence of sulfate-reducing bacteria (SRB) and a suitable substrate. SRB and sulfate-reduction are well-documented topics in the wastewater field and in industry but the intense generation of H_2S gas in stormwater retention ponds is not common. Thus, creating a need for better understanding of the factors causing intense these H_2S gas production events, to improve stormwater retention pond design guidelines and to develop mitigation strategies for H_2S generation problems in existing facilities. The present study focuses on the occurrence of these intense H_2S gas production events and studies two stormwater retention ponds situated in the Riverside South region of Ottawa, Ontario, Canada.

1.2. AIM OF STUDY

The objective of this study is to develop a fundamental understanding of H_2S production in stormwater retention ponds by investigating two ponds currently in operation in Ottawa, Ontario, Canada. This study analyzes the water quality parameters and the microbial community

structure two stormwater retention ponds, RSP1 and RSP2. The specific objectives of this research are as follows:

- Verify if correlation between sulfide production and various water quality parameters (including hypoxia) exists, in an attempt to identify critical parameters and operation that initiated large H_2S production events;
- Verify if there is a correlation between total sulfide concentrations, hypoxia and bacterial analysis of sediment (using ddPCR enumeration and genetic sequencing techniques);
- Characterize the bacterial communities within benthic sediment collected from both stormwater ponds, and determine the effects of bulk water, temperature and seasonal conditions on the microbiota present in the sediment;
- Quantify the kinetics of the sediment oxygen demand due to carbonaceous and nitrogenous oxidation along with total ammonia oxidation and total sulfide production in stormwater sediment;
- Quantify the effects of temperatures at 20°C and 4°C on the kinetics of sediment oxygen demand, ammonification, sulfate-reduction and nitrogenous oxidation;

1.3. THESIS ORGANIZATION

Chapter 1 presents brief background information on the topic of stormwater management infrastructure, stormwater retention ponds, cold-weather operation of stormwater retention ponds, sulfate-reducing bacteria and events of intense H_2S gas production in stormwater retention ponds. Chapter 1 also presents the significance of the research and the objectives of the study. Chapter 2 presents a literature review of SRB, SRB kinetics, operating guidelines and recommendations for stormwater retention ponds and current techniques to mitigate H_2S in various settings. Chapter 3 describes the experimental overview, a description of the field and laboratory experiments and methodologies used to perform the herein study.

The relationships between hypoxic conditions in stormwater retention ponds, the associated water quality parameters and the production of total sulfides during both warm and cold weather are investigated in Chapter 4. Additionally, we investigate and characterize the microbial populations present in the sediment of two distinct stormwater retention ponds, compare these microbial communities and investigate correlation between hypoxia and total sulfide production events and SRB population shifts. This work will be submitted to Environmental Science: Water Research & Technology under the following title: *Hydrogen sulfide production in municipal stormwater retention ponds under ice and non-ice covered conditions* by P. M. D'Aoust, R. Delatolla, A. Poulain, R. Wang, C. Rennie, L. Chen and F. Pick.

In Chapter 5, the key kinetic parameters significant to important H_2S production are studied via field and laboratory experiments, at 20°C, 5°C and 4°C. In addition, the study investigates the suitability of laboratory experiments to predict results in the field and analyzes the predominating microbes found in the benthic sediment of a stormwater pond experience intense H_2S gas production. This work will be submitted to the Journal of Environmental

Engineering (ASCE) under the following title: *Determination of Hydrogen Sulfide Kinetic Parameters in Stormwater Retention Ponds* by P. M. D'Aoust, R. Wang, F. Pick, A. Poulain, C. Rennie, L. Chen and R. Delatolla.

Finally, Chapter 6 will present all conclusions resulting from this study in regards to total sulfide production in stormwater retention basins.

1.4. CONTRIBUTION OF AUTHORS

The following two manuscripts, which are based directly on the findings of this study, have been prepared for submission to peer-reviewed journals. The author`s contributions to the work is described below.

Article 1:

P. M. D'Aoust, R. Delatolla, A. Poulain, R. Wang, C. Rennie, L. Chen, F. Pick *Hydrogen sulfide production in municipal stormwater retention ponds under ice and non-ice covered conditions.* In preparation for submission to Environmental Science: Water Research & Technology.

P. M. D'Aoust: Conducted literature review, performed and optimized field sampling and laboratory analytical procedures, provided technical and logistical support, collected samples, analyzed results and wrote the manuscript.

R. Delatolla: Provided supervision in the development of experimental procedure, analysis of results and revision of the manuscript.

A. Poulain: Provided expertise, supervision and guidance in the microbial analytical methods.

C. Rennie: Provided expertise, supervision and guidance in the hydraulic methods.

L. Chen: Provided assistance in the collection of samples.

R. Wang: Performed microbial analyses, contributed to the analysis of the microbial results and assisted in the collection of the samples.

F. Pick: Provided supervision, expertise in the analyses of the water quality parameters and reviewed the manuscript.

Article 2:

P. M. D'Aoust, R. Wang, F. Pick, A. Poulain, C. Rennie, L. Chen, R. Delatolla. *Determination of Hydrogen Sulfide Kinetic Parameters in Stormwater Retention Ponds*. In preparation for submission to Journal of Environmental Engineering (ASCE).

P. M. D'Aoust: Conducted literature review, performed and optimized field sampling and laboratory analytical procedures, provided technical and logistical support, collected samples, analyzed results and wrote the manuscript.

R. Wang: Performed microbial analyses, contributed to the analysis of the microbial results and assisted in the collection of the samples.

A. Poulain: Provided expertise, supervision and guidance in the microbial analytical methods.

F. Pick: Provided supervision, expertise in the analyses of the water quality parameters and reviewed the manuscript.

C. Rennie: C. Rennie: Provided expertise, supervision and guidance in the hydraulic methods.

L. Chen: Provided assistance in the collection of samples.

R. Delatolla: Provided supervision in the development of experimental procedure, analysis of results and revision of the manuscript.

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CHAPTER 2 : LITERATURE REVIEW

2.1. STORMWATER SYSTEMS

Stormwater management systems typically have two main objectives: (i) control and contain stormwater, (ii) to prevent flooding and infrastructure damage, and (iii) prevent pollutants which are carried by stormwater from negatively impacting receiving waters. Stormwater management systems are comprised of many different systems aimed at achieving one or both of these objectives. Stormwater ponds and constructed wetlands are common options considered when constructing additional stormwater infrastructure, and both function in similar ways.

Stormwater management in Ontario relies greatly on systems referred to as "end-of-pipe" treatment systems, meaning systems which treat water following its capture and conveyance to another location. These end-of-pipe systems usually act as an intermediary between stormwater collected from roads and urban areas and receiving waters. The most popular options in Ontario are the following:

- Wet ponds (retention ponds)
- Artificial wetlands
- Dry ponds (detention ponds)
- Infiltration basins
- Filters and oil/grit separators

Not all systems are suitable for every situation, and Ontario's Ministry of the Environment (MOE) published their recommendations for the use of end-of-pipe systems. Table 2.1 below shows the suitability of each system (adapted from Ontario Ministry of the

Environment, 2003). Stormwater retention ponds (wet ponds) are good options as they provide good overall performance while limiting the quantity of land real-estate required, making them an economically sound option in urban areas (Wossink and Hunt, 2003).

Table 2.1: Suitability of typical end-of-pipe controls in Ontario

	Water Balance	**Water Quality**	**Erosion**	**Water Quantity**
Wet pond	L	H	H	H
Artificial Wetland	L	H	H	H
Dry pond	L	M	H	H
Infiltration basin	M	H	M	F
Filters	L	H	L	L
Oil/grit separators	L	M	L	L
End-of-Pipe Controls (H = High suitability, M = Medium suitability, L = Low suitability)				

2.1.1. Stormwater retention ponds

Stormwater retention ponds are the cornerstone of stormwater management policies in North America. They are capable of significantly reducing concentrations of the most common contaminants, nutrients and pathogens such as *Escherichia coli*, total suspended solids, nitrogens and total phosphorus (Makepeace et al., 1995). The main objectives of stormwater retention ponds are to limit the impact of urbanization on the water quality of receiving waters, by letting solids and organic matter decant out and by reducing nutrient (nitrogen, phosphorus) loading, mostly via biological processes. These facilities also provide water holding capacity within the stormwater system, and by design the facilities will often regulate outflow regardless of pond volume, reducing the risk of flooding or damage to downstream infrastructure (Bradford and

Gharabaghi, 2004, Asano et al., 2007). This is especially important in locations where combined sewers are still in operation. Combined sewers are mixed sewers, conveying both stormwater and sanitary waste, and are common in parts of older cities such as Ottawa, Halifax and Toronto. Combined sewers located in older sections of cities are often cost-prohibitive to replace due to hard to reach locations. Due to the disruption caused by construction activity (Bradford and Gharabaghi, 2004). If the capacity of combined sewers is exceeded, municipalities face two options: let the combined sewage overflow into streets, or let the combined sewers overflow into natural waters (Field and Jr, 1972, Tarr, 1979). The overflow of sewage into natural waters typically will lead to fines issued by the local/provincial/federal government, in addition to the very negative press generated by the events, due to health concerns from citizens living near the overflows. Therefore, municipalities in Canada have a strong incentive to overhaul stormwater management systems, due to economical (fines) and political pressures. Stormwater retention pond have the ability to limit outflow regardless of the holding volume, which can prevent exceeding the capacity of older infrastructure downstream (Searle, 2014).

2.2. TYPICAL STORMWATER RETENTION POND WATER CHARACTERISTICS

2.2.1. Physical characteristics

The design of stormwater retention ponds is a crucial process which requires sound engineering judgment and experience. Typically, the ponds will be built with one or more goals in mind, such as: (i) suitability for frequent but intense precipitations or discharges, (ii) suitability for rare, but very intense precipitation events, and (iii) suitability for treatment, or

removal, of specific contaminants (suspended solids, colloidal, or other dissolved pollutants and some bacteria).

Retention ponds should generally have a depth of at least 0.7m to promote wind-induced mixing and reaeration (Lawrence and Breen, 1998). To function adequately, retention ponds should have a length to width ratio of at least 3:1 to 5:1 (Lawrence and Breen, 1998; Ontario Ministry of the Environment, 2003). If ponds do not have a streamlined flow path, sufficient baffling or flow-directing mechanisms, short-circuiting could occur. Short-circuiting in stormwater retention is a significant problem as it effectively renders sometimes large portions of the stormwater pond ineffective due a lack of water flow, while at the same time causing stagnation.

In addition, the pond must have adequate drainage area, to ensure that the pond will have sufficient flow to maintain the water volume in the pond. Insufficient base flow can lead to localized hypoxia and unsatisfactory operation. The volume of the pond should also be of a size sufficient for the achievement of treatment objectives, and should also take into account for the potential volume of ice cover in winter.

2.2.2. Water chemical characteristics

Stormwater retention ponds must be of a certain size and volume to effectively mitigate flooding risk, and the design volumes are typically based on historical storm events. Prediction of effluent water chemical characteristics within said ponds is more difficult. Characteristics of the watershed and land use, along with the mineralogy of the soil are all factors which can significantly affect water quality characteristics within stormwater retention ponds. Coupled with the dynamic nature of operation in regions of the world which suffer long and cold winters and

use of road salt (Semadeni-Davies, 2006), designing and constructing a robust stormwater retention pond is difficult. Table 2.2, shown below, demonstrates average concentrations of different common stormwater constituents in both influent and effluent concentrations using data from reports archived by the ISBMP. All measured constituents below showed a decrease, except sulfate, which does not seem to be reduced by stormwater retention ponds.

Table 2.2: Average influent and effluent concentrations of water constituents from various North American studies

Constituent	**Influent Concentration (mg/L)**	**Effluent Concentration (mg/L)**
Ammonia as N	0.217	0.176
Nitrate as N	0.695	0.742
Nitrite as N	0.082	0.043
Sulfate	114	133
Chemical Oxygen Demand	90	57
Total Phosphorus as P	0.481	0.242

2.2.3. Major benthic zone biological processes

Many important and significant biological processes occur in the benthic zone of stormwater retention ponds. The most common processes which typically occur in the bottom sediments include ammonification, nitrification, sulfate reduction and phosphorus cycling.

Ammonification is the conversion of organic nitrogen to ammonia. This process is typically indicative of protein, amino acid and nucleotide decomposition (M. T. Madigan et al.,

2012) in an anaerobic environment (Dorland and Beauchamp, 1991). In stormwater retention ponds, ammonification occurs in the hypoxic portion of sediments and is linked to increases in the bulk water concentration of ammonia during ice covered periods, due to widespread and persistent hypoxia. The most common bacteria which produce ammonia from the decomposition of organic compounds are the following: *Bacillus, Clostridium, Proteus, Pseudomonas* and *Streptomyces* (Bisen et al., 2012). Ammonification can occur in many different routes, but some of the most common processes are shown below:

a) Conversion of urea to ammonium via hydrolysis, adapted from Bundy (2016).

$$NH_2CONH_2 + 2H_2O \xrightarrow[Urease]{} (NH_4)_2CO_3$$

$$(NH_4)_2CO_3 + 2H^+ \rightarrow 2NH_4^+ + CO_2 + H_2O$$

$$NH_4^+ + OH^- \underset{pH\ dependant}{\Longleftrightarrow} NH_3 + H_2O$$

b) Bacterial nitrogen fixation process, adapted from Yang et al. (2014).

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

Nitrification is the biological oxidation of ammonia (NH_3), to nitrite (NO_2^-) and then nitrate (NO_3^-). This two-step process occurs in aerobic environments and is not typically seen in hypoxic environments due to the oxygen requirement (Beutel, 2006). Nitrifying bacteria are sensitive to inhibition by toxic substances. In stormwater retention ponds, nitrification can be inhibited due to hypoxia and H_2S (Joye and Hollibaugh, 1995a). The most common nitrifying bacteria (for the step converting ammonia to nitrite) is *Nitrosomonas* (Schimel and Bennett, 2004). The inhibition of nitrification is believe to be partially responsible for ammonia

concentration increases in the bulk water during ice covered periods. The simplified process of nitrification is shown below (Schimel and Bennett, 2004).

a) Step 1: Conversion of ammonia to nitrite

$$NH_3 + O_2 \rightarrow NO_2^- + \mathrm{H}^+ + \mathrm{H_2O}$$

b) Step 2: Conversion of nitrite to nitrate

$$NO_2^- + O_2 \rightarrow 2NO_3^- + 2\mathrm{H}^+ + 2\mathrm{e}^-$$

Sulfate reduction is a biological process where sulfate is utilized by bacterium as a terminal electron acceptor. Sulfate reduction is an obligatorily anoxic process and sulfate-reducing bacteria (SRB) are inhibited by oxygen, yet some SRB are aerotolerant and so will not die rapidly due to oxygen exposure (Hardy and Hamilton, 1981), and are able to resume sulfate reduction rapidly when conditions become favourable again. In stormwater retention ponds, sulfate reduction (and the subsequent production of H_2S) is highly problematic as the H_2S produced will exacerbate pond hypoxia (Chen and Morris, 1972), inhibit some bacterial processes and lead to significantly impoverished water quality. The most common and simplified sulfate-reduction pathways are shown below:

a) General reaction (Christensen et al., 2000)

$$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$$

b) Sulfate-reduction utilizing organic carbon (D E Canfield, 2001)

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^-$$

c) Sulfate-reduction utilizing H_2 (Donald Eugene Canfield, 2001)

$$SO_4^{2-} + 2H^+ + 4H_2 \rightarrow H_2S + 4H_2O$$

Phosphorus cycling is an important process which typically occurs at the water-sediment interface within the stormwater retention pond. Iron phosphate is one of the more common forms of phosphorus encountered in freshwater benthic sediment (Hyacinthe and Van Cappellen, 2004). Depending on environmental conditions, there can either be a net increase or loss of phosphorus. Boström et al. (1988) identify 6 major phosphorus transfer mechanisms for an increase in phosphorus within the benthic sediment, such as sedimentation precipitation or absorption. In the context of hydrogen sulfide production in stormwater ponds, hydrogen sulfide generated during periods of hypoxia and subsequent sulfate-reduction will "attack" iron phosphate to form iron sulfide and in the process, release phosphorus to the bulk water.

2.3. COLD CLIMATE STORMWATER RETENTION POND DESIGN AND PERFORMANCE

It is well known that stormwater ponds do not function optimally under cold weather conditions, as biological processes slow down and salinity (due to road salt use) worsen stratification (McEnroe et al., 2012) and precipitations accumulate in form of snow instead of melting and entering the conveyance systems, causing flows within the pond to stagnate. In addition, under sub-zero temperatures, an ice surface will form across the pond, significantly hindering reaeration processes (German et al., 2003). As stormwater retention ponds are also often heavily loaded with organic matter due to algae and vegetation, cold weather will cause the accumulation of organic material at the bottom of the ponds due to plant die-off, and exacerbate oxygen demand, leading to hypoxic conditions within the facilities.

Cold seasonalweather hypoxia is a significant hazard in slow flowing stormwater retention ponds as any wildlife present in the ponds (arthropods, fish and insects) are at risk of suffocating due to decreasing dissolved oxygen (DO) concentrations. Cold weather hypoxia witnessed in stormwater retention ponds is very similar to winter fish kills in lakes (Mathias and Barica, 1980). During periods of low DO, ammonia-oxidizing bacterial processes are inhibited due to low DO, and ammonification, an anaerobic process, begins, usually leading to significantly higher concentrations of ammonia in the water, well over the acutely lethal limit of 1.25 mg NH_3-N/L established by Environment and Climate Change Canada (Environment and Climate Change Canada, 2014) for treated wastewater effluent. Since stormwater retention ponds are often home to a variety of animals and insects, this can be a problem.

Under hypoxic condition, another process which can occur is sulfate-reduction. Sulfate-reduction is a process in which sulfate is utilized as a terminal electron acceptor by bacteria to get energy, and as a result typically output hydrogen sulfide (H_2S).

Cold weather hypoxia is difficult to curb by pond design only, as flow rates will become stagnant during winter time, due to ice cover formation and a lack of significant liquid precipitation or snowmelt for several weeks at a time. In hypereutrophic lakes and stormwater ponds, a reduction in the loading of nitrogen and phosphorus could potentially mitigate oxygen demand during late summer and fall periods (Hawley et al., 2006). In wintertime, an engineering solutions such as the mechanical aeration or mixing can be appropriate to prevent or mitigate hypoxia (Cowell et al., 1987; Price and Tillman, 1991).

Retention ponds are regulated under legislation around the world. In Canada, stormwater retention ponds will fall under the Canadian Water Act, while in the EU, retention ponds will fall under the European Water Framework Directive. Similarly, retention ponds in the United States

will fall under the guidelines of the Environmental Protection Agency's (USEPA) National Pollutant Discharge Elimination System. In Ontario, stormwater retention ponds' performance are typically monitored for a set amount of time, based on the installation, the watershed and even the MOE official issuing the Environmental Compliance Approval (ECA) (Melanson, Personal communication, 2016).

2.4. HYDROGEN SULFIDE

Hydrogen sulfide is a colourless, noxious and corrosive gas. It is easily identifiable by its characteristic rotten egg smell (Burton and Pitt, 2001). When encountered in stormwater retention ponds, it is indicative of widespread hypoxic conditions. H_2S exposure is harmful to fish health (Torrans and Clemens, 1982), fish development (Van Leeuwen et al., 1986) and invertebrates (Oseid and Smith, 1974; Smith Jr. and Oseid, 1974). H_2S may also significantly harm biodiversity in urban stormwater retention ponds (Le Viol et al., 2009).

While it is undesirable, H_2S is not currently a regulated pollutant in Canadian stormwater ponds or natural waters. The only standard which exists in regards to H_2S in water is within the Canadian Drinking Water Guidelines, setting the limit at 0.05 mg/L (Government of Canada, 2012), for palatability purposes.

As outlined in **Error! Not a valid bookmark self-reference.**, the distribution of sulfide species is heavily reliant on pH. Indeed, at a pH value of 5.5 or lower, nearly all sulfides (95%+) are in the form of H_2S (the volatile form) while at a neutral pH of 7, the sulfides are divided equally between the H_2S (volatile) and HS^- (non-volatile) species.

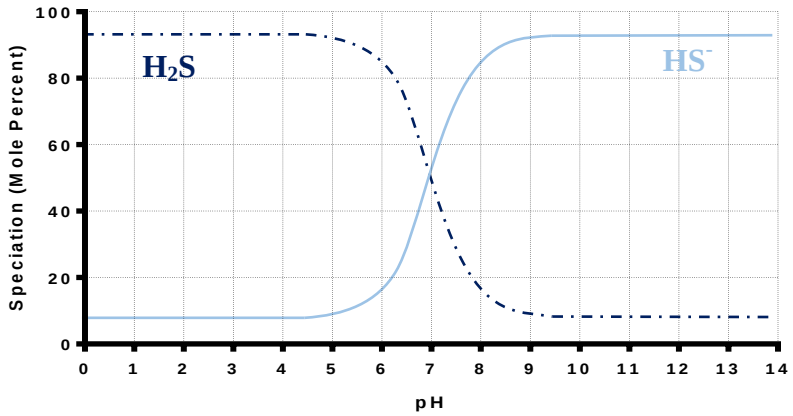

Figure 2.1: Speciation of H_2S and HS^- in water, depending on solution pH. (Adapted from Speight, 2005)

2.4.1. Sulfate-reducing bacteria

In the past, the bacteria believed to have been responsible for most sulfate-reduction has been *Desulfovibrio desulfuricans*, identified via cultures in the laboratory (Jørgensen, 1977; Nissenbaum et al., 1972; Okabe et al., 1999). Present-day genomics however have identified other SRB to predominate, likely due to the growth substrate. Bacteria belonging to the *Desulfovibrio* genus generally do not utilize acetate (Hao et al., 1996), leading to the conclusion that acetate-rich environments are likely to see other SRB predominate. Robador et al., (2009) also demonstrated that SRB communities subject to cyclical seasonal changes acclimatized to changing temperatures while SRB communities which did not experience seasonal temperature changes (such as SRB in arctic sediment, which only experience cold temperatures) instead underwent important community shifts. Some other commong SRB found in aquatic sediment are from the family *Desulfobulbaceae (unspecified genus)*, *Desulfococcus sp.*, family *Desulfobactareceae (unspecified genus)* (Zhang et al., 2016) The most common traits shared by SRB are an anoxic metabolism, varying degrees of aerotolerance (Hardy and Hamilton, 1981)

and the capacity to produce H_2S. Unlike some other anaerobic microbes, the presence of oxygen is not toxic to SRB, but rather inactivating, due to the aerotolerance. SRB will generally perform two types of sulfate-reduction: dissimilative and assimilative (M. T. Madigan et al., 2012). Assimilative sulfate-reduction does not output H_2S, rather it assimilates all produced H_2S into other organic sulfur compounds and sulfur-containing amino acids. Dissimilative sulfate-reduction on the other hand is the main source of H_2S output to the environment.

Dissimilative sulfate-reduction:

$$SO_4^{-2} + Organic\ matter \underset{\emptyset\ DO}{\Longrightarrow} H_2S + CO_2 + energy$$

Assimilative sulfate-reduction:

$$SO_4^{-2} + Organic\ matter \underset{\emptyset\ DO}{\Longrightarrow} H_2S + CO_2 + energy$$

$$H_2S \underset{assimilated\ by\ SRB}{\Longrightarrow} \text{amino acids} + \text{organic sulfur compounds}$$

Unless planned, the presence of SRB in aqueous environments is troublesome as they will produce hydrogen sulfide. Hypoxic environments capable of sustaining SRB growth will generally be found in biofilms or in benthic sediment. Therefore, to avoid intense H_2S production, oxic conditions should be maintained, to inactivate SRB.

2.4.2. Hydrogen sulfide production in natural waters and stormwater ponds

H_2S production is natural waters has been reported for the last four decades in hypereutrophic and eutrophic lakes across North America (Babin and Prepas, 1985; Ingvorsen et al., 1981), Asia (Maeda and Kawai, 1988) and Europe (Cappenberg, 1974), where intense stratification led to the development of hypoxic conditions and the unrestrained growth of SRB, leading to worsened hypoxic conditions due to an increase in oxygen demand stemming from

H_2S oxidation (Effler et al., 1988). Research on the phenomena of sulfate-reduction and the production of H_2S in stormwater retention ponds has been relatively scarce, however many of the processes occurring during the winter time in shallow lakes under ice cover also occur in stormwater retention ponds, allowing for the transfer of some of the knowledge over to stormwater retention ponds.

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CHAPTER 3 : MATERIALS AND METHODS

3.1. EXPERIMENTAL DESIGN

This study involves the Riverside South Pond #1 (RSP1) and Riverside South Pond #2 (RSP2) stormwater retention ponds, in Ottawa, Ontario, Canada. The stormwater ponds are situated in close proximity to each other and serve distinct but adjacent rain catchment areas. The facilities are designed to provide a constant flow rate downstream to reduce and mitigate the effects of spring snowmelt and high-rainfall events on the downstream combined sewers. RSP1 is a stormwater retention pond constructed in 1996 with a flow path approximately 1,000 m long, a permanent pool surface area of approximately 5.10 hectares and an average depth of 1.50 m. RSP2 is a retention pond that was constructed in 2007, it has a total length of approximately 290 m, a permanent pool surface area of approximately 0.97 hectares and an average depth of 1.41 m, and a depth of 2.49 m at the outlet. In order to compare both ponds over winter and summer operations, water samples were collected on a regular basis over a period of 448 days, from June 3^{rd,} 2014 to August 25^{th,} 2015. The sampling frequency was bi-weekly during summer time and aimed to be every 10 days during the rest of the study. All collected water samples were characterized for pH, soluble ammonia as NH_3-N, soluble nitrite as NO_2-N, soluble nitrate as NO_3-N, soluble chemical oxygen demand (sCOD), soluble total phosphorus as PO_4^{3-}P, soluble sulfate as SO_4^{2-} and total sulfides as S_2^-. In addition, in-situ dissolved oxygen and temperature measurements were taken while sampling.

In addition to extensive field testing of the stormwater retention ponds, laboratory experiments were also conducted in order to measure rates of change in bulk water due to sediment bacterial activity. Using sediment collected from the outlet of RSP2, five different experiments were conducted in BOD bottles under different sets of conditions. These bench-top

experiments allowed measuring and comparing rates of change observed in the field and in the laboratory experiments.

3.2. ANALYTICAL METHODS

3.2.1. pH determination

The pH of all samples was measured with a Corning Pinnacle 530 pH meter (Corning, NY) with a glass electrode. Prior to measurements, the pH was regularly calibrated using three buffer solutions with standard pH of 4, 7 and 10, while rinsing with distilled water and drying with delicate task wipes in between every measurement. In between measurements, the pH meter's electrode was stored in a pH 7 buffer solution and was rinsed and dried following the procedure above before measurements were taken. The range of this unit was from -2.00 to 19,99 pH, and the resolution was of ± 0.01 pH units.

3.2.2. Ammonium/ammonia

The soluble ammonia concentration of all samples was measured using a HACH TNT 830 (HACH Method 10205) [0.015 – 2.00 mg/L NH_3-N] kit. Samples were filtered through a 1.5 µm filter via a filtering apparatus connected to a Marathon Electric 110-115V ¼ HP vacuum pump (distributed by Thermo Fisher Scientific, Waltham, MA). The filtrate was then transferred to a clean sample containment bottle and gently stirred. 5.0 ml of sample were then pipetted from the container and into a HACH TNT 830 vial. The protective foil on the cap was removed, the cap was inverted, screwed on and the sample was then immediately shaken 5 times to dissolve the reagent in the cap. The vial was then left aside in a vial tray for 15 minutes. After the reaction took place, the vial was wiped using a delicate task wipe and inserted into a calibrated HACH DR 6000 spectrophotometer. The spectrophotometer has an internal calibration which delivers

NH_3-N concentrations in mg/L directly. Samples were stored at 4°C and tested within 24 hours of sampling to avoid degradation and when not possible, they were frozen at -20°C until analyzed. The HACH TNT 830 reagent vials were stored in the refrigerator at 4°C as outlined in this particular reagent's operation manual.

3.2.3. Nitrite

The soluble nitrite concentration of all samples was measured following Standard Methods 4500-NO_2^- B. Samples were filtered through a 1.5 µm filter via a filtering apparatus connected to a Marathon Electric 110-115V ¼ HP vacuum pump (distributed by Thermo Fisher Scientific, Waltham, MA). The filtrate was then transferred to a clean sample containment bottle and gently stirred. 10.0 ml of sample were then pipetted from the container and into a clean 25 ml Pyrex glass Erlenmeyer flask. 0.4 ml of nitrite color reagent was then added; the sample was stirred and then left to sit for 30 minutes while the reaction took place. After the reaction finished, approximately 3.5 ml of the solution was pipetted via a glass pipette (and rubber bulb) to a 10mm HACH Co. glass cuvette to condition the cuvette. The cuvette was then emptied into a liquid waste container and then filled once again with the same solution. The cuvette's exterior was then wiped with a delicate task wipe and inserted into a calibrated HACH DR 6000 spectrophotometer and read at an optical wavelength of 543 nm. The absorbance values were converted into NO_2 concentrations using a previously prepared calibration curve. Samples were stored at 4°C and tested within 24 hours of sampling to avoid degradation and when not possible, they were frozen at -20°C until analyzed. The nitrite color reagent was stored in a dark glass container, in the refrigerator, at 4°C as best practice measure. The range of this method is from 0.01 to 1.00 mg/L NO_2^-.

3.2.4. Nitrate

The soluble nitrate concentration of all samples was measured following Standard Methods 4500-NO_3^- B. Samples were filtered through a 1.5 µm filter via a filtering apparatus connected to a Marathon Electric 110-115V ¼ HP vacuum pump (distributed by Thermo Fisher Scientific, Waltham, MA). The filtrate was then transferred to a clean sample containment bottle and gently stirred. 10.0 ml of sample was then pipetted from the container and into a clean 25 ml Pyrex glass Erlenmeyer flask. 0.4 ml of nitrite color reagent was then added; the sample was stirred and then left to sit for 30 minutes while the reaction took place. After the reaction finished, approximately 3.5 ml was pipetted via a glass pipette (and rubber bulb) to a 10mm HACH Co. quartz cuvette to condition the cuvette. The cuvette was then emptied into a liquid waste container and then filled once again with the same solution. The cuvette's exterior was then wiped with a delicate task wipe and inserted into a calibrated HACH DR 6000 spectrophotometer and read at an optical wavelength of 543 nm. The instrument measured the absorbance, and the absorbance values were converted into NO_3 concentrations using a previously prepared calibration curve. Samples were stored at 4°C and tested within 24 hours of sampling to avoid degradation and when not possible, they were frozen at -20°C until analyzed. The nitrite color reagent was stored in a dark glass container, in the refrigerator, at 4°C as best practice measure. The range of this method is from 0.01 to 11.00 mg/L NO_3^-.

3.2.5. Soluble chemical oxygen demand

The soluble chemical oxygen demand concentration of all samples was measured following the HACH Method 8000 (a kit version of Standard Methods 5220 D). A HACH DRB200 reactor/digester was preheated to 150 °C prior to the start of the experiment. Samples were filtered through a 1.5 µm filter via a filtering apparatus connected to a Marathon Electric

110-115V ¼ HP vacuum pump (distributed by Thermo Fisher Scientific, Waltham, MA). The filtrate was then transferred to a clean sample bottle and gently stirred. 2.0 ml of sample was then pipetted from the container and into a HACH COD LR vial. The vial was then tightly sealed and inverted 10 times to ensure consistent mixing. The vials were then added to the digester and its timer was started (for the COD routine). After 140 minutes elapsed, the samples were then removed from the digester and left to cool to ambient temperature in a test tube rack. Samples were then wiped down and read in a calibrated HACH DR 6000 spectrophotometer using the 430 COD LR program to obtain the soluble chemical oxygen demand concentration. Samples were stored at 4°C and tested within 24 hours of sampling to avoid degradation, and when there was going to be a longer delay, they were frozen at -20°C until analyzed. The range of this method is from 3to 150 mg/L COD^-.

3.2.6. Soluble total phosphorus

The soluble total phosphorus concentration of all samples was measured following the HACH Method 8190 (a kit version of Standard Methods 4500-P B). A HACH DRB200 reactor/digester was preheated to 150 °C prior to the start of the experiment. Samples were filtered through a 1.5 µm filter via a filtering apparatus connected to a Marathon Electric 110-115V ¼ HP vacuum pump (distributed by Fisher Scientific). The filtrate was then transferred to a clean sample bottle and gently stirred. A 5.0 ml sample was then pipetted from the container and into a HACH PhosVer® 3 TNT vial. The content of one Potassium Persulfate Powder Pillow was then added to the vial and it was then tightly sealed and shaken 12 times to ensure consistent mixing. The vials were then added to the digester and its timer was started (for the 150 °C, 30-minute routine). After 30 minutes elapsed, the samples were then removed from the digester and left to cool to ambient temperature in a test tube rack. Once the samples were cooled, 2.0 ml of

1.54 N Sodium Hydroxide Standard Solution was then added to the sample, and the vial was then recapped and inverted 12 times. The samples were then wiped and read in a calibrated HACH DR 6000 spectrophotometer using the 536 P Total/AH PV TNT program, to obtain a sample blank value. The vials were then removed and the contents of one PhosVer® 3 Powder Pillow was then added to the vial and shaken for approximately 20 seconds to mix well. A two-minute timer was then started on the instrument and the vials were left to sit in a test tube rack until the two minutes expired. Following this, the samples were immediately wiped down again and read using the same program to obtain the soluble total phosphorus concentration. Samples were stored at 4°C and tested within 24 hours of sampling to avoid degradation and when not possible, they were frozen at -20°C until analyzed. The range of this method is from 0.06 to 3.50 mg/L PO_4^{3-}.

3.2.7. Soluble sulfate

The soluble sulfate concentration of all samples was measured following the HACH Method 8051 (equivalent to USEPA method 375.4 for wastewater). Samples were filtered through a 1.5 µm filter via a filtering apparatus connected to a Marathon Electric 110-115V ¼ HP vacuum pump (distributed by Thermo Fisher Scientific, Waltham, MA). The filtrate was then transferred to a clean sample bottle and gently stirred. 2.5 ml of sample was then pipetted from the container and into a 10 ml quartz sample cell. 7.5 ml of deionized water was then pipetted to the quartz sample cell and the sample was lightly stirred to obtain sample homogeneity with the dilution. The content of one SulfaVer 4® powder pillow was then added to each sample cell and they were stirred and then left to sit for 5 minutes while the reaction took place. After the 5-minute timer elapsed, the samples were wiped and the sample absorbance was read in a calibrated HACH DR 6000 spectrophotometer using the 680 Sulfate program. Samples were

stored at 4°C and tested within 7 days of sampling to avoid degradation, when there was going to be a longer delay the samples were frozen at -20°C until they could be analyzed. The range of this method is from 2 to 70 mg/L SO_4.

3.2.8. Total sulfides

The total sulfides concentration of all samples was measured following the HACH Method 8131 (equivalent to Standard Methods 4500-S_2^- D). Preserved samples (see description in section 3.2.9) were pipetted promptly to clean 10 ml quartz sample cells and immediately stoppered using rubber stoppers. One-by-one, samples were then rapidly unstoppered, 0.5 ml of Sulfide Reagent #1 was added and the samples were then rapidly restoppered. The samples were stirred lightly to ensure homogeneity. Sulfide Reagent #1 lowers the pH of the sample and "releases" the sulfide which has been previously complexed with Zinc Acetate to prevent sulfide volatility. The samples were then unsealed once again, 0.5 ml of Sulfide Reagent #2 was added after what the samples were rapidly stoppered. The samples were gently stirred and a timer of 5 minutes was started on the HACH DR 6000 spectrophotometer. After the 5-minute timer elapsed, the samples were wiped and read in the calibrated HACH DR 6000 spectrophotometer using the 690 Sulfide program. Samples were stored at 4°C and tested within 24 days of sampling to avoid degradation. When there was going to be a longer delay the samples were kept in the refrigerator at 4°C for a maximum of 7 days and tested as soon as possible. The range of this method is from 5 to 800 μg/L S^{2-}.

3.2.9. Preservation of sulfide samples

In order to ensure sample sulfide sample integrity, it was necessary to prepare the reagents to be added to water samples destined to total sulfides measurements. At normal surface

water pH values (6.5-7.5), sulfides in aqueous solutions are readily capable of escaping to the atmosphere, therefore, chemical stabilization is necessary.

The first addition is a 6N sodium hydroxide (NaOH) solution to samples. The addition of the NaOH solution to samples raises their pH (typically to the pH range of 12-13.5), affecting the speciation of sulfides present in the sample. Typically, 1.5 ml of 6N NaOH solution was added to 250 ml water samples. To prepare the 6N NaOH solution, 239.28 g of NaOH crystals were slowly added a beaker containing 1 L of distilled water and dissolved using a magnetic stirrer and stir bar. Care must be taken to let the solution cool down between the additions of NaOH crystals, as this reaction is exothermic and generates a moderate amount of heat. Once cooled, the solution was transferred to and stored in a capped glass bottle and kept out of direct light at normal room temperature.

The second chemical addition necessary for stabilization of sulfides is the addition of a 2N zinc acetate solution. Zinc acetate reacts with hydrogen sulfide to form a precipitate and prevents its escape. Typically, 1.5 ml of 2N zinc acetate solution was added to 250 ml water samples. To prepare the 2N zinc acetate solution, 220 g of $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ were added to 1 L of distilled water and slowly dissolved using a magnetic stirrer and stir bar. The solution was transferred to and stored in a capped glass bottle and kept out of direct light at normal room temperature.

3.2.10. Measurement of dissolved oxygen

The dissolved oxygen concentration of the stormwater ponds was determined in-situ by using a YSI ProODO DO meter to measure DO concentration at 0.20 m and 1.50 m of depth under the water surface. To perform laboratory measurements of DO concentrations, a Hach

HQ40d optical DO meter was used instead. Units were periodically calibrated according to manufacturer's guidelines and recommendations to maintain measurement accuracy and precision. For the YSI unit, the range and resolution were from 0 to 200% air saturation ± 1% of the reading or ± 1% air saturation, whichever one is greater. For the Hach unit, the range was from 0.01 - 20 mg/L and the resolution was of 0.01 mg/L.

3.3. LABORATORY EXPERIMENTS

In order to measure the changes in oxygen, ammonia and sulfide concentrations within the bulk water phase of the pond, small 300 ml glass reactors (BOD bottles) were utilized to run batch experiments. The experiments were performed using the author's adaptation of Standard Methods 5210 A, B, & C (APHA, WEF, 2012), and resembled procedures outlined by Wang (1980) for the fractionation of sediment oxygen demand (SOD). The modified procedure is described below:

3.3.1. Reagent and sample preparation

A 3 g/L allylthiourea solution was prepared by dissolving 2.0 g allylthiourea ($C_4H_8N_2S$) in 500 ml of distilled water and then diluting to 1 L. This solution was prepared 5 days (or less) before being utilized and stored in a glass container at 4°C until needed. Typically, 1 ml of allylthiourea solution was added to vessels to inhibit nitrification.

67 glass vessels were utilized in this series of experiments. Their distribution is shown in Table 3.1 below. The testing vessels were first filled with 53.52g ± 3.70g of sediment originating from the pond benthic zone at location RSP2-4 and filled up to the 300 ml mark (fully filled up to the bottom of the neck) with water from the pond collected at the same location. Samples

bottles which required the addition of nitrification inhibitors received 1 ml of the allylthiourea solution prior to the addition of the pond water, but after the addition of sediment.

15 additional glass vessels were utilized to measure the pond water BOD_5, as per SM 5210 A, B & C methodology. These vessels only contained stormwater from the pond, to monitor oxygen consumption in the water phase without any effect from sediment. 15 additional glass vessels were utilized as blanks and were filled with distilled water, at 20°C, as control blanks.

DO starved testing was conducted to attempt to simulate real-world pond conditions under ice-cover and to jump-start the reaction, as preliminary testing showed that the time required to consume most of the oxygen in the reactor vessel was sometimes greater to 30 days.

Table 3.1: Outline of experiments conducted with stormwater pond sediment and water

Trial #1 (27 sample bottles total)
• **4°C** • **No nitrification inhibition** • **Sampling on days 0, 14, 16, 18, 20, 22, 24, 26, 28, 30**
Trial #2 (10 sample bottles total)
• **20°C** • **No nitrification inhibition** • **Sampling on days 0, 1, 2, 3, 4, 5**
Trial #3 (10 sample bottles total)
• **20°C** • **Nitrification inhibition with allylthiourea** • **Sampling on days 0, 1, 2, 3, 4, 5**
Trial #4 (10 sample bottles total)
• **4°C** • **No nitrification inhibition, DO below 1 mg/L** • **Sampling on days 0, 1, 2, 3, 4, 5**
Trial #5 (10 sample bottles total)
• **4°C** • **Nitrification inhibition with allylthiourea, DO below 1 mg/L** • **Sampling on days 0, 1, 2, 3, 4, 5**

3.3.2. Testing procedure

After samples have been prepared, DO is measured using a Hach HQ40d optical DO meter. Once the initial DO reading has been taken, ammonia and total sulfides are measured, the bottles are stoppered, and a proper seal is ensured by adding a small amount of distilled water around the neck of the stopper, and then covering the stopper and neck of the bottle with aluminum paper foil, to prevent evaporation.

Samples are then put into their respective incubation locations, shielded from light using aluminum foil and behind closed door, in a 20°C temperature controlled incubator or at 4°C in a refrigerator. Once samples were due for testing, they were sacrificed, i.e. they were unsealed and tested, and were not reused again in the experiment. Once samples are unsealed, water samples are taken immediately for the testing of sulfide (as per the procedure outlined in section 3.2).

Following this, the DO is then measured, followed by other constituents. Samples are then discarded.

3.4. FIELD MEASUREMENTS

For the full-scale study, water quality samples were collected at two specific depths (0.20 m and 1.50 m from the surface) and at four locations in RSP2 and at one location in RSP1. Sample locations are shown below in Figure 3.1. The sampling locations were chosen for the following reasons: it was decided that two of the sampling location should be near the inlet (RSP2-2) and outlet (RSP2-4) of the pond, another point was located near the secondary inlet location in case of very intense flow (RSP2-1) and the fourth point was placed in the deeper portion of the pond, where we expected some of the sediment to deposit (RSP2-3). To reach the sampling locations, two aluminum boats supplied by the City of Ottawa were utilized to navigate both ponds. The research group utilized oars, two 6.8 kg anchors, a Minn Kota 30 Endura C2 electric motor and a MotoMaster Nautilus 800A battery pack to navigate the boats on the ponds. During ice covered periods, which did not allow for the use of boats, the group physically went out on the ice and drilled holes with an ice auger to give access to the bulk water phase and allow for the collection of water and sediment samples. Water samples were collected using a Wildco 1520 C25 Kemmerer 2.2L TT water sampler (Yulee, FL). The water sampler was modified and a 0.40 m long, 12.7 mm inner diameter silicone hose was added to the decanting valve of the sampler (as shown in Figure 3.2), a measure to help restrict air entrainment into the sample containers during the collection of water at depth in the pond. For general water samples not destined to sulfide determination, samples were collected using clear 500 mL PETE (polyethylene) bottles, with wide-mouth plastic screw caps. For sulfides, necked 250 mL LDPE or HDPE (low-density polyethylene or high-density polyethylene), semi-opaque bottles with

screw cap were utilized. Necked bottles were utilized for sulfide measurements, as the tapering of the neck allowed for more effective removal of air from the sampling bottle during filling, reducing the stripping of sulfides and improving the overall testing accuracy.

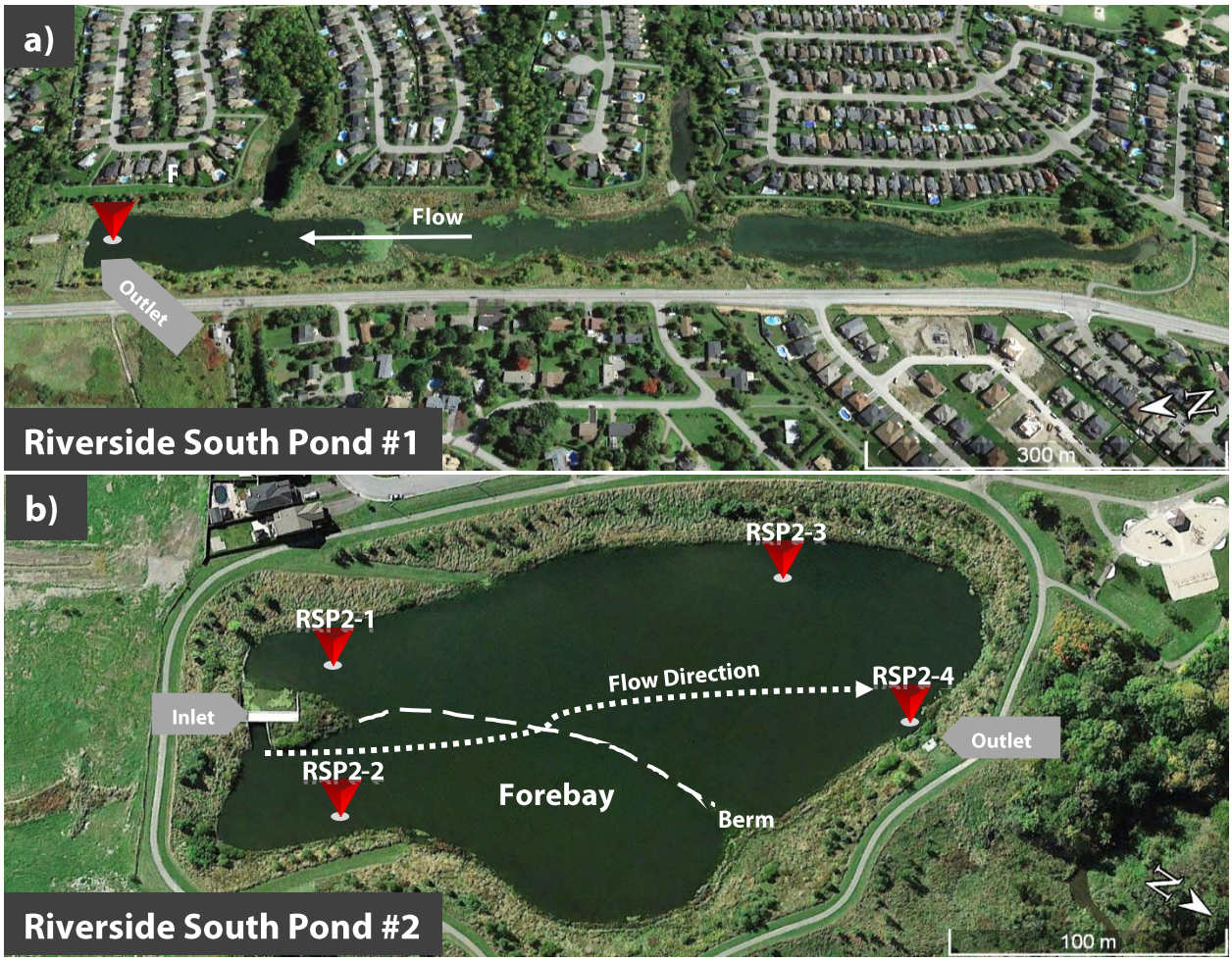

Figure 3.1: Sampling locations, a) RSP1 and b) RSP2

Total sulfide samples were immediately preserved with the consecutive addition of a 2N zinc acetate and 6N sodium hydroxide solution after collection. The minimization of air entrainment into the water samples reduced the effect of oxygen on the total sulfide

concentration of the water samples and ultimately increased the precision of the total sulfide measurements.

Figure 3.2 Collection of water samples in winter, with the modified hose attachment installed on the water sampler

3.4.1. Sample collection for kinetics experiment

For the laboratory study, bulk water and sediment samples were collected from the sediment near RSP2-4 (outlet of the pond), approximately 1.5m from the outlet pipe. Approximately 15 liters of sediment were collected from RSP2-4 using a stainless steel Ekman Dredge and stored at 4°C for a maximum for 14 days before it was used. Likewise, approximately 20 liters of bulk water was collected from RSP2-4 at a depth of 1.50 m.

3.4.2. Statistical analysis

Statistical analysis techniques were employed to ensure statistical relevance of data. Locations in the ponds were intermittently sampled in triplicates on an alternating schedule, allowing all five sampling locations to be sampled in triplicates over a period of 5 weeks (1 location in triplicate, per week). In addition, a regression analysis was performed between total sulfides and DO, ammonia, sulfate, nitrate, nitrite, chemical oxygen demand, total phosphorus and pH values, and also between DO and the same list of constituents, to evaluate the relevance of each parameter to changes in sulfides and DO, respectively. Regression analyses were performed on a depth and location basis. Regression results were then corrected for familywise error rate (FWER) using the Bonferroni correction (Abdi, 2007).

3.5. CALCULATIONS OF KINETIC PARAMETERS

3.5.1. Arrhenius' temperature coefficient

Arrhenius' temperature coefficient is a very common parameter in water quality models, as is widely accepted as a parameter used to approximate the effects of temperature on enzymatic, chemical and bacteria reactions rates. To calculate Arrhenius' temperature coefficient using results from the rates of change experiments performed in the laboratory, one must utilize the following formula (Walker and Snodgrass, 1986):

$$k_T = k_{20}\theta^{T-20} \qquad \textbf{Equation 3.1}$$

Where k_T is the volumetric rate constant at the target temperature (h^{-1}), k_{20} is the volumetric rate constant at 20°C (h^{-1}), T is the target temperature (°C) and Θ is Arrhenius' temperature coefficient.

By plotting concentrations of different constituents over time at 20°C and at another known temperature, one can determine the slope of each curve, and by inputting the k values into Equation 3.1 it is possible to determine Arrhenius' temperature coefficient.

3.5.2. Determination of rates of change in bulk water concentrations

By utilizing data obtained from the BOD bottle experiments, it was possible to determine rates of change values (k) by plotting the change per unit of time (Δ mg/L) of constituents over time (day). For the laboratory experiments, the volume of water, sediment and vessel dimensions were known therefore calculations to determine the rate of production or consumption of different constituents was intuitive, as demonstrated in equation 3.2 below.

$$Rate_{Production/Cconsumption} = \Delta_{concentration}/(sediment\ area) \qquad \textbf{Equation 3.1}$$

For the field study, the rates of change of total sulfides, dissolved oxygen and ammonia concentration on a per day basis were first calculated. Afterwards, utilizing bathymetric information of the RSP2 pond, the volume of water present based on the ice cover thickness at specific dates was calculated. From this, and knowing the surface area of sediment throughout the entire RSP2 pond, it was possible to determine the daily rate of production or consumption of total sulfides, dissolved oxygen and ammonia, normalized by surface area of sediment (g/m^2/day).

3.6. BACTERIAL ANALYSIS

Sediment samples collected in triplicate from the benthic zone of the ponds were washed using 5 ml of buffer solution to remove potential PCR inhibitors. DNA was extracted from the sediment samples using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA)

and stored at -80°C in Tris(hydroxymethyl)aminomethane buffer until the DNA samples were extracted. Extraction quality was determined using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

Extracted DNA was amplified using a BIO-RAD S1000TM Thermal Cycler (Saint-Laurent, QC). The primers used (forward 27F and reverse 907R) targeted the hypervariable region of the 16s rRNA. The primers were acquired from Integrated DNA Technologies (Coralville, IA). The amplified product was 1000bp. The primers utilized for the amplification process are primer 27F Sequencing was performed by Molecular Research LP (Shallowater, TX), which amplified DNA using a two-step polymerase chain reaction (PCR) targeting the V6 hypervariable region of the 16s rRNA. The primers used were custom in-house primers developed and prepared by Molecular Research LP. Each sample was then sequenced as a 2x300bp run on an Illumina MiSeq sequencer (San Diego, CA). The DNA sequencing results were analysed using the Bio-Linux (Field et al., 2006) operating system. The QIIME software was utilized to perform operational taxonomical unit (OTU) grouping (Tanja Magoč and Salzberg, 2011) and the determination of the organisms present in the sediment of the ponds. ddPCR evaluation was conducted using a BIO-RAD QX200TM ddPCR system (Hercules, CA). Count data from the ddPCR was acquired using the Quantasoft software, developed by BIO-RAD (Hercules, CA). The expected amplicon size for SRB and methanogens were of 221bp and 491bp, respectively. Table 4.4.1 outlines the primers utilized for ddPCR. All primers were purchased from Integrated DNA Technologies (Coralville, IA).

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CHAPTER 4 : HYDROGEN SULFIDE PRODUCTION IN MUNICIPAL STORMWATER RETENTION PONDS UNDER ICE AND NON-ICE COVERED CONDITIONS

4.1. SETTING THE CONTEXT

The article presented in Chapter 4 is titled *Hydrogen sulfide production in municipal stormwater retention ponds under ice and non-ice covered conditions* by P. M. D'Aoust, R. Delatolla, A. Poulain, R. Wang, C. Rennie, L. Chen, F. Pick. This article is in preparation for publication in Environmental Technology. This article describes the characterization of conditions present in stormwater retention ponds experiencing sulfate-reduction and significant sulfide production, and identifies the main water quality conditions associated with the initiation of significant sulfide production. Additionally, this study quantified the percent abundance of the dominant sulfate-reducing bacteria (via next generation genomic methods) in a non-sulfide producing reference pond and a pond that exhibits significant sulfide production. The percent abundance data is used to identify whether distinct sulfate-reducing bacterial populations are observed in stormwater ponds that experience significant hydrogen sulfide production events.

4.2. ABSTRACT

Stormwater retention ponds are an increasingly integral component of stormwater management policies across the world. Under prolonged hypoxia, some of these ponds are capable of releasing large quantities of hydrogen sulfide (H_2S) gas. This study monitored water quality constituents in two stormwater retention ponds, RSP1 (reference pond) and RSP2 (problematic pond), over a period of 448 days to identify factors driving hydrogen sulfide generation in stormwater ponds. It was found that background total sulfide concentrations were not statistically different during summer periods (RSP2-1 to 4: 0.012 ± 0.001 mg/L-S; RSP1-1: 0.010 ± 0.001 mg/L-S). It was found that there was a link with sediment deposition locations within RSP2 and the likelihood of having H_2S gas production. There was a strong correlation between low dissolved oxygen (DO) at depth and intense production of H_2S gas production events in RSP2 ($p < 0.006$, $R > 0.58$). Both RSP1 and RSP2 were found to be unstratified during periods which did not have significant H_2S gas production. During winter, low DO conditions (hypoxia) were first witnessed in RSP2, before being witnessed approximately a month later in RSP1. Additionally, pH was found to fluctuate depending on pond oxic levels. Finally, it was found that seasonal changes did not promote the proliferation of any specific organism, and the intense sulfide production in RSP2 is a result of increased SRB activity, but not of a community shift.

4.3. INTRODUCTION

The management of rainfall and run-off is a significant concern in heavily urbanized North American and European communities, where stormwater is the leading cause of surface water pollution (Eriksson et al., 2007; National Research Council, 2008). A popular method to

manage stormwater in Canada is the installation of a wet retention pond (Drake and Guo, 2008). Stormwater retention ponds have been shown to be economical options (Weiss et al., 2007; Wossink and Hunt, 2003), capable of mitigating the effects of increased urbanization and the increase in the quantity of impervious surfaces, which impacts both the quality and the quantity of water that must be captured, stored, treated and discharged (Ontario Ministry of the Environment, 2003). Proper implementation of these facilities will often increase the quality of receiving waters. These facilities can also often be turned into attractive water features (Polta, 2004). Retention ponds therefore play an important role in stormwater management plans across the globe and are frequently considered to be the "backbone of urban stormwater quantity-quality management" (Novotny, 2003).

The European Water Framework Directive (EU-WFD) and the Canadian Water Act provide guidelines for the design of stormwater retention ponds for all member-states/provinces (European Comission - Community research, 2008), where every individual member-state/province also hold their own set of regulations and guidelines (European Comission - Community research, 2008). As municipalities attempt to mitigate flooding risks, infrastructure damage, land washouts and negative water quality impacts on the receiving waters, many have now adopted stormwater retention ponds as a main tool to mitigate the environmental impact of increased urbanization. The International Stormwater Best Management Practices (BMP) Database (ISBMPD), which collects and repertories government-submitted data and studies of such facilities, reports that there are over 530 BMP studies, investigating over 16,000 stormwater management facilities. These studies investigated performance and treatment efficiency, of at least 57 of the facilities were stormwater retention ponds. An analysis of the 2014 summary report reveals that numerous stormwater retention ponds failed to meet their treatment objectives

for total dissolved solids, and failed to reduce the loading of certain dissolved metals, such as nickel. It is a given than not all facilities will always operate optimally and will not always meet their treatment objectives, this can ultimately result in the retrofitting of the retention ponds (Borne et al., 2013; Khan et al., 2011; Roy et al., 2008). Further, climate change is projected to change global weather patterns (Moss et al., 2010; Oreskes, 2005) and have significant effects on the hydrological cycle at various locations across the world (National Research Council, 2008). Locales situated in northern temperate climates, such as Southern Canada (Lemmen et al., 2008), the Northern United States (Palmer and Räisänen, 2002) and Northern Europe (Beniston et al., 2007) are expected to experience climate change in the form of increased, more intense or frequent precipitation (Kay et al., 2006; Knutson et al., 2010) and warmer daily minimum temperatures (Lemmen et al., 2008; Meehl et al., 2007). In response, some governments are striving to improve their urban stormwater management planning, practices and policies. As a consequence, stormwater retention ponds are becoming increasingly common and increasingly larger, to accommodate the heightened precipitation in the near future (Semadeni-Davies et al., 2008). Although larger pond design guidelines will improve the retention capacity for large rain events, this large retention capacity may also impact the water quality of the ponds and increase the occurrence of hydrogen sulfide (H_2S) generation at these facilities due to the more frequent occurrence of dead zones and low dissolved oxygen conditions.

H_2S is a noxious and toxic gas produced by sulfate-reducing bacteria (SRB). SRBs are anaerobic microorganisms that facultatively or obligatorily reduce sulfate (SO_4^{2-}) to H_2S to obtain energy (M. T. M. Madigan et al., 2012). The occurrence of H_2S gas in stormwater retention ponds is an indicator of sub-optimal facility design or operational problems as it is produced during periods of significant hypoxia (Ku et al., 2016). Although Makepeace

(Makepeace et al., 1995) recognized H_2S production as a potential problem in stormwater retention ponds, literature on the occurrence of H_2S gas in stormwater systems is currently limited. There is presently a fundamental lack of knowledge and understanding of the processes and factors affecting the initiation and sustained production of H_2S in stormwater retention ponds.

Current efforts to mitigate surface water pollution are at risk of falling short of expectations if policies and practices do not take into account events like H_2S production in stormwater infrastructure. Hence, climate change and the need for increased capacity in stormwater retention ponds concomitantly combined with an evident lack of knowledge on H_2S production in stormwater retention ponds has led to the need to better understand the production of H_2S in stormwater retention ponds. The aim of the study is to identify and quantify the key parameters influencing H_2S generation and total sulfide presence in stormwater retention ponds during various seasons of operation, including ice covered operation during winter months. In particular, water quality parameters and the microbial community of sediment collected from the benthic zone of two stormwater retention pond facilities in Ottawa, Ontario, Canada were studied and compared across a full year of operation. Water samples were collected in a manner permitting the analysis of spatial and depth variations throughout the facilities. The Riverside South Pond #2 (RSP2) was observed to generate H_2S during specific periods of operation prior to this work while the reference pond of Riverside South Pond #1 (RSP1), which is located in close proximity to RSP2, was not shown to generate H_2S.

4.4. MATERIALS AND METHODS

4.4.1. Experimental plan

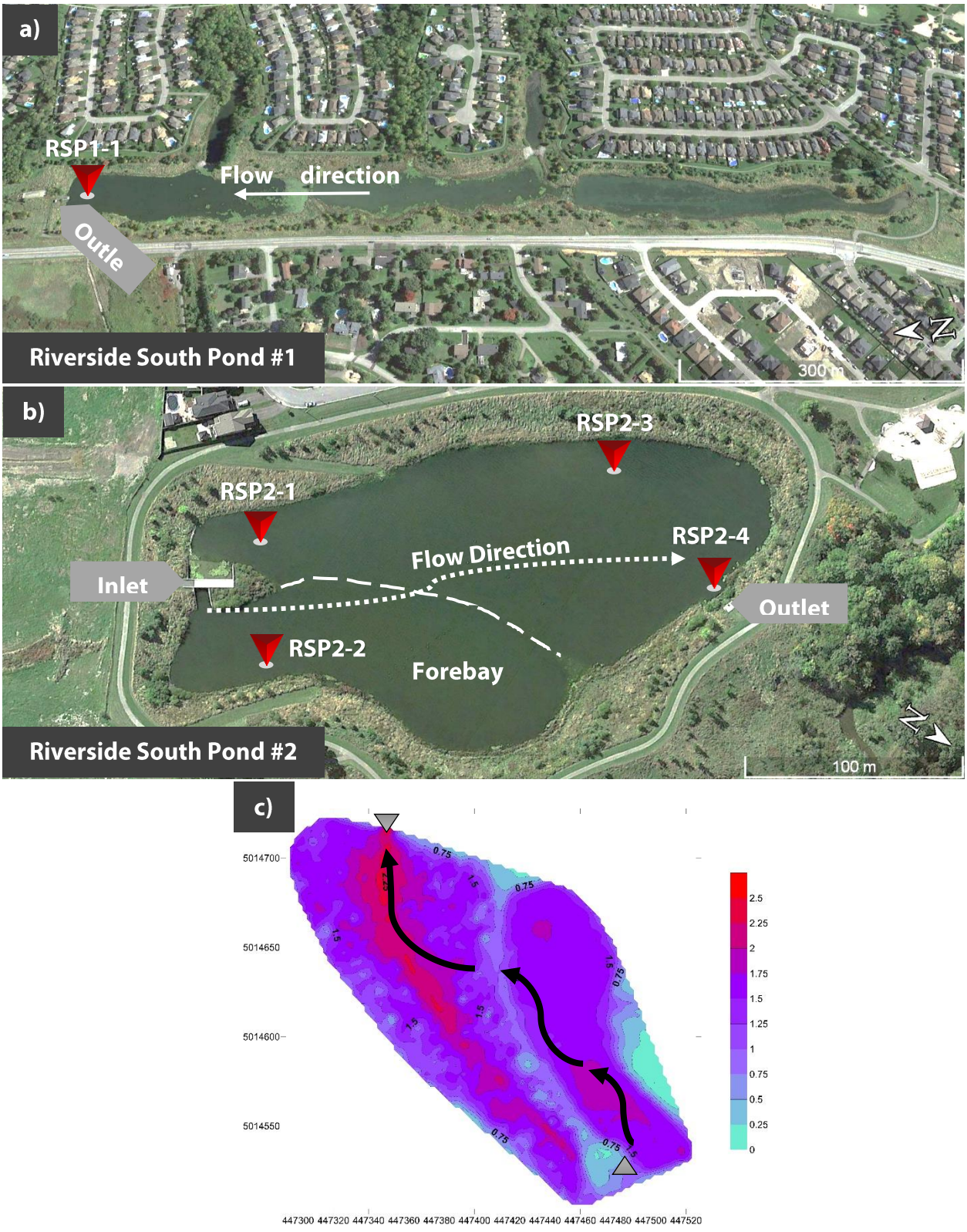

Figure 4.4.1 Sampling locations, a) RSP1, b) RSP2, c) contour plot of the bathymetry of RSP2, with the flow path highlighted

Water samples were collected at the locations indicated in Figure 4.4.1. Samples were collected using a small boat during non-ice covered conditions and by auguring through the ice during ice covered conditions.

From December 31st 2014 until April 8th 2015, both ponds were fully covered with ice, allowing access and samples to be collected using an ice-auger to drill through the ice. Ice cover persisted until April 8th 2015 and proceeded to melt from April 8th to May 12th, 2015. During periods of ice formation and melt, sampling frequency was decreased as pond conditions were not adequate to allow for safe access.

4.4.2. Water quality sampling, analysis and in-situ measurements

In-situ dissolved oxygen (DO) measurements were acquired using a handheld field optical YSI ProODO DO meter (Yellow Springs, OH). The field optical DO meters were calibrated by following manufacturer instructions at three different occasions during the study. DO was measured at 1.50 m and 0.20 m below the surface of the water with the handheld units. Samples were collected at the locations shown in Figure 4.4.1 a) and b). In addition to weekly in-situ measurements performed with the handheld unit, two YSI 6600 V2 datasondes (Yellow Springs, OH) were installed at a depth of approximately 1.00 m from the bottom at the outlets (Figure 4.4.1) of both RSP1 and RSP2. The datasondes provided continuous DO, pH, water level and temperature measurements.

Water quality samples were collected at two specific depths (0.20 m and 1.50 m from the surface) and at four locations in RSP2 and at one location in RSP1. Samples were collected using a Wildco 1520 C25 Kemmerer 2.2L TT water sampler (Yulee, FL). A simple modification to the 1520 C25 water sampler was performed by adding a 0.40 m long, 12.7 mm inner diameter piece

of silicone tubing on the decanting valve of the sampler; with the added tubing restricted air entrainment into the sample containers during the collection of water at depth in the pond. The minimization of air entrainment into the water samples reduced the effect of oxygen on the total sulfide concentration of the water samples and ultimately increased the precision of the total sulfide measurements. Total sulfide samples were also immediately preserved on-site with the addition of a 2N zinc acetate and 6N sodium hydroxide solution.

The following water quality concentrations were measured in accordance with standard methods (APHA, WEF, 2012) and US EPA methods (USEPA, 1978): i) total sulfides (SM 4500-S^{2-}D), ii) soluble ammonia (SM 4500-NH_3 B), iii) soluble sulfate (US EPA 375.4 US), iv) soluble nitrate (SM 4500-NO_3^- B), v) soluble nitrite (SM 4500-NO_2^- B), vi) soluble chemical oxygen demand (SM 5220 D), vii) soluble total phosphorus (SM 4500-P E) and viii) pH (SM 4500-H+B).

4.4.3. Sediment sample collection

Sediment samples were collected using an Ekman dredge at the outlets of RSP1 and RSP2. The sediment was transferred from the dredge to sterile, gamma-irradiated 15 ml Falcon tubes and frozen at -20°C for preservation until further processing. The dredge was washed with a 10% bleach solution, followed by a 99% ethanol solution, to clean and disinfect the dredge to avoid cross-contamination of samples destined for microbial community analyzes.

4.4.4. DNA extraction, amplification, ddPCR and sequencing

Sediment samples collected in triplicate from the benthic zone of the ponds were washed using 5 ml of buffer solution to remove potential PCR inhibitors. DNA was extracted from the sediment samples using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA)

and stored at -80°C in Tris(hydroxymethyl)aminomethane buffer until the samples were shipped for sequencing. Extraction quality was determined using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

Extracted DNA was amplified using a BIO-RAD S1000TM Thermal Cycler (Saint-Laurent, QC). The primers used (forward 27F and reverse 907R) targeted the hypervariable region of the 16s rRNA. The primers were acquired from Integrated DNA Technologies (Coralville, IA). The amplified product was 1000bp. The primers utilized for the amplification process are primer 27F Sequencing was performed by Molecular Research LP (Shallowater, TX), which amplified DNA using a two-step polymerase chain reaction (PCR) targeting the V6 hypervariable region of the 16s rRNA. The primers used were custom in-house primers developed and prepared by Molecular Research LP. Each sample was then sequenced as a 2x300bp run on an Illumina MiSeq sequencer (San Diego, CA). The DNA sequencing results were analysed using the Bio-Linux (Field et al., 2006) operating system. The QIIME software was utilized to perform operational taxonomical unit (OTU) grouping (Tanja Magoč and Salzberg, 2011) and the determination of the organisms present in the sediment of the ponds. ddPCR evaluation was conducted using a BIO-RAD QX200TM ddPCR system (Hercules, CA). Count data from the ddPCR was acquired using the Quantasoft software, developed by BIO-RAD (Hercules, CA). The expected amplicon size for SRB and methanogens were of 221bp and 491bp, respectively. Table 4.4.1 outlines the primers utilized for ddPCR. All primers were purchased from Integrated DNA Technologies (Coralville, IA).

Table 4.4.1: Droplet digital PCR primers for SRB and methanogen counts

Microorganism	Primer	Sequence
Sulfate-reducing bacteria	dsr1-F RT	5'-ACS CAC TGG AAG CAC GGC GG-3'
	dsr-R RT	5'-GTG GMR CCG TGC AKR TTG G-3'
Methanogens	mcrA R	5'-CGT TCA TBG CGT AGT TVG GRT AGT-3'
	mlas F	5' GGT GGT GTM GGD TTC ACM CAR TA-3'

4.4.5. Statistical analysis

Water quality constituent statistical analysis was based on rotating triplicates. Locations were intermittently sampled in triplicate on an alternating pattern, allowing for all locations to be sampled in triplicate every 5 sampling rounds. Regression analyses were performed between total sulfides and DO, ammonia, sulfate, nitrate, nitrite, chemical oxygen demand, total phosphorus and pH, and also between DO and the same list of constituents to evaluate statistical significance between the data sets (p-values < 0.05 and Pearson's $R > 0.35$ signifying significance). Regression analyses were performed on a depth and location basis. Regression results were then corrected for familywise error rate (FWER) using the Bonferroni correction (Abdi, 2007).

4.5. RESULTS AND DISCUSSION

4.5.1. Total sulfides

The study demonstrated significant increases in total sulfides in the water column of RSP2 during the winter ice covered period of December 15th, 2014 to April 8th, 2015, a trend

which was also observed in RSP1 across a smaller time period and to a significantly lower maximum total sulfide concentrations (Figure 4.5.1). Concentrations at the RSP2 outlet averaged 6.375 ± 1.135 mg/L-S during this period and the maximum recorded concentration was of 11.51 mg/L. These concentrations were approximately 400 times greater than the observed concentrations in the adjacent reference pond, RSP1 (0.016 ± 0.009 mg/L-S), during the same period of operation. The absolute peak in total sulfide concentrations in RSP2 occurred from March 10th to March 30th, 2015. During this peak period, high concentrations of total sulfides migrated from the bottom of the pond up the water column at locations RSP2-1, RSP2-2 and RSP2-3. This sulfide migration was not significant at locations RSP2-4 and RSP1-1 as RSP1-1 did not experience such an elevated sulfide concentration increase ice covered conditions.

High total sulfides concentrations are often a result of sulfate-reduction (Hem, 1985). Previous studies confirm that the measured concentrations of total sulfides in this study are not out of range compared to cold, deep, and heavily stratified aquatic environments. These include stormwater retention pond studied in Edmonton, Canada which experienced 1.4-3.6 mg/L-S (Ku et al., 2016); the Onondaga lake study, NY, US which demonstrated 56.23 mg/L-S (Effler et al., 1988) and the Torquay Canal study, DE, US, which measured ≥ 40.90 mg/L-S (Luther et al., 2004).

Additionally, as seen in Figure 4.5.1, during summer operation between June 12th and June 25th, 2015 an H_2S release event lasting approximately two weeks was measured at the outlet of RSP2 (i.e. RSP2-4). This summer event showed a maximum increase of total sulfides at the RSP2 outlet to 0.628 ± 0.007 mg/L-S, while concentrations during the same period in RSP1 were measured at 0.025 ± 0.002 mg/L-S. Hence, the concentration of total sulfides in RSP2 was significantly greater than the measured concentration of total sulfides in RSP1 during this event.

The production of sulfides found at the RSP2 outlet during the summer production event is similar to other reported cases of sulfide generation in warm lakes, such as in the Lake Brooker study, FL, US where concentrations were of 0.176 ± 0.069 mg/L-S (Cowell et al., 1987).

Background, the daily total sulfide concentrations at all sampling locations in the two ponds (RSP2-1 to 4: 0.012 ± 0.001 mg/L-S; RSP1-1: 0.010 ± 0.001 mg/L-S) were not statistically different for the periods outside of periods of high total sulfides production. Further, the calculated average background total sulfides concentrations at depths of 0.20 m and 1.50 m along with the maximum concentrations of total sulfides measured in RSP2 were shown to not be statistically different at the two sampling depths and were shown not be different spatially throughout the year of operation. As such, both RSP1 and RSP2 were predominately not chemically stratified throughout the year, with the exception of during ice cover (December 15th, 2014 to April 8th, 2015) and during the sulfide production event at the outlet of RSP2 (June 12th to June 25th 2015).

Although the daily, average and maximum total sulfide concentrations did not show differences spatially or at depth, two distinctions were observed between RSP2-4 as compared to other spatial locations sampled in the pond. These include the summer sulfide production event that was isolated to RSP2-4 and the lack of statistically validated stratification of H_2S with depth during the ice covered event at RSP2-4. Sampling location RSP2-4 is located in close proximity to the outlet of the pond, is located in the area of the deepest waters of the pond and was qualitatively observed in the field to accumulate the greatest quantity of sediment as compared to other locations in the pond outside of the forebay. Based on the summer sulfide production event isolated to RSP2-4 and the saturated water column with hydrogen sulfide during ice covered conditions, it can be concluded that the deepest portion of the pond with the greatest accumulated

quantity of sediment was the most likely location for initial sulfide production. Sediment seemed to accumulate in the greatest quantities in the deepest portions of the pond, in proximity to the outlet and the preceding depression. This area therefore showed the highest propensity for the production of hydrogen sulfide in pond.

4.5.2. Dissolved oxygen

Dissolved oxygen was confirmed in this study to be the most critical parameter that is limited initializes high rates of benthic hydrogen sulfide generation in stormwater ponds. A regression analysis was performed to evaluate its significance. The threshold for significance was corrected for familywise error rate (FWER) using the Bonferroni correction (Abdi, 2007). A linear regression analysis demonstrates a significant statistical correlation between low (< 2.0 mg/L) DO concentrations at depth and the increase in total sulfides concentrations ($p < 0.02$, $R > 0.58$), where decreases in DO result in the generation of total sulfides (Figure 4.5.1). The critical DO concentration measured at depth that was observed in this study is approximately 1.0 to 2.0 mg/L, which is somewhat to reported critical DO ranges (0.1 and 1.0 mg/L) (EPA, 1985; Hao et al., 1996) in wastewater where there is risk of hydrogen sulfide generation. It should be reiterated that the reported DO concentration of 2.0 mg/L was measured at a depth of 1.50 m below the water surface, with the DO concentration expected to decrease within the sediment layer.

There was no measured lag period between the onset of hypoxic conditions and a significant increase in total sulfides at warmer temperatures between June 12th, 2015 and June 25th, 2015 in RSP2-4 or under ice cover at all locations in RSP2 or RSP1. Low DO concentrations (< 2.0 mg/L) at depth (1.50 m) in RSP2 were first observed at RSP2-4 on January 7th, 2015, followed by RSP2-2 and RSP2-3 on January 9th, 2015, and finally at RSP2-1 on January 21st, 2015. Low DO concentrations at RSP1-1 were only first observed approximately a

month later, on February 12th, 2015. DO concentrations < 2.0 mg/L near the surface (0.20 m) were first observed at all locations (in RSP2 on February 12th, 2015. DO concentrations < 2.0 mg/L in RSP1-1 near the surface occurred, again at a later date compared to RSP2, on March 3rd, 2015. Additionally, there was periodic stratification of DO concentrations during the ice covered period at all locations with stratification occurring at a later date at RSP1-1, as shown in Figure 4.5.1, starting at the end of December 2015 and continuing during January and February 2016.

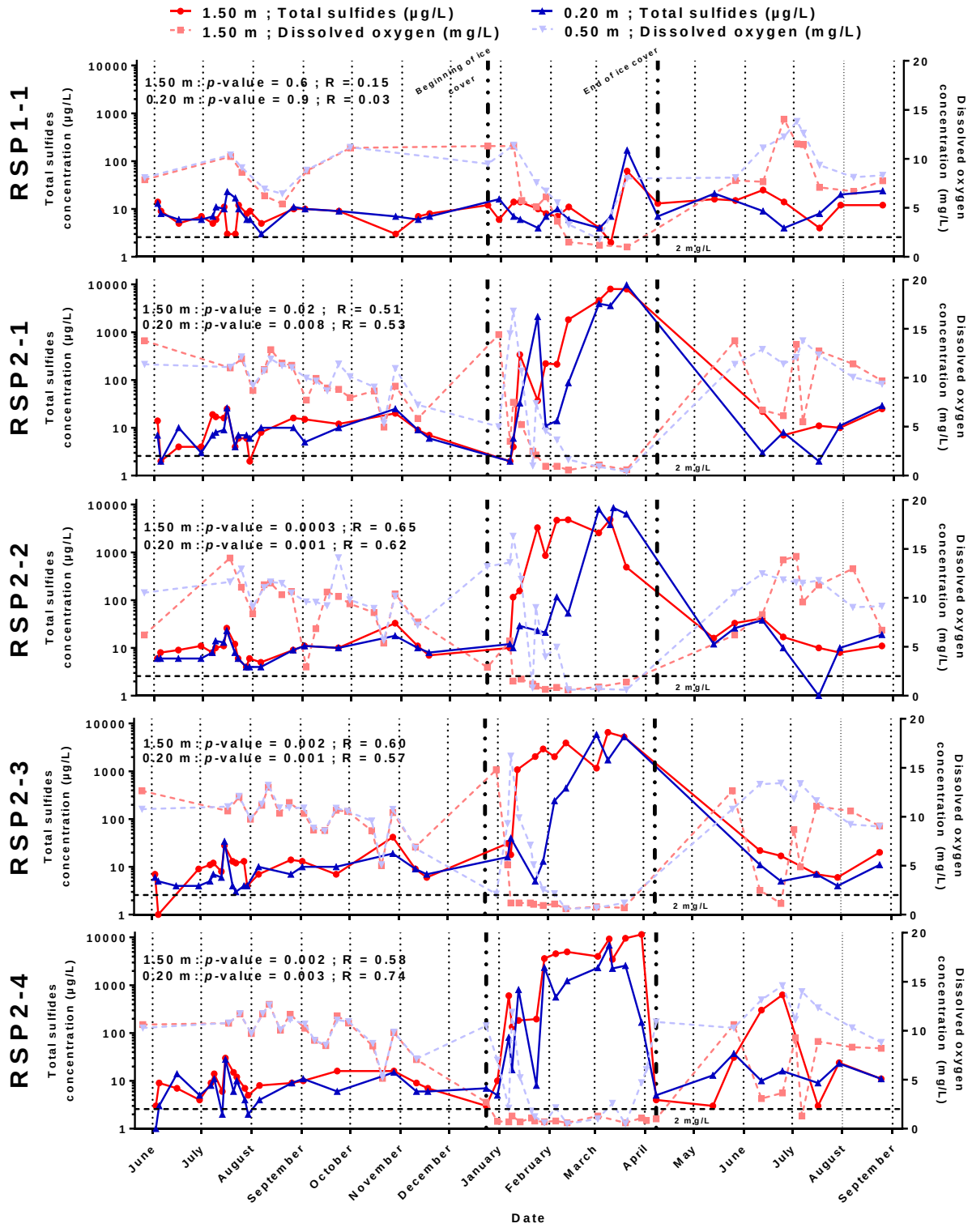

Figure 4.5.1: Total sulfides and DO concentrations at the following sampling locations: RSP1-1, RSP2-1, RSP2-2, RSP2-3 and RSP2-4

4.5.3. Total ammonia

The presence of total ammonia (NH_3/NH_4^+) in stormwater ponds can lead to the consumption of DO through microbially mediated nitrification (oxidation of NH_3/NH_4^+ to NO_2^- and NO_3^-) (Dorland and Beauchamp, 1991). Decreases in DO concentrations below 2.0 mg/L correlated strongly ($p < 0.03$, $R > 0.68$) with increases in NH_3/NH_4^+ concentrations (Figure 4.5.2). Nitrogenous biological oxygen demand in the sediment (Burton and Pitt, 2001) can reduce dissolved oxygen concentrations and create conditions more favourable for SRB proliferation. NH_3/NH_4^+ concentrations exhibit a seasonal pattern in both RSP2 and RSP1, with low concentrations (< 0.50 mg/L NH_3-N) during non-ice covered periods and higher concentrations (> 1.50 mg/L NH_3-N) during ice covered periods (Figure 4.5.2). The increase in NH_3/NH_4^+ concentrations observed during ice cover may be caused by an increase in the rate of ammonification due to low DO concentration (anaerobic biological conversion of organic matter to NH_3/NH_4^+) and/or the loss of nitrification due to low DO concentration, low temperature (Cheremisinoff, 2001) and/or H_2S inhibition (Bejarano Ortiz et al., 2013) .

During the ice covered period, NH_3/NH_4^+ concentrations began to increasing, reaching their peak at all locations on March 20th to March 30th 2015. Similar to sulfide, there was a slow progression of high ammonia concentrations at depth which progressed to 0.20 m. Initially, concentrations were determined to be statistically different at depth than near the surface, but towards the end of the ice covered period (March 2015), concentrations were similar at all locations and at all depths within RSP2 (1.59 ± 0.52 mg/L NH_3-N). During the same time period (March 2015), concentrations in RSP1-1 were slightly lower at (1.23 ± 0.48 mg/L NH_3-N). It is hypothesized that much of the increase in ammonia during winter months is due to breakdown plant material.

During summer periods, ammonia concentrations were low and similar at all locations. The average concentrations measured in RSP1 and RSP2 at 1.50 m of depth were 0.32 ± 0.27 mg/L NH_3-N and 0.25 ± 0.28 mg/L-NH_3-N, respectively. It is hypothesized that low ammonia concentrations measured during the summer period are due to the potential use of ammonium by aquatic plants as a building block. There was no stratification which could be perceived during the summer period.

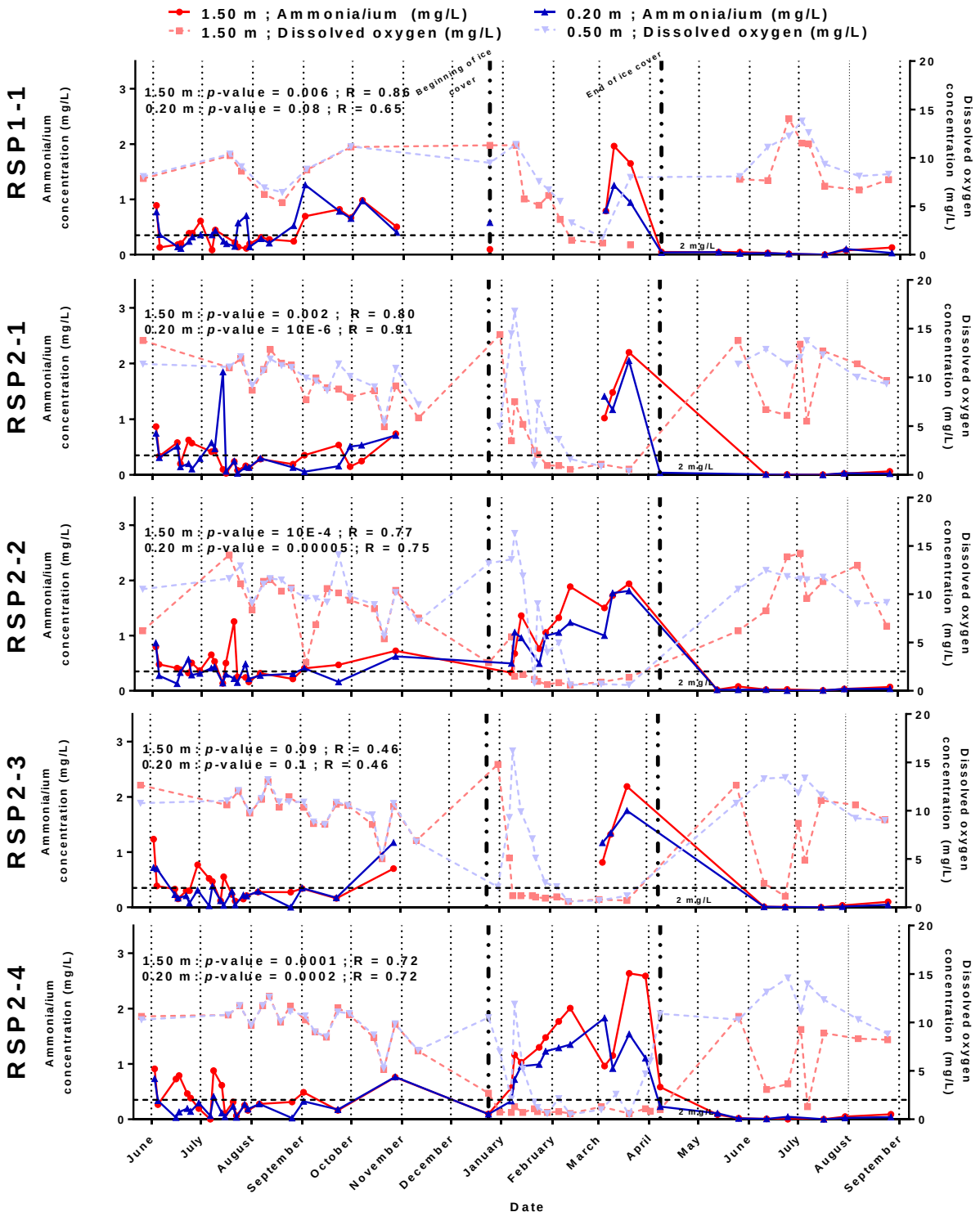

Figure 4.5.2: Soluble NH_3/NH_4^+ and DO concentrations at the following sampling locations: RSP1-1, RSP2-1, RSP2-2, RSP2-3 and RSP2-4

4.5.4. Temperature & pH

Temperature and pH are also critical parameter to monitor in environments where the production of H_2S is possible. Temperature affects the microbial kinetics of DO consumption and total sulfides production along with the pH of the water (Lower, 1996), which will in turn have an effect on the speciation of sulfide (H_2S, HS^- or S^-) (Wang et al., 2007). Figure 4.5.3 shows the pH values recorded throughout the study, along with the air and water temperatures. The average pH value measured in RSP1-1 is 7.43 ± 0.38. The average value in RSP2 is 7.78 ± 0.46 and the average value at RSP2-4 is 7.85 ± 0.47.

During the ice covered period, the average pH value in RSP2 decreased to approximately 7.27 ± 0.28, while values in RSP1-1 remained stable, with an average winter pH value of 7.48 ± 0.20. The measured decrease in pH is believed to occur due to an increase in the anaerobic activity of the benthic sediment of RSP2 (Soetaert et al., 2007), and appears to coincide with the decrease in DO and production of sulfides in RSP2 (Figure 4.5.1, Figure 4.5.3).

During summer periods, the average pH value in RSP2 was 7.90 ± 0.41, while values in RSP1-1 were of 7.35 ± 0.44. It is hypothesized that the higher pH values in RSP2 are the result of higher primary production rates in RSP2 than at in RSP1-1 (Cerco et al., 2013) due to the removal of pH from the water by plant respiration, in turn causing a decrease in carbonic acid in the bulk water.

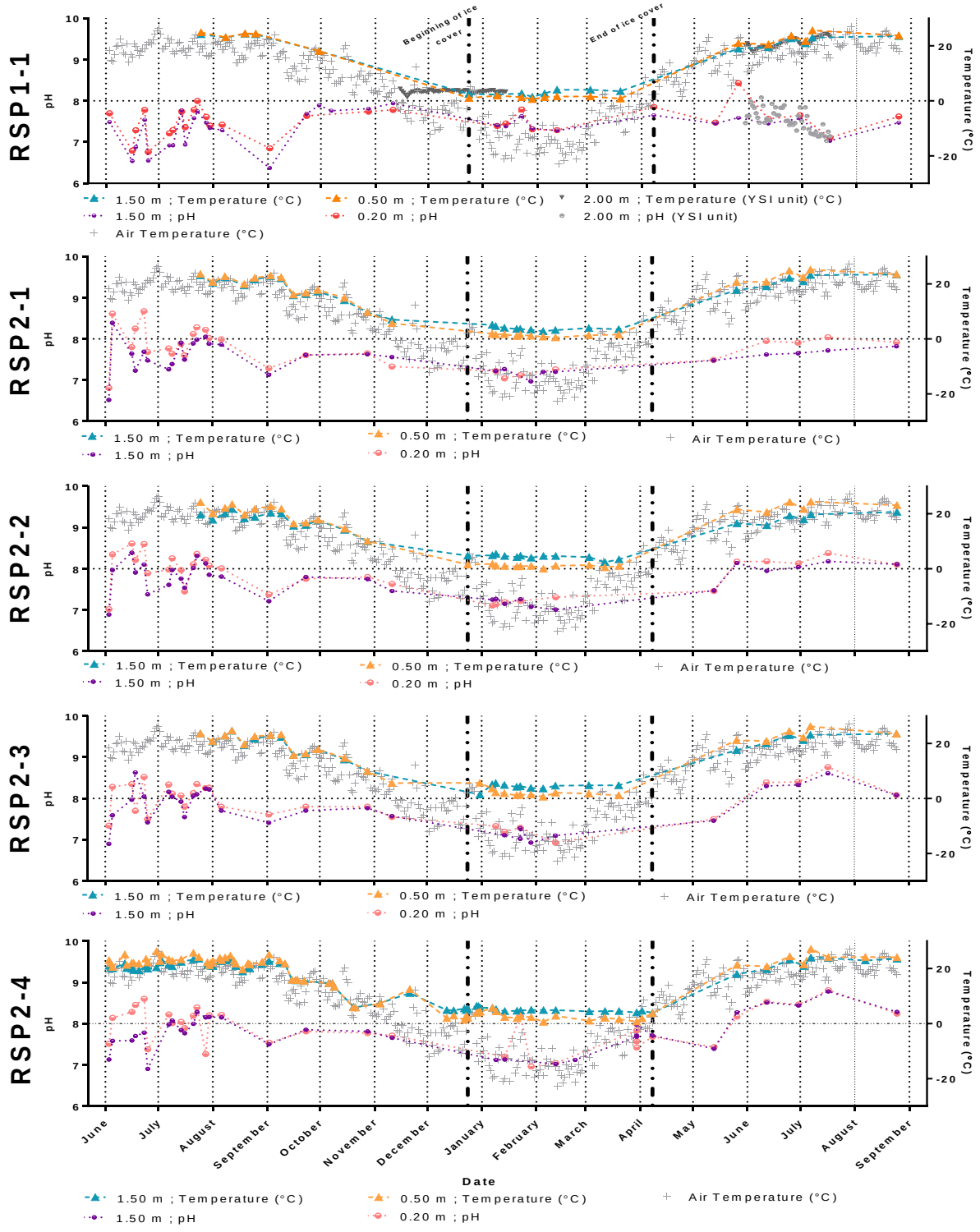

Figure 4.5.3: pH, air and water temperature at the following sampling locations: RSP1-1, RSP2-1, RSP2-2, RSP2-3 and RSP2-4

4.5.5. Additional water quality measurements

The following water quality measurements were recorded throughout the study to determine statistical correlation with the production of H_2S in stormwater ponds. The additional constituents are the following: sulfate, soluble chemical oxygen demand, nitrate, nitrite and soluble total phosphorus. The average concentrations measured throughout the study in both ponds are outlined in Table 4.5.1. Previous work on reclaimed water and its suitability for irrigation purposes reported that sulfide generation was a concern when, in addition to low DO conditions, SO_4^{2-} concentrations were 50 mg/L or greater and COD concentrations were 20 mg/L or greater (Asano et al., 2007), as these parameters were capable of sustaining H_2S production.

Sulfate, soluble chemical oxygen demand, nitrate and soluble total phosphorous concentrations were all stable spatially and at various depths in RSP2 throughout the entire study period. The measured sulfate concentrations indicate that sufficient sulfate was present to allow for significant SRB activity (Asano et al., 2007). The soluble chemical oxygen demand concentrations are also within the required ranges for significant to SRB activity (Asano et al., 2007). Soluble nitrate concentrations were stable spatially and at various depths throughout the entire study period, with average values of 0.92 ± 0.38 mg N/L in RSP1-1 and 1.04 ± 0.20 mg N/L in RSP2. Soluble total phosphorus concentrations were indicative of non-limited phosphorus conditions for microbial activity and hence SRB activity (allowing SRB activity). It is estimated that phosphorus concentrations should be maintained below 0.010 to 0.015 mg/L-P to prevent or limit algal blooms (Davis and Masten, 2004). Nitrite concentrations were below the practical quantification limit (PQL) of 0.012 mg N/L for the majority of the tested samples throughout the study with concentrations always being below 0.090 mg N/L, when quantifiable.

Table 4.5.1: Average concentrations of water quality parameters

Water Quality Constituent	Average RSP2	Average RSP2 Outlet (RSP2-4)	Average RSP1 Outlet (RSP1-1)
Sulfate (mg SO_4/L)	50.1 ± 10.9	49.51 ± 12.6	46.5 ± 8.5
Soluble chemical oxygen demand (mg/L)	20.2 ± 12.4	21.2 ± 14.6	16.6 ± 8.0
Nitrate (mg-N/L)	1.04 ± 0.20	1.04 ± 0.23	0.92 ± 0.38
Nitrite (mg-N/L)	<0.012	<0.012	<0.012
Soluble total phosphorus (mg-P/L)	0.13 ± 0.13	0.15 ± 0.15	0.09 ± 0.08

4.5.6. Investigation of microbial communities

The relative abundance of the microbial communities of the outlets of RSP1 and RSP2 were investigated in the study. The percent abundance of SRB in the microbial community of the RSP1 and RSP2 outlet sediment do not appear to be statistically different (Figure 4.5.4). Further, the top 10 dominant SRB organisms identified in the outlet sediment of both ponds (shown in Table 4.5.2**)**, along with their average relative abundance in the sediment biota also are similar in both ponds. The following results indicate that organisms belonging to the *Desulfovibrio* genus (such as *Desulfovibrio vulgaris* and *Desulfovibrio desulfuricans*) were not present and contributing to sulfate-reducing processes in the studied stormwater ponds.

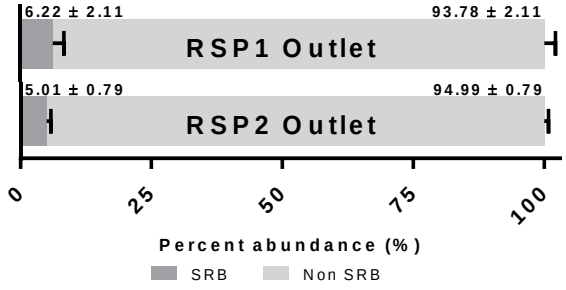

Figure 4.5.4: Bacterial genus distribution at the outlets of the RSP1 and RSP2 ponds, comparing SRB and non-SRB organisms

Table 4.5.2: List of the most abundant SRB-type organisms found in outlet sediment of both studied ponds, at the L6 (Genus) Operational Taxonomic Unit

	RSP1 Outlet	**RSP2 Outlet**
Organism	**Percent organisms (%)**	**Percent organisms (%)**
Family Desulfobulbaceae, Unclassified Genus	2.39 ± 1.58	1.98 ± 0.49
Desulfococcus	1.45 ± 0.92	1.14 ± 0.26
Family Desulfobacteraceae, Unclassified Genus	1.25 ± 0.65	1.04 ± 0.26
Geobacter	0.38 ± 0.07	0.29 ± 0.11
Desulfobulbus	0.16 ± 0.12	0.17 ± 0.02
Desulfomonile	0.15 ± 0.08	0.12 ± 0.10
Synthrophobacter	0.11 ± 0.06	0.06 ± 0.01
Family Desulfuromonadales, Unclassified Genus	0.07 ± 0.03	0.05 ± 0.03
Desulfobacca	0.17 ± 0.23	0.05 ± 0.04
Desulfomicrobium	0.04 ± 0.02	0.04 ± 0.01

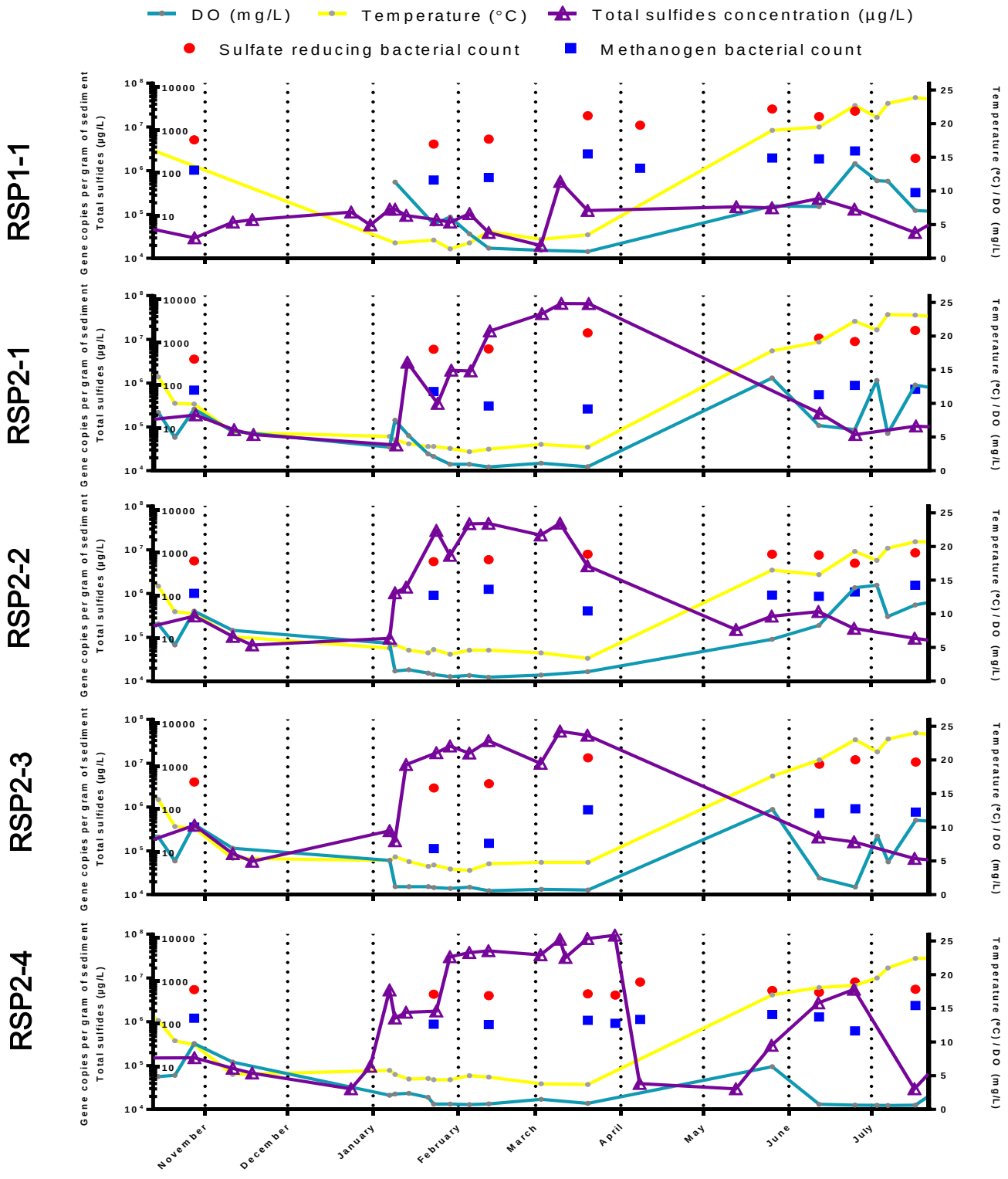

Figure 4.5.5: Sulfide, ddPCR bacterial counts, water temperature and DO at the following sampling locations: RSP1-1, RSP2-1, RSP2-2, RSP2-3 and RSP2-4

This study also quantified the counts of the SRB and methanogenic populations in the outlet sediment of RSP1 and RSP2 (Figure 4.5.5 above and Table 4.5.3 below). In general, the counts of SRB and methanogens were similar between both studied ponds. Furthermore, it was not evident that individual SRB or methanogen bacterial counts at individual locations varied significantly over time. Additionally, it was not possible to establish any statistical correlations were found to exist between SRB counts and DO, total sulfide concentrations or temperature at RSP2-1 (p-value = 0.391), RSP2-2 (p-value = 0.313), RSP2-3 (p-value = 0.996) or RSP2-4 (p-value = 0.134). There is a perceived statistical correlation at RSP1-1 (p-value = 0.034), however that is likely based on coincidental small magnitude changes and is likely not actually indicative of a relationship. SRB counts were shown to be higher than methanogen bacterial counts, at all locations, regardless of season or temperature.

Table 4.5.3: Sulfate-reducing and methanogenic bacterial counts in benthic sediment at RSP1 and RSP2

Location	Sulfate-reducing bacterial counts (copies g sediment^{-1} ± standard deviation)	Methanogenic bacterial counts (copies g sediment^{-1} ± standard deviation)
RSP1-1	$1.24x10^7 \pm 2.96x10^6$	$1.46x10^6 \pm 2.92x10^5$
RSP2-1	$9.34x10^6 \pm 1.73x10^6$	$5.79x10^6 \pm 8.71x10^4$
RSP2-2	$6.74x10^6 \pm 4.97x10^5$	$1.00x10^6 \pm 1.17x10^5$
RSP2-3	$7.92x10^6 \pm 1.70x10^6$	$5.55x10^6 \pm 1.29x10^5$
RSP2-4	$5.35x10^6 \pm 4.85x10^5$	$1.18x10^6 \pm 1.52x10^5$

Combined, the bacterial results demonstrate that the microbial SRB and methanogenic populations were similar in overall percent abundance, in percent abundance of dominant SRB organisms along with in SRB and methanogenic population counts in a pond that demonstrated significant total sulfide production as compared to a pond that showed limited total sulfide production. Further, season change and bulk water quality changes of the retention ponds across the period of over a year, and specifically during periods of significant total sulfides production, did not promote the proliferation of SRB nor methanogens. Hence, total sulfide production in RSP2, while limited in RSP1, is a result of a higher SRB activity in RSP2 as compared to RSP1 and not a symptom of a higher concentration of SRB or the proliferation of differing dominant species of SRB in the pond.

4.6. CONCLUSIONS

This study aimed to identify and quantify the factors influencing H_2S gas generation and total sulfide presence in stormwater retention ponds in two stormwater retention ponds. It was shown that hypoxia (DO concentrations < 2.0 mg/L) is the single most important factor in both initiating and sustaining hydrogen sulfide production events in stormwater retention ponds (p < 0.006, R > 0.58). This problem is compounded in regions where sub-zero weather is experienced in winter, where ice cover will forms over the ponds, hindering reaeration processes and prolonging hypoxic conditions. Furthermore, there are indications that sulfides are travelling up the water column of the pond over time, as there is a lag phase between the drop in the DO concentrations at depth and the increase in total sulfides concentrations near the surface of the pond. It was also established that outside of periods of intense H_2S production, background total sulfide concentrations were not statistically different in both ponds.

Additionally, it was found that the deepest portion of the RSP2 pond, and locations with the most accumulated sediment had the highest propensity for the production of H_2S gas. It is believed that pH change can be a good indicator of pond oxic conditions. A raise in pH during summer periods is indicative of high rates of primary production, while inversely, a drop in pH indicates an increase in anaerobic activity, due to the release of CO_2 gas, causing an acidification of the bulk water of the stormwater retention pond.

Meanwhile, it was found that there was a rapid increase in total sulfides concentrations at depth when hypoxia occurred in RSP2, suggesting that the SRB present in the sediment are fully adapted and acclimatized to temperature changes and periodical oxygen exposure. Furthermore, DNA sequencing and microbial analyses also show that the microbial communities at both stormwater ponds did not undergo a community shift, further making the point for increased

microbial activity, and suggests that sulfide production events are due solely to environmental conditions present in the ponds. Seasonal changes did not appear to promote SRB, or methanogen proliferation within either of the stormwater ponds. It is therefore clear for the authors that sulfide production is a result of increased bacterial activity, and not indicative of SRB proliferation, or the population shift towards a specific SRB species.

4.7. ACKNOWLEDGEMENTS

The authors acknowledge the support of the City of Ottawa and the Natural Sciences and Engineering Research Council of Canada (NSERC). This research project was funded by a collaborative research and development (CRD) grant between the Natural Sciences and Engineering Research Council of Canada (NSERC) and the University of Ottawa in collaboration with the City of Ottawa (CRDPJ 469596–14).

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CHAPTER 5 : DETERMINATION OF HYDROGEN SULFIDE KINETIC PARAMETERS IN STORMWATER RETENTION PONDS

5.1. SETTING THE CONTEXT

The article presented in Chapter 5 is entitled *Determination of modeling parameters for hydrogen sulfide producing stormwater retention ponds* by P. M. D'Aoust, R. Wang, F. Pick, A. Poulain, C. Rennie, L. Chen, and R. Delatolla. This article is in preparation for publication in Journal of Environmental Engineering (ASCE). This article presents key kinetic parameters that are significant in the understanding and modeling of hydrogen sulfide producing stormwater retention ponds. The study presents bacterial kinetics determined for both field experiments and laboratory bench top experiments. In particular, this work quantifies rates of sediment oxygen demand, ammonification rates of the sediment, nitrification rates of the sediment, sulfate-reduction rates of the sediment and the biological oxygen demand of the water column at 20°C and 4°C. Furthermore, the work investigates the suitability of laboratory experiments to predict results in the field.

5.2. ABSTRACT

The production of hydrogen sulfide in stormwater retention ponds is a serious issue but there is currently a fundamental lack of knowledge about these events and most modern stormwater computer models do not predict sulfide production. Laboratory experiments at 4°C identified total SOD, ammonification and sulfate-reduction rates to be 0.023 g/m^2/day, 0.027 g N/m^2/day and 0.004 g S/m^2/day, respectively. Meanwhile, rates calculated from the field study of stormwater retention ponds for total SOD, ammonification and sulfate-reduction were of 0.491 g/m^2/day, 0.120 g N/m^2/day and 0.147 g S/m^2/day, respectively. The discrepancy between lab and field results were attributed to the inadequacy of sediment area-normalized production/consumption kinetics (g/m^2/day) in comparing lab and field studies, due to the depth of active sediment and its impact on measureable kinetics. Furthermore, it was found that sulfate-reducing bacteria (SRB) had a relative abundance of 5.01% in the benthic sediment of a sulfide producing stormwater pond and that *Desulfobulbaceae* (39.5%), *Desulfococcus* (22.8%) and *Desulfobactaraceae* (20.8%) were the dominant SRB in the top 30 cm of the benthic sediment. Finally, this study also provided supplementary kinetic parameters such as Arrhenius' coefficient and half saturation coefficients for SOD, ammonification, sulfate-reduction and nitrification, to build a better understanding of sulfate-reduction in stormwater retention ponds.

5.3. INTRODUCTION

Proper stormwater management is crucial to limit the negative effects of urbanization on surface water quality (Eriksson et al., 2007; National Research Council, 2008). As part of an effort to mitigate the effect of surface water pollution, stormwater retention ponds have become a common element of cities' municipal plans (Drake and Guo, 2008). However, improperly

designed facilities run the risk of encountering operational problems. Stormwater retention ponds experiencing low flowrate and high organic loads are at risk of periodically becoming hypoxic in colder climates, due to ice cover hindering reaeration processes, leading to hypoxia and increased anaerobic bacterial activity in the benthic sediment of the ponds. When prolonged hypoxia is encountered, sulfate-reduction can occur in the benthic sediment. Sulfate-reducing bacteria (SRB) utilize sulfate as a terminal electron acceptor to produce hydrogen sulfide (H_2S), which is a highly undesirable pollutant. H_2S and related sulfide compounds have a strong and characteristic rotten-egg odor (Crittenden et al., 2012), are harmful to wildlife (Smith Jr. and Oseid, 1974) and accelerate the deterioration of infrastructure and in particular the inlet and outlet structures of the ponds (Ma et al., 2000; United States Environmental Protection Agency, 1991). Sulfide production in stormwater is almost always the result of widespread hypoxia in stormwater retention ponds. Sulfide generation can also exacerbate oxygen demand, due to sulfide oxidation (Chen and Morris, 1972). Previous research shows that in some cases, a large proportion (more than 50 %) of the oxygen consumed by sediment in natural water bodies could be attributed to sulfide oxidation (Wang, 1980).

There is currently a lack of literature and a fundamental lack of understanding of sulfide production processes in stormwater retention ponds in cold climates. As such, water quality models are frequently utilized in the design stages of stormwater retention ponds to predict operating conditions, optimize performance and avoid unsatisfactory operation lack the capability to model and predict sulfide production by default, providing designs which may in fact not be suitable for operation in cold climates. This study aims to characterize the critical kinetics of consumption and production in benthic sediment and the bulk water column in hypoxic, sulfate-reducing stormwater retention ponds in addition to providing the necessary

information to integrate sulfide production subroutines into existing and future stormwater retention pond models. A review of SOD measurement techniques by Bowman and Delfino (1980) found that many studies comparing in-situ and laboratory SOD rates reported significantly different rates, but stressed the fact that laboratory experiments were necessary nonetheless due to the repeatability of experiments and the standardization of parameters such as temperature, light and water movement. This spurred interest in comparing how other kinetics such as sulfate-reduction and ammonification. This study incorporates a laboratory study utilizing sediment and water collected from RSP2 to characterize the kinetic parameters of the benthic sediment. In addition, this study also moved beyond simple laboratory experiments and included field testing of two stormwater retention ponds for a period of 15 months. To enhance the knowledge of sulfide generation in stormwater retention ponds, two ponds in Ottawa, ON, Canada were studied, and it was found that during periods of low flow (summer droughts and winter ice cover), hypoxia developed throughout Riverside South Pond #2 (RSP2) and promoted sulfide generation and the release of hydrogen sulfide (H_2S) gas to the atmosphere. The second pond, Riverside South Pond #1 (RSP1) which did not develop hypoxic conditions or experience significant H_2S release, served as a reference pond to the field portion of the study. In addition, the study incorporated microbial analysis of the RSP2 sediment to understand the bacterial communities and in particular the SRB populations in the two ponds.

5.4. METHODS

5.4.1. Description of stormwater ponds

RSP1 and RSP2 are two stormwater ponds located in Ottawa, Canada. RSP1 is a stormwater pond which was built in 1996 and does not typically suffer from any significant H_2S

production. RSP2 is a stormwater pond which was built in 2007 and has experienced H_2S gas generation for several years, typically during winter under ice covered periods and during summer periods of drought. Satellite photographs of the two ponds with sampling locations identified are shown below in Figure 5.4.1 a) and b).

5.4.2. Field study

5.4.2.1. Sample collection in field study

Bulk water samples were collected from four locations in RSP2 (RSP2-1, RSP2-2, RSP2-3 and RSP2-4, shown in Fig. 5.4.1) at a depth of 1.50 m, using a Wildo 1520 C25 Kemmerer 2.2L TT water sampler (Yulee, FL) over a period of 15 months.

5.4.2.2. Bulk water analysis

In the field study, the water samples were assayed for the following parameters; i) total sulfides (SM 4500-S^{2-}D) (APHA, WEF, 2012), ii) ammonia (SM 4500-NH_3 B) (APHA, WEF, 2012), iii) soluble sulfate (US EPA 375.4 US) (USEPA, 1978), iv) soluble nitrate (SM 4500-NO_3^- B) (APHA, WEF, 2012), v) soluble nitrite (SM 4500-NO_2^- B) (APHA, WEF, 2012), vi) soluble chemical oxygen demand (SM 5220 D) (APHA, WEF, 2012), vii) soluble total phosphorus (SM 4500-P E) (APHA, WEF, 2012) and viii) pH, using a Corning Pinnacle 530 glass electrode pH meter (Corning, NY). Additionally, in-situ measurements of dissolved oxygen (DO) and temperature we recorded using a YSI ProODO optical DO meter.

5.4.2.3. Sample collection for microbial community analysis

Sediment samples were collected at RSP2-4 using a sanitized Ekman dredge, to prevent cross-contamination of samples. Sediment samples were collected on October 28th 2014, February 12th 2015, March 20th 2015, March 30th 2015, April 8th 2015, May 26th 2016, June 12th 2015, June 25th 2015 and July 17th 2015.

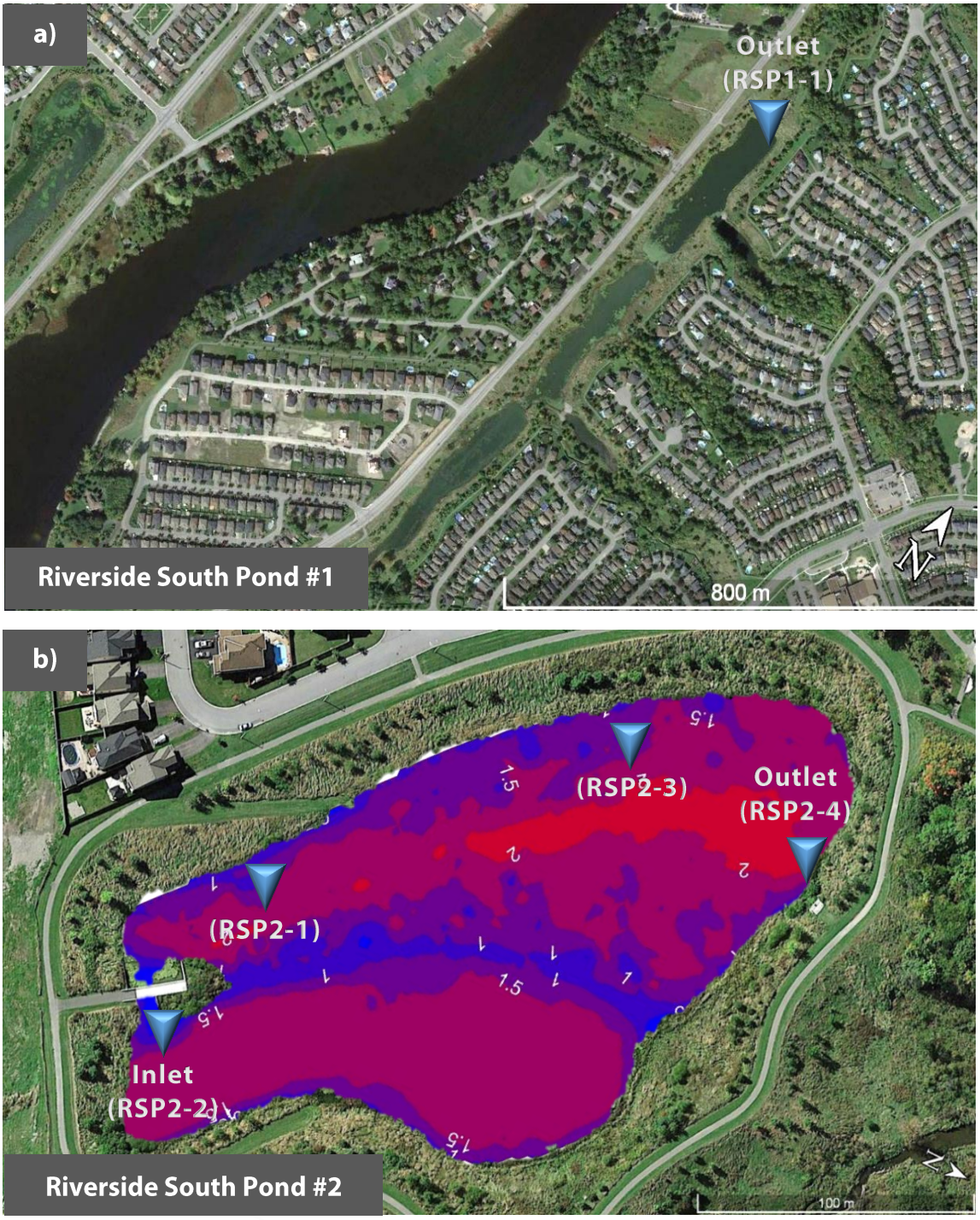

Figure 5.4.1: Sampling locations of a) Riverside South stormwater pond #1, and b) Riverside South stormwater pond #2, with deepest sections outlined in red

5.4.3. Laboratory kinetics study

5.4.3.1. Sample collection for the kinetics study

Water and benthic sediment samples were collected from the outlet of the RSP2 stormwater pond (shown in Figure 5.4.1). Approximately 15 liters of sediment was collected for kinetics testing using an Ekman dredge at the outlet of the pond, which is the deepest location of the pond (approx. 2.49 m). To provide the bulk water for the kinetics testing, approximately 30 liters of water was also collected at the outlet of the pond, at a depth of 1.0 m under the water surface, using a Wildco 1520 C25 Kemmerer 2.2L TT water sampler (Yulee, FL). Unused samples were discarded.

5.4.3.2. Characterization of sediment moisture content and pore water analysis

The determination of water content within the bottom sediment in the pond was performed as per ASTM Standard Test Methods D2216 (ASTM, 2005). Additionally, 100 grams of sediment collected from RSP2-4 was filtered through a 1.5 µm nominal pore-size glass-fiber filter connected to a vacuum pump apparatus in order to extract some of the pore-water for analysis. The filtrate was then tested for ammonia, sulfate and chemical oxygen demand, as described in section 5.4.2.2.

5.4.3.3. Experimental set-up

Five sets of bench top experiments were performed as part of the kinetics study. These five sub-experiments monitored total sulfides, ammonia and dissolved oxygen concentration changes over time in BOD bottles, which acted as small individually sealed batch reactors. Some of these reactors had their nitrification inhibited via the addition of allylthiourea, to enable the measurement of the impacts of nitrification on the dissolved oxygen concentrations.

Sediment samples were harvested from RSP2-4 in this pond during Fall of 2015. The sediment was stored for a maximum period of 14 days prior to use in the kinetic experiments. Test vessels (300 ml glass BOD bottles) were prepared for each experimental trial by adding 53.52 ± 3.70 g of sediment and then slowly filling them with aerated pond water, careful not to disturb the sediment. The BOD bottles were allowed to sit for 15 minutes, and the initial DO concentrations were recorded using a Hach HQ30d optical DO meter (Loveland, Colorado). The BOD bottles' bulk water concentrations of ammonia, soluble sulfate, soluble chemical oxygen demand and total sulfides were then measured, as described in section 5.4.2.2. The bottles were finally filled with a small volume of distilled water (<10 ml) to ensure a proper seal with glass stoppers, and once sealed, covered with aluminum foil.

The testing regimen is outlined in Table 5.4.1. Trials 1, 2 and 4 did not include the addition of a nitrification inhibitor while trials 3 and 5 included the addition of an inhibitor (allylthiourea), in an attempt to measure the impacts of temperature change on nitrification by performing the assays with and without nitrification inhibition, to measure the contribution of nitrification alone. 27 samples (9 sets of triplicates) were prepared and placed at 4°C to perform Trial #1, VWR 2020 Low Temperature Incubator (Mississauga, ON). Similarly, Trial #2 and #3 were performed by respectively preparing and placing 20 samples at 20°C (2 runs; 5 sets of

duplicates; one run with no nitrification inhibition and the other run with nitrification inhibition). For Trial #4 and #5, respectively, 20 samples were prepared and placed at 4°C in a VWR 2020 Low Temperature Incubator (Mississauga, ON) (2 runs; 5 sets of duplicates; O_2 starved with N_2 gas; one run with no nitrification inhibition and the other with nitrification inhibition). The decision to test at 4°C stemmed from the fact that at the start of the kinetic experiments, the ice-covered period was not yet over and temperature data had not been analyzed, leading to the conservation assumption that water temperatures would reach this temperature.

Samples from Trial #1 were tested at time = 0, at time 336h (14 days) and then every 48 ± 0.5h until 30 days had elapsed in total. The decision to test only after 14 days had elapsed stemmed from previous preliminary trials at 4°C, where the magnitude of the change in concentrations with time were not sufficiently high to accurately measure. Samples from Trial #2, #3, #4 and #5 were tested at time = 0, and then every 24 ± 0.5h, for 5 days. Testing of time = 0 occurred before the initial sealing of the samples and once a sample was unsealed and tested, it was discarded. Nitrification inhibition in samples was performed via the addition of an allylthiourea solution, as per SM 5210 A (APHA, WEF, 2012).

Table 5.4.1: Kinetic experiments conducted with stormwater pond sediment and water

Trial #1 (27 sample bottles total)
• 4°C • No nitrification inhibition • Testing on days 0, 14, 16, 18, 20, 22, 24, 26, 28, 30
Trial #2 (10 sample bottles total)
• 20°C • No nitrification inhibition • Testing on days 0, 1, 2, 3, 4, 5
Trial #3 (10 sample bottles total)
• 20°C • Nitrification inhibition with allylthiourea • Testing on days 0, 1, 2, 3, 4, 5
Trial #4 (10 sample bottles total)

• 4°C • No nitrification inhibition, DO < 1 mg/L • Testing on days 0, 1, 2, 3, 4, 5
Trial #5 (10 sample bottles total)
• 4°C • Nitrification inhibition with allylthiourea, DO < 1 mg/L • Testing on days 0, 1, 2, 3, 4, 5

The Arrhenius temperature coefficient, Θ, was determined for sediment oxygen demand (SOD), ammonification, sulfate-reduction by analyzing kinetics data acquired during the laboratory experiment and inputting the measured k_T and k_{20} values into Equation 2.1. The k values are the slopes of dissolved oxygen curves. The half-saturation constants were also determined by analyzing measured kinetics data acquired during the laboratory experiments and applying a methodology outlined by Ghimire (2012). By doing so, it was possible to determine the half-saturation coefficients for SOD, ammonification, nitrification and BOD at 20°C, as well as the half-saturation coefficients for SOD and ammonification at 4°C. Additionally, it is also possible to determine the rates of change (increase or decrease) of total SOD, aerobic-heterotrophic SOD, aerobic-autotrophic SOD, sediment nitrogen production, sediment nitrogen consumption, and sediment sulfide production, on a gram per square meter per day basis ($g/m^2/day$).

It is widely accepted that temperature has an effect on the rates of enzymatic and chemical reactions, which can be approximated by the Arrhenius equation:

$$k_T = k_{20}\theta^{T-20} \qquad \textbf{Equation 5.1}$$

Where k_T is the volumetric rate constant at the target temperature (h^{-1}), k_{20} is the volumetric rate constant at 20°C (h^{-1}), T is the target temperature (°C) and Θ is Arrhenius' temperature coefficient.

5.4.4. Determination of the rates of production and consumption of bulk water constituents

By utilizing data obtained from the BOD bottle experiments, it was possible to determine rates of change values (k) by plotting the change per unit of time (Δ mg/L) of constituents over time (day). For the laboratory experiments, the volume of water, sediment and vessel dimensions were known therefore calculations to determine the rate of production or consumption of different constituents was intuitive as the difference in concentrations were measured and exact reactor vessel dimensions and sediment volume/mass were recorded. The calculation of a rate of production or consumption was performed by dividing the change in concentration (mg/L) by the sediment cross-section within the reactor vessel.

For the field study, the rate of change of total sulfides, dissolved oxygen and ammonia concentration on a per day basis were calculated using field study data. These rates accounted for the loss of water volume due to ice-buildup, by utilizing bathymetric information of the RSP2 pond and calculating the volume of water present based on the ice cover thickness at specific dates. From this, and knowing the surface area of sediment throughout the entire RSP2 pond, it was possible to determine the daily rate of production or consumption of total sulfides, dissolved oxygen and ammonia, normalized by surface area of sediment ($g/m^2/day$). The simplified equation is shown below, as Equation 5.2.

$$q = (\alpha)(V)/(A) \qquad \textbf{Equation 5.2}$$

Where q is the rate of production or consumption of total sulfides, dissolved oxygen or ammonia per surface area per day ($g/m^2/day$), α is the daily change in concentration within the stormwater pond (average change considering all locations in the pond), ($\Delta\ g/m^3 \cdot d^{-1}$); V is the

volume of liquid water at that moment (total pond volume minus ice volume, in m^3; volume of ice is calculated using equation 5.4), and A is the total benthic sediment surface area in contact with the bulk water. Due to continual sediment deposition, as-built bathymetric information may differ slightly, so we chose to apply a factor of scaling to the permanent pond water surface area instead. In this instance, the scaling factor chosen was one of 15% (1.15 x permanent pool surface area), to estimate the surface area of sediment in contact with the bulk water within the pond.

Using data obtained from the BOD bottle experiments, it was also possible to determine some half-saturation coefficients. The BOD, ammonification, SOD and nitrification rates of change (mg/L/day) were plotted against DO (and SOD in the case of ammonification) and the resulting curve was utilized to determine the half-saturation coefficients for the respective parameters. The calculation methodology employed is similar to the one described by Ghimire (2012) but in the present study, less data points were collected due to the lack of automatic measurement systems and the slower experiment occurring in our study, compared to Ghimire's experiment (they employed mixed liquor and oxygen was completely consumed within minutes, whereas we employed stormwater pond bulk water and benthic sediment). A simple resume of the calculation process for half-saturation coefficients is as follows: the rate of change of a constituent (DO, for example, in mg/L/day; y-axis) is plotted against the average dissolved oxygen constituent concentration (mg/L; x-axis). The maximum change in the constituent is then divided by 2, to obtain the half saturation rate of change. Under the assumption that the data will be linear, the half saturation rate of change is divided by the slope of the plotted data. This yields a very crude half-saturation coefficient. An example of the crude calculation is shown below in Equation 5.3.

$$HS\ coefficient = ((\Delta max) \cdot 0.5)/slope_{\Delta\ vs.\ \mu\ value} \quad \textbf{Equation 5.3}$$

Where: HS coefficient is the half saturation coefficient, Δmax is the maximum rate of change (mg/L/day), and $slope_{\Delta \text{ vs. } \mu \text{ value}}$ is the slope of the plot of rate of change (in mg/L/day) vs. average constituent concentration. It is important to note that the x-axis will differ based on the half-saturation coefficient which is sought. Processes which require oxygen (such as SOD) will use DO on the x-axis, while processes such as ammonification and sulfate-reduction, which should theoretically be more active processes at low oxygen concentrations, will instead use SOD on the x-axis.

5.4.5. Determination of ice cover thickness

The ice cover thickness readily affects the bulk water volume, hence affecting the calculations mentioned in 5.4.4.. The depth of ice was measured at the boreholes used for sampling the pond under ice covered conditions. The measured ice cover depths were also compared in this study to the predicted Stefan's equation (Ashton, 1989) values.

$$h = \beta\left(D_f\right)^{0.5} \qquad \textbf{Equation 5.4}$$

Where h is ice thickness (mm), α is the coefficient of ice growth (mm $°C^{-0.5}$ $d^{-0.5}$) and D_f is the sum of freezing degree-days (d). Values collected by Davar et al. (1996) (Table 5.4.2) were used to estimate the β value of the pond.

Table 5.4.2: Coefficients of ice growth (Davar et al, 1996)

Water body condition	β (mm $°C^{-0.5}$ $d^{-0.5}$)
Theoretical maximum	34
Windy lakes with no snow	27
Average lake with snow	17-24
Average river with snow	14-17
Shelter river with rapid flow	7-14

5.4.6. Characterization of microbial communities

In order to characterize the microbial communities present in the sediment of the sulfide producing stormwater pond, sediment samples were harvested in triplicates at the outlet of RSP2. The harvested samples were washed using 5 ml of buffer to remove potential polymerase chain reaction (PCR) inhibitors. DNA was extracted from the sediment samples using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) and stored at -80°C in

Tris(hydroxymethyl)aminomethane buffer. Extraction quality was measured using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE).

Sequencing was performed by the Molecular Research LP (Shallowater, TX), which amplified DNA using a two-step PCR targeting the V6 hypervariable region of the 16s rRNA. The primers used were custom in-house primers developed and prepared by Molecular Research LP. Each sample was sequenced as a 2x300bp run on an Illumina MiSeq sequencer. The DNA sequencing results were analyzed using the Bio-Linux (Field et al., 2006) operating system. The QIIME software was used to perform operational taxonomical unit (OTU) grouping (T. Magoč and Salzberg, 2011). This allowed for the determination of the bacterial taxa present in the benthic sediment of both ponds.

5.5. RESULTS AND DISCUSSION

5.5.1. Benthic sediment characterization

The benthic sediment of the RSP2 stormwater pond outlet was characterized from harvested samples. The sediment has an average water content of 52.35 ± 7.00 % and a bulk density of approximately 1060 ± 10 kg/m^3. The bottom of the studied pond is a natural clay liner, and the calculated density of the sediment is consistent with bulk density values for clay, with fines (1121 kg/m^3) (Anval Valves Pvt Ltd, 2015).

Table 5.5.1 displays the concentrations of NH_3/NH_4-N, SO_4 and sCOD in the pore water of the sediment samples collected throughout the study. The measured pore water concentration of ammonia of 16.37 ± 0.65 mg/L-N is higher than typical freshwater sediment pore water concentrations (0.028 mg/L-N to 12.57 mg/L-N, Lomans et al. 1997), and in fact is slightly lower than marine sediment concentrations (*Brandford Harbor, CT*, 16.80 mg/L-N to 21.00

mg/L-N, Berner et al. 1969). The pore water concentration of sulfate of 208.67 ± 9.87 mg/L is at the higher end of the range of typical freshwater sediment pore water concentrations (*Brandford Harbor, CT*, 1.15 mg/L SO_4^{2-} to 203.65 mg/L SO_4^{2-}, Lomans et al. 1997). The third column of Table 5.5.1 illustrates concentrations of the same constituents in the bulk water of RSP2. As expected, concentrations of NH_3/NH_4^+ in the sediment were significantly higher than those measured in the bulk water of the pond. The high NH_3/NH_4^+ concentrations observed in the pore water are characteristic of ammonification occurring in benthic sediment (Burton and Pitt, 2001). sCOD concentration in the sediment were also higher than in the bulk water. The higher sCOD concentrations in the sediment are attributed to particulate matter in the benthic zone undergoing hydrolysis. The measured BOD in the bulk water of the RSP2 pond was 3.18 ± 1.06 mg/L. This is within the average bulk water BOD concentrations from 48 different studies recorded by the International Stormwater Best Management Practices Database (ISBMPD) (International Stormwater BMP Database, 2004) which was 5.20 ± 3.81 mg/L.

Table 5.5.1: Characteristics of the benthic sediment and bulk water at the outlet of RSP2

Chemical characteristics	***Pore water concentration (mg/L)***	***Bulk water concentration (mg/L)***
NH_3/NH_4^+-N	16.37 ± 0.65	0.64 ± 0.69 *(Max.: 2.64, Min.: 0.01)*
SO_4^{2-}	208.67 ± 9.87	155.94 ± 31.56 *(Max.: 208, Min.: 96)*
sCOD	81.33 ± 2.08	20.79 ± 14.08 *(Max.: 95.67, Min.: 4.00)*

5.5.2. Sediment kinetics at 20°C, 5°C and 4°C, comparison between field and laboratory

Table 5.5.2 shows the different calculated rates of oxygen consumption by the sediment (Total SOD, carbonaceous SOD, and nitrogenous SOD), and the sediment production and consumption rates of nitrogen. It also shows the sulfide production rate of the sediment. With the help of the addition of nitrification inhibitor, it was possible to fractionate SOD into total, carbonaceous and nitrogenous SOD. Kinetics in the field were calculated by looking at the change in concentration of the different constituents over time, to obtain a daily rate of change. The methodology to calculate the kinetics in the pond are further explained in detail in section 5.4.4. The period of study considered for the determination of in-pond kinetics is exclusively during periods of ice cover development across RSP2. During this period, there was a decrease in dissolved oxygen in the pond, the SOD, ammonification and sulfide production were measured. To supplement the calculations described in 5.4.4, it was necessary to calculate the ice-cover thickness over the pond in order to estimate the bulk water volume of water which no longer contributed to the bulk water volume. Measurements were periodically taken throughout the study period in winter but to get a daily rate of change, Stefan's equation was employed, as described in section 5.4.5. Measurements in the field could only be acquired at 5°C as this was the average temperature recorded at 1.50 m of depth during the onset of ice cover, which was the period of rapid DO change. Typically, production or consumption of different bulk water constituents by microbial processes can be expressed in terms of g of substance produced and/or consumed per square meter of surface area of sediment, per day.

Table 5.5.2: Measured rates of SOD, nitrogen, and sulfide production/consumption in field and laboratory experiments

	Total SOD ($g/m^2/day$)	Carbonaceous SOD ($g/m^2/day$)	Nitrogenous SOD ($g/m^2/day$)	Sediment ammonia production ($g\ N/m^2/day$)	Sediment ammonia oxidation ($g\ N/m^2/day$)	Sediment sulfide production ($g\ S/m^2/day$)
20°C	0.481	0.342	0.139	0.076	0.017	0.055
4°C	0.023	0.012	0.011	0.027	0.013	0.004
Pond (5°C)	0.491	-	-	0.120	-	0.147

Total SOD measured in the laboratory was strongly affected by the temperature and was measured to be 0.481 $g/m^2/day$ at 20°C and 0.023 $g/m^2/day$ at 4°C. The portion of SOD attributable to aerobic-heterotrophic organisms (determined by inhibiting nitrifying bacteria) was 0.342 $g/m^2/day$ at 20°C and 0.012 $g/m^2/day$ at 4°C. The portion of SOD attributable to aerobic-autotrophic organisms was 0.139 $g/m^2/day$ at 20°C and 0.011 $g/m^2/day$ at 4°C. The total SOD rate in this study is in the same range as other similar studies (Zison et al., 1978).

At 20°C, the sediment was observed to produce 0.076 g NH_3/NH_4^+-$N/m^2/day$, consume 0.017 g NH_3/NH_4^+-$N/m^2/day$ and produce 0.055 g $S/m^2/day$. At 4°C, the sediment was calculated to produce 0.027 g NH_3/NH_4^+-$N/m^2/day$, consume 0.013 g NH_3/NH_4^+-$N/m^2/day$ and produce 0.004 g $S/m^2/day$. The rate at which nitrogen is consumed in the laboratory experiments at 20°C did not appear to be drastically different from the rate at 4°C (0.017 g $N/m^2/d$ vs. 0.013 g $N/m^2/day$), which leads the authors to conclude that nitrification is likely restricted at 20C or at both temperatures due to hypoxia induced by carbonaceous oxidation or by inhibition of nitrifying bacteria by sulfide exposure (Joye and Hollibaugh, 1995b).

The rates of total SOD, sediment ammonia production, and sediment sulfide production measured in RSP2 were 0.491 $g/m^2/d$, 0.120 g NH_3/NH_4^+-N $/m^2/d$ and 0.147 g $S/m^2/day$, respectively. The SOD, ammonia and sulfide rates of consumption/production measured in the bench top experiments were less than those measured in the full-scale study. This concurs with findings from Edberg and Hofsten (1973), Patterson et al. (1975) and the USEPA (1976). The significantly higher rates of change observed in the full-scale pond are possibly due to the depth of sediment playing a factor in the specific rates of output of the various constituents. Unit normalization per an area-only basis is conventional and valuable due to its ease of use and comparison with past work, however the study demonstrates that the depth of sediment is likely a key contributing factor to anaerobic kinetics. Ammonification and sulfate-reduction processes have been shown to continue to occur several meters below the benthic surface (Haglund et al., 2003).

Table 5.5.3 displays the experimental Arrhenius' temperature correction coefficients, alongside the measured rates of change of SOD, ammonification, and sulfate-reduction. The temperature coefficients found in this study are slightly higher than reported literature. Values measured with and without nitrification inhibition did not change significantly. The SOD's Arrhenius temperature coefficient found in this study (1.152) is in accordance with findings by Walker and Snodgrass (1986) (1.16) and Edberg and Hofsten (1973) (1.13). The Arrhenius' temperature coefficient for SOD and sulfate-reduction were not appreciably different from the ammonification's Arrhenius coefficient, suggesting that bacteria causing SOD, sulfate-reduction and ammonification are similarly affected by temperature changes in the 4°C to 20°C range.

Table 5.5.3: Experimental Arrhenius' temperature coefficients

Parameter	*Arrhenius' coefficient, Θ*
SOD *(mg O_2/l/day)*	1.152
Ammonification *(mg NH_3-N/l/day)*	1.154
Sulfate-reduction *(mg S/l/day)*	1.155
Nitrification *(mg N/l/day)*	1.131

The half-saturation coefficient for SOD, ammonification, nitrification and BOD were also determined as they can be useful modeling parameters which can be incorporated into many commercial models, to aid in accurately modeling sulfide production. Table 5.5.4 contains the experimental half-saturation coefficients determined using data obtained during from the BOD bottles experiments. These results, while not directly applicable, are crucial to build a better understanding of sulfate-reducing processes in stormwater retention ponds and can aid in the modelling of water quality parameters.

Table 5.5.4: Experimental half-saturation coefficients

Temperature	*Parameter*	*Half-saturation coefficient*
20℃	SOD half-saturation coefficient	3.51 mg O_2 / L
	Ammonification half-saturation coefficient	5.20 mg SOD / L
	Nitrification half-saturation coefficient	0.48 mg O_2 / L
	BOD half-saturation coefficient	2.36 mg O_2 / L
4℃	SOD half-saturation coefficient	1.09 mg O_2 / L
	Ammonification half-saturation coefficient	2.19 mg SOD / L

The calculated SOD half-saturation coefficient at 20°C is 3.51 mg O_2/l. This value is

unsurprising and is comparable to values reported in literature (Jorgensen, 1980). This value is significantly higher than the one calculated at 4°C (1.09 mg O_2/L), which is expected, due to decreased bacterial activity at lower temperatures. The calculated ammonification half-saturation coefficient at 20°C is 3.81 mg SOD/L. Likewise, this value is also higher than the half-saturation coefficient at 4°C (2.83 mg SOD/L). The calculated nitrification half-saturation coefficient at 20°C is 0.48 mg O_2/L. This value is similar to the value of 0.31 ± 0.10 mg O_2/L found by Ghimire (2012). These findings seem to indicate that ammonifying bacteria are less affected by temperature gradient than aerobic organisms, which is not what was observed with the Arrhenius coefficients.

5.5.3. Ice cover thickness comparison

Using Stefan's equation (Eq. 5.3) it is possible to evaluate the ice thickness. Comparing the thickness at the end of the ice covered period, but before the initiation of ice melt, it is apparent that the use of Stefan's equation coupled with coefficients of ice growth found by Davar (1996) (Table 5.4.2) provide very reasonable estimates of the ice cover thickness on stormwater retention ponds. Table 5.5.5 demonstrates calculated theoretical ice thicknesses using Stefan's equation coupled with parameters found by Davar (Average lake with snow, $\beta = 17$) compared to the measured, in-situ ice thickness in the full scale stormwater pond study. This method of estimating ice cover thickness therefore seems very appropriate and acceptable. Back calculations revealed that a β-value of 17.39 provided the best fit of estimates to field measurements.

Table 5.5.5: Comparison of theoretical and experiment ice cover thickness

March 19, 2015 theoretical ice thickness (mm)	March 19, 2015 experimental ice thickness (mm)
546.1 – 771.0	546.1

Using these values, it is possible to estimate the average ice thickness throughout the study and obtain the bulk water to sediment ratio and compare it to the lab bench experiments. Knowing the ice thickness, it becomes possible to calculate the portion of bulk water which will be affected by bacterial processes in relation to the depth of active sediment in the stormwater pond, and compare these results to the lab bench experiments. Rough estimates indicate that during the ice covered period, the volume of liquid water in RSP2 decreased by at least 13.7%, or slightly more than 4,200,000 liters.

5.5.4. Analysis of bacterial communities

Figure 5.5.1 is a graphical interpretation of the DNA sequencing results, and illustrates the proportion of organisms which are SRB and the proportion which are not. At the L6 taxonomic level (Genus), 5.01% of all positively identified organisms (with 97% confidence) were sulfate reducers, while the other 94.99% were not. It was found that *Desulfobulbaceae* (39.5%), *Desulfococcus* (22.8%) and *Desulfobactaraceae* (20.8%) predominated amongst the SRB in the benthic sediment of the stormwater retention pond, which is in accordance with other studies investigating SRB populations in benthic sediment (Gittel et al., 2008; Zhang et al., 2016). Table 5.5.6 shows the top 10 list of SRB and non SRB organisms, sorted by relative abundance (%), at the L6 taxonomic level, in the sediment of RSP2's outlet. It was found that *Desulfobulbaceae* (39.5%), *Desulfococcus* (22.8%) and *Desulfobactaraceae* (20.8%)

predominated amongst the SRB in the benthic sediment of the stormwater retention pond, which is in accordance with other studies investigating SRB populations in benthic sediment (Gittel et al., 2008; Zhang et al., 2016). The results seem to indicate that organisms belonging to the *Desulfovibrio* genus (such as *Desulfovibrio vulgaris* and *Desulfovibrio desulfuricans*) were not present in great numbers and were unlikely to be contributing to sulfate-reducing processes in the studied stormwater ponds.

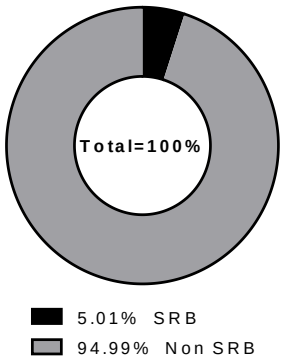

Figure 5.5.1: Distribution of SRB and non SRB organisms found in the outlet sediment of RSP2

Table 5.5.6: Distribution of the top 10 SRB and non SRB organisms found in the benthic sediment of RSP2-4, at the L6 taxonomic level (Genus)

Sulfate-reducing bacteria		**Non sulfate-reducing bacteria**	
Organism	**R.A. (%)**	**Organism**	**R.A. (%)**
Family Desulfobulbaceae, Unclassified Genus	1.98 ± 0.49	*Order Bacteroidales, Unclassified Family, Unclassified Genus*	10.39 ± 3.74
Desulfococcus	1.14 ± 0.26	*Thiobacillus*	8.02 ± 1.47
Family Desulfobacteraceae, Unclassified Genus	1.04 ± 0.26	*Family Sinobacteraceae, Unclassified Genus*	6.33 ± 1.98
Geobacter	0.29 ± 0.11	*Family Rhodocyclaceae, Unclassified Genus*	5.08 ± 2.29
Desulfobulbus	0.17 ± 0.02	*SHD-231*	3.05 ± 1.55
Desulfomonile	0.12 ± 0.10	*Family Anaerolinaceae, Unclassified Genus*	2.86 ± 1.17
Synthrophobacter	0.06 ± 0.01	*Dechloromonas*	2.58 ± 0.72
Family Desulfuromonadales, Unclassified Genus	0.05 ± 0.03	*Family Flavobacteriaceae, Unclassified Genus*	2.39 ± 2.42
Desulfobacca	0.05 ± 0.04	*Family Comamonadaceae, Unclassified Genus*	2.32 ± 1.15
Desulfomicrobium	0.04 ± 0.01	*Order GCA004, Unclassified Family, Unclassified Genus*	2.14 ± 0.80

5.6. CONCLUSIONS

This study aims to advance fundamental kinetic understanding of sulfate-reduction processes in stormwater retentions ponds and as there currently is a lack of literature and fundamental knowledge about H_2S gas production in stormwater retention ponds.

The study indicates that the bacterial populations responsible for sulfate-reduction and ammonification likely remain active several meters deep into the sediment. Hence it is likely that sulfate-reduction and ammonification processes in benthic sediment are not only dependent on sediment surface area but also on sediment depth and substrate concentrations of sulfate and nitrogen, respectively. SRB represented on average 5.01 % of all bacterium present in the top 30 cm of benthic sediment at the outlet of RSP2, and that genuses of the family *Desulfobulbaceae*, *Desulfobacteraceae* and genus *Desulfococcus* predominated the SRB in the benthic sediment, regardless of environmental conditions or season, demonstrating that SRB in temperate climates can develop acclimatization mechanisms, rather than undergo community shifts, as observed by Robador et al. (2009). Additionally, staple SRB which could be expected to drive some of the sulfate-reduction activity were not present in significant proportions, which was very interesting. Finally, supplementary kinetic parameters were determined for SOD, ammonification, sulfate-reduction and nitrification processes. Arrhenius's temperature coefficients were found to be similar for all processes, with values of 1.15 for SOD, ammonification and sulfate-reduction, and 1.13 for nitrification.

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CHAPTER 6 : CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Two stormwater retention ponds located in Ottawa, Ontario, Canada, were thoroughly investigated to develop a fundamental understanding of the hydrogen sulfide (H_2S) production process in these facilities. The main conclusions that were drawn from this work are the following:

1. Hypoxia (dissolved oxygen < 2.0 mg/L) was directly correlated to the production of H_2S ($p < 0.006$, $R > 0.58$);
2. Locations with the most accumulated sediment had the highest propensity for the production of H_2S gas;
3. Significant hydrogen sulfide production events did not demonstrate SRB proliferation and were not indicative of an SRB community shift, rather the production of hydrogen sulfide is likely due to increased SRB bacterial activity stemming from favourable environmental conditions;
4. Microbial analyses show that the microbial communities found in both stormwater ponds did not undergo a community shift, further making the point for increased activity, and suggests that sulfide production events are due solely to environmental conditions present in the ponds;
5. Seasonal changes did not appear to be related to the promotion of SRB or methanogen proliferation within either of the stormwater ponds. Additionally, ice cover of stormwater ponds exacerbated hypoxia in stormwater retention ponds during winter periods.

6. Area-normalized daily rates of change of bulk water concentrations ($g/m^2/day$) measured in laboratory experiments are poor indicators of actual field rates. Rates measured in field studies are higher, due to the active depth of sediment which can be of several meters or more. Active sediment depth is relevant because reactions such as ammonification and sulfate-reduction will keep occurring deep in the sediment column as long as the conditions and the substrate are suitable for the bacteria.
7. Laboratory experiments at 4°C identified total SOD, ammonification and sulfate-reduction kinetics to be 0.023 $g/m^2/day$, 0.027 g $N/m^2/day$ and 0.004 g $S/m^2/day$, respectively. Meanwhile, kinetics calculated from the field study of stormwater retention ponds for total SOD, ammonification and sulfate-reduction were of 0.491 $g/m^2/day$, 0.120 g $N/m^2/day$ and 0.147 g $S/m^2/day$, respectively.
8. Arrhenius's coefficient was calculated for SOD, ammonification and sulfate-reduction and nitrification. This revealed that all SOD, ammonification and sulfate-reduction processes were similarly affected by temperature change ($\emptyset$ = 1.15). Nitrification was slightly less affected by temperature change ($\emptyset$ = 1.13).

6.2. RECOMMENDATIONS

The following recommendations are intended to assist researchers working on topics which were presented in the thesis in order to expand the knowledge of bacterial reaction rates and hydrogen sulfide production in stormwater retention ponds:

- Active depth of microbes in sediment columns is not clearly defined and should be studied further. Looking at sediment cores from different facilities with different bottom materials could reveal trends and potential propensity to sulfide production.
- The effects of intermittent aeration at the very beginning of the winter season should be further studied to evaluate if intermittent aeration can be a cheap and effective mitigation method.

APPENDIX A: CALCULATION EXAMPLE

Below are more detailed sample calculations of different kinetic parameters discussed in the thesis.

Sample calculation of sulfide pond-wide rate of production (as seen in Table 5.5.2):

Knowns/Assumptions:

Design pond volume: 31,104 m^3

Using Eq. 5.4, it is possible to determine the volume of ice on the pond at a certain date. Using environmental data collected from an Environment Canada logging station at the Ottawa International Airport (closest logging station); It was determined that the sum of frozen degree days throughout the winter period at RSP2 was of -1032 (1032) frozen degree days (FDD). This value is calculated by calculating the sum of degrees (Celcius) below the point of freezing (0°C) for water. An example of the FDD calculation is shown in the table below:

Total days where temperature is freezing	Average daily temperature (°C)	Degrees °C below freezing point (0°C)	Cumulative freezing degree days (FDD)
1	-6.2	6.2	6.2
2	-10.8	10.8	17.0
3	-20.1	20.1	37.1

For RSP2 (whole pond), the FDD value was of 1032 for the winter period spanning from Dec. 2015 until March 17th 2016 (date at which ice thickness was measured).

Now using equation 5.3: $$h = \beta\left(D_f\right)^{0.5}$$

Where β = 17 (Average lake with snow)

D_f (March 17th, 2016) =1032

Gives us h = 546.12 mm ice thickness.

Compared to actual data, the measured ice thickness was of 559 mm. The equation therefore provides a good estimate of ice cover. Now, since the measurements of rate of change are occurring throughout the whole study period (and ice cover varies during winter time, getting thicker as time passes), it is necessary to take the dynamic nature of ice cover, and as such the thickness is averaged for the whole winter period. Since temperatures were colder in the beginning of winter (December, January) than towards the end (March, April), it was hypothesized that the average ice cover thickness could not simply be half of the total calculated seasonal thickness, rather it was found that at the halfway mark (66.5 days out of 127 consecutive freezing days), 66.6% of the FDD had already been achieved, therefore it was decided that the average ice thickness would be considered as 66.6% of the maximum thickness (559.71 mm with an FDD of 1084). The hypothetical average pond ice thickness was therefore set to 373 mm.

It was known that the area of the pond was of 0.97 ha, or 9,700 m^2. As mentioned earlier, there was a factor of 1.15 added on to account to slight sloping to estimate sediment area (or pond bed, essentially) for the pond. This led to the assumption that the area would be of approximately 11500 m^2.

The volume of water which was to be used in our rates of change calculations was therefore of 31,104 m^3 – (11,500 m^2 x 0.373 m) = 26,815 m^3

The integral constituent concentration data sheet for total sulfides was then utilized and the average total sulfides daily change in concentration throughout the winter period was

calculated throughout the entire RSP2 pond. This amounted to 60 µg/L/day, or 0.06 mg/L/day total sulfides, as the average change in total sulfides concentration throughout the RSP2 pond during the whole during the ice-covered period.

In order to get the rate of production/consumption/change in a (g/m^2/day form), it was necessary to do the following calculation:

$$\frac{0.06\frac{mg}{L*day}*\frac{1\,g}{1000\,mg}*26{,}815\,m^3*\frac{1000\,l}{1\,m^3}}{11{,}500\,m^2}=\mathbf{0.147}\frac{\boldsymbol{g}}{\boldsymbol{m^2*day}}$$