PHOSPHORUS REMOVAL IN PASSIVE COLD REGION WASTE STABILIZATION PONDS

by

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ABSTRACT

A majority of communities in the Canadian territory of Nunavut rely on passive waste stabilization ponds (WSP) for domestic wastewater treatment. Little research has been conducted on the treatment performance of these systems. In response to impending federal wastewater regulations, a research program was conducted in order to characterize contaminant removal. Due to its role in receiving water eutrophication, phosphorus is a contaminant of particular concern. Phosphorus is removed in WSPs both chemically (precipitation, adsorption) and biologically (microalgae and bacteria uptake). Due to extended photoperiods during the ice-free summer treatment season, it is hypothesized that microalgae play an important role in phosphorus removal.

The objectives of this research were to (i) characterize phosphorus removal and identify promising removal mechanisms occurring in full-scale WSPs in Nunavut, Canada, (ii) quantify microalgae growth rates and phosphorus uptake, and identify microalgae uptake mechanisms under simulated cold region summer conditions, and (iii) develop a predictive stochastic ecological model for microalgae growth and effluent phosphorus concentration in WSPs in various geographical locations in Nunavut, Canada. Full-scale WSP evaluations occurred at four communities (Kugaaruk, Pond Inlet, Grise Fiord and Clyde River) over a period of four years (2011-2014). Phosphorus removal was highly variable. Most of the WSPs operated anoxically with minimal microalgae growth; however, the highest removal was observed during a microalgae bloom. A factorial laboratory experiment was then done to quantify microalgae growth rates and phosphorus uptake under varying climate conditions (temperature, photosynthetically active radiation) and phosphorus concentrations. Growth rates were similar to those found at temperate climates. Biomass phosphorus concentrations were found to be 45% greater than previously observed in studies at temperate climates. A stochastic ecological model with integrated equilibrium temperature model was then developed to predict microalgae growth and phosphorus removal in WSPs at various geographical locations in Nunavut. The model utilized a Monte Carlo Simulation to account for parameter uncertainty. The model showed that July temperature and summer treatment season temperature were the best predictors of microalgae growth. Modeled phosphorus removal was consistent with secondary treatment if WSP depth was less than 2 m and the WSP is operating facultatively.

LIST OF ABBREVIATIONS AND SYMBOLS USED

/h	Per hour
°C	Degrees celsius
a	Slope of the equilibrium temperature vs air temperature regression
Al	Aluminum
$AlCl_3•6H_2O$	Aluminum chloride hexahydrate
APHA	American Public Health Association
b	y-intercept of the equilibrium temperature vs air temperature regression
BOD	Biochemical oxygen demand
c	Specific heat capacity of water
C	Cloud cover
$C_{10}H_{14}N_2Na_2O_8•2H_2O$	Sodium ethylenediaminetetraacetate dihydrate
$CaCl_2•2H_2O$	Calcium chloride dihydrate
$CBOD_5$	5-day Carbonaceous Biochemical Oxygen Demand
CCME	Canadian Council of Ministers of the Environment
cm	Centimeter
$CoCl_2•6H_2O$	Cobalt chloride hexahydrate
$CuSO_4$	Copper sulfate
d	Day
e_a	Air water vapour pressure
EDTA	Ethylenediaminetetraacetate
e_s	Saturated water vapour pressure
f	Microalgae biomass phosphorus percentage
Fe	Iron
$FeSO_4•7H_2O$	Ferrous sulfate heptahydrate
g P/g dry biomass	Grams of phosphorus per gram of dry biomass
g P/g P	Gram of phosphorus per gram of phosphorus
H	Total heat flux at the water surface
h	Hour

h_0	Dimensionless Baranyi-Roberts model parameter
ha	Hectares
HRAP	High-rate algal pond
H_{si}	Incoming solar radiation
J	Joule
K	Thermal exchange coefficient
K_2HPO_4	Potassium orthophosphate
kg	Kilogram
kg BOD/ha/d	Kilograms of biochemical oxygen demand per hectare per day
kJ	Kilojoules
k_L	Light attenuation coefficient
km	Kilometer
L	Litres
L/h	Litres per hour
LED	Light emitting diode
light	Water surface photosynthetically active radiation
$light_{optimal}$	Optimal microalgae growth photosynthetically active radiation
$light_z$	Mean subsurface photosynthetically active radiation at depth *z*
m	Meters
M	Mole per litre
m	Grouped microalgae death/settling coefficient
m^2	Square meters
m^3/d	Cubic meters per day
mg P/ g dry sediment	Milligrams of phosphorus per gram of dry sediment
mg P/L	Milligrams of phosphorus per litre
mg/L	Milligram per litre
$MgCl_2$	Magnesium chloride
mL	Milliliter
Mn	Manganese
$MnCl_2•4H_2O$	Manganese chloride tetrahydrate
n	Number of samples
$Na_2MoO_4•2H_2O$	Sodium molybdate dihydrate

$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NE	Northeast
NH_4Cl	Ammonium chloride
P	Phosphorus concentration
PAR	Photosynthetically active radiation
R^2	Coefficient of determination
rpm	Revolutions per minute
s	Second
SD	Standard deviation
SE	Southeast
SF	Shading factor
SRP	Soluble reactive phosphorus
SW	Southwest
t	Time
T_a	Air temperature
T_d	Dew point temperature
T_e	Equilibrium temperature
$T_{minimum}$	Minimum microalgae growth temperature
$T_{optimal}$	Optimal microalgae growth temperature
TP	Total phosphorus
TSS	Total suspended solids
T_w	Water temperature
V	Wind speed
W	Watt
W/m^2	Watt per square meter
WSP	Waste stabilization pond
X	Microalgae biomass concentration
$X_{background}$	Background microalgae biomass concentration
X_{max}	Maximum microalgae biomass concentration
y	Depth
z	Depth

$ZnSO_4$	Zinc sulfate
β	Atmospheric emissivity
λ	Wavelength
μ_{20}	Maximum specific growth rate at 20°C
µg/L	Micrograms per litre
µm	Micrometer
μ_{max}	Maximum specific growth rate
µmol/m^2/s	Micromole per square meter per second
ρ	Density

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1 Introduction

1.1 Research Rationale

On February 17, 2009, The Canadian Council of Ministers of the Environment (CCME) endorsed the development of a Canada-Wide Strategy for the Management of Municipal Wastewater Effluent. The goal of this strategy is to create a national regulatory framework. However, the Canada-Wide Strategy has special provisions for Canada's Far North (Northwest Territories, Nunavut, Nunavik region of Quebec and Nunatsiavik region of Newfoundland/Labrador) citing, "due to the extreme climatic conditions and remoteness, a viable means to improve human and environmental health protection needs to be carefully considered." A five-year window was allotted these regions to allow for research into the performance of existing wastewater facilities and factors affecting their performance (CCME, 2009).

Passive single cell waste stabilization ponds (WSPs) are the primary wastewater treatment system used in Nunavut (Johnson, 2008). However, published studies pertaining to WSPs in Canada's northern regions are essentially non-existent and a comprehensive understanding of the treatment mechanisms that occur in WSPs in these regions has not been attained. Phosphorus is a contaminant of particular concern due to its role in receiving water eutrophication. Localized nutrient enrichments have previously been documented in WSP effluent receiving waters in several communities in Nunavut (Krumhansl *et al.*, 2015). While most communities in Nunavut discharge into marine environments, which are typically nitrogen limited, there are communities in northern

Canada that discharge into freshwater environments, which are typically phosphorus limited. Therefore, this research looks to begin to address the knowledge gap associated with phosphorus removal in cold region WSPs. While a majority of this research is conducted in Nunavut, it has application to other cold climate regions that experience similar climatic challenges.

1.2 Current State of Knowledge

The level and complexity of wastewater treatment in cold regions varies greatly. Strategies include passive wetlands and/or WSPs (Prince *et al.*, 1995; Rockne and Brezonik, 2006; Hayward *et al.*, 2014; Ragush *et al.*, 2015; Yates *et al.*, 2012), chemical precipitation ponds or fellingsdams (Hanaeus, 1987; Hanaeus *et al.*, 2010; Ødegaard *et al.*, 1987), and mechanical treatment plants (moving bed biofilm reactor, activated sludge, membrane bioreactor, etc.) (Grönlund *et al.*, 2004; Di Trapani *et al.*, 2013). Direct discharge is also practiced in cold regions such as Greenland (Gunnarsdóttir *et al.*, 2013). There are advantages and disadvantages of each treatment strategy. Passive systems are inexpensive to operate, do not require significant technical expertise and do not depend on mechanical equipment or chemicals, which is a definite advantage for remote communities (Heaven *et al.*, 2003). However, passive systems are subject to climate conditions, and therefore have lower treatment efficiencies during colder months (Hanaeus, 1987). They also require a large footprint (Heaven *et al.*, 2003). Fellingsdams are similar to passive WSPs, but are able to achieve high treatment efficiency year round due to the addition of chemical precipitants (alum salts, ferric salts and/or lime). Reliance on chemical precipitants and high sludge generation rates resulting in frequent desludging

are the main disadvantages of fellingsdams (Ødegaard *et al.*, 1987). Mechanical plants are capable of high treatment efficiency and require a relatively small footprint. However, mechanical plants often require technical expertise to operate and require a reasonably consistent influent in terms of flow rate and wastewater composition (Hanaeus, 1987). For remote communities, getting replacement parts for mechanical equipment and/or chemicals for treatment processes may be challenging.

Passive WSPs are the most common form of wastewater treatment in Nunavut (Johnson, 2008). Communities in Nunavut are very remote, only accessible by air and sea. Therefore, passive systems are advantageous as there is limited reliance on mechanical equipment and chemicals, which would be potentially financially difficult to transport. Most communities utilize a trucked sewage collection system because of continuous permafrost (Johnson, 2013). This results in inconsistent system flow rates, which is also not ideal for mechanical treatment systems.

WSPs in Nunavut are designed with 12 months of storage capacity to allow for extended periods of ice cover (Johnson *et al.*, 1998; Johnson, 2008). An example of the monthly air temperature trends in Pond Inlet, Nunavut is shown in Figure 1-1. Temperatures are well below freezing from October through May, ranging from -9°C to -34°C. Temperatures then increase above freezing from June to early September, allowing the WSP to thaw.

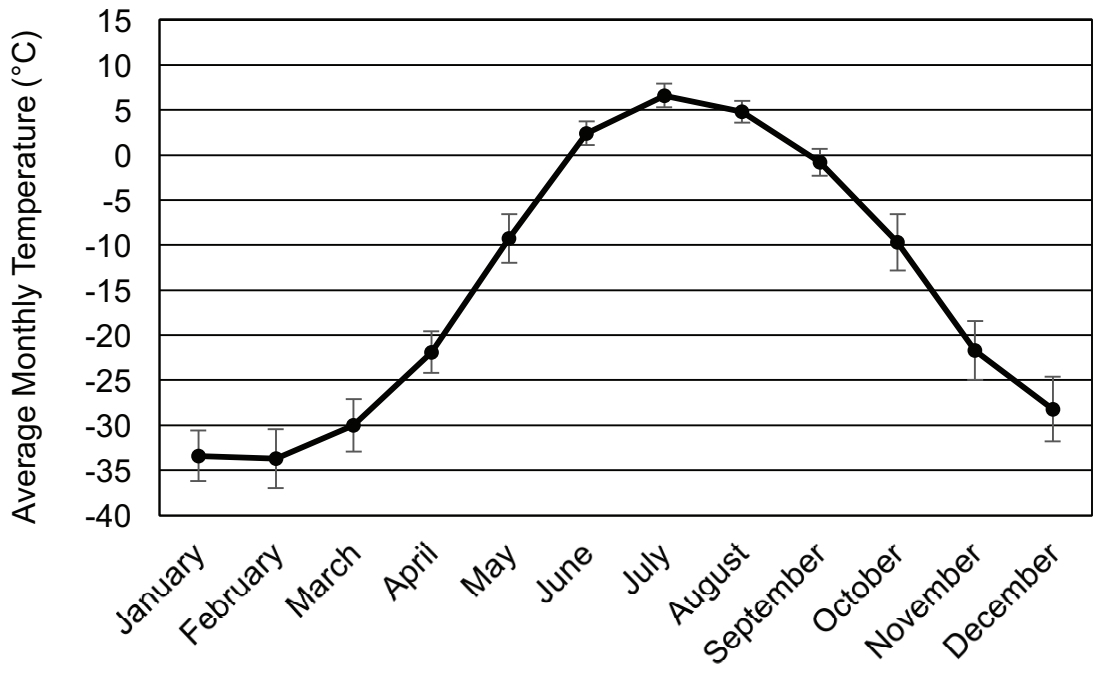


Figure 1-1: Mean monthly air temperature in Pond Inlet, Nunavut (Environment Canada, 2014). Error bars represent standard deviation.

Generally, it is assumed that WSPs designed for extended storage function facultatively during ice-free periods (Prince *et al.*, 1995). In facultative WSPs, microalgae communities are relied on to provide aeration, thereby allowing for the aerobic treatment of nutrients and organic matter. In Nunavut, extended photoperiods coincide to when the WSPs are ice-free (approximately June – September). It has been hypothesized these extended photoperiods accelerate microalgae growth, allowing for aerobic conditions within the WSP. Limited research has been done to confirm that WSP operate facultatively in the ice-free treatment season.

Phosphorus can be removed in WSPs chemically (precipitation, adsorption) and biologically (bacteria and microalgae uptake). Calcium, iron and aluminum are the three most common metals associated with the chemical removal of phosphorus. Calcium precipitates form at alkaline pH (>10) while iron and aluminum precipitates form at slightly acidic-neutral pH (5-7) (Tchobanoglous *et. al.*, 2003). WSPs in Nunavut are not dosed with precipitants, so chemical removal is limited to metals that are naturally occurring in the drinking water source or from the dissolution of premise plumbing and the distribution/collection truck tank. Both bacteria and microalgae are capable of biologically removing phosphorus. Bacteria take up and utilize phosphorus for growth. Enhanced phosphorus removal by bacteria can be achieved through the use of specific phosphorus accumulating organisms in conjunction with anaerobic and aerobic tanks/zones in series with mixed liquor recycling (Tchobanoglous *et. al.*, 2003). However, since most WSPs in Nunavut are passive single cells, it is unlikely that phosphorus accumulating organisms will significantly contribute to overall phosphorus removal. Microalgae remove phosphorus through two mechanisms. The first is assimilation into the microalgae biomass through the construction of cellular components such as phospholipids. The second is luxury uptake, where phosphorus, in excess of what is required for growth, is stored within microalgae cells as inorganic polyphosphate granules. Previous research in WSPs at temperate climates has shown that luxury uptake can play a significant role in phosphorus removal efficiency (Powell *et al.,* 2011a). The role of microalgae removal mechanisms has not been documented in cold region WSPs.

Limited research has been conducted on microalgae-based wastewater treatment systems in cold climates. Grönlund *et al.* (2010) evaluated the use of high-rate algal ponds (HRAP) in Östersund, Sweden (63.2°N). A pilot HRAP was constructed beside a municipal fellingsdams treatment system and operated in the Fall of 2002 and 2003. The HRAP received effluent from a presedimentation pond prior to precipitant dosing. Total phosphorus removal in the HRAP ranged from 11 to 49% during the pilot operation. This was much lower than the performance of the fellingsdams which achieved a mean total phosphorus removal of 85 to 89% in the summer of 2001 and 2002 (Hanaeus *et al.*, 2010).

There is no specific performance standard stipulated by the Canada-Wide Strategy for the Management of Municipal Wastewater Effluent for phosphorus; however, there is a requirement to provide adequate environmental protection through localized effluent discharge objectives (as determined through an environmental risk assessment). It is foreseeable that phosphorus effluent concentrations may be regulated in the future. Therefore, based on the potential for changing regulations and the general knowledge gap associated with cold region WSP performance, this research looks to address the following questions:

- What are the expected phosphorus removal efficiencies of full-scale WSPs in Nunavut and what are the primary removal mechanisms?
- What are the microalgae growth rates and phosphorus uptake rates under cold region conditions?

- What phosphorus uptake mechanisms exists for microalgae in cold region WSPs? Does luxury uptake play a significant role?
- Can the probability of a WSP operating facultatively be predicted using climate data? Can this be used as a tool to screen communities to determine whether facultative WSPs are an appropriate treatment option?

1.3 Research Objectives

The main research purpose was to evaluate the use of passive WSPs for phosphorus removal in cold climates. This was addressed through several objectives:

Objective 1: Characterize phosphorus removal in full-scale passive WSPs in cold climates (Nunavut, Canada) and identify promising removal pathways using sediment fractionation.

Objective 2: Quantify microalgae growth rates and phosphorus uptake, and identify microalgae phosphorus uptake mechanisms under simulated cold regions summer conditions.

Objective 3: Develop a predictive stochastic ecological model for microalgae growth and effluent phosphorus concentrations in WSPs in various geographical locations in Nunavut, Canada.

1.4 Organization of Thesis

This thesis was organized such that each objective was presented as a chapter in the style of a refereed journal article. Each of the objective chapters (Chapters 2-4) contain their

own abstract, introduction, materials and methods, results and discussion, and conclusion. The literature review for this study is presented in the introduction sections of the objective chapters (Chapters 2-4).

Chapter 1 describes the research rationale, research objectives and the organization of the thesis.

Chapter 2 presents the findings of objective 1, which involved evaluating phosphorus removal from four full-scale WSPs in Nunavut, Canada from 2011 to 2014. Sediment fractionation at two of the WSPs allowed for inferences on predominant phosphorus removal mechanisms. This work has been published in the journal *Arctic Science*.

Chapter 3 presents the findings of objective 2, which involved quantifying microalgae growth rates and phosphorus uptake under simulated cold region summer conditions. Phosphorus uptake pathways (luxury uptake, assimilation) were also quantified. This work has been published in the journal *Ecological Engineering*.

Chapter 4 presents the findings of objective 3, which involved developing a predictive stochastic ecological model for microalgae growth and effluent phosphorus concentration in Nunavut, Canada. The model was parameterized using field data as well as historic climate data. Predictive equations for the probability of microalgae growth based on local climate information were developed.

Chapter 5 presents an overall synthesis of key finding and conclusions. Recommendations for further work on phosphorus removal in cold climate WSPs is provided.

2 Characterizing Phosphorus Removal in Passive Waste Stabilization Ponds in Arctic Communities

This chapter has been published in *Arctic Science*:

Schmidt, J.J., Ragush, C.M., Krkosek, W.H., Gagnon, G.A., Jamieson, R.C. 2016. Characterizing phosphorus removal in passive waste stabilization ponds in Arctic communities. *Arctic Science*, 2(1), 1-14.

2.1 Abstract

A majority of communities in the Canadian territory of Nunavut rely on passive waste stabilization ponds (WSPs) for domestic wastewater treatment. Little research has been conducted on the treatment performance of these systems. Therefore, in response to impending federal wastewater regulations, a research program was conducted in order to characterize contaminant removal, with phosphorus a contaminant of particular concern. The performance of WSPs in the Arctic communities of Kugaaruk, Pond Inlet, Grise Fiord and Clyde River was evaluated from 2011 to 2014. Removal of total phosphorus was highly variable ranging from 24% (Pond Inlet, 2014) to 76% (Grise Fiord, 2011). The average removal efficiency was 44%. Effluent total phosphorus concentrations generally exceeded 7 mg P/L, partly due to elevated raw wastewater concentrations. Over the course of the treatment season (defined as June to September, when the WSP is thawed), limited additional total phosphorus removal was observed. A fractionation analysis of WSP sediments showed that organic phosphorus and phosphorus bound to

aluminum and iron were the predominant forms of sediment phosphorus, which provided insight into primary treatment mechanisms. Further studies on these mechanisms are needed in order to optimize Arctic WSP treatment.

2.2 Introduction

Passive systems are the most common form of municipal wastewater treatment in the Canadian Arctic territory of Nunavut. Of the territory's 25 municipal wastewater treatment systems, 21 use passive systems such as WSPs and/or wetlands. Passive systems have several advantages including low operating costs, minimal required technical expertise, and long retention periods (Heaven *et al.*, 2003). However, their performance can be variable (Hanaeus *et al.*, 2010).

Little research has previously been conducted on the performance of passive systems in northern environments and a comprehensive understanding of the treatment mechanisms occurring in these systems has not been obtained. Therefore, in 2009, when the Canadian Council of Ministers of the Environment (CCME) endorsed the development of a Canada-Wide Strategy for the Management of Municipal Wastewater Effluent, special provisions were allotted for Canada's 'Far North'. Northern regions were allotted 5 years in which to conduct research into the performance of existing wastewater facilities and factors affecting their performance (CCME, 2009).

In Nunavut, passive WSPs used for municipal wastewater treatment are typically designed to provide storage for 365 days of wastewater generation. They are generally ice-free from June to September. Most commonly, WSPs have a controlled decant at the end of the ice-free period. It is generally accepted that any treatment provided occurs primarily during the ice-free period. Therefore, for the purpose of this study, the ice-free

period is referred to as the treatment season. Raw wastewater is characterized by high organic/nutrient concentrations attributed to a low per capita water usage.

The Canada-Wide Strategy set out National Performance Standards for total suspended solids (TSS), 5-day carbonaceous biochemical oxygen demand ($CBOD_5$), and total residual chlorine. Effluent discharge objectives for other parameters, such as nutrients, can also be implemented on a site-by-site basis in order to adequately protect human health and the receiving water body. Due to its role in eutrophication, phosphorus is an important parameter for consideration. An initial study of receiving water benthic environments in Nunavut found that effluent from WSPs was causing localized nutrient enrichment (Krumhansl *et al.*, 2015)

Phosphorus can be removed by both biological and chemical mechanisms in a WSP. Biological removal involves the assimilation of phosphorus into bacteria or microalgae biomass. Promoting microalgae growth is considered a promising mechanism for Arctic climates due to extended summer photoperiods. Microalgae consume phosphorus to construct cellular components such as nucleic acids and phospholipids. Phosphorus can also be stored in algae as polyphosphate through a mechanism referred to as luxury uptake (Miyachi *et al.*, 1964). Chemical removal mechanisms involve the precipitation of orthophosphate with calcium, magnesium, aluminum, or iron. The complexes formed depend on pH, alkalinity and temperature, as well as orthophosphate and cation concentration. Phosphate can also adsorb to hydroxides of calcium, iron, and aluminum (Moutin *et al.*, 1992; Peng *et al.*, 2007; Wilsenach *et al.*, 2007). Both biological and

chemical removal mechanisms ultimately rely on sedimentation for removal from the water column of a WSP.

Highly variable phosphorus removal efficiencies have been demonstrated in northern climates, depending on the WSP design and operation. Fellingsdams, common in Scandinavian countries, use slaked lime, aluminum sulfate (alum) or ferric chloride as precipitants. Fellingsdams in northern Sweden have demonstrated mean phosphorus removals of 84-96% (Hanaeus, 1987; Hanaeus *et al.*, 2010). The main disadvantage of fellingsdams is increased sludge generation, which results in yearly dredging (Hanaeus, 1987). Experiments on biological treatment options have also been conducted. Pilot-scale high-rate algal ponds operated in northern Sweden achieved 11-49% removal efficiency before experiments ceased in October due to ice formation (Grönlund *et al.*, 2010).

The objective of this study was to characterize phosphorus removal in passive WSPs in northern environments and to identify promising removal pathways. Field research was conducted on four wastewater systems located above the Arctic Circle in Nunavut, Canada. All systems were located in small, remote communities only accessible by aircraft. Frequency of scheduled flights varied from daily to weekly, depending on the community. Relying on aircraft for transportation creates a unique set of challenges including limited ability to ship samples for analysis, inability to access research supplies deemed hazardous, and extremely high costs. Within the systems, phosphorus concentrations were measured at various points over the course of 4 years. Since both chemical and biological removal mechanisms require sedimentation for removal, a phosphorus fractionation analysis was conducted on sediments from two WSPs.

Sediment phosphorus fractionation is a method commonly used to study natural ecosystems, but is not widely used in engineered systems. Fractionation methods generally apportion phosphorus into multiple organic and inorganic pools (Goltermann, 1996; Lukkari *et al.*, 2007). The fractionation method used in this study, as described by Lukkari *et al.* (2007), separates total sediment phosphorus into the following pools: loosely bound and pore water phosphorus, redox sensitive iron and manganese bound phosphorus, phosphorus bound to aluminum and non-reducible iron oxides, calcium bound phosphorus, and organic phosphorus. The results of the sedimentation analysis were used to hypothesize which removal mechanisms are occurring in the WSP environment.

2.3 Materials and Methods

2.3.1 Description of sites

Research was conducted in Kugaaruk, Pond Inlet, Clyde River and Grise Fiord, Nunavut. A map of the sites is shown in Figure 2-1.

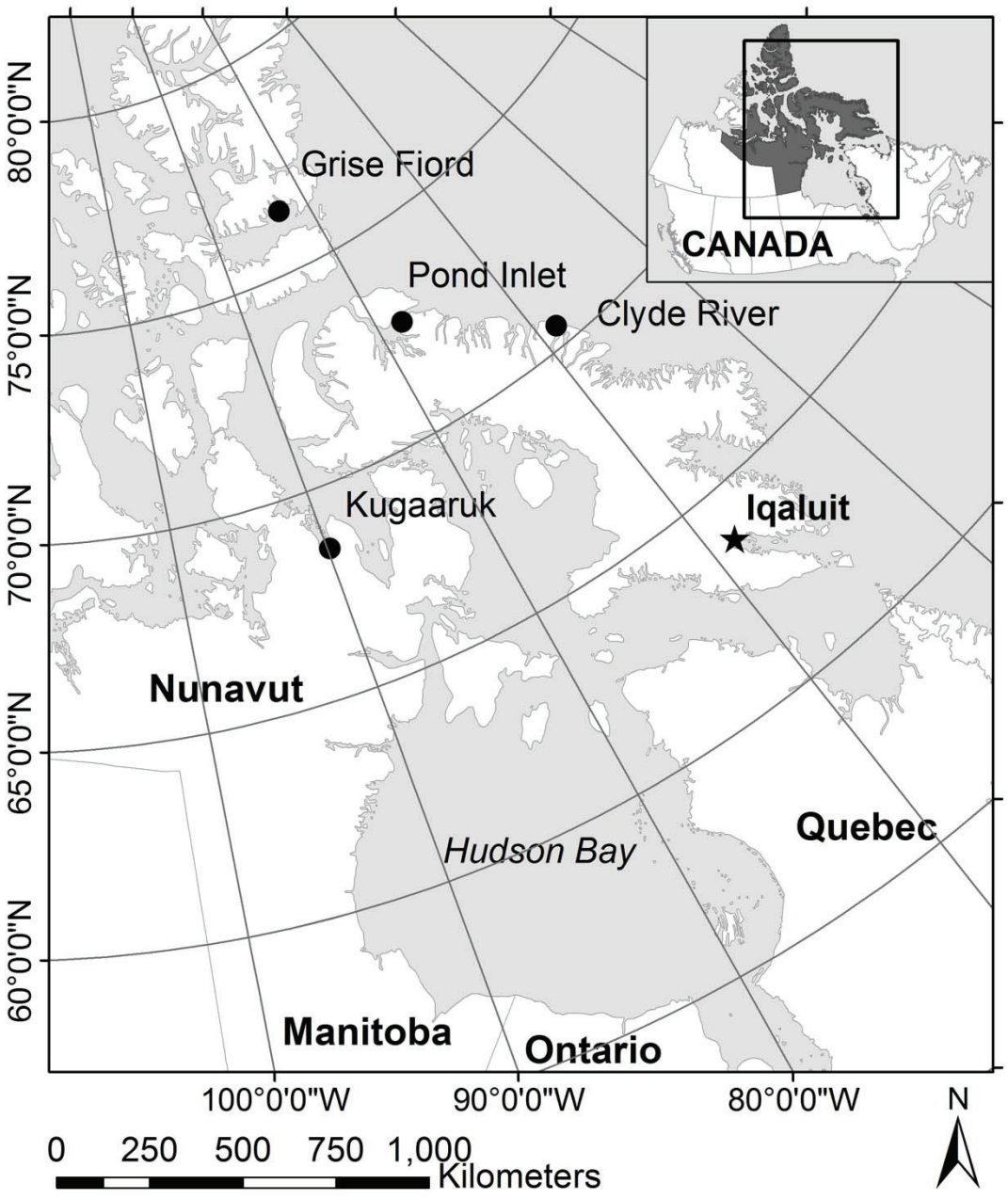


Figure 2-1: Locations of the four communities (Kugaaruk, Grise Fiord, Pond Inlet, and Clyde River) in Nunavut where WSP sampling took place between 2011 and 2014.

2.3.1.1 Kugaaruk (68°31'59" N, 89°49'36" W)

Kugaaruk is located in central Nunavut and has a population of approximately 878 (Nunavut Bureau of Statistics, 2013). Kugaaruk has a daily average temperature (± standard deviation) of -13.5 ± 1.5°C with an average temperature during July and August of 7.9°C (Environment Canada, 2014). Kugaaruk's wastewater treatment system consists of a single cell WSP with an estimated surface area of 10188 m^2 and average operating depth of 5.4 m during the treatment season. The WSP has an approximate volumetric and organic loading rate of 76 m^3/d and 28 kg BOD/ha/d, respectively. The WSP is decanted annually from July-October, depending on the weather. During the decant, wastewater is pumped from the WSP into a smaller pond with a downstream permeable berm. Wastewater seeps through the berm into a natural tundra wetland. The purpose of the decant pond is to spread the wastewater flow over the width of the wetland. The outfall of the wetland is a marine receiving environment approximately 650 m from the community.

2.3.1.2 Pond Inlet (72°41'57" N, 77°57'33" W)

Pond Inlet is located on northern Baffin Island and has a population of approximately 1612 (Nunavut Bureau of Statistics, 2013). Pond Inlet has a daily average temperature (± standard deviation) of -14.6 ± 4.9°C with an average temperature during July and August of 5.7°C (Environment Canada, 2014). Pond Inlet's system consists of a single cell WSP with an estimated surface area of 40000 m^2 and an average operating depth of 1.9 m during the treatment season. The WSP has an approximate volumetric and organic loading rate of 112 m^3/d and 15 kg BOD/ha/d, respectively. The WSP is decanted in

September. During the decant, wastewater is pumped from the WSP into a gravel channel. The outfall of the channel is a marine receiving environment approximately 2 km from the community.

2.3.1.3 Clyde River (70°28'26" N, 68°35'10" W)

Clyde River is located on northern Baffin Island and has a population of approximately 1004 (Nunavut Bureau of Statistics, 2013). Clyde River has a daily average temperature (± standard deviation) of -12.6 ± 3.5°C with an average temperature during July and August of 4.7°C (Environment Canada, 2014). Clyde River's system consists of a two cell WSP. The primary cell has an estimated surface area of 6000 m^2 and an average operating depth of 1.1 m during the treatment season. The secondary cell has an estimated surface area of 15000 m^2 and an average operating depth of 2.3 m. The primary cell has an approximate volumetric and organic loading rate of 93 m^3/d and 57 kg BOD/ha/d, respectively. The system is designed to decant the secondary cell biennially, with wastewater being transferred from the primary to secondary cell yearly. However, due to operation issues, raw wastewater is occasionally discharged into the secondary cell. During the decant, wastewater is pumped from the secondary cell into an engineered vegetated filter strip. The outfall of the vegetated filter strip is a marine receiving environment approximately 1.2 km from the community.

2.3.1.4 Grise Fiord (76°25'3" N, 82°53'38" W)

Grise Fiord is located on southern Ellesmere Island and has a population of approximately 157 (Nunavut Bureau of Statistics, 2013). Limited historical climate data

is available for Grise Fiord. From 2011 to 2014, the daily average temperature was -13.3°C and the average daily temperature during July and August was 4.2°C (Environment Canada, 2015). Grise Fiord's system consists of a single cell WSP with an estimated surface area of 4100 m^2 and an estimated average operating depth during the treatment season of 1.5 m. The WSP has an approximate volumetric and organic loading rate of 16 m^3/d and 25 kg BOD/ha/d, respectively. The exact decant schedule varies and is highly weather dependent, but occurs during the ice-free season. During the decant, wastewater is pumped into a natural tundra wetland. The outfall of the wetland is a marine receiving environment approximately 620 m from the community.

2.3.1.5 Wastewater collection

Due to continuous permafrost, all of the communities utilize vacuum trucks to collect wastewater directly from household storage tanks, as opposed to a conventional piped system. The wastewater generated by the communities is exclusively from domestic sources.

2.3.2 Field Data Collection Timeline

Field data collection occurred from June to September in 2011 through 2014. Due to logistical constraints associated with northern research, such as weather, cost and lack of facilities, each site was not visited an equal number of times. Field visits fell into three ranges during the treatment season: start (June 15-July 7), middle (July 20-August 7), and end (August 25-September 15). The start of the treatment season coincides with the WSP thawing; often there is still limited ice coverage during this period. The end of the

treatment season coincides with the decanting of the WSP prior to freezing. A summary of the sampling plan is shown in Table 2-1.

Table 2-1: Sampling plan for wastewater systems in four Arctic communities.

	2011			2012			2013			2014		
	Start	**Middle**	**End**	**Start**	**Middle**	**End**	**Start**	**Middle**	**End**	**Start**	**Middle**	**End**
Grise Fiord		+										
Pond Inlet				+	+	+	+	+	+	+, o, §	+, o, §	+, o, §
Clyde River				+		+	+	+	+	+		+
Kugaaruk				+		+	+, o		+, o, §			

\+ Total Phosphorus, metal parameters (Aluminum, iron, manganese, calcium)

o Phosphorus Species Analysis: Total Phosphorus, Total Soluble Phosphorus, Soluble Reactive Phosphorus

§ Sediment Analysis

Note: Start (June 15-July 7), middle (July 20-August 7) and end (August 25-September 15) refer to the time during the treatment season, with start corresponding to the WSP thaw and the end corresponding to the WSP freezing

2.3.3 Environmental Monitoring

The WSP environment and ambient climatic conditions were continuously monitored in Kugaaruk, Pond Inlet and Clyde River. Dissolved oxygen, water temperature, conductivity, pH, pressure and relative light were measured in the WSP throughout the treatment season using 6-Series and EXO in-situ multi-parameter sondes (YSI Inc., Yellow Springs, Ohio), as well as HOBO temperature/light pendants (Onset Computer Corporation, Cape Cod, Massachusetts). Air temperature, barometric pressure, solar radiation and photosynthetically active radiation were measured using a weather station (Onset Computer Corporation, Cape Cod, Massachusetts) placed beside the WSP.

Temperature, dissolved oxygen and pH were measured as spot samples in the Grise Fiord WSP using a handheld multi-parameter water quality sonde (YSI Inc., Yellow Springs, Ohio).

2.3.4 Sample Collection and Analysis

2.3.4.1 Water Collection and Analysis

Grab samples were collected using clean, Milli-Q rinsed, plastic sample bottles. WSP grab samples were taken from shore or from an inflatable boat using a sub-surface pole sampler (Environmental Remediation Equipment, Inc., Montreal, Quebec) or an acrylic bacon bomb sampler (Koehler Instrument Company, Inc., Bohemia, New York). Occasionally, surface samples were taken by hand.

As shown in Table 2-1, water sample analysis consisted of two sampling scenarios. The first was an analysis of total phosphorus (TP) and metals (aluminum, iron, manganese, and calcium) in the WSP. The second was an analysis of phosphorus species in the WSP, consisting of TP, total soluble phosphorus, and soluble reactive phosphorus (SRP). A summary of the sampling locations and depths for each analysis is shown in Table 2-2. Raw wastewater was analyzed for TP and metals. Raw wastewater was not sampled at every sampling event shown in Table 2-1.

Table 2-2: Summary of waste stabilization pond sampling locations and depths for each analysis. Surface and bottom refer to approximate depths.

Location	Sampling Locations	Total Phosphorus and Metals Analysis Depths	Phosphorus Species Analysis Depths
Grise Fiord	4 (corners)	Surface Only	
Pond Inlet	5 (corners + middle)	Surface and Bottom	Corners - 0, 50, and 100 cm Middle – 0, 50, 100 and 250 cm
Clyde River	2 in each cell	Surface and Bottom	
Kugaaruk	5 (corners + middle)	Surface and Bottom	Surface and Bottom

Samples were stored cooled and transported by aircraft to either Dalhousie University's Clean Water Laboratory in Halifax, Nova Scotia, or Dalhousie University's Northern Water Quality Laboratory in Iqaluit, Nunavut. Samples from Kugaaruk were analyzed at Taiga Environmental Laboratory, an accredited commercial lab located in Yellowknife, Northwest Territories.

Samples were analyzed according to APHA and/or manufacturer specifications within their respective hold times.

SRP and TP were analyzed using the ascorbic acid method with Hach® TNT™ or TNTplus™ test kits. SRP samples were filtered prior to analysis using a 0.45 µm polysulfone filter (GVS Life Sciences, Rome, Italy). Metals were digested with nitric acid according to APHA and analyzed using inductively coupled plasma mass spectrometry (XSeries 2 ICPMS, Thermo Fisher Scientific, Inc., Waltham, Massachusetts).

2.3.4.2 Sediment Analysis

Sediments were collected from Kugaaruk in 2013 and Pond Inlet in 2014. Sediments were not collected in Grise Fiord and Clyde River due to logistical travel constraints. Sediments were collected in Kugaaruk using custom-built buckets. Three buckets were suspended in the water column approximately 3 m from the water surface using an anchor and buoy near the truck discharge location. The bucket had holes in the bottom with a non-woven geotextile glued over the top. This allowed for sediments to be retained by the geotextile while allowing water to flow through. The buckets were installed at the start of the treatment season and removed at the end of the treatment season. Upon removal, only one bucket was successfully retrieved. The remaining buckets either flipped or were placed in areas without considerable sedimentation. Sediments were collected in Pond Inlet in 2014 using an acrylic bacon bomb sampler (Koehler Instrument Company, Inc., Bohemia, New York). Single sediment samples were collected at the middle and end of the treatment season from four locations (inlet, outlet, center and the

southeast corner). Sediments were stored cooled and transported by aircraft to Dalhousie University's Clean Water Laboratory in Halifax, Nova Scotia and analyzed within 96 hours, with an average hold time less than 48 hours.

Sediments were analyzed for water content, TP, calcium, manganese, magnesium, iron and aluminum. Water content was analyzed according to APHA Standard Methods. Sediments were digested in 50% nitric acid at 105°C. Digestion occurred for 3-4 hours, to the point where only inert material was remaining. Digested sediment was then analyzed for metals using inductively coupled plasma mass spectrometry (XSeries 2 ICPMS, Thermo Fisher Scientific, Inc., Waltham, Massachusetts). TP was also confirmed using the ascorbic acid method with acid persulfate digestion (TNT™ or TNTplus™, Hach Company, Loveland, Colorado).

Phosphorus in the sediment was fractionated using the method described by Lukkari *et al.* (2007). The method is a further modification of the method developed by Psenner and Pucsko (1988) and modified by Jensen and Thamdrup (1993). It allows for the extraction of six phosphorus pools: loosely bound phosphorus, redox sensitive iron and manganese bound phosphorus, aluminum oxide or non-reducible iron bound phosphorus, calcium bound phosphorus and organic phosphorus. Organic phosphorus includes cellular components such as orthophosphate monoesters and diesters, phosphosaccharides, phytate, nucleic acids and phospholipids. Five different extractants were used in conjunction with filtration with 0.4 μm polycarbonate membranes (Nuclepore™, General Electric Healthcare Life Sciences, Little Chalfont, United Kingdom) to extract the pools. A summary of the extractants and their associated pools are shown in Table 2-3. Each

extractant was analyzed for TP using the ascorbic acid method with acid persulfate digestion (TNT™ or TNTplus™, Hach Company, Loveland, Colorado). No modifications were made from the procedure described by Lukkari *et al.* (2007).

Table 2-3: Summary of the extractants used and their associated pools of phosphorus, as described by Lukkari *et al.* (2007).

Extractant	Pool
0.46 M sodium chloride	Loosely bound, pore water phosphorus, organic phosphorus
0.11 M sodium dithionite solution buffered with 0.11 M sodium bicarbonate	Redox sensitive iron and manganese bound phosphorus, organic phosphorus
0.1 M sodium hydroxide	Aluminum and non-reducible iron bound phosphorus, organic phosphorus
0.5 M hydrochloric acid	Calcium bound phosphorus, organic phosphorus
1 M hydrochloric acid	Refractory organic phosphorus

2.3.5 Data Analysis

Sampling events (WSP and raw wastewater) were tested for significance using analysis of variance or a Student's t-test depending on the number of events being evaluated. All of the sampling events were independent of one another. Assumptions of normality and equal variance were tested using the Shapiro-Wilk Normality Test and the F-test, respectively. Some sampling events failed ($p<0.05$) the normality and/or equal variance test, in which case, an appropriate test was chosen based on the findings of Skovlund and Fenstad (2001). For instance, if the sampling events had unequal variances, a non-normal distribution (heavy tailed) and unequal sample sizes, then Welch's U test was performed.

In cases of non-normality, the data were always heavy tailed, as opposed to skewed. A confidence level of 95% was used for determining significance of all tests. Statistical tests were conducted using Minitab 17 statistical software or R v3.2.2 statistical programming language (R Core Team 2015).

2.4 Results and Discussion

2.4.1 Temperature and pH Conditions

A summary of the air and water temperatures and pH for Grise Fiord, Kugaaruk, Pond Inlet, and Clyde River over the course of the treatment season is shown in Table 2-4. Kugaaruk, Pond Inlet, and Clyde River experienced similar trends. Average air and water temperatures were consistently below 10°C. Minimum temperatures below 0°C were experienced at the start and end of the treatment season. The pH was consistently near neutral with a range of 6.8 to 8.0. The temperature and pH followed a similar trend for the three sites. An example of the temperature and pH patterns over a sample year (Kugaaruk, 2013) is shown in Figure 2-2.

During the site visit to Grise Fiord, a very high pH (10.8) was observed, possibly due to an algae bloom occurring in the WSP. The average temperature was also higher, because samples were only taken over a few days during the warmest period of the treatment season, as opposed to over the entire treatment season. The water temperature (14°C) was similar to the maximum temperatures at the other research sites.

Table 2-4: A summary of mean (± standard deviation), maximum and minimum air temperatures, water temperatures and pH for WSPs located in Kugaaruk, Pond Inlet and Clyde River during the treatment season (June-September).

Site		Year	Air Temperature (°C)			Water Temperature (°C)			pH		
			Average	Max	Min	Average	Max	Min	Average	Max	Min
Grise Fiord		2011				14.2 ± 1.0[1]			10.8 ± 0.1[1]		
Kugaaruk		2012	8.9 ± 4.4	24.3	-3.2	7.8 ± 0.9	10	4.1	7.6 ± 0.1	7.8	7.4
		2013	7.9 ± 5.2	24.0	-1.7	6.7 ± 3.0	12.6	0.3	7.2 ± 0.1	7.4	6.8
Pond Inlet		2012	6.8 ± 3.5	17.9	-1.5	8.4 ± 1.5	12.6	5.2	7.6 ± 0.1	7.8	7.2
		2013	6.4 ± 3.5	18.3	-2.0	7.3 ± 3.7	14.8	1.0	7.5 ± 0.1	7.7	7.1
		2014	5.9 ± 4.1	18.3	-5.6	8.5 ± 2.2	13.6	4.0	7.8 ± 0.1	8.0	7.6
Clyde River	Primary	2012	7.0 ± 3.5	22.3	-0.9				7.2[2]		
	Secondary					8.6 ± 2.8	13.3	4.0	7.4[2]		
	Primary	2013	5.2 ± 3.3	15.6	-1.4	7.9 ± 3.4	17.5	0.6	7.5 ± 0.1	7.7	7.2
	Secondary					8.1 ± 2.5	13.9	2.7	7.4 ± 0.1	7.6	7.2
	Primary	2014	6.2 ± 3.6	18.0	-1.4	7.2 ± 3.7	16.4	0.0	7.3 ± 0.2	7.6	6.9
	Secondary					7.9 ± 2.7	12.7	0.7	7.4 ± 0.1	8.0	7.3

[1] Spot samples (n=8)

[2] Spot samples (n=2)

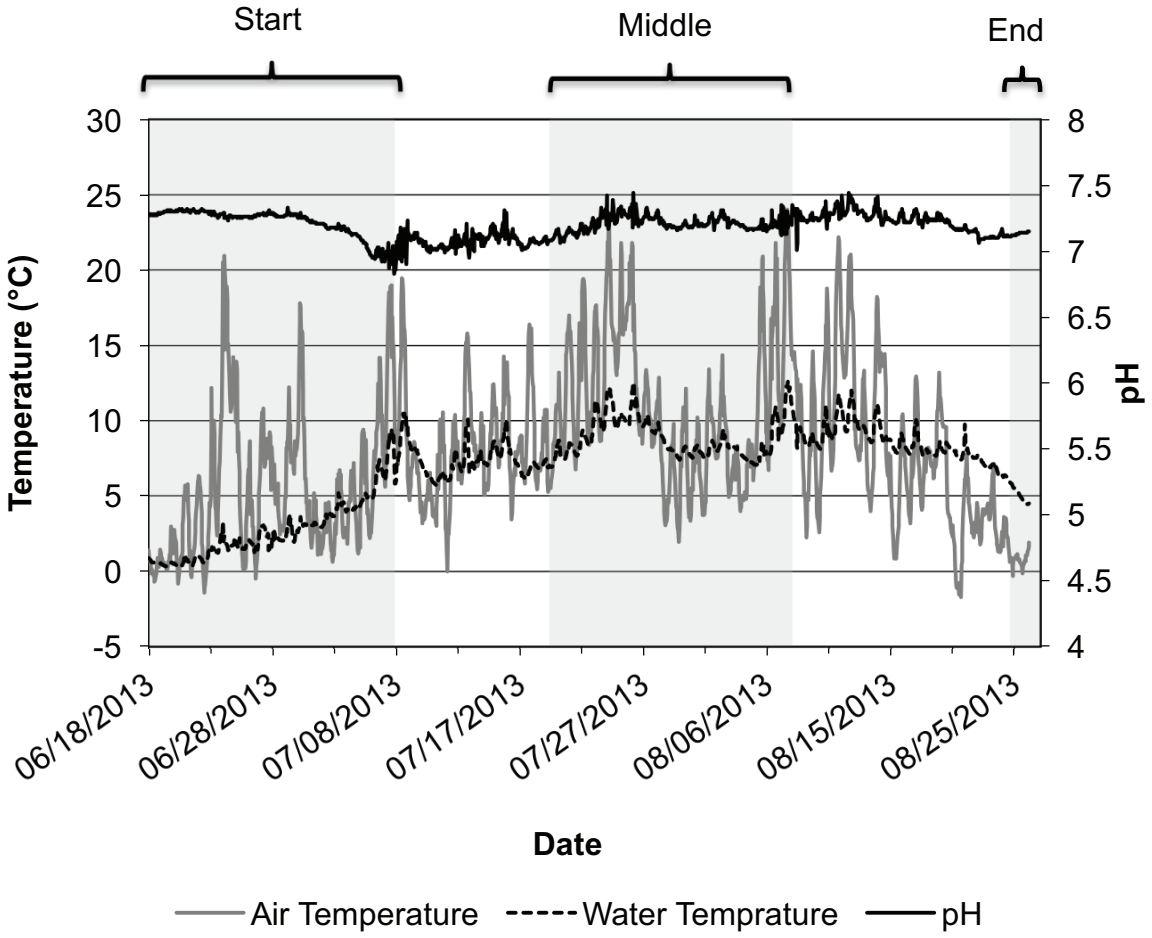
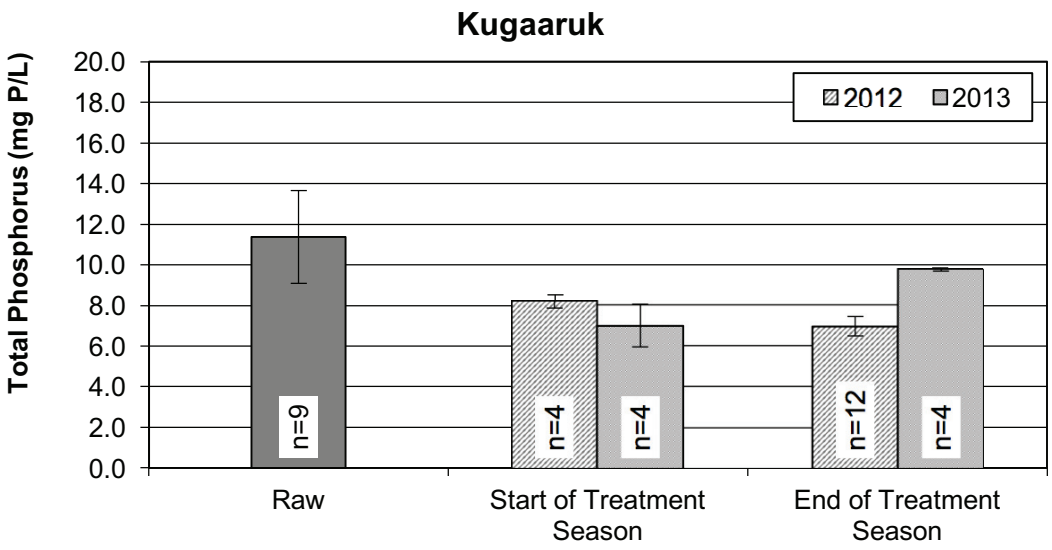


Figure 2-2: . Air temperature, water temperature, and pH measurement collected in the Kugaaruk WSP in 2013. The grey boxes refer to the different periods of the treatment season.

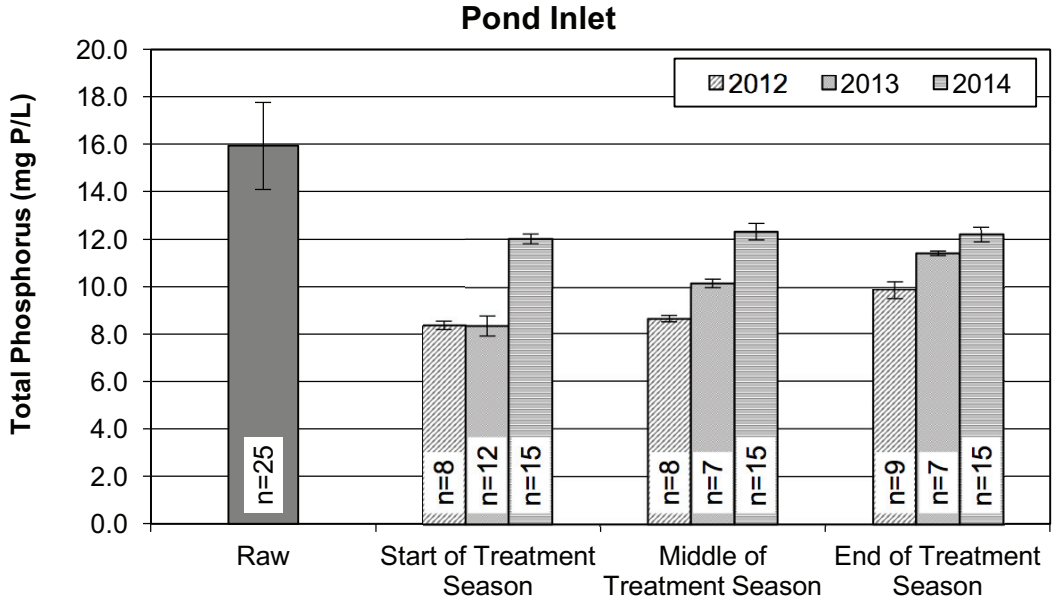
Total Phosphorus Results for Kugaaruk, Pond Inlet, Grise Fiord and Clyde River

The treatment performance for each system, with respect to TP, was highly variable. WSP effluent concentrations ranged from 3.5 to 12.2 mg P/L. A summary of the TP concentrations observed for each system is shown in Figure 2-3.

a)


Kugaaruk
Total Phosphorus (mg P/L)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
2012
2013
n=9
n=4
n=4
n=12
n=4
Raw
Start of Treatment Season
End of Treatment Season

b)


Pond Inlet
Total Phosphorus (mg P/L)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
2012
2013
2014
n=25
n=8
n=12
n=15
n=8
n=7
n=15
n=9
n=7
n=15
Raw
Start of Treatment Season
Middle of Treatment Season
End of Treatment Season

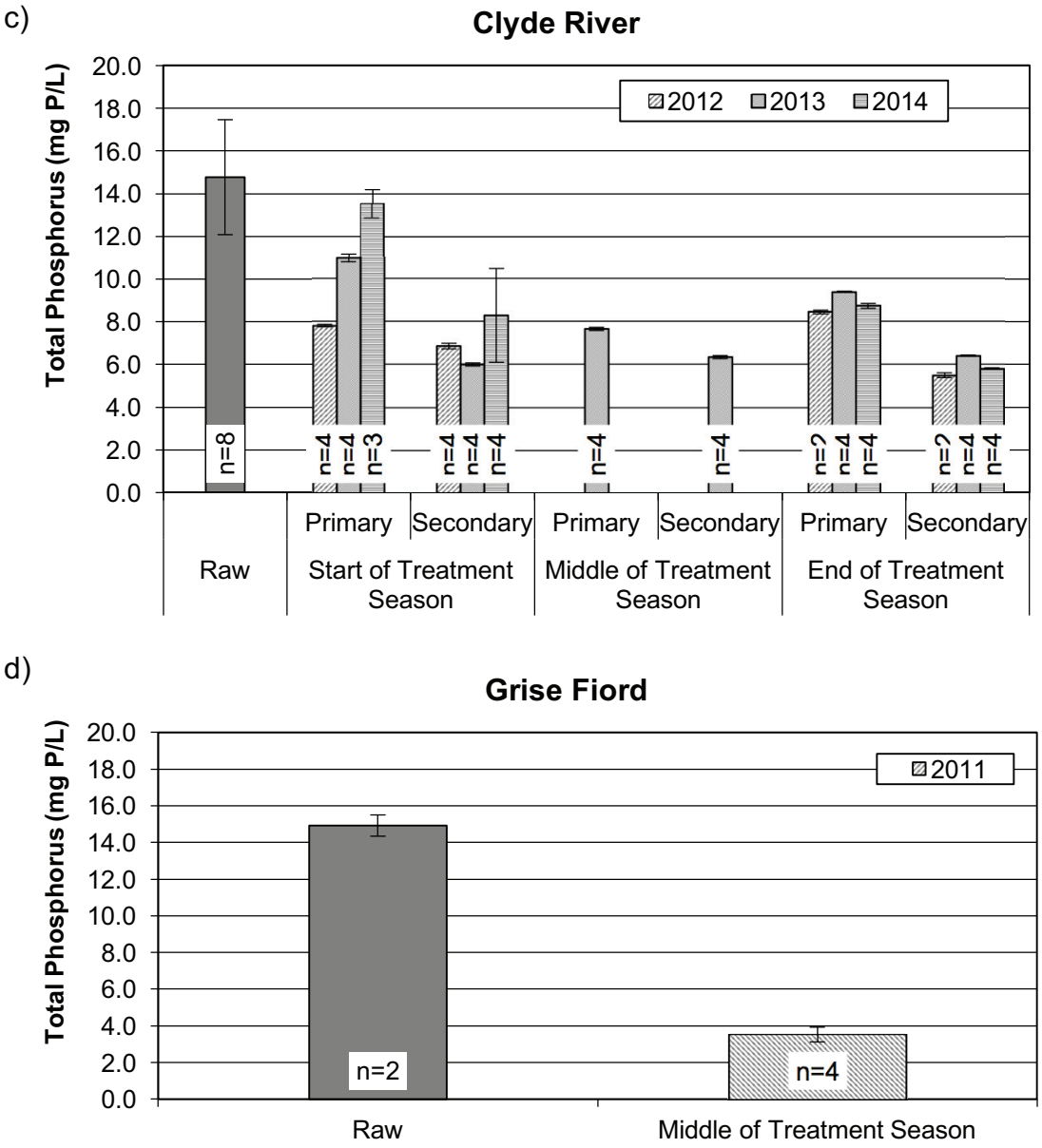


Figure 2-3: TP concentration found for raw wastewater and in WSPs during the summer treatment season at (a) Kugaaruk, (b) Pond Inlet, (c) Clyde River, and (d) Grise Fiord. Data are means with 95% confidence intervals and the number of samples is shown at the bottom of each bar.

Raw wastewater concentrations were not taken consistently and are not available for each year. Since wastewater in each community is solely from domestic sources, it was

assumed that raw wastewater did not change significantly from year to year. Therefore, raw wastewater data were pooled for each site. This assumption was tested using data from Pond Inlet from 2012 (n=17) and 2013 (n=8). The mean raw wastewater concentration for each year was confirmed to not be significantly different ($p>0.05$). There was variation in the raw wastewater concentrations between each site (Figure 2-3). At 11.4 mg P/L, the lowest average concentration was observed in Kugaaruk. Pond Inlet had the highest raw wastewater concentration at 15.9 mg P/L. With the exception of Grise Fiord, a high variability was observed for the raw wastewater concentrations. The low variability in Grise Fiord is attributed to a smaller sample size compared to other sites that were sampled multiple times per year for 2-3 years. Overall, the concentrations were much higher than what is typically observed in southern Canada (below the 60^{th} parallel) where TP concentrations in raw domestic wastewater of 7 mg P/L (deemed medium strength) are typical (Tchobanoglous *et al.*, 2003). This discrepancy is due to a low per capita water usage in Nunavut. The average water consumption in the four research communities was approximately 87 L per capita per day whereas in southern Canada, the average usage is 274 L per capita per day (Environment Canada, 2011). In effect, the low water consumption concentrates wastewater constituents.

In Kugaaruk, reduction in TP was highly variable (Figure 2-3a). In 2012, TP concentrations at the start and end of the treatment season were found to be significantly different ($p<0.05$). Throughout the treatment season, TP concentrations decreased from 8.2 to 6.9 mg P/L. TP concentrations at the start and end of the treatment season were significantly different from the raw wastewater TP concentrations ($p<0.05$). In 2013, TP

concentrations were also significantly different ($p<0.05$) at the start and end of the treatment season. However, during the 2013 treatment season, TP concentrations increased from 7.0 to 9.8 mg/L. By the end of the 2013 treatment season TP concentrations were not significantly different from the raw wastewater TP concentration ($p>0.05$). The reason for the variable treatment between years is unknown. However, it could be due to several factors such as increased rainfall in 2012 or variability in decant volumes leading to longer retention times.

In Pond Inlet, some reductions in TP concentrations were seen in the WSP relative to the raw wastewater (Figure 2-3b). TP concentrations throughout the treatment season (2012-2014) were lower than the raw wastewater TP concentration ($p<0.05$). However, TP concentrations at the end of the treatment season were either higher (2012, 2013; $p<0.05$) or not significantly different (2014, $p>0.05$) from TP concentrations at the start of the treatment season. This shows that little or no additional treatment was occurring during the summer treatment season. At the end of the treatment season, TP concentrations ranged from 9.8 (2012) to 12.2 mg P/L (2014). The reason for lower TP concentrations at the start of the treatment season in 2012 and 2013 is unknown, however it could be due to several factors including increased dilution from snowmelt.

Clyde River was the only two-cell WSP studied (Figure 2-3c). TP concentrations in the primary cell at the start of each treatment season were significantly different for each year ($p<0.05$). TP concentrations ranged from 7.8 (2012) to 13.5 mg P/L (2014). In 2014, the TP concentration in the primary cell at the start of the treatment season was not significantly different from the raw wastewater TP concentration ($p>0.05$). In 2012 and

2013, TP concentrations were lower than the raw wastewater concentration ($p<0.05$). At the end of the treatment season TP concentrations in the primary cell either increased (2012: $p<0.05$) or decreased (2013, 2014: $p<0.05$) compared to TP concentrations at the start of the treatment season. In all years, TP concentrations at the end of the treatment season were lower than the raw wastewater TP concentrations ($p<0.05$). There was much less variation in TP concentrations in the primary cell at the end of the treatment season compared to the start of the treatment season, however concentrations were significantly different among years ($p<0.05$). TP concentrations ranged from 8.3 (2012) to 9.4 mg P/L (2013). Over 3 years of measurement, the primary cell was able to achieve a mean removal of 40%. TP concentrations in the secondary cell were lower than in the primary cell ($p<0.05$). However, there was minimal reduction (14%) over the treatment season. The mean difference in TP concentrations at the end of the treatment season between the primary and secondary cell was 33%. Overall, the system was able to achieve a mean removal of 60%.

Inter-seasonal comparisons could not be made in Grise Fiord (Figure 2-3d). However, at 3.5 mg P/L, the lowest WSP TP concentration was observed in Grise Fiord. When this sample was taken, an abundant population of algae was observed in the WSP. It is hypothesized that the increased removal efficiency was due to a combination of two mechanisms. The first is direct biological uptake of phosphorus by algae. The second is chemical precipitation of phosphorus with calcium facilitated by an increase in the WSP pH caused by the algae bloom. Algae blooms cause pH increases by consuming carbon dioxide during photosynthesis. A pH of 10.8 was observed in the WSP, which is ideal for

calcium phosphate formation. Moutin *et al.* (1992) found that 93% of phosphate deposits in a high-rate algal pond with natural calcium concentrations ranging from 50-150 mg/L were calcium phosphate precipitates. The system studied by Moutin *et al.* (1992) had a pH of 8.7 and was only able to achieve a phosphorus removal of ~25%. Calcium concentrations in the raw wastewater and WSP in Grise Fiord were 18.2 ± 0.5 and 21.1 ± 4.9 mg/L, respectively. Therefore, it is possible that some of the TP removal in Grise Fiord could be attributed to calcium precipitation. Understanding the exact removal mechanisms in Grise Fiord, as well as the long-term phosphorus storage in the sediment layer, requires further research.

In their current configuration, the WSPs are unable to consistently achieve high removals of phosphorus. Single-cell WSPs in Kugaaruk and Pond Inlet achieved removal efficiencies of 27% and 31%, respectively. Higher removals were seen in the Grise Fiord single-cell WSP in 2011; however, these results were likely a function of algae growth and favourable climate conditions that were not indicative of an average treatment season. It does show that if WSPs could be reconfigured to optimize algae growth, high percent removals could be achieved in a passive system. The two-cell configuration studied in Clyde River achieved removal efficiencies of 60%, performing much better than the other multi-year study sites (Kugaaruk and Pond Inlet). Ultimately, TP concentrations in the effluent from the studied WSPs were comparable to typical medium strength raw wastewaters (7 mg P/L) in southern Canada.

2.4.2 Soluble Reactive Phosphorus Results for Kugaaruk and Pond Inlet

Phosphorus species were analyzed in Kugaaruk in 2013 and Pond Inlet in 2014. Analyses revealed that SRP was the predominant aqueous species. Results from each site showed similar trends. SRP represented 81% of TP in both Kugaaruk and Pond Inlet. A summary of the TP and SRP concentrations in Kugaaruk and Pond Inlet are shown in Table 2-5.

Table 2-5: Total and soluble reactive phosphorus concentrations at various points in the treatment systems in Kugaaruk (2013) and Pond Inlet (2014). Data shown are means ± standard deviations.

Location	Sample Information	Total Phosphorus (mg P/L)	Soluble Reactive Phosphorus (mg P/L)
Kugaaruk	Start of the Treatment Season	7.0 ± 0.1	5.6 ± 0.1
	End of the Treatment Season	9.8 ± 0.1	8.1 ± 0.2
Pond Inlet	Start of the Treatment Season	12.0 ± 0.1	10.0 ± 0.2
	Middle of the Treatment Season	12.2 ± 0.1	10.2 ± 0.4
	End of the Treatment Season	12.2 ± 0.2	9.7 ± 0.2

Similar to TP concentrations, SRP concentrations in Kugaaruk increased from 5.6 to 8.1 mg P/L ($p<0.05$) over the course of the treatment season. SRP concentrations in Pond Inlet stayed constant; no significant difference ($p>0.05$) between concentrations at the start, middle, and end of the treatment season was observed.

The large concentration of SRP could be advantageous, as it represents the fraction of TP that could be removed with additional treatment such as coagulation or WSP reconfiguration to optimize algae growth. If WSPs could be optimized for SRP removal, it is expected that effluent TP concentrations equal to the soluble unreactive phosphorus

(< 2 mg P/L) could be achieved. Considerations would have to be made to ensure that algae can be removed from the system through settling, filtration, or another process. The re-release of phosphorus from settled algae under the extreme conditions experienced in these WSPs requires further investigation.

2.4.3 Sediment Analysis Results for Kugaaruk and Pond Inlet

The results of the sediment analysis from Kugaaruk and Pond Inlet showed that sediment phosphorus concentrations varied spatially and temporally. Sediments were not collected in Clyde River and Grise Fiord due to logistical travel constraints. A summary of the phosphorus concentration for each fraction is shown in Table 2-6.

Table 2-6: Phosphorus concentrations (expressed in mg P/g dry sediment) for various fractions (as described in Lukkari *et al.* (2007)) from sediments collected from Kugaaruk (inlet, end of season, 2013, collected using custom buckets) and Pond Inlet (4 locations, middle and end of the treatment season, 2014, collected using acrylic bacon bomb sampler).

Phosphorus Fraction	Kugaaruk	Pond Inlet							
	Inlet	SW Corner (Inlet)		NE Corner (Outlet)		SE Corner		Center	
		Middle	End	Middle	End	Middle	End	Middle	End
Pore water and loosely bound	0.30	0.24	0.17	0.16	0.05	0.10	0.19	0.33	0.36
Redox sensitive Fe and Mn oxide bound	0.60	0.69	0.68	0.76	0.14	0.35	0.63	1.08	1.18
Bound to Al and non-reducible Fe oxides	0.50	1.10	2.14	2.44	0.30	0.70	2.16	1.83	3.25
Calcium bound	0.05	0.13	0.40	0.02	0.09	0.01	0.14	0.16	0.28
Organic	1.40	1.72	1.65	2.43	0.72	1.16	3.28	2.27	3.91
Total	2.84	3.87	5.04	5.82	1.30	2.33	6.39	5.67	8.98
Mean (± SD)		Middle: 4.42 ± 1.65 mg P/g dry sediment End: 5.43 ± 3.20 mg P/g dry sediment							

Only one sediment sample was taken from Kugaaruk (Table 2-6). The sample had a phosphorus concentration of 2.84 mg P/g dry sediment. Organic phosphorus (49%) was the largest fraction followed by phosphorus bound to redox sensitive iron and manganese oxides (21%) and phosphorus bound to aluminum and non-reducible iron oxides (18%). Calcium bound phosphorus was the smallest fraction (2%).

Samples were taken from the Pond Inlet WSP at four points: the inlet, outlet, center and the southeast corner (Table 2-6). The center of the WSP had the highest sediment phosphorus concentration at both the mid-point and end of the treatment season. At the inlet, center and southeast corner, sediment phosphorus concentrations increased through the treatment season. However, at the outlet, concentrations decreased. This difference is likely due to sample variation. Fractionation results followed a similar trend to Kugaaruk. The largest fraction was consistently either organic phosphorus or phosphorus bound to aluminum and non-reducible iron oxides. Phosphorus bound to redox sensitive iron and manganese oxides was the third largest fraction in all of the samples. An analysis in Pond Inlet of the metal concentrations in raw wastewater found approximately 8 mg/L of calcium, 1.0 mg/L of iron, 2.8 mg/L of aluminum and 0.05 mg/L of manganese. Drinking water in Pond Inlet comes from a water reservoir that fills from either snowmelt water or water pumped from a nearby stream. Surface water in Nunavut is generally considered to be pristine and therefore disinfection is the only treatment provided. Metals present in the wastewater would have to come from this low impacted water source or through dissolution of premise plumbing.

Sediments from the Pond Inlet WSP had a pooled mean of 4.42 ± 1.65 and 5.43 ± 3.20 mg P/g dry sediment at the middle and end of the treatment season, respectively. These concentrations were not significantly different from one another ($p>0.05$).

The sediment sample from Kugaaruk had a relatively low phosphorus concentration compared to samples from Pond Inlet (Table 2-6). The Kugaaruk sample was collected from within the water column, as opposed to from the bottom sludge layer where the Pond Inlet sample was taken. Therefore, the Kugaaruk sample represents sediments deposited in one treatment season, as opposed to long-term sediment consolidation and storage.

At both sites, calcium bound phosphorus made up a relatively small portion of the sediment phosphorus. Therefore, is it expected that while there are calcium ions present, the WSP pH is limiting calcium phosphate formation. The average pH observed in Pond Inlet and Kugaaruk WSP was 7.4 ± 0.2 with a maximum value of 8.0. Diaz *et al.* (1994) found that phosphorus solubility was not affected at a pH less than 9 when calcium concentrations were below 50 mg/L.

The most promising removal mechanisms were biological and precipitation/adsorption with iron (redox sensitive or non-reducible) or aluminum. These mechanisms were able to occur in passive WSPs and most importantly, the particulate was able to persist in the sediment layer resulting in long-term storage. Three possible options for system modification in order to optimize these removal mechanisms are as follows: (1) the WSP could be reconfigured to increase surface area to help support algae growth, (2) iron or aluminum could be added to increase chemical precipitation, or (3) an integrated

approach combining both strategies could be used. While these strategies could increase phosphorus removal, they are not without disadvantages. WSP reconfiguration would have significant capital costs and land requirements. Chemical addition would also have associated capital and operating costs. It would also increase sludge production, therefore requiring sludge dredging and disposal.

2.5 Conclusions

Research in these Arctic communities provided a unique set of challenging conditions including weather and facility constraints. However, after 4 years of field monitoring, the following conclusions were made:

- TP removal in Kugaaruk, Pond Inlet and Clyde River ranged from 14 to 63%. The average effluent concentrations for Kugaaruk, Pond Inlet and Clyde River were 8.4, 11.2 and 5.9 mg P/L, respectively. Even at the highest percent removal (63%), effluent concentrations were high relative to concentrations seen in southern Canada. High effluent concentrations are partly due to elevated raw wastewater concentrations attributed to low per capita water usage.
- Grise Fiord exhibited much higher removal (76%); however, this was only observed once and was not representative of the rest of the data gathered. Therefore, the results in Grise Fiord represent an opportunity for further research rather than an expected result for other systems.
- SRP represented 81% of the total phosphorus present in the Kugaaruk and Pond Inlet WSP. Therefore, it is expected that high phosphorus removal could be

achieved if a new treatment design, such as coagulation addition or WSP reconfiguration, were implemented in order to target this fraction.

- Sediment analysis in Kugaaruk and Pond Inlet show that organic phosphorus and phosphorus bound to iron or aluminum represented the highest fraction of sediment phosphorus. These two fractions represented between 88 and 97% by dry mass. Therefore, these removal mechanisms should be further examined in order to determine if they could be optimized for increased removal.

3 Microalgae Growth and Phosphorus Uptake in Wastewater Under Simulated Cold Region Conditions

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3.1 Abstract

Facultative waste stabilization ponds (WSP) are a common form of wastewater treatment in cold regions. However, cold region WSPs have been found to have highly variable and inconsistent microalgae growth and phosphorus removal. This study investigated whether facultative WSPs can be used to provide biological phosphorus removal in cold regions by evaluating maximum specific growth rates and phosphorus removal pathways under simulated cold region summer (ice-free) conditions. A factorial experiment was conducted in order to determine the main effects and interactions of temperature (10, 15°C), photosynthetically active radiation (PAR) (100, 150 μmol/m^2/s) and initial phosphorus concentration (7.5, 15 mg P/L) on microalgae growth and phosphorus uptake. Maximum specific growth rates varied from 0.029 to 0.058/h. PAR and temperature had a statistically significant negative and positive effect, respectively, on growth rates. Initial phosphorus concentration had no statistical effect on growth rates under the studied ranges. Growth rates were similar to those observed at temperate climates. Luxury uptake

was a significant phosphorus removal mechanism as it accounted for 53 ± 8% (g P/g P) of biomass phosphorus. Biomass phosphorus concentrations were positively affected by PAR and initial phosphorus concentration while temperature had no effect. A crossover interaction between temperature and initial phosphorus concentration was found to have a negative effect on biomass phosphorus concentration. Under cold region conditions biomass phosphorus concentrations were 45% greater than under warm climate conditions. Ultimately, it is expected that climate should not hinder microalgae production in cold region WSPs during the summer months when temperatures exceed 10°C and the surface is ice-free. Cold region conditions appear to aid in phosphorus removal by increasing biomass phosphorus concentrations.

3.2 Introduction

Facultative WSPs are a common wastewater treatment strategy for communities in cold regions. This is due to their low operating cost, minimal required technical proficiency, and low energy and chemical demand. WSPs can also be designed for long retention periods (~6-12 months) with intermittent discharge, which are necessary in communities that cannot have continuous discharge due to extended ice cover during the winter (Heaven *et al.*, 2003). Facultative WSPs rely on microalgae to provide aeration. Microalgae are directly or indirectly related to the removal of a number of parameters including organics, nitrogen and phosphorus.

Recent research conducted in the Canadian territory of Nunavut showed that there was significant variance in the design, operation and treatment performance of four WSPs (Ragush *et al.*, 2015). WSPs were effective at removing suspended solids but were not capable of achieving secondary wastewater treatment objectives for biochemical oxygen demand. Three of the four WSPs studied were operating anaerobically, with little microalgae production. The lack of microalgae production was found to be associated with design (depth), operational parameters (organic loading rate) and climate. Ultimately, Ragush *et al.* (2015) concluded that it is possible to operate a WSP facultatively in cold regions, if different design guidelines were implemented.

The removal of phosphorus is of particular interest due to its role in receiving water eutrophication. Localized nutrient enrichment has been documented in cold regions where WSP effluent enters marine receiving water environments (Krumhansl *et al.,*

2015). Limited research has been conducted on phosphorus concentrations and removal rates in cold region treatment systems. Total phosphorus concentrations in WSPs in cold regions have been shown to exceed 7 mg P/L, while lower effluent concentrations (3.5 mg P/L) have been observed during a microalgae bloom (Schmidt *et al.*, 2016a). In Scandinavian countries, use of WSPs has been limited since the 1960s. Precipitation ponds, or fellingsdams, are a common treatment option for small, remote communities. However, due to economic and environmental disadvantages associated with chemical precipitants there has been a renewed interest in biological treatment during ice-free periods (Ødegaard *et al.,* 1987; Hanaeus *et al.,* 2010). Pilot scale high-rate algal ponds evaluated in a sub-arctic climate in Sweden achieved phosphorus removal of approximately 20% while daily mean temperatures were below 10°C (Grönlund *et al.,* 2010).

Phosphorus uptake by microalgae occurs through two mechanisms. In the first mechanism, phosphorus is assimilated into the microalgae biomass through the construction of organic cellular components such as phospholipids. The second microalgae mechanism is referred to as luxury uptake. Luxury uptake occurs when microalgae take up and store excess phosphorus as inorganic polyphosphate granules. Polyphosphate granules can be acid soluble or insoluble. Acid soluble polyphosphate is associated with metabolism while acid insoluble polyphosphate is considered to be a storage product for when external phosphorus is limiting (Miyachi *et al.*, 1964). Limited research has been conducted on luxury uptake in wastewater systems, however its potential has been documented. For example, Powell *et al.* (2008) found that by

manipulating temperature (15 to 25°C) and light intensity (60 to 150 μmol/m^2/s) biomass phosphorus percentage can be increased from 0.4 to 3.2%. Previous studies have shown that the critical growth level for microalgae is 1% phosphorus (Borchardt and Azad, 1968). The difference from this critical value represents the potential increase in removal performance due to luxury uptake. The biomass phosphorus concentrations shown by Powell *et al.* (2008) below the critical level may be related to the composition of the microalgae community studied. The microalgae community studied by Powell *et al.* (2008) was dominated by *Scenedesmus* spp., a genus of microalgae previously found to have a relatively low minimum phosphorus quota (Gotham and Rhee, 1981).

Light, temperature and external phosphorus concentration have been shown to affect biomass phosphorus concentrations (Brown and Shilton, 2014). Light has been shown to have negative (Hessen *et al.,* 2002; Powell *et al.*, 2008; Sterner *et al.*, 1997) or no (Frost and Elser, 2002) effect on biomass phosphorus concentrations. Based on previous studies, light effects depend on other interactions such as external phosphorus concentration and stage of growth. For instance, under low external phosphorus concentrations, as light increases, phosphorus becomes limiting and cells become carbon rich (Sterner *et al.*, 1997). While it is not expected that this will occur in wastewater systems, as nutrient availability is usually high, previous research has suggested a negative effect may still occur (Powell *et al.*, 2008). It has also been hypothesized that higher light intensities lead to higher growth rates and quicker consumption of stored polyphosphates leading to lower biomass phosphorus concentrations (Powell *et al.*, 2009). Temperature has been shown to have a positive effect on biomass phosphorus concentrations, however previous

studies were conducted outside the range experienced in cold regions (Powell *et al.*, 2008). External phosphorus concentrations have been shown to have a positive (Frost and Elser, 2002; Hessen *et al.,* 2002) or no (Powell *et al.*, 2008) effect on biomass phosphorus concentrations. While current research in this area demonstrated the potential for biological phosphorus removal in WSPs, the ranges of tested conditions for temperature and external phosphorus concentration were outside the typical ranges experienced by communities in cold regions (Ragush *et al.*, 2015; Schmidt *et al.*, 2016a). Therefore, exact effects and interactions need to be experimentally determined in order to effectively create predictive tools to determine system reliability.

Current studies have shown that microalgae growth and phosphorus removal in cold region wastewater systems are inconsistent and not well understood (Ragush *et al.*, 2015; Schmidt *et al.*, 2016a). Therefore, the objective of this study is to determine microalgae growth rates and phosphorus uptake under simulated cold regions conditions. A lab scale factorial experiment was used to evaluate multiple conditions and their associated interactions. The conditions studied were temperature, photosynthetically active radiation (PAR) and phosphorus concentration. Luxury uptake was quantified in order to determine predominate removal mechanisms. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* were used, as they were previously identified as two prominent microalgae species in facultative WSPs operated in cold climates (unpublished data, identified in a WSP in Pond Inlet, Nunavut).

3.3 Materials and Methods

3.3.1 Algae Cultivation

Chlorella vulgaris and *Chlamydomonas reinhardtii* strains were obtained from the National Research Council of Canada. Strains were cultivated in 250-mL erlenmeyer flasks under constant illumination using a modified Bold 3N medium developed by UTEX. These cultures were used to seed a 10 L chemostat receiving simulated raw wastewater as growth medium. A chemostat was used in order to maintain a consistent inoculant for experiments. The simulated wastewater recipe is shown in Table 3-1. The recipe was adapted from the one described by Davis and Wilcomb (1967) in order to match typical raw wastewater composition in Nunavut (unpublished data). No organic carbon was added in order to minimize bacterial growth. Sodium EDTA was added as a chelating agent to prevent metal precipitation. The chemostat was under constant illumination using fluorescent lights resulting in a PAR of approximately 150 $\mu mol/m^2/s$ on the immediate surface.

Table 3-1: Simulated wastewater recipe for microalgae cultivation and growth experiments.

Compound	Concentration (mg/L)
K_2HPO_4	84[a], 42[b]
$MgCl_2$	45
NH_4Cl	350
$CaCl_2•2H_20$	38
$NaHCO_3$	47
$C_{10}H_{14}N_2Na_2O_8•2H_2O$ (Na-EDTA)	280
Trace Metals Solution	1 mL Stock/L
Trace Metals Solution (x1000 Stock)	
$MnCl_2•4H_2O$	300
$AlCl_3•6H_2O$	1700
$ZnSO_4$	200
$Na_2MoO_4•2H_2O$	24
$CoCl_2•6H_2O$	12
$CuSO_4$	20
$FeSO_4•7H_20$	3000
$C_{10}H_{14}N_2Na_2O_8•2H_2O$ (Na-EDTA)	5000

[a] Concentration for cultivation and high phosphorus condition

[b] Low phosphorus condition

3.3.2 Experimental Approach

Temperature, initial phosphorus concentration and PAR were tested to determine their influence on microalgae growth rates and phosphorus uptake. A summary of the levels used for each factor is shown in Table 3-2.

Table 3-2: The tested levels/concentrations for each experimental factor.

Factor	High	Low
Temperature	15°C	10°C
Initial Phosphorus Concentration	15 mg P/L	7.5 mg P/L
Photosynthetically Active Radiation	150 μmol/m^2/s	100 μmol/m^2/s

PAR and temperature levels were representative of cold region summers when WSPs are ice free and biological activity may occur. PAR levels were based on monitoring conducted from 2012-2014 at WSPs located in Pond Inlet (72°41'57" N, 77°57'33" W) and Kugaaruk (68°31'59" N, 89°49'36" W), Nunavut, Canada (Chapter 2). Temperature values were based on typical maximum (~15°C) and mean (10°C) values found by Schmidt *et al.* (2016a) in a study of four WSPs in Nunavut from 2011 to 2014. Schmidt *et al.* (2016a) also reported lower temperatures (< 5°C) for the WSPs. However, growth did not occur under the high PAR and high phosphorus condition with temperature of 5°C; accordingly, further laboratory testing was not conducted at temperatures of 5°C or lower.

The high phosphorus concentration was based on the maximum concentrations found by Schmidt *et al.* (2016a). The low phosphorus condition mimics primary cell effluent in a multi-celled WSP scenario, as suggested by Ragush *et al.* (2015). Under this scenario, a deep anaerobic primary cell provides approximately 50% phosphorus removal and feeds a shallower secondary cell capable of biological activity. The estimation of 50% removal from a deep primary cell is based on the findings of Schmidt *et al.* (2016a).

Synthetic wastewater was prepared in a 4 litre bottle according to the recipe shown in Table 3-1 using tap water further processed by reverse osmosis. Organic carbon was not fed into the system to minimize bacterial growth and sodium EDTA was used to prevent metal precipitation to allow for accurate quantification of biological activity. The solution was adjusted to a starting pH of approximately 7.3 using 1M NaOH. Microalgae from the chemostat was used to inoculate the solution. Water was taken from the cultivation chemostat and centrifuged at 3000 rpm for 15 minutes until a microalgal pellet formed. The supernatant was poured off and the microalgal pellet was added to the solution bottle. Microalgae was added to yield a target initial optical density of 0.010. Synthetic wastewater was added to twenty autoclaved 250-mL Erlenmeyer flasks capped with cotton plugs. Flasks were placed on a shaker table (150 rpm) under programmable LED lights (Orphek, Fairmount, Indiana). Cotton plugs were used to allow for air exchange while limiting contamination. PAR was measured at the water surface using a handheld sensor (Apogee, Logan, Utah).

Sampling occurred every 1 to 5 days, with an average time between samples of 35 hours. Sampling occurred for an average of 18 days. At each sampling event, one flask was sacrificed and analyzed. The sampling period varied in order to ensure that adequate samples were taken during the initial lag, growth and stationary phases. Flasks were weighed at the start of the experiment and at each sampling event. Any evaporation was accounted for by adding deionized water. Samples were analyzed for pH, total and dissolved phosphorus, optical density (λ=680 nm) and total suspended solids (TSS). Total and dissolved metals (calcium, magnesium, manganese, iron and aluminum) were

periodically measured to ensure metal precipitation was not occurring. Dissolved samples were obtained after filtration with 0.45 μm polysulfone filter membrane (GVS Life Sciences, Rome, Italy). The wavelength for optical density was determined experimentally by conducting wavelength scans (190-800 nm, 1 nm increments) on samples from the chemostat using a spectrophotometer (Hach Company, Loveland, Colorado).

All samples, unless otherwise stated, were measured according to Standard Methods (APHA, 2012). Total and dissolved phosphorus was measured using the ascorbic acid method with acid persulfate digestion (TNTplus™, Hach Company, Loveland, Colorado). pH was measured using a benchtop meter (Thermo Scientific™ Orion ™ Star™, Waltham, Massachusetts) and associated probe. Metals samples were digested with concentrated nitric acid and measured using inductively coupled plasma mass spectrometry (XSeries 2 ICPMS, Thermo Fisher Scientific, Inc., Waltham, Massachusetts). Optical density was measured using a spectrophotometer (Hach Company, Loveland, Colorado). TSS analysis was performed using glass fiber filters (GE Whatman, Little Chalfont, United Kingdom) according to Standard Methods.

During the exponential growth stage, microalgae samples were also analyzed for polyphosphate in order to quantify and confirm luxury uptake mechanisms. The method used to extract polyphosphate was previously described by Eixler *et al.* (2005). In short, samples were centrifuged at 3000 rpm for 15 minutes in 50-mL centrifuge tubes until a microalgal pellet formed. The supernatant was then replaced with deionized water and the microalgal pellet was resuspended. Samples were then autoclaved at 100°C for 20

minutes to rupture the microalgae cell wall while keeping the polyphosphate granules intact. After the samples cooled, they were filtered with 1.5-µm glass fiber filters (GE Whatman, Little Chalfont, United Kingdom) to remove the microalgae biomass. The filtrate was analyzed for soluble reactive and total phosphorus. The difference between these two concentrations represented the polyphosphate concentration.

3.3.3 Growth Model

Microalgae growth was modeled using a first order differential equation developed by Baranyi & Roberts (1994). The model, originally designed to predict bacterial growth in food, has also been found to adequately describe microalgae growth (Mohamed *et al.*, 2014; Tevatia *et al.*, 2012). The model describes microalgae growth as:

$$\frac{dX}{dt} = \mu_{max}\alpha(t)k(t)X(t) \qquad \text{Eq. 3-1}$$

where

X is the microalgae biomass concentration at time t

μ_{max} is the maximum specific growth rate

$\alpha(t)$ is an adjustment function (Eq. 3-2)

$k(t)$ is an inhibition function (Eq. 3-3)

The adjustment function accounts for the "bottle neck" of substances required for growth as cells acclimatize to a new environment. The effect the bottle neck substance has on

growth is described by Michaelis-Menten kinetics. The inhibition function governs the end-of-growth inhibition allowing for a transition from the growth phase to the stationary phase.

The mathematical expressions for the adjustment (Eq. 3-2) and inhibition functions (Eq. 3-3), as expressed by Perni *et al.* (2005), are:

$$\alpha(t) = \frac{e^{-h_0}}{e^{-\mu_{max}t} + e^{-h_0} - e^{-\mu_{max}t-h_0}} \quad \text{Eq. 3-2}$$

$$k(t) = 1 - \frac{X}{X_{max}} \quad \text{Eq. 3-3}$$

where

h_0 is the dimensionless Baranyi-Roberts model parameter

X_{max} is the maximum microalgae biomass concentration.

Model fits were generated using non-linear regression in ComBase DMFit web edition (ComBase, 2015). Optical density was used as the measure for microalgae biomass (X). This was due to limitations measuring low TSS concentrations in a 200 mL sample volume during the initial lag phase.

3.3.4 Statistics

For growth rates, main effects were tested using an additive analysis of variance. An additive model was required as there was only one growth rate calculated per

combination of factors. The assumption of normality was confirmed using the Shapiro-Wilk test. The assumption of equal variance was confirmed for each factor using the F-test.

For biomass phosphorus concentrations, main effects and interaction were tested using an analysis of variance. The assumption of normality was tested using the Shapiro-Wilk test. Biomass phosphorus concentrations required log-transformation to confirm the assumption of normality. The assumption of equal variance was confirmed using the Bartlett's test.

Statistical tests were conducted using R v3.2.2 statistical programming language (R Core Team 2015).

3.4 Results and Discussion

3.4.1 Microalgae Growth Rates

The Baranyi and Roberts (1994) model was used to determine maximum specific growth rates. A summary of the maximum specific growth rates for each experimental scenario is shown in Table 3-3. Maximum specific rates varied from 0.029 to 0.058/h. PAR had a statistically significant negative effect (photoinhibition) on growth rates ($p<0.05$). Photoinhibition in microalgae has been shown to vary depending on the species and temperature (Dauta *et al.*, 1990, Talbot *et al.*, 1991). Dauta *et al.* (1990) grew four microalgae species (*Chlorella vulgaris*, *Fragilaria crotonensis*, *Staurastrum pingue*, *Synechocystis minima*) under a range of temperatures (10-35°C) and PAR (5-800 $\mu mol/m^2/s$). Typically, optimal PAR increased with temperature until an optimal

temperature was reached, optimal PAR then remained constant or decreased with increasing temperature. The optimal PAR found by Dauta *et al.* (1990) for *Chlorella vulgaris* (one of the species used in this study) was less than 100 µmol/m^2/s at both temperatures tested in this study (10, 15°C). This confirms that photoinhibition likely occurred.

As expected, temperature had a positive effect on growth rates; however, the level of significance was less ($p<0.10$). Total phosphorus concentration had no statistically significant effect on growth rates ($p>0.10$). Therefore, at both levels phosphorus was not the limiting nutrient/factor.

Table 3-3: The maximum specific growth rate and associated 95% confidence interval of microalgae in simulated wastewater grown under simulated cold region summer conditions.

PAR (µmol/m^2/s)	Temperature (°C)	Total Phosphorus (mg P/L)	μ_{max} (1/h)
150	15	15	0.044 ± 0.008
		7.5	0.032 ± 0.006
	10	15	0.029 ± 0.005
		7.5	0.032 ± 0.009
100	15	15	0.049 ± 0.007
		7.5	0.058 ± 0.011
	10	15	0.039 ± 0.017
		7.5	0.042 ± 0.017

An example of the model fit is shown in Figure 3-1. The example represents the high PAR (150 µmol/m^2/s), temperature (15°C) and initial phosphorus concentration (15 mg P/L) scenario. Linear regression of the predicted and observed data for each experimental scenario yielded R-squared values ranging from 0.96 to 0.99.

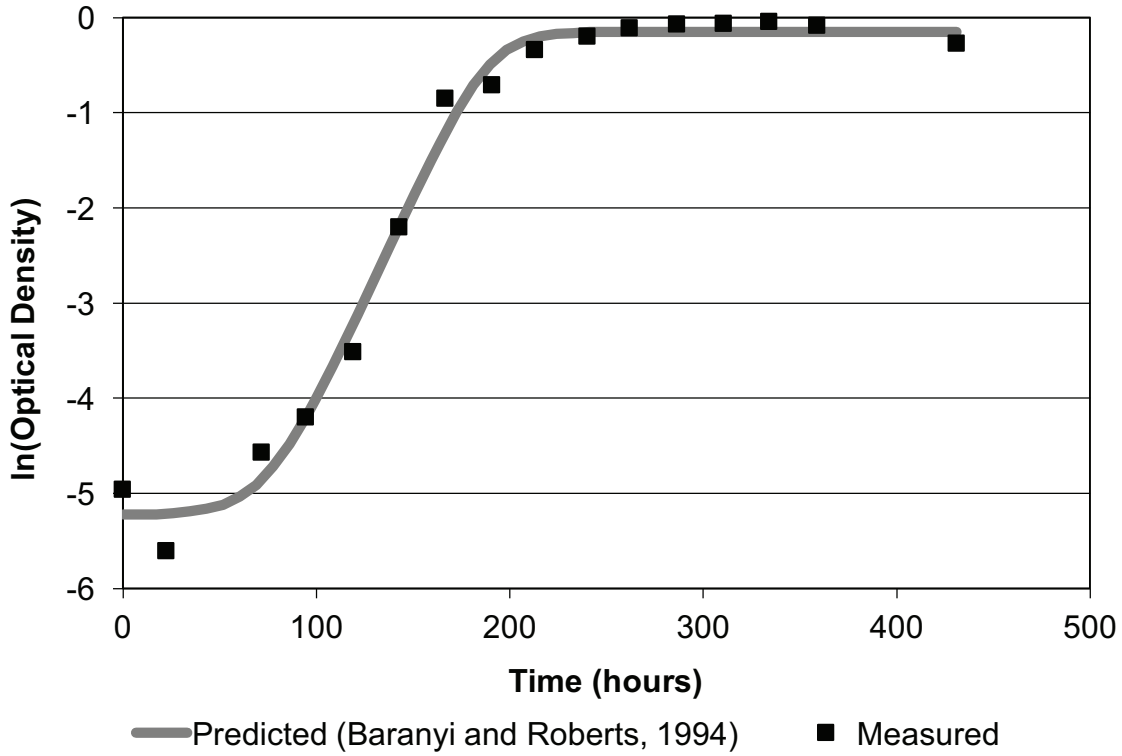

Figure 3-1: Optical density measurements and Baranyi and Roberts (1994) model predicted values for microalgae grown in batch conditions in simulated wastewater (total phosphorus – 15 mg P/L) under simulated cold region summer conditions (Temperature – 15°C, Photosynthetically Active Radiation – 150 µmol/m^2/s).

Growth rates determined by this study were generally within the range of rates calculated by Grönlund *et al.* (2010) in a batch open-air high-rate algal pond in Sweden (0.007 to 0.042/h). The lower range of growth rates was found during colder temperatures in April and May. Similarly, growth rates found during this study were also equal to or greater than those measured at temperatures characteristic of warmer climates. Wang *et al.* (2010) cultivated microalgae (*Chlorella* sp.) in wastewater from several points in a municipal activated sludge treatment plant. Cultivation occurred at 25°C with a PAR of

200 μmol/m^2/s. Mean specific growth rates ranged from 0.017 to 0.040/h. Similar mean specific rates (0.008-0.017/h) were found for two microalgae strains (*Scenedesmus obliquus* and *Chlorella vulgaris*) grown in simulated and domestic wastewater at 25°C with a PAR of 136 μmol/m^2/s (Ruiz-Marin *et al.,* 2010). Therefore, it is expected that microalgae production will not be hindered in cold region WSPs during the summer months when water temperature exceed 10°C. It is worth noting that this study was conducted under idealized laboratory conditions (ie. constant light, no background TSS, etc.). It is expected that growth rates found in this study will over-estimate growth rates found in full-scale wastewater treatment systems.

3.4.2 Luxury Uptake and Biomass Phosphorus

A summary of the biomass polyphosphate and organic phosphorus concentration for each test scenario is shown in Table 3-4. The mean biomass organic phosphorus was 1.1% (g P/g dry biomass). This was consistent with a previous estimate that found that the critical growth level for microalgae was 1% phosphorus (Borchardt and Azad, 1968). One of the trials (PAR-150 μmol/m^2/s, Temperature-10°C, Total Phosphorus-15 mg P/L) resulted in an elevated biomass organic phosphorus concentration (2%). The reasoning for the elevated concentration is unclear, but it is not representative of the rest of the dataset. Biomass polyphosphate, which represents luxury uptake, ranged from 0.9 to 2.3%. Therefore, the accumulation of polyphosphate is a significant removal mechanism as it represented 53 ± 8% of microalgae biomass phosphorus. Similar polyphosphate concentrations have been found at temperate climates. Powell *et al.* (2011b) found the

phosphorus fraction of sludge from three WSPs in New Zealand consisted of 33 to 73% polyphosphate.

Table 3-4: Biomass polyphosphate and organic phosphorus percentages during maximum specific growth in simulated wastewater under simulated cold region summer conditions. Samples were taken and extracted once during the growth phase.

PAR ($\mu mol/m^2/s$)	Temperature (°C)	Total Phosphorus (mg P/L)	Biomass Polyphosphate (%)	Biomass Organic Phosphorus (%)
150	15	15	1.2	1.3
		7.5	1.8	0.9
	10	15	2.3	2.0
		7.5	1.1	0.9
100	15	15	0.9	1.0
		7.5	0.7	0.9
	10	15	1.2	1.1
		7.5	0.9	0.7

A summary of mean total biomass phosphorus concentrations is shown in Figure 3-2. The mean ranged from 1.5% (100 $\mu mol/m^2/s$, 10°C, 7.5 mg P/L) to 3.3% (150 $\mu mol/m^2/s$, 10°C, 15 mg P/L). On average, microalgae biomass phosphorus concentrations were 45% greater in cold climate conditions compared to microalgae (mixed culture, *Scenedesmus* spp. dominated) grown in temperate climate conditions (Powell *et al.,* 2008). It is possible that this difference is due to microalgae community composition rather than environmental conditions. *Scenedesmus* spp. has previously been found to have a relatively low minimum phosphorus quota (Gotham and Rhee, 1981)

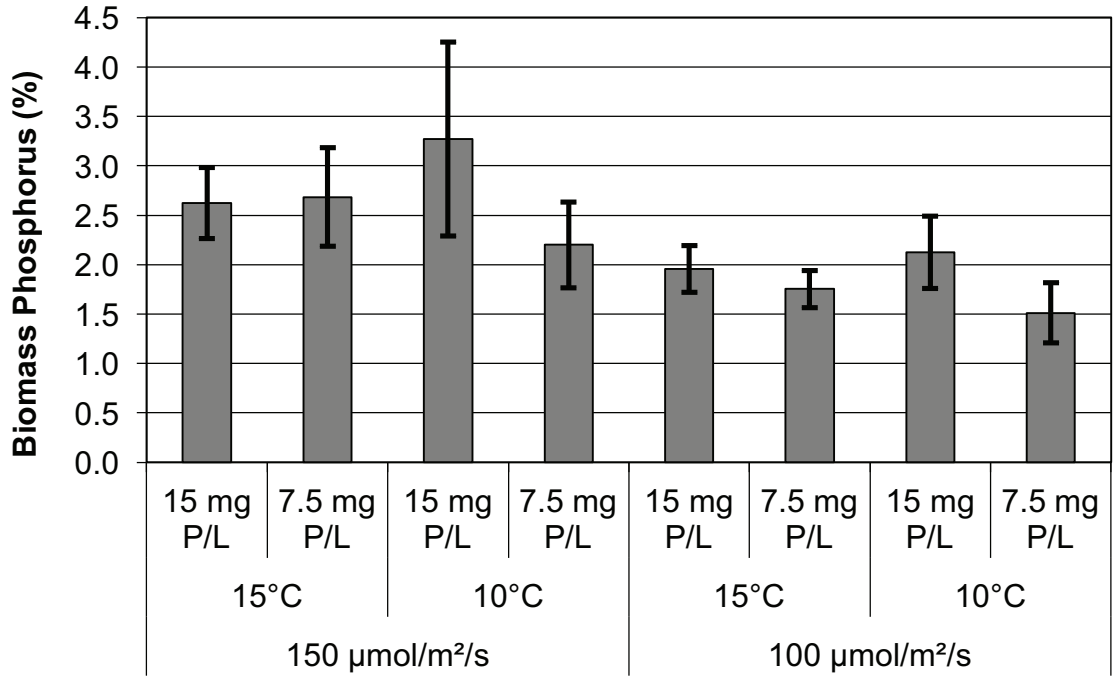

Figure 3-2: Phosphorus biomass concentration for microalgae grown in simulated wastewater under varied initial phosphorus concentration (15, 7.5 mg P/L), temperature (15, 10°C) and photosynthetically active radiation (150, 100 µmol/m^2/s). Samples were taken multiple times (n=6-10) during the growth phase. Error bars represent the 95% confidence interval.

A statistical analysis of the factors influencing biomass phosphorus concentrations is shown in Table 3-5. PAR and initial phosphorus concentration were found to have a significant positive effect on biomass phosphorus. The interaction between temperature and initial phosphorus concentration was found to have a significant negative effect. Previous experiments conducted under temperate climate conditions concluded that temperature had a positive effect, PAR had a negative effect, and initial phosphorus concentration was not significant (Powell *et al.,* 2008).

Table 3-5: Statistical analysis of microalgae total biomass phosphorus under variable photosynthetically active radiation (150, 100 µmol/m^2/s), temperature (15, 10°C) and initial phosphorus concentration (15, 7.5 mg P/L).

Factor	*p*-value	Effect
Photosynthetically Active Radiation	<0.001	+0.88
Temperature	0.607	-
Initial Phosphorus Concentration	0.004	+0.46
Photosynthetically Active Radiation x Temperature	0.717	-
Photosynthetically Active Radiation x Initial Phosphorus Concentration	0.475	-
Temperature x Initial Phosphorus Concentration	0.034	-0.37

It was hypothesized that the difference in the temperature effect is due to short-term accumulation of acid insoluble polyphosphate at higher temperatures (25°C). Previous studies (Powell *et al.*, 2009) have shown that insoluble polyphosphate had a short term peak (~3 days) at a temperature of 25°C. Beyond the initial peak, acid insoluble polyphosphate was utilized and within 4 days biomass acid insoluble polyphosphate concentrations were not significantly different from microalgae grown at 15°C. An initial peak was not observed in microalgae grown at 15°C (Powell *et al.*, 2009). Under the tested temperatures (10, 15°C) it is therefore unlikely that a peak of acid insoluble polyphosphate would occur. Temperature has been shown to not affect acid soluble polyphosphate (Powell *et al.*, 2009). A negative crossover interaction effect between temperature and initial phosphorus concentration was found. At 10°C, as the initial phosphorus concentration increases, biomass phosphorus concentrations also increase. At 15°C, as the initial phosphorus concentration increases, biomass phosphorus concentrations do not significantly change ($p>0.10$). This may be associated with the

positive effect temperature has on growth rates. It is possible that at higher growth rates additional phosphorus is utilized for growth rather than being stored as polyphosphate. The main effect of temperature is still insignificant.

The reasoning for discrepancies in PAR is more difficult to determine. Under the light:nutrient hypothesis, as described by Sterner *et al.* (1997), as PAR increases, microalgae nutrient content decreases. Previous studies have both confirmed (Hessen *et al.,* 2002) and rejected (Frost and Elser, 2002; Hill *et al.*, 2009) the hypothesis. Studies that rejected the light:nutrient hypothesis found that light did not affect nutrient content. Powell *et al.* (2008) used studies that confirmed the light:nutrient hypothesis, where microalgae become carbon rich and nutrient poor when light energy is in abundance relative to nutrient availability, to partially explain their findings. However, in a WSP, nutrient availability is generally always high even as lighting conditions change, which is further illustrated by Hessen *et al.* (2002) who found that light only had a negative effect on biomass phosphorus when external phosphorus was 31 to 310 µg/L. When external phosphorus was increased to 1.6 mg/L, no negative effect was observed. Therefore, it is not expected that PAR would have a negative effect on biomass phosphorus particularly on the basis of the light:nutrient hypothesis. This study did show a photoinhibition effect on specific growth rates. Therefore, it is possible that a higher growth rate also corresponds to a utilization of stored polyphosphates and a decrease in biomass phosphorus despite the high phosphorus concentration. This finding requires further study to properly understand.

The positive observed effect of initial phosphorus concentration and biomass phosphorus was expected, as this effect has also been demonstrated in natural environments by others (e.g., Hessen *et al.,* 2002; Frost and Elser, 2002).

3.5 Conclusions

Few previous studies have focused on microalgae growth and phosphorus accumulation in cold region WSPs. This study addressed this knowledge gap, and the following conclusions were made:

- Maximum specific growth rates varied from 0.029 to 0.058/h. Photosynthetically active radiation (100, 150 μmol/m^2/s) had a statistically significant negative effect on growth rates ($p<0.05$). Temperature (10, 15°C) had a statistically significant positive effect on growth rates ($p<0.10$). Initial phosphorus concentration (7.5, 15 mg P/L) had no statistical effect on growth rates. Growth rates were similar to those observed at temperate climates.
- The model developed by Baranyi and Roberts (1994) accurately described microalgae growth under the tested conditions.
- Luxury uptake was an important phosphorus removal mechanism. Polyphosphate accounted for 53 ± 8% of biomass phosphorus.
- Photosynthetically active radiation and initial phosphorus concentration had a positive effect on biomass phosphorus concentrations. The interaction between temperature and initial phosphorus concentration had a negative effect on biomass

phosphorus concentrations. Future models should account for these effects in order to accurately assess and predict phosphorus removal.

- Biomass phosphorus concentrations were 45% greater than those observed in previous studies conducted in temperate climates.

4 Predicting Microalgae Growth and Phosphorus Removal in Cold Region Waste Stabilization Ponds Using a Stochastic Modeling Approach

4.1 Abstract

A stochastic ecological model with an integrated equilibrium temperature model was developed to predict microalgae growth and phosphorus removal in cold region waste stabilization ponds (WSP). The model utilized a Monte Carlo Simulation to account for parameter uncertainty. An equilibrium temperature model was developed to model water temperature as a function of air temperature. The equilibrium temperature model was parameterized using field data collected from two WSPs in Nunavut, Canada from 2012-2014. The equilibrium temperature model provided good agreement with field data on a daily time step. The full model was run using historic (1956-2005) temperature and solar radiation data from five communities (Baker Lake, Cambridge Bay, Coral Harbour, Hall Beach, Resolute) in Nunavut, Canada. The communities represented a range of geographical locations and environmental conditions. Logistic regression on pooled model outputs showed that mean July temperature and mean treatment season temperature (June 1 – September 15, ice-free period) provided the best predictors for microalgae growth. They had a predictive success rate of 93 and 88%, respectively. The modelled threshold (ie. 50% probability from the logistic regression) for microalgae growth was 8.7 and 5.6°C for the July temperature and mean treatment season temperature, respectively. The logistic regression was applied to each community (except

Sanikiluaq) in Nunavut using historic climate data and a probability of microalgae growth was calculated. Based on the model results, soluble phosphorus concentrations consistent with secondary treatment could be achieved if WSP depth is less than 2 m. The model demonstrated a robust method to predict whether a microalgae bloom will occur under a range of model parameters.

4.2 Introduction

Facultative waste stabilization ponds (WSP) are commonly used for wastewater treatment in the Canadian territory of Nunavut. Generally, WSPs in Nunavut are designed based on local siting and required storage volume. WSPs are often operated with a yearly controlled discharge due to extended periods of ice coverage (Johnson *et al.*, 1998; Johnson, 2008). In facultative WSPs, microalgae communities are relied on to provide aeration allowing for the aerobic treatment of nutrients and organic matter. However, differences in design and operation, such as high organic loading rates and WSP depths, has contributed to inconsistent microalgae growth and treatment performance in Nunavut WSPs (Ragush *et al.*, 2015; Schmidt *et al.*, 2016a). Phosphorus was a particular contaminant of concern due to its receiving water impacts (Krumhansl *et al.*, 2015) and its limited removal in previously studied Nunavut WSPs (Schmidt *et al.*, 2016a).

The role climate plays in cold region WSP treatment performance is not clearly understood. It is also unclear whether an appropriately designed WSP (ie. organic loading and depth) would operate facultatively under the climate constraints observed in cold regions. Therefore, the objective of this study was to develop a predictive model for microalgae growth and effluent phosphorus concentrations in WSPs in various geographical locations in Nunavut over multiple years (1956-2005). The aim of the model is to create a screening tool to assess geographical climatic constraints, allowing for inferences on whether WSPs could operate facultatively assuming they are designed appropriately.

4.3 Model Development

The model consisted of two components. The first component is an equilibrium temperature model relating air temperature to water temperature. The model was parameterized using meteorological parameters including wind speed, solar radiation and dew point temperature. The equilibrium temperature model allows for application of the ecological model to locations without water temperature data. The second component is an ecological model relating various parameters, including light intensity, water temperature and WSP depth, to microalgae production and phosphorus uptake. Ecological models have previously been used to model WSPs by several authors (Beran and Kargi, 2005; Fritz *et al.*, 1979; Moreno-Grau *et al.*, 1996). A Monte Carlo Simulation was used to account for variation and uncertainty in various parameter values for both model components. The parameters used in the Monte Carlo Simulation are discussed in Section 4.3.3.

A general diagram of the models is shown in Figure 4-1.

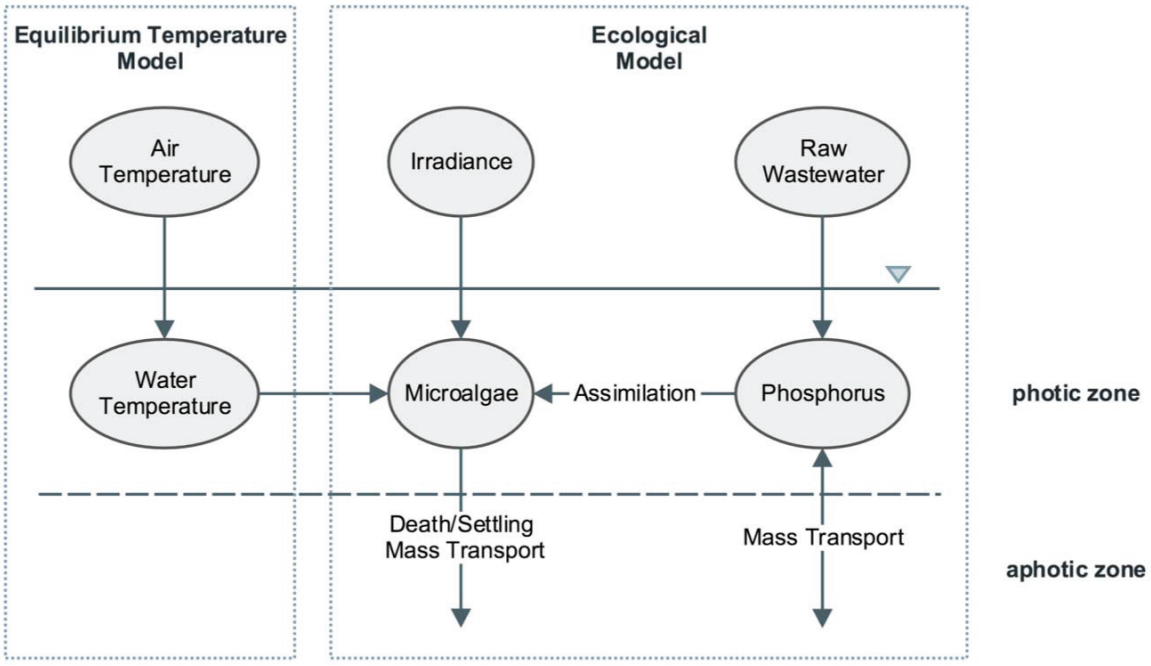

Figure 4-1: Diagram of the two models used in this study

4.3.1 Equilibrium Temperature Model

Water temperature was modeled using an equilibrium temperature model. Equilibrium temperature models use the concept of equilibrium temperature in order to describe heat flux between air and water. They have previously been shown to accurately predict water temperature at an hourly time step (Herbert *et al.*, 2015). The general form of the model is shown below:

$$\frac{dT_w}{dt} = \frac{H}{c\rho y} = \frac{K(T_e - T_w)}{c\rho y} \qquad \text{Eq. 4-1}$$

where:

T_w is water temperature (°C)

t is time (h)

H is the total heat flux at the water surface

c is the specific heat capacity of water (4.19×10^{-3} MJ kg^{-1} $°C^{-1}$)

ρ is the density of water (1000 kg m^{-3})

y is the mean water depth (m)

K is the thermal exchange coefficient (W m^{-2} $°C^{-1}$)

T_e is equilibrium temperature (°C)

Thermal exchange coefficients (K) have been shown to be specific to individual water bodies and therefore need to be determined experimentally. Equilibrium temperature represents the water temperature such that the total heat flux at the air-water interface is zero. Heat fluxes considered in this model were net short and long wave radiation, convection and evaporation (Caissie *et al.*, 2005). Heat flux associated with raw wastewater loading was assumed negligible, as the volume added is very small compared to the total WSP volume. Equilibrium temperature can therefore be calculating according to the equation previously described by Caissie *et al.* (2005) as follows:

$$T_e = \frac{0.97H_{si}(1 - SF) + (0.97\beta A_1 + 0.03V)T_a + 0.07V\eta T_d + 0.097A_2(\beta - 1)}{0.97A_1 + 0.07V\eta + 0.03V} \quad \text{Eq. 4-2}$$

where:

T_e is equilibrium temperature (°C)

H_{si} is incoming solar radiation (MJ m^{-2} day^{-1})

SF is a shading factor ranging from 0 to 1 depending on forest cover and topography (assumed 0 for this study),

β is atmospheric emissivity (Eq. 4-3)

A_1 is a constant (0.46 MJ m^{-2} day^{-1} $°C^{-1}$)

V is wind speed (km h^{-1})

T_a is air temperature (°C)

η is the relationship between vapour pressure and temperature (Eq. 4-4)

T_d is the dew point temperature (°C)

A_2 is a constant (28.38 MJ m^{-2} day^{-1} $°C^{-1}$)

$$\beta = 0.74 + 0.0065e_a(1 + 0.17C^2) \quad \text{Eq. 4-3}$$

where:

e_a is air water vapour pressure (mm Hg)

C is cloud cover, 0=clear sky, 1=total cloud cover (assumed 0 as cloud cover data is not readily available)

$$\eta = \frac{e_s - e_a}{T_w - T_d} \quad \text{Eq. 4-4}$$

where:

e_s is the saturated water vapour pressure at water temperature (mm Hg)

e_a is the air water vapour pressure (mm Hg)

T_w is the water temperature (°C)

T_d is the dew point temperature (°C)

A linear relation between equilibrium (T_e) and air (T_a) temperature has been previously shown (Caissie *et al.*, 2005; Hebert *et al.*, 2015), and therefore was used as a further simplification. This is advantageous, as water temperature is then only a function of air temperature, which is a widely available parameter. Finally, the equation for water temperature change in a well-mixed water body is as follows:

$$\frac{dT_w}{dt} = \frac{K((aT_a + b) - T_w)}{c\rho y} \quad \text{Eq. 4-5}$$

where:

T_w is water temperature (°C)

t is time (h)

K is the thermal exchange coefficient (W m^{-2} $°C^{-1}$)

T_a is air temperature (°C)

a and b are linear regression coefficients

c is the specific heat capacity of water (4.19 x 10^{-3} MJ kg^{-1} $°C^{-1}$)

ρ is the density of water (1000 kg m^{-3})

y is the mean water depth (m)

The model was parameterized using data collected from WSPs in Pond Inlet (2011, 2012 and 2013) and Kugaaruk (2012, 2013). First, equilibrium temperatures were calculated using data collected onsite (water temperature, solar radiation) and historic weather data from Environment Canada (dew point temperature, air temperature, wind speed). Water temperature was measured using temperature/light pendants (Onset Computer Corporation, Cape Cod, Massachusetts). Solar radiation was measured using a weather station with an associated sensor (Onset Computer Corporation, Cape Cod, Massachusetts). Equilibrium temperatures were then regressed against air temperature. The regression is shown in Figure 4-2. Since equilibrium temperature accounts for convection, radiation and evaporation, values are much higher than the measured air

temperature. Using the regression results, a time series was generated with Eq. 4-5 using an estimate of the thermal exchange coefficient. A new thermal exchange coefficient was then calculated for each site and year by minimizing the mean squared error between the predicted and measured water temperatures. A final model thermal exchange coefficient (50 W m^{-2} $°C^{-1}$) was then calculated as the mean of the individual values for each WSP and year. A mean water depth of 10 cm was used for both locations, as this is the estimate of the photic zone depth.

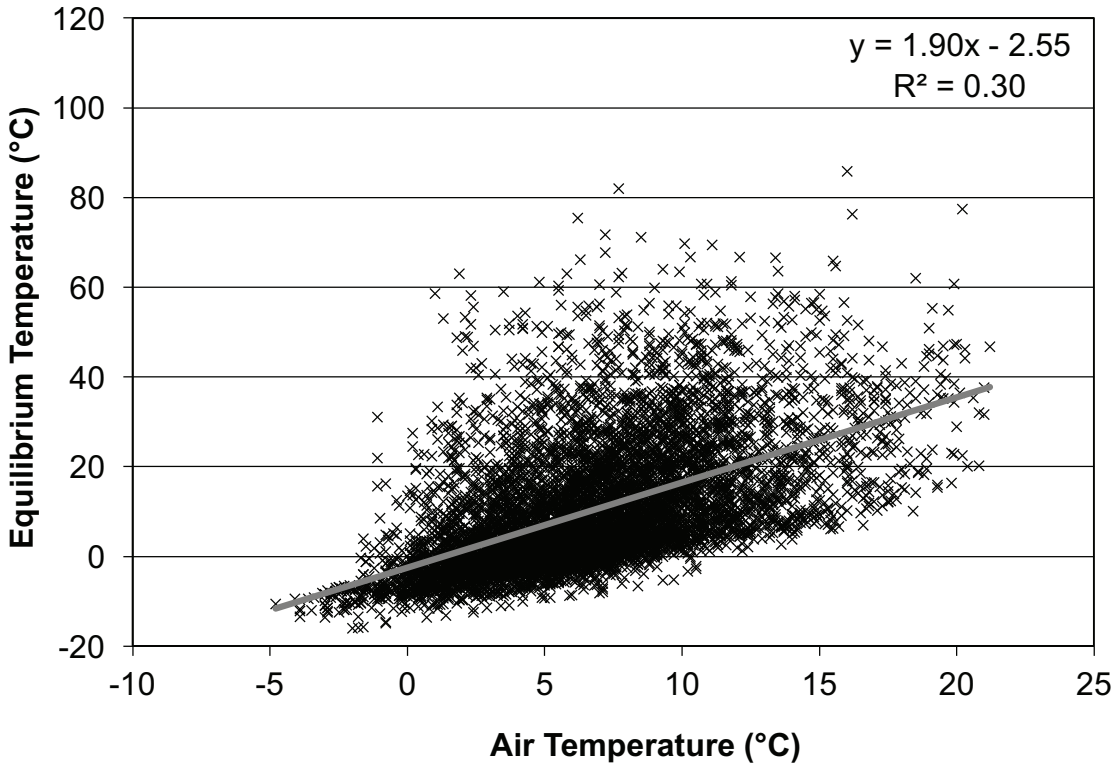

Figure 4-2: Equilibrium versus air temperature for Pond Inlet (2011, 2012, 2013) and Kugaaruk (2012, 2013) (n=7751)

An example (Kugaaruk 2013) of the model predicted values and the measured field values using the average thermal exchange coefficient is shown in Figure 4-3. The model

is able to predict general seasonal temperature trends but is not able to capture short term (sub-daily) increases/decreases. For the purposes of this study however, the model performance is sufficient. Uncertainty in model parameters was addressed by including the three governing parameters (*K*, *a*, *b*) in the Monte Carlo Simulation (Table 4-2).

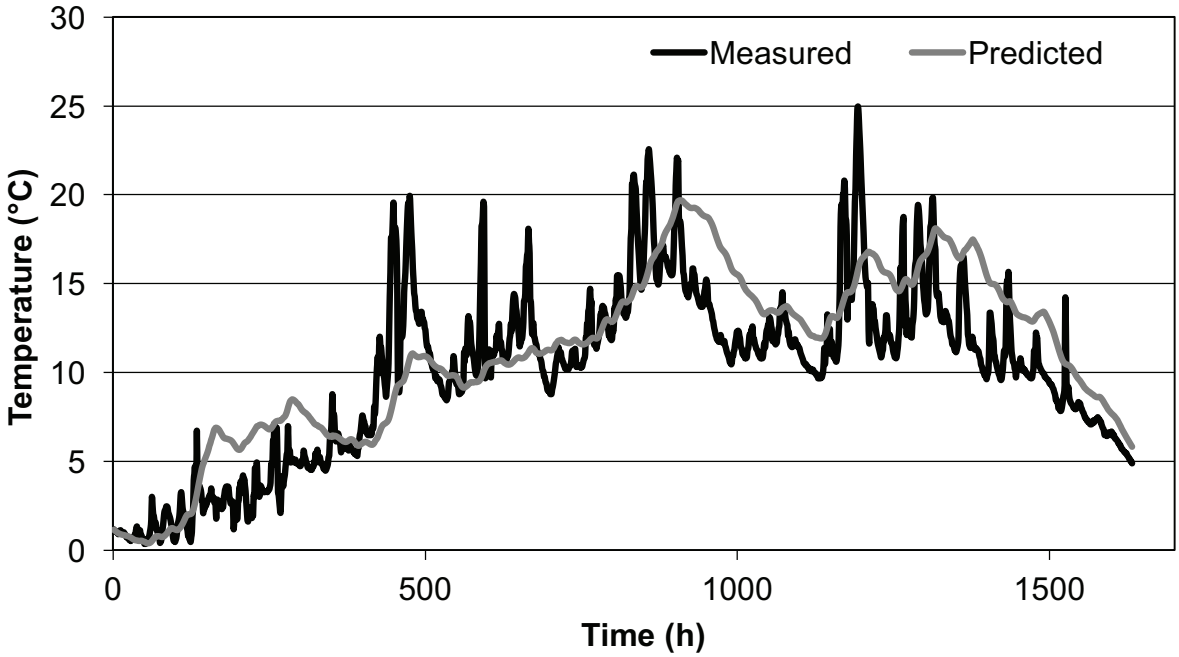

Figure 4-3: The modelled and measured values for water surface temperature in the Kugaaruk WSP in 2013.

4.3.2 Ecological Model

WSP performance was predicted at five communities in Nunavut (Baker Lake, Cambridge Bay, Coral Harbour, Hall Beach, Resolute) using a multi-year ecological model with a Monte Carlo Simulation. A Monte Carlo Simulation was used to account for uncertainty in model parameters. The sites were picked based on the availability of

environmental data. The sites represent a range of geographical locations and environmental conditions.

4.3.2.1 Environmental Data

Environmental Canada's Canadian Weather Energy and Engineering Data Sets were used to gather hourly environmental data for each site. The number of years of data for each site varied from 42 to 49. Global horizontal irradiance was used to estimate photosynthetically active radiation. Global horizontal irradiance measures the total sum of irradiance over an hour in kJ/m^2. Global horizontal irradiance was converted to photosynthetically active radiation (PAR, unit: μmol/m^2/s) using Eq. 4-6.

$$Global\ Horizontal\ Irradiance\ (kJ/m^2/h) \times \frac{h}{3600\ s} \times \frac{1000\ J}{1\ kJ} \times \frac{1\ W}{1\ J/s} \times \frac{1\ \ \mu mol/m^2/s}{0.42\ \ W/m^2} = PAR\ (\mu mol/m^2/s) \quad \text{Eq. 4-6}$$

The relationship between μmol/m^2/s and W/m^2 was determined using data collected in Kugaaruk in 2013. Solar radiation and PAR were measured in Kugaaruk using a weather station and associated sensors (Onset Computer Corporation, Cape Cod, Massachusetts). The relationship is shown in Figure 4-4.

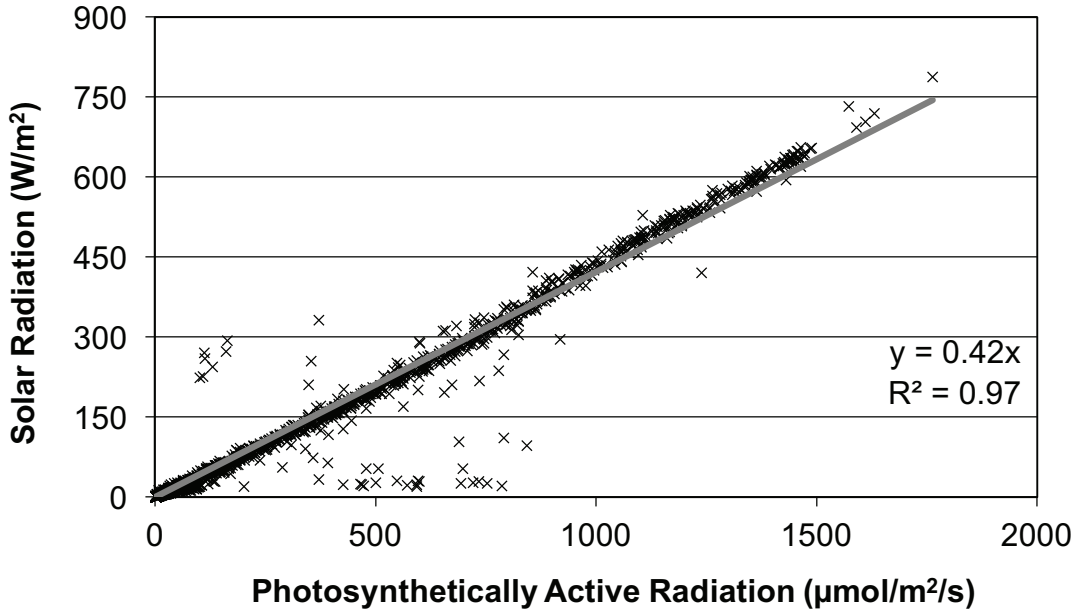

Figure 4-4: Relationship between photosynthetically active radiation and solar radiation in Kugaaruk in 2013

Water surface PAR can be converted to a subsurface reading at any depth using Eq. 4-7. The light attenuation coefficient was calculated using field data from Kugaaruk in 2013. PAR measurements were collected at three depths (0, 10, 30 cm) using temperature/light pendants (Onset Computer Corporation, Cape Cod, Massachusetts). Data were plotted using the linearized form of the Beer-Lambert Law in order to determine the light attenuation coefficient. The light attenuation coefficient will change depending on the microalgae concentration due to self-shading, however this is an adequate estimate. The data and linear regression are shown in Figure 4-5.

$$light_z = light\frac{(1 - e^{-k_L z})}{k_L z} \qquad \text{Eq. 4-7}$$

where

$light_z$ is the mean subsurface PAR to a depth of z (µmol/m^2/s)

light is the water surface PAR (µmol/m^2/s)

k_L is the light attenuation coefficient (23.8 m^{-1})

z is depth (m)

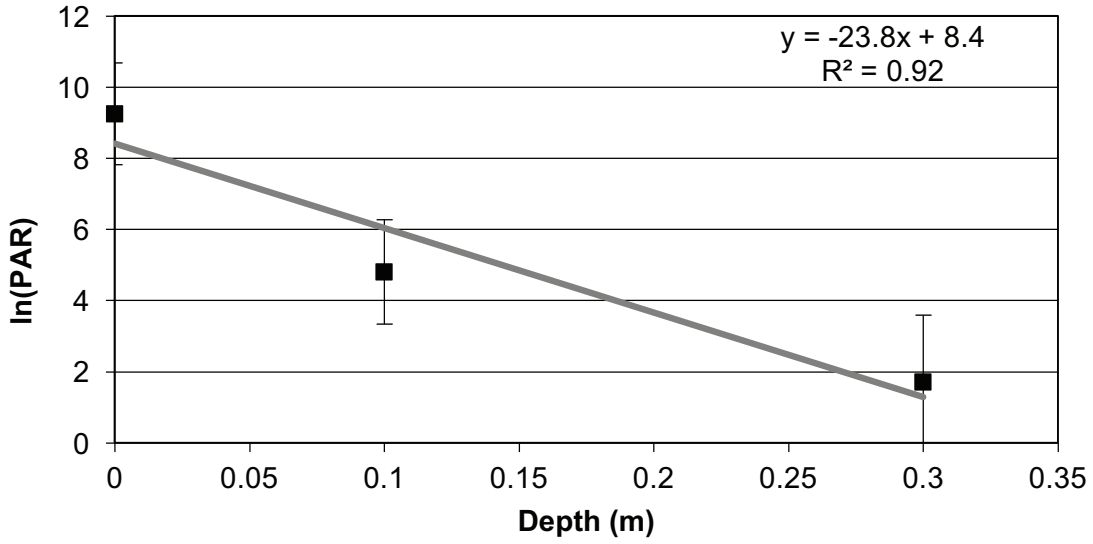

Figure 4-5: Regression of ln(PAR) on depth in order to determination of the light attenuation coefficient using field data from Kugaaruk 2013 (n=885 per depth). Error bars represent the standard deviation.

4.3.2.2 WSP Physical Characteristics

The physical characteristics of the Kugaaruk WSP were used to develop standardized WSP characteristics that were applied in each geographical location. A summary of the characteristics is shown in Table 4-1. Multiple depths were tested in order to evaluate whether there is an optimal design depth.

Table 4-1: Physical characteristics of modeled waste stabilization pond

Volume	55015 m^3
Depth	1, 2, 3, 4, 5 m
Volumetric Loading Rate	3167 L/h

4.3.2.3 Microalgae

The general equation for changes in microalgae concentrations is shown in Eq. 4-8. Growth was influenced by temperature and light limitations. Growth rates were temperature adjusted using a function (Eq. 4-9) similar to a Gaussian distribution (Cerco and Cole, 1995). The temperature correction coefficient was calculated using temperature specific growth rates measured in Schmidt *et al.* (2016b). Light limitation (Eq. 4-10) was modeled using a modified Steele's equation (Steele, 1962). Photoinhibition was not modelled as it was assumed that microalgae would move vertically in the photic zone to achieve optimal light intensities. A lumped death/settling rate was used to describe the transport of microalgae out of the photic zone. The model was initialized with a microalgae concentration of 5 mg/L. Minimum microalgae concentrations were controlled by not allowing concentrations to decrease below background concentrations ($X_{background}$).

$$\frac{dX}{dt} = \mu_{20}\text{lim(temp)lim(light)}X - mX \quad \text{Eq. 4-8}$$

where:

X is microalgae concentration (mg/L)

t is time (h)

μ_{20} is the maximum specific growth rate at 20°C (h^{-1})

lim(temp) is the temperature limitation on growth (Eq. 4-9)

lim(light) is the light limitation on growth (Eq. 4-10)

m is a grouped death/settling coefficient (h^{-1})

$$\text{lim(temp)} = \begin{cases} e^{-k_T(T_w - T_{optimal})^2}; T \geq T_{minimum} \\ \text{lim}(temp) = 0; T < T_{minimum} \end{cases} \quad \text{Eq. 4-9}$$

where:

k_T is the temperature correction coefficient (0.0034 $°C^{-2}$)

T_w is the water temperature (°C)

$T_{optimal}$ is the optimal growth temperature (20°C)

$T_{minimum}$ is the minimum growth temperature (°C)

$$\lim(\text{temp}) = \begin{cases} \dfrac{light_z}{light_{optimal}} e^{1-\left(\frac{light_z}{light_{optimal}}\right)}; light_z < light_{optimal} \\ 1; light_z \geq light_{optimal} \end{cases} \quad \text{Eq. 4-10}$$

where:

$light_z$ is the PAR concentration (μmol/m^2/s) at the active depth (z)

$light_{optimal}$ is the optimal growth PAR concentration (μmol/m^2/s)

4.3.2.4 Phosphorus Removal

The primary phosphorus removal mechanism modelled was microalgae uptake and assimilation/storage. While other mechanisms, such as precipitation with iron, do contribute to phosphorus removal, the model only considered biological removal mechanisms. Metals concentrations are generally low in Nunavut WSPs as no coagulants are added. Microalgae uptake was modelled by assuming phosphorus comprises a fixed percentage of microalgae. The mathematical representation of uptake is shown in Eq. 4-11.

$$\frac{dP}{dt} = -f\frac{dX}{dt} \quad \text{Eq. 4-11}$$

where:

P is phosphorus concentration (mg/L)

t is time (h)

f is the microalgae biomass phosphorus percentage

X is microalgae concentration (mg/L)

The modelled WSP had an initial phosphorus concentration of 7.5 mg P/L. Phosphorus concentrations in the raw wastewater had a concentration of 12 mg P/L. These values are based on results found in Kugaaruk in 2012 and 2013 by Schmidt *et al.* (2016a).

4.3.2.5 Mass Transport

Mass transport into the photic zone from raw water and between the photic and aphotic zone was modelled. A diagram of the mass transport is shown in Figure 4-1. The photic zone had a fixed volume, so any transport into the photic zone (from raw wastewater) is counteracted by an equal transport out (into the aphotic zone).

Phosphorus was input into the WSP at a rate equal to the displacement rate of the photic zone (ie. the raw wastewater flow rate divided by the photic zone volume). Phosphorus from the photic zone was also transported to the aphotic zone at an equivalent rate. Raw wastewater was assumed to not be a significant source of microalgae. However, similarly to phosphorus, as raw wastewater is input into the photic zone, wastewater with microalgae is transported to the aphotic zone at an equivalent rate to the raw wastewater input. Microalgae transport due to death and subsequent settling was previously introduced in Eq. 4-8.

Phosphorus was also transported between the photic and aphotic zone through mixing. Field studies conducted in Pond Inlet and Kugaaruk showed that Nunavut WSPs are chemically well mixed. Therefore, at each modelled time step, phosphorus concentrations in the photic and aphotic zone were equalized.

4.3.3 Monte Carlo Simulation Parameters

In ecological models, assumptions are often made for the values of various parameters. In order to understand and account for parameter uncertainty, a Monte Carlo Simulation was used. By using a Monte Carlo Simulation, a range of parameter values could be tested and the most likely result could be determined. Nine parameters were included in the Monte Carlo Simulation. A summary of the parameters and the range of values tested are shown in Table 4-2.

Table 4-2: Summary of the parameters used in the Monte Carlo Simulation

Parameter	Description	Range of Values	Units
K	thermal exchange coefficient (Eq. 4-1, Eq. 4-5)	33.3-66.7	W m^{-2} $°C^{-1}$
a	slope of the T_e vs T_a regression line (Eq. 4-5, Figure 4-2)	1.4-2.4	unitless
b	y-intercept of the T_e vs T_a regression line (Eq. 4-5, Figure 4-2)	1-4	°C
μ_{20}	microalgae maximum specific growth rate at 20°C (Eq. 4-8)	0.04-0.05	h^{-1}
m	microalgae death/settling rate (Eq. 4-8)	0.02-0.03	h^{-1}
$T_{minimum}$	minimum temperature for microalgae growth (Eq. 4-9)	5-8	°C
$light_{optimal}$	optimal irradiation for microalgae growth (Eq. 4-10)	75-125	µmol/m^2/s
f	microalgae biomass phosphorus concentration (Eq. 4-11)	0.016-0.027	unitless
$X_{background}$	background microalgae concentration	1-5	mg/L

The range of values for the thermal exchange coefficient and the T_e vs T_a regression parameters were set to reflect the variability seen in the single year parameterizations. Parameters associated with microalgae have a wide range of reported values. The growth rate range was set to reflect the rates found in Schmidt *et al.* (2016b) as well as reported values from literature. For instance, Dauta *et al.* (1990) found that *C. vulgaris*, a previously identified microalgae species in Nunavut WSPs, had a maximum growth rate of 0.05/h under optimal lab conditions. Furthermore, previous modelling of microalgae has used maximum growth rates ranging from 0.04-0.08/h (Asaeda and Van Bon, 1997; Diehl, 2002; Fritz *et al.*, 1979). Reported mortality/settling rates also vary greatly. Obayashi and Tanoue (2002) found that microalgae sampled in the North Pacific Ocean had a mortality rate of 0.01-0.04/h. The modelled range was narrowed slightly to eliminate outliers/unrealistic results.

Results from Schmidt *et al.* (2016b) were used to set the ranges for the minimum growth temperature, optimal light intensity and biomass phosphorus concentration. For instance, no microalgae growth was observed at 5°C while growth was observed at 10°C. Temperatures between these values were not tested. Therefore, a range was selected to reflect this uncertainty. Slight photoinhibition occurred at 150 μmol/m^2/s relative to growth at 100 μmol/m^2/s. No other intensities were tested. Therefore, the upper range was selected as the midway point between these two intensities. The lower range was selected such that the centre of the range was 100 μmol/m^2/s. Microalgae biomass phosphorus concentrations ranged from 0.015 to 0.033%. The range used was decreased slightly in order to be more representative of the entire data set. The range of background microalgae concentrations

was set to ensure that microalgae concentration could rebound from extreme temperatures but did not contribute significantly to overall growth.

4.3.4 Model Simulation

The equilibrium temperature and ecological model had a time step of 1 hour from June 1 to September 15 (defined as the treatment season, when WSPs are ice-free). This time step was selected as it is the minimum time step of the available data. The Monte Carlo parameters were simulated 1000 times for each unique combination of location, year and depth. The Monte Carlo parameter values were determined using a uniform distribution. For each simulation the maximum microalgae concentration, the total microalgae production and the final total phosphorus concentration were stored, along with the values of the Monte Carlo parameters.

The model was run and analyzed using R v3.2.2 statistical programming language (R Core Team 2015). The code used to run the model is provided in Appendix A: R code for ecological model with integrated equilibrium temperature model

4.3.5 Model Post Analysis

4.3.5.1 Microalgae Growth

Maximum microalgae concentration was used as an analog for WSP performance. It is expected that a relatively low maximum concentration would result in an anaerobic WSP while a higher concentration would result in an aerobic WSP. In order to determine the most likely maximum microalgae concentration for each location, year and depth,

maximum concentrations were rounded to the nearest 10 mg/L and the mode was taken. Maximum concentrations were capped at 400 mg/L, which would correspond to a highly productive WSP. Any concentrations exceeding 400 mg/L were rounded down. An example of a histogram showing the maximum concentrations in Baker Lake in 1975 is shown in Figure 4-6. In this simulation, the most likely maximum concentration is 400 mg/L. The code to post process microalgae data and determine the most common concentration is provided in Appendix B.

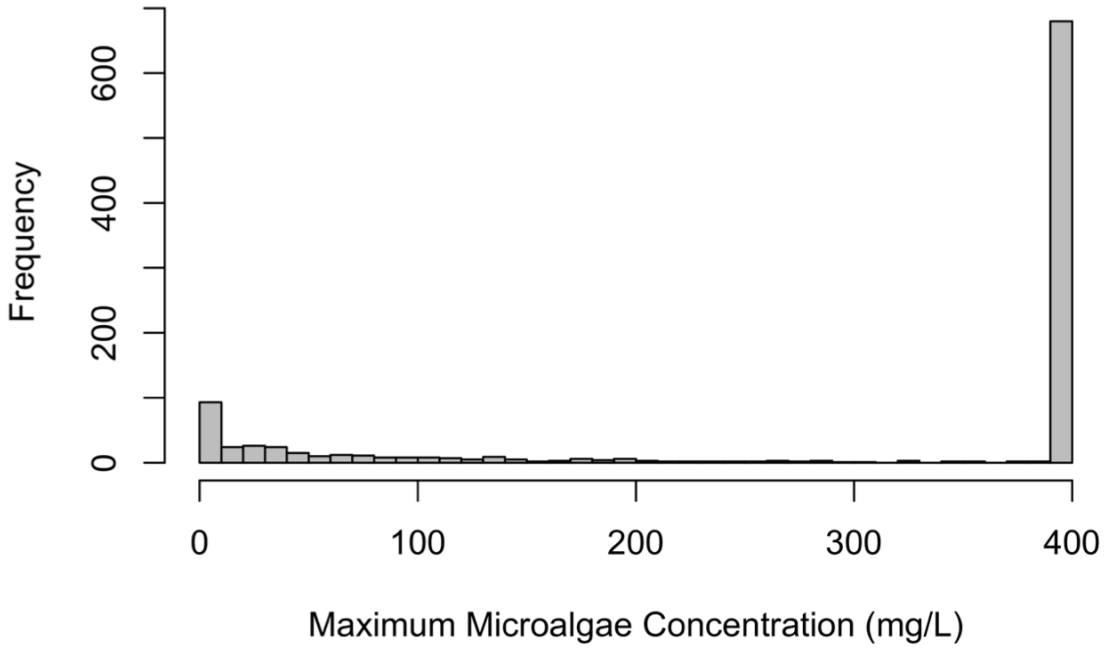

Figure 4-6: Histogram of the simulations for Baker Lake in 1975

For all locations and years, it was found that maximum concentrations were either 0, 10 or 400 mg/L. In other words, the WSP either had no microalgae growth or significant

growth. Based on these results, it was determined that a logistic regression could be applied to the entire dataset in order determine the probability of growth. Various predictors were tested including mean treatment season temperature, mean temperature for each simulated month (June, July, August, September), mean treatment season solar radiation and mean solar radiation for each simulated month (June, July, August, September). Three pseudo R^2 calculations (Tjur's Coefficient of Discrimination, Cox-Snell's and Nagelkerke) as well as success rate were used the determine which predictor (or combination of predictors) produced the best fit. Multiple regression was only conducted with one temperature predictor and one solar radiation predictor.

4.3.5.2 Phosphorus Removal

Phosphorus removal varied as a function of depth due to differences in microalgae production. As depth increases, the surface area of the WSP decreases to maintain the same total volume. This also results in a decrease in the photic zone volume which decreases the total amount of microalgae production. Since phosphorus removal is directly proportional to microalgae production (through *f*), as depth increases, phosphorus removal decreases. Phosphorus removal was evaluated for scenarios where microalgae growth was observed. The criteria for growth was that the maximum microalgae concentration was between 350 and 450 mg/L. In contrast to the evaluation of microalgae growth (Section 4.3.5.1), maximum microalgae concentrations exceeding 450 mg/L were not rounded down. This is because phosphorus removal is a cumulative parameter while maximum microalgae concentration is an instantaneous parameter.

In order to determine the most likely effluent phosphorus concentration for each location, year and depth, effluent phosphorus concentrations were rounded to the nearest 0.5 mg P/L and the mode was taken.

4.3.6 Model Post Analysis Results

4.3.6.1 Microalgae Growth

By comparing pseudo R^2 values, it was found that temperature was the best predictor of microalgae growth. The addition of solar radiation as a predictor provided minimal improvement of the model fit. Due to minimal model improvement, and the fact that solar radiation is not a readily available historic climate parameter, it was decided that temperature would be the only predictor included in the logistic regression.

A summary of the model parameters and pseudo R^2 values for each temperature predictor is shown in Table 4-3. The best predictor was mean July temperature followed by mean treatment season temperature. The intercept and slope were found to be significant for all predictors ($p<0.001$). The logistic regression with July temperature as the predictor had a 93% success rate. Success rate was defined as the percentage of successful prediction where a model probability of 0.50 and greater corresponds to microalgae growth.

Table 4-3: Logistic regression (probability of microalgae growth vs. temperature) pseudo R^2, success rate and model parameters for five mean temperature predictors. Success rate is defined as the percentage of correct predictions where a model probability of 0.50 and greater corresponds to microalgae growth.

Predictor	Pseudo R^2			Success Rate	Intercept	Slope
	Tjur	Cox-Snell	Nagelkerke			
Mean Treatment Season Temperature	0.65	0.55	0.74	88%	-8.50	1.52
June Temperature	0.51	0.45	0.61	85%	-2.79	1.07
July Temperature	0.72	0.57	0.77	93%	-12.40	1.43
August Temperature	0.50	0.46	0.62	82%	-6.60	0.95
September Temperature (Note: September 1 – 15)	0.27	0.26	0.36	73%	-1.56	0.52

A comparison of the logistic regression plots for mean July temperature and mean treatment season temperature are shown in Figure 4-7. The threshold (50% probability) for microalgae growth was 8.7 and 5.6°C for July temperature and mean treatment season temperature, respectively.

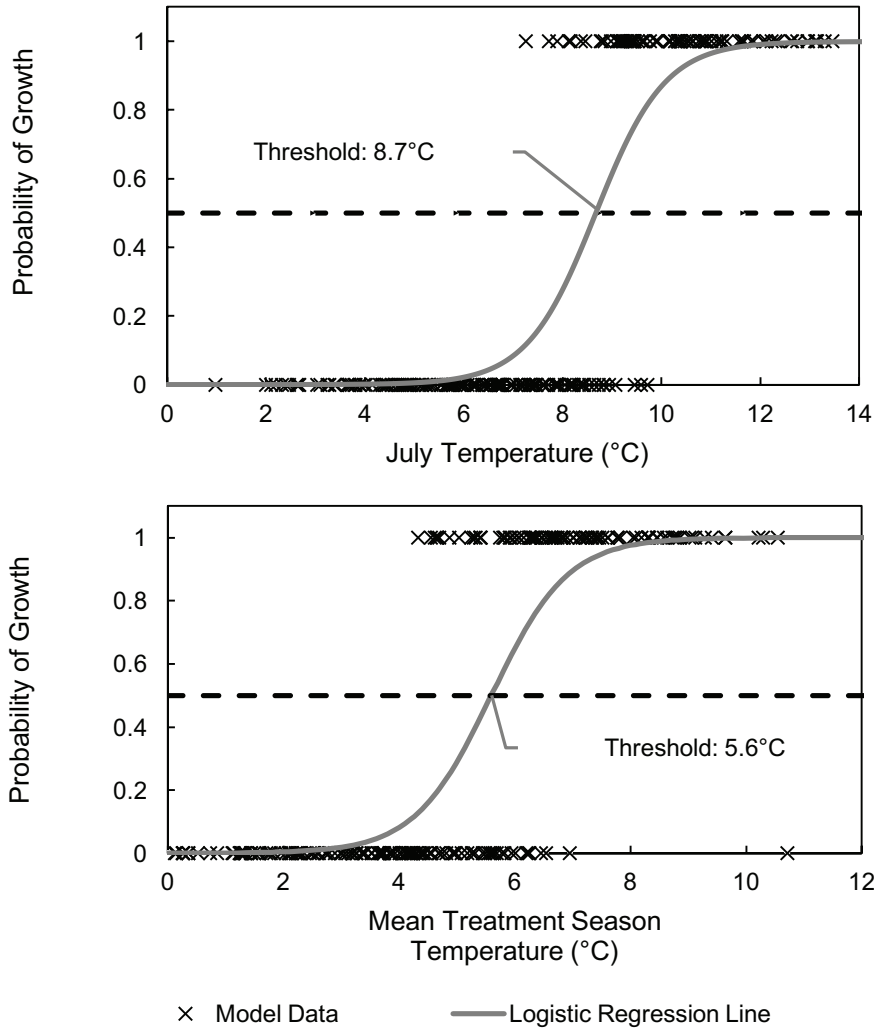

Figure 4-7: Model data and logistic regression lines for two predictors (July temperature and mean treatment season temperature). Growth is represented as a binary variable where 1 represents growth (≥400 mg/L) and 0 represents no growth (≤10 mg/L). Dotted line represents the threshold for growth (ie. 50% probability).

4.3.6.2 Phosphorus Removal

A boxplot of the expected effluent phosphorus concentrations by depth is shown in Figure 4-8. Median concentrations ranged from <0.5 mg P/L to 5.5 mg P/L. At a depth of

1 m, 86% of the sample runs that fit the analysis criteria (350 mg/L < maximum microalgae concentration < 450 mg/L) had effluent phosphorus concentrations <0.5 mg P/L. There were some outliers that represent elevated effluent phosphorus concentrations. This shows that even if the maximum microalgae concentration is high (~400 mg/L), the total microalgae production, and therefore phosphorus removal, during the treatment season may be low. At a depth of 2 m, the median effluent phosphorus concentration was 2.5 mg P/L with maximum concentrations as high at 6 mg P/L. At depths of 3 to 5 m, some reduction was seen, with median concentrations ranging from 4.5 to 5.5 mg P/L. In reality, it is unlikely that WSPs of this depth would operate aerobically with considerable microalgae concentrations. Effluent phosphorus concentrations exceeding 7 mg P/L, as shown in Schmidt *et al.* (2016a), are more likely.

The effluent total phosphorus concentrations showed in Figure 4-8 represent soluble phosphorus only. In scenarios where there is considerable microalgae growth, actual total phosphorus would be much higher due to the particulate (microalgae) portion. A filtration or sedimentation process could be considered to remove the particulate portion.

Nunavut does not have a guideline for effluent total phosphorus concentrations. Canada's Wastewater Systems Effluent Regulations (WSER), which does not currently apply to Nunavut, also does not have a guideline for effluent total phosphorus concentrations. Some provinces (ie. British Columbia, Manitoba) have implemented guidelines of 1 mg P/L for discharge into freshwater environments. Generally, the guidelines either do not apply or are more lenient for small systems. Nunavut consists primarily of small systems discharging into marine environments, so it is unlikely that stringent guidelines will be

applied. However, for a comparison, it is expected that a 1 m deep WSP operating facultatively would be capable of achieving a 1 mg P/L guideline if a polishing step was used to remove the particulate phosphorus portion.

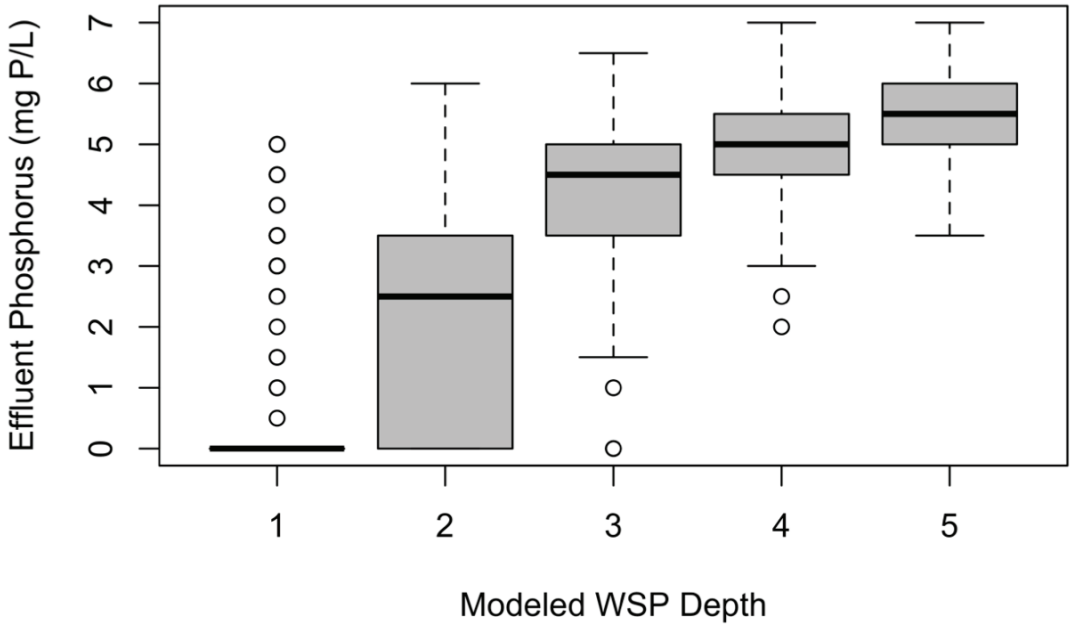

Figure 4-8: Predicted effluent soluble phosphorus concentration for each modelled depth (n=192 per depth). Open circles represent outliers (>1.5 times the interquartile range).

4.4 Comparison of Field Observations and Model Predictions

WSPs in four communities (Clyde River, Grise Fiord, Kugaaruk and Pond Inlet) were characterized over four years (2011-2014). Findings are summarized in Ragush *et al.* (2015) and Schmidt *et al.*, (2016a).

4.4.1 Microalgae Growth

Three of the WSPs operated anaerobically with little microalgae production. The exception is Grise Fiord in 2011 where the WSP was aerobic and significant microalgae growth occurred. Limited data also showed that a microalgae bloom occurred in the Pond Inlet WSP in 2011. Prior to the bloom in Pond Inlet (Fall 2010 to Spring 2011) the WSP was not loaded due to the installation of a HDPE liner.

A summary of mean July and treatment season temperature for each of the studied communities and years is shown in Table 4-4. The oxygen state and probability of microalgae growth for each predictor is also shown.

Table 4-4: Mean treatment season and July temperature and the predicted probability of microalgae growth for four communities in Nunavut. Dissolved oxygen state is based on field observations.

Community	Year	Dissolved Oxygen State	Mean Treatment Season Temp (°C)	Mean July Temp (°C)	Probability of Microalgae Growth	
					Predictor: Mean Treatment Season Temp	Predictor: Mean July Temp
Clyde River	2012	Anaerobic	4.9	7.2	26%	11%
	2013	Anaerobic	3.8	4.7	6%	0%
	2014	Anaerobic	4.1	6.1	10%	2%
Grise Fiord	2011	Aerobic	4.6	7.2	19%	11%
Kugaaruk	2012	Anaerobic	6.3	9.5	75%	76%
	2013	Anaerobic	5.5	8.1	47%	31%
Pond Inlet	2011	Aerobic	5.8	8.2	58%	34%
	2012	Anaerobic	5.2	8.1	36%	31%
	2013	Anaerobic	4.0	5.8	8%	2%
	2014	Anaerobic	4.4	7.3	13%	13%

In Clyde River and Pond Inlet, the observed dissolved oxygen state was accurately predicted in 2012-2014. These results are further corroborated with an analysis of historical mean temperatures. Clyde River has a historical mean July and mean treatment season temperature of 4.7 ± 0.3°C and 3.1 ± 0.2°C, respectively. These historical temperatures are less than the threshold temperatures identified (Figure 4-7). Pond Inlet has slightly higher historical temperatures, but they are still well below the threshold temperatures. It is expected WSPs in Pond Inlet and Clyde River are unlikely to operate facultatively. In 2011, a microalgae bloom was observed in Pond Inlet; this is likely due to an unseasonably warm treatment season compared to historical means (5.8°C vs 3.9 ± 0.3°C) and/or changes in operation associated with HDPE liner installation. The WSP was not loaded from Fall 2010 to Spring 2011, and this caused a decreased total volume and depth. This may have resulted in more amenable conditions for microalgae growth.

Kugaaruk had conflicting results. In 2012, the model predicted microalgae growth but the WSP was anaerobic. The difference is likely attributed to the WSP design. The Kugaaruk WSP has a mean depth of 5.4 m which is too deep for a facultative WSP and is more indicative of an anaerobic WSP (Shilton, 2005). Kugaaruk has a historical mean July and mean treatment season temperature of 7.9 ± 0.6°C and 4.4 ± 0.5°C, respectively. Since the historical mean temperatures are below the threshold temperatures, it is expected that, even if a new WSP was built with more appropriate dimensions, it is expected that a WSP is unlikely to operate facultatively in Kugaaruk.

Based on the model results, it was not expected that the Grise Fiord WSP would be operating aerobically in 2011. The microalgae growth may be due to unseasonably warm

temperatures. Grise Fiord has a historical mean treatment season and July temperature of 2.3 ± 0.9°C and 4.0 ± 1.0°C, respectively. In 2011 the mean treatment season and July temperature was 4.6°C and 7.2°C, respectively. Arctic lakes have been shown to be extremely sensitive to climate change. This is due to their thermal proximity to freezing. Even slight climate warming lengthens the ice-free growing season and increases primary production (Smol *et al.*, 2005; Quayle *et al.*, 2002). The shift to warmer temperatures may have also resulted in the proliferation of cold-adapted microalgae. Microalgae cold adaptation has previously been demonstrated in literature (Blanc *et al.*, 2012; Seaburg *et al.*, 1981; Teoh *et al.*, 2004). Teoh *et al.* (2004) isolated microalgae from seawater, freshwater, soil and snow in Antarctica and grew them under a range of temperatures (4-30°C). Multiple species were capable of growth in temperatures as low as 4°C. Growth rates for *Chlorella* UMACC 234 effectively doubled (0.09 ± 0.02 to 0.20 ± 0.01) when temperatures increased from 4 to 6°C. It is possible that a similar shift occurred in Grise Fiord. If and how cold-adapted microalgae grow in WSPs is unknown and requires further investigation.

4.4.2 Phosphorus Removal

Microalgae growth was only observed twice during field sampling (Table 4-4) so comparisons and validation of modelled results are limited. Phosphorus data was also not available in Pond Inlet in 2011. In Grise Fiord in 2011, total phosphorus concentrations were 3.5 mg P/L. Soluble phosphorus can be calculated by subtracting an estimate of microalgae particulate phosphorus. The Grise Fiord WSP had a mean (± standard deviation) total suspended solids (TSS) concentration of 439 ± 142 mg/L. Assuming the

mean TSS concentration in the Clyde River primary cell (58 mg/L) is a reasonable estimate of non-microalgae TSS, microalgae concentrations were 381 mg/L. Using a conservative estimate of biomass phosphorus concentration of 1% results in a microalgae particulate phosphorus concentration of 3.8 mg P/L. The high variation in the mean TSS concentration is likely the reason why microalgae particulate phosphorus exceeded total phosphorus. Ultimately, it is expected that there was little soluble phosphorus remaining in the WSP. The WSP had an estimated depth of 1.5 m. Based on the model results, the median predicted soluble phosphorus concentrations would range from <0.5 to 2.5 mg P/L. Therefore, there is reasonable agreement between the field data (Grise Fiord 2011, only) and the modelled results. More field data is required to effectively evaluate and verify the model.

4.5 Model Application: Probability of microalgae growth in other Nunavut communities

Historic climate data was gathered from Environment Canada for 25 of the 26 communities in Nunavut. There was not sufficient climate data available for Sanikiluaq. The number of years of data ranged from 7 to 79. There were considerable missing data entries, therefore, data was only gathered for years when there was 10 or fewer missing data entries from June 1 to September 15 (ie. the treatment season). A summary of data from each community and their associated probability of microalgae growth is shown in Table 4-5. In general, predictions based on mean July temperature and mean treatment season temperature produced similar results. Kimmirut, Iqaluit and Pangnirtung were exceptions, where using mean treatment season temperature as a predictor resulted in an

increased (>30%) probability of microalgae growth. These communities were characterized by relatively warmer June and September temperatures and relatively cooler July temperatures. In these communities, there is not enough evidence to suggest which predictor is more accurate. The communities that showed that highest probability of microalgae growth were Arviat, Baker Lake, Bathurst Inlet, Chesterfield Inlet, Kugluktuk and Rankin Inlet. Communities with the lowest probability of growth were Arctic Bay, Clyde River, Grise Fiord, Hall Beach, Pond Inlet, Qikiqtarjuaq and Resolute.

Table 4-5: A summary of historic climate data and the probability of microalgae growth (using mean treatment season temperature and mean July temperature as predictors) for 25 communities in Nunavut. Temperature data shown are means ± standard deviations.

Community	Historic Climate Data				Probability of Microalgae Growth	
	Number of Years	First Year of Data	Mean Treatment Season Temp (°C)	Mean July Temp (°C)	Predictor: Mean Treatment Season Temp	Predictor: Mean July Temp
Arctic Bay	33	1939	3.8 ± 0.3	5.8 ± 0.3	6%	2%
Arviat	29	1973	8.5 ± 0.4	11.0 ± 0.6	99%	97%
Baker Lake	65	1946	8.1 ± 0.3	11.3 ± 0.4	98%	98%
Bathurst Inlet	7	1958	9.3 ± 1.6	12.1 ± 1.7	100%	99%
Cambridge Bay	76	1930	5.3 ± 0.3	8.6 ± 0.4	41%	45%
Cape Dorset	31	1963	5.1 ± 0.3	7.7 ± 0.6	31%	20%
Chesterfield Inlet	26	1923	7.4 ± 0.5	10.1 ± 0.6	94%	89%
Clyde River	64	1943	3.1 ± 0.2	4.7 ± 0.3	2%	0%
Coral Harbour	72	1934	6.0 ± 0.3	9.2 ± 0.4	67%	68%
Gjoa Haven	25	1986	4.7 ± 0.5	7.9 ± 0.5	22%	24%
Grise Fiord	11	1974	2.3 ± 0.9	4.0 ± 1.0	1%	0%
Hall Beach	57	1958	3.6 ± 0.3	6.2 ± 0.4	5%	3%
Igloolik	31	1978	4.3 ± 0.4	7.3 ± 0.6	13%	12%
Iqaluit	68	1946	5.9 ± 0.2	8.0 ± 0.3	63%	27%
Kimmirut	15	1914	6.2 ± 0.5	8.1 ± 0.8	73%	31%
Kugaaruk	56	1959	4.4 ± 0.5	7.9 ± 0.6	14%	25%
Kugluktuk	79	1933	7.3 ± 0.3	10.1 ± 0.4	94%	88%
Naujaat	20	1974	5.8 ± 0.5	8.9 ± 0.8	57%	58%
Pangnirtung	23	1931	6.4 ± 0.4	8.4 ± 0.5	77%	38%
Pond Inlet	55	1924	3.9 ± 0.3	6.1 ± 0.4	7%	2%
Qikiqtarjuaq	53	1959	2.7 ± 0.4	4.7 ± 0.4	1%	0%
Rankin Inlet	34	1981	7.9 ± 0.4	10.6 ± 0.5	97%	94%
Resolute	67	1948	1.6 ± 0.3	4.5 ± 0.3	0%	0%
Taloyoak	46	1953	4.9 ± 0.4	8.0 ± 0.4	25%	28%
Whale Cove	27	1978	7.2 ± 0.5	9.3 ± 0.7	92%	71%

An analysis of community latitude versus microalgae growth was conducted to determine the influence of geographical location. The results are shown in Figure 4-9. Latitude was not a good predictor of microalgae growth for latitudes below 70°N. For communities above 70°N, it is expected that microalgae growth is not probable.

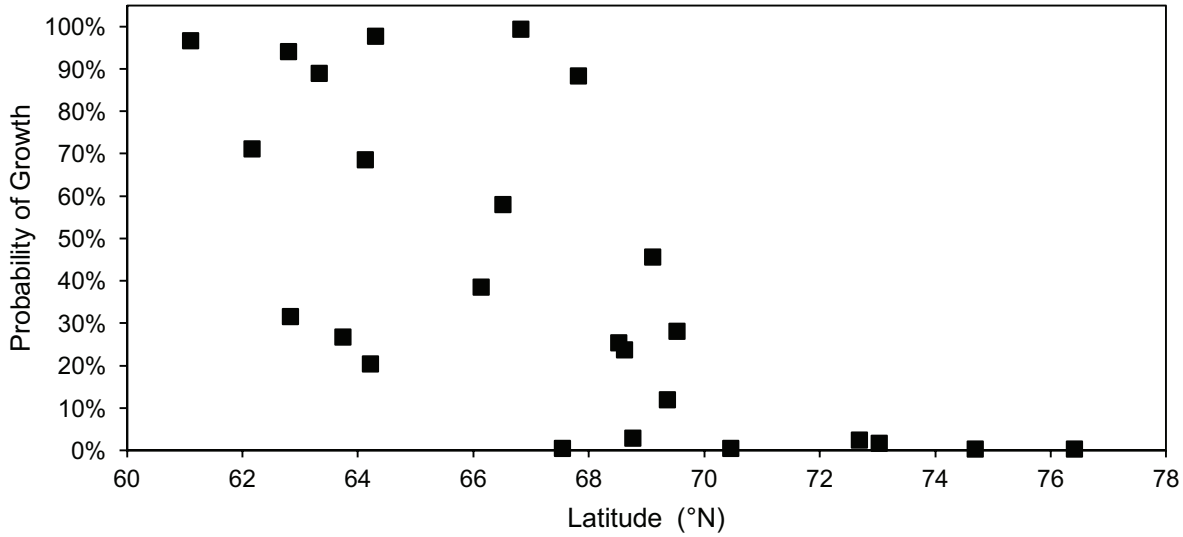

Figure 4-9: Probability of microalgae growth versus the latitude of the communities evaluated

4.6 Conclusions

A stochastic ecological model with an integrated equilibrium temperature model was developed to predict microalgae growth and phosphorus removal in cold region WSPs. Based on the modelled results, the following conclusions were drawn:

- An equilibrium temperature model provided adequate predictive capabilities for water temperature on a daily time step.
- A Monte Carlo Simulation provided a realistic and robust method to account for model parameter uncertainty.
- The mean July temperature and the mean treatment season temperature were determined to be the best individual predictors of the probability of microalgae growth using a logistical regression. They had predictive success rates of 93 and 88%, respectively.
- It is likely that microalgae growth can consistently occur in a cold region WSP provided sufficient temperatures are reached. The threshold (50% probability) for microalgae growth was 8.7 and 5.6°C for July temperature and mean treatment season temperature, respectively.
- Soluble phosphorus concentrations consistent with secondary treatment could be achieved if WSP depth is less than 2 m and the WSP is operating facultatively. Modelled concentrations were consistent with the field data found in Grise Fiord in 2011.

The model was used as a screening tool to assess geographical climatic constraints. Based on the results, it is likely that WSPs can be operated facultatively in some communities in Nunavut assuming they are designed appropriately in terms of organic loading rates and depth. While the model provided promising results, further field data is required to validate its predictive capabilities.

5 Conclusions and Recommendations

5.1 Summary and Conclusions

The purpose of this research was to evaluate the use of passive WSPs for phosphorus removal in arctic climates. This research was conducted in response to impending federal wastewater regulations in Canada and looks to address previous knowledge gaps associated with the performance of cold region WSPs with regards to phosphorus removal.

Monitoring of full scale WSP systems was conducted in four arctic communities (Kugaaruk, Pond Inlet, Clyde River and Grise Fiord, Nunavut) over a period of four years. During the study period, WSPs in Kugaaruk, Pond Inlet and Clyde River had high effluent phosphorus concentrations. Mean effluent concentrations ranged from 5.9 to 11.2 mg P/L. This was partially due to elevated raw wastewater concentrations attributed to low per capita water usage in the communities. Over the study period, these systems operated anoxically with little microalgae growth. The Grise Fiord WSP differed from the other studied WSP. It operated aerobically with significant microalgae production during a July 2011 site visit. During the microalgae bloom phosphorus effluent concentrations were 3.5 mg P/L. It was estimated that the phosphorus concentration was comprised mainly of microalgae particulate phosphorus. While in Kugaaruk and Pond Inlet, it was found that the effluent phosphorus was comprised mainly of SRP. Sediment analysis in Kugaaruk and Pond Inlet showed that organic phosphorus and phosphorus bound to iron and aluminum represented the highest fraction of sediment phosphorus.

To better understand microalgae growth in cold region WSPs, a laboratory investigation was done to quantify microalgae growth and phosphorus uptake, and identify microalgae phosphorus uptake mechanisms under simulated summer (ie. ice free) conditions. Maximum specific growth rates ranged from 0.029 to 0.058/h and were similar to those previously observed at temperate climates. Biomass phosphorus concentrations ranged from 1.5 to 3.3% which is 45% greater than concentrations observed at temperate climates. It was concluded that microalgae growth, and subsequently phosphorus uptake, should not be hindered in cold regions when temperatures exceed 10°C.

Taking into account the field and laboratory findings, a stochastic ecological model with an integrated equilibrium temperature model was developed. The aim of the model was to predict the total phosphorus effluent concentrations and the probability of microalgae growth in various communities in Nunavut over multiple years. The model included an equilibrium temperature model, which related various climate parameters to water temperature, to expand the dataset of available climate data needed to drive the model. The equilibrium temperature was adequate for predicting water temperatures on a daily time step. To account for parameter uncertainty a Monte Carlo Simulation approach was used for both the ecological model and the equilibrium temperature model. Mean July and mean treatment season (June 1-September 15) temperature were determined to be the best individual predictors of the probability of microalgae growth using logistic regression. They had predictive success rates of 93 and 88%, respectively. The threshold temperature for 50% probability of microalgae growth was 8.7°C for mean July temperature and 5.6°C for mean treatment season temperature. The model results

indicated that soluble phosphorus concentrations consistent with secondary treatment could be achieved if WSP depth is less than 2 m. This was consistent with field data found in Grise Fiord in 2011. Limited data was available to validate the findings of the model, therefore further verification is required before the model can used for design purposes.

5.2 Recommendations for Future Research

Based on the findings and limitations of this research, there are several recommendations for future field and modelling research.

5.2.1 Recommended Field Research

A microalgae bloom was only observed once during the study period. Further in depth investigations are required in order to fully understand phosphorus removal during these events. Investigations could include:

- Tracking phosphorus concentrations throughout a treatment season when a microalgae bloom occurs. This would provide further insight into the relationship between microalgae growth and phosphorus removal. Investigations could include evaluating whether phosphorus becomes a limiting nutrient for microalgae growth and determining whether there is an optimal time and/or strategy to decant the WSP. Since the WSPs are operated as batch reactors, it is possible that decanting should occur earlier and/or more often to mitigate phosphorus release from the sediment layer.

- Quantifying the fraction of soluble and particulate phosphorus during a bloom. In this study, soluble phosphorus was only able to be estimated after the site visit. This would demonstrate the effect of particulate/microalgae removal (e.g. settling pond, filtration).
- Identifying luxury uptake biomass percentages. This would allow for verification of laboratory results and model assumptions.
- Investigating sediment phosphorus stability under varying seasonal redox and pH conditions. This could include similar fractionation experiments as shown in Chapter 2 or potentially other laboratory based experiments.
- Investigating the role/presence of cold adapted microalgae. This could help explain the results found in Grise Fiord and look at the impacts of climate change.

An expansion of the dataset gathered during this research project will allow for further verification of the model presented in Chapter 4. Investigations should focus on shallow WSPs (< 2 m) in climates that can consistently produce the threshold temperatures determined in Chapter 4. Other potential field research could investigate microalgae removal and/or harvesting strategies to mitigate transmission to receiving waters and potential development of useful byproducts such as fertilizer or biofuel.

5.2.2 Recommended Model Research

The model presented in Chapter 4 was limited by the lack of verification data available. Further verification could be integrating into future field studies. Another option is to adapt the model for other regions where data is available, allowing for further

verification. Adapting the model for other regions does not verify the model for use in Nunavut, however it would help justify the continued use of the modelling framework.

The model only evaluated biological phosphorus removal, however the integration of chemical phosphorus removal would allow for a more complete understanding of treatment performance.

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Appendix A: R code for ecological model with integrated equilibrium temperature model

```
## This is an ecological model with integrated equilibrium
 temperature model to predict maximum microalgae
 concentrations and effluent phosphorus concentrations in
 intermittent discharge waste stabilization pond with
 seasonal ice cover. The model was developed using data from
 Nunavut, Canada.

## Monte Carlo Parameters
n <- 1:1000 ##number of runs
Kt <- runif(length(n), 0.002, 0.004) ## Temperature Model K
alpha <- runif(length(n), 1.4, 2.4) ## slope in Temp Model
beta <- runif(length(n), 1, 4) ## intercept from Temp Model
mort <- runif(length(n), 0.02, 0.030) ## Death and Settling
 rate
maxu <- runif(length(n), 0.04, 0.05) ## Growth rate
nogrow <- runif(length(n), 5, 8) ## minimum growth
 temperature
background <- runif(length(n), 1, 5) ## Background/min TSS
P <- runif(length(n), 0.016, 0.027) ## biomass phosphorus
 concentration
Light <- runif(length(n), 75, 125) ## optimal light
parameters <- data.frame(Kt, alpha, beta, mort, maxu,
 nogrow, background, P, Light)

## Climate data, form: [Year; Month; Day; Hour; Temperature
 (°C); Solar Radiation (W/m²)]
climate <- read.csv("climateData.csv")

years <- unique(climate$Year)
numYears <- length(years)
Final <- data.frame()
loc <- 1
```

```
## Loop through all Monte Carlo Parameter values
for (j in n) {

loc <- 1

## Initialize parameter value for run 'j'
K_Temp <- parameters[j,1] ## Temperature Model K
a <- parameters[j,2] ## slope in Temp Model
b <- parameters[j,3] ## intercept from Temp Model
Out <- parameters[j,4] ## Death and Settling rate
u <- parameters[j,5] ## Growth rate
Tmin <- parameters[j,6] ## minimum growth temperature
TSS_background <- parameters[j,7] ## Background/min TSS
BioP <- parameters[j,8] ## biomass phosphorus concentration
OptLight <- parameters[j,9] ## optimal light

## Loop through each year of data using parameter values
 for run 'j'
for (i in years) {

## Determine temperature and solar radiation for year 'i'
climate_year <- subset(climate, Year == i)

## Set start depth, will loop through for 1, 2, 3, 4, 5 m
 later
depth <- 1

simlength <- length(climate_year[,1])
airTemp <- data.frame(climate_year[,5])

## Determine water temperature, set initial values = air
 temperature
Temp <- data.frame()
Temp[1,1] <- airTemp[1,1]
m <- 2

## Determine remain water temperature values using
 equilibrium model
while (m < (simlength+1)) {
```

```
dTemp <- (K_Temp/(1000*0.1*0.00419))*(((a*airTemp[m-1,1])-
b)-Temp[m-1,1])
Temp[m,1] <- Temp[m-1,1] + dTemp
m <- m+1
}


## Run ecological model for each depth
while (depth<6) {

## Initial conditions
TSSinitial <- 5
TPinitial <- 7.5
t <- 1
Qin <- 3167 ##Kugaaruk, units L/hr
TPinflow <- 12

## WSP size
Volume <- 55015 ##units: m^3
Area <- Volume/depth ##units: m^2
ActiveDepth <- 0.1
ActiveVolume <- (Area*ActiveDepth*1000*1000)  ##units: L
RemainingVolume <- (Area*(depth-ActiveDepth)*1000*1000)
 ##units: L


## Calculate/Set rates
Input <- Qin/ActiveVolume  ##units: 1/hr
TSSout <- Out ##death and sinking, units: 1/hr
u20 <- u ##1/hr
k <- 23.789 ##light attentuation units:m
biomassP <- BioP

## Results
TSS <- data.frame()
TSS[1,1] <- TSSinitial
P <- data.frame() ## Active/Photic Layer P
P[1,1] <- TPinitial
Pbottom <- data.frame() ## Non-active/Aphotic layer
```

```
Pbottom[1,1] <- TPinitial
time <- data.frame()
time[1,1] <-t/24
Production <- 0

while(t<simlength) {

## Determine temperature and solar radiation
SolarRadiation <- climate_year[t,6]
temp <- Temp[t,1]

TSS[t,1] <- TSS[t,1]

## Determine growth rate
u_now <- ifelse(temp<Tmin, 0, u20*exp(-0.00338212*(temp-
 20)^2))
light <- (SolarRadiation/0.42)*(1-exp(-
 k*ActiveDepth))/(k*ActiveDepth)
if(light>OptLight) light_lim = 1 else light_lim =
 (light/OptLight)*exp(1-(light/OptLight))
grow <- u_now*light_lim

## Determine microalgae growth, total production and
 death/settling
TSSgrowth <- TSS[t,1]*grow
Production <- Production+TSSgrowth
TSSdeath <- -TSS[t,1]*TSSout

## Determing P uptake
uptakeP <- TSSgrowth*biomassP

## Inflow/Outflow
TSSflux <- -TSS[t,1]*(Input)
Pflux <- (TPinflow-P[t,1])*Input

## Next time step
TSS[t+1,1] <- TSS[t,1] + TSSgrowth + TSSflux + TSSdeath
if(TSS[t+1,1]<TSS_background) TSS[t+1,1] = TSS_background
 else TSS[t+1,1] = TSS[t+1,1]
```

```
P[t+1,1] <- P[t,1] + Pflux - uptakeP
P[t+1,1] <- ifelse((P[t+1,1]<0),0,P[t+1,1])
Pbottom[t+1,1] <- Pbottom[t,1]

## Equalize photic and aphotic zone P
P[t+1,1] <- ((P[t+1,1]*ActiveVolume) +
 (Pbottom[t+1,1]*RemainingVolume))/(ActiveVolume+RemainingVo
 lume)
Pbottom[t+1,1] <- P[t+1,1]

t<- t+1

}

## Determine position in Final data frame to save data
 without overwriting
pos <- (5*numYears)*(j-1)+loc

Final[pos,1] <- i
Final[pos,2] <- depth
Final[pos,3] <- Production*ActiveVolume
Final[pos,4] <- P[simlength,1]
Final[pos,5] <- max(TSS)
Final[pos,6] <- K_Temp
Final[pos,7] <- a
Final[pos,8] <- b
Final[pos,9] <- Out
Final[pos,10] <- u
Final[pos,11] <- Tmin
Final[pos,12] <- TSS_background
Final[pos,13] <- BioP
Final[pos,14] <- OptLight

loc <- loc+1
depth <- depth+1

}

}
```

```
}

## Final data frame column names
colnames(Final) <- c("Year", "Depth", "Production", "TP",
 "Max Microalgae", "Temp Model K", "Temp Model alpha", "Temp
 Model beta", "Death/Settling rate", "Growth rate", "Min
 Growth Temp", "Background TSS", "Biomass P", "Optimal
 Light")

write.csv(Final, file="Results.csv")
```

Appendix B: R code for post processing microalgae results

```
## Read in climate data

climate <- read.csv('climateData.csv')

## Determine predictor values for future analysis

agg_averageTemp <- aggregate(list(Temp = climate$Temp),
 list(Year = climate$Year), FUN=mean)
agg_JuneTemp <- subset(climate, Month==6)
agg_JuneTemp <- aggregate(list(Temp = agg_JuneTemp$Temp),
 list(Year = agg_JuneTemp$Year), FUN=mean)
agg_JulyTemp <- subset(climate, Month==7)
agg_JulyTemp <- aggregate(list(Temp = agg_JulyTemp$Temp),
 list(Year = agg_JulyTemp$Year), FUN=mean)
agg_AugTemp <- subset(climate, Month==8)
agg_AugTemp <- aggregate(list(Temp = agg_AugTemp$Temp),
 list(Year = agg_AugTemp$Year), FUN=mean)
agg_SeptTemp <- subset(climate, Month==9)
agg_SeptTemp <- aggregate(list(Temp = agg_SeptTemp$Temp),
 list(Year = agg_SeptTemp$Year), FUN=mean)

agg_averageSolar <- aggregate(list(Solar = climate$Solar),
 list(Year = climate$Year), FUN=mean)
agg_JuneSolar <- subset(climate, Month==6)
agg_JuneSolar <- aggregate(list(Solar =
 agg_JuneSolar$Solar), list(Year = agg_JuneSolar$Year),
 FUN=mean)
agg_JulySolar <- subset(climate, Month==7)
agg_JulySolar <- aggregate(list(Solar =
 agg_JulySolar$Solar), list(Year = agg_JulySolar$Year),
 FUN=mean)
agg_AugSolar <- subset(climate, Month==8)
agg_AugSolar <- aggregate(list(Solar = agg_AugSolar$Solar),
 list(Year = agg_AugSolar$Year), FUN=mean)
agg_SeptSolar <- subset(climate, Month==9)
agg_SeptSolar <- aggregate(list(Solar =
```

```
 agg_SeptSolar$Solar), list(Year = agg_SeptSolar$Year),
 FUN=mean)

## Read in results
results <- read.csv('Results.csv')

## Remove results columns that are not needed
results <- subset(results, Depth==1)
results <- subset(results, select = -
 c(Temp.Model.K:Optimal.Light))
results <- subset(results, select = -c(Depth:TP))
results <- subset(results, select = -X)

x <- length(results$Max.Microalgae)


## Set max allowable microalgae concentration

for (i in 1:x) {

 results[i,2] <- ifelse(results[i,2]>400, 400, results[i,2])

 year <- subset(agg_averageTemp, Year==results[i,1])

 results[i,3] <- year[1,2]

}

colnames(results) <- c("Year", "Algae", "Average Temp")

## Round microalgae to nearest 10

results$Algae <- round(results$Algae,-1)

## Write function to determine the mode

Mode <- function(x) {
  ux <- unique(x)
  ux[which.max(tabulate(match(x, ux)))]
```

```
}

## Calculate most common microalgae concentration

agg_algae <- aggregate(list(Algae = results$Algae),
 list(Year = results$Year), FUN=Mode)

y <- unique(agg_algae$Year)
z <- 1
num <- data.frame()

## Calculate fraction of most common microalgae
 concentration

for (i in y) {

 dat <- subset(results, Year==i)
 num[z,1] <- sum(dat$Algae == agg_algae[z,2])
 num[z,1] <- num[z,1]*length(y)/x
 z <- z+1

}

final <- data.frame(agg_algae$Year, agg_averageTemp$Temp,
 agg_JuneTemp$Temp, agg_JulyTemp$Temp, agg_AugTemp$Temp,
 agg_SeptTemp$Temp, agg_averageSolar$Solar,
 agg_JuneSolar$Solar, agg_JulySolar$Solar,
 agg_AugSolar$Solar, agg_SeptSolar$Solar, agg_algae$Algae,
 num$V1)
colnames(final) <- c('Year','Average Temp', 'Average June
 Temp', 'Average July Temp','Average Aug Temp','Average Sept
 Temp', 'Average Solar', 'Average Solar June', 'Average
 Solar July','Average Solar Aug','Average Solar
 Sept','Algae', 'Probability')

write.csv(final, file='PostResults.csv')
```

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Appendix D: Chapter 3 Copyright Information

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