Open Access Theses & Dissertations

2012-01-01

Recent Changes In The Growth And Nutrient Limitation Of Benthic And Pelagic Algae In Arctic Tundra Ponds

Christina Hernandez
University of Texas at El Paso, chernandez8@miners.utep.edu

Follow this and additional works at: https://digitalcommons.utep.edu/open_etd

Part of the Ecology and Evolutionary Biology Commons, Environmental Sciences Commons, and the Fresh Water Studies Commons

RECENT CHANGES IN THE GROWTH AND NUTRIENT LIMITATION OF BENTHIC AND PELAGIC ALGAE IN ARCTIC TUNDRA PONDS.

CHRISTINA HERNANDEZ

Environmental Science Program

APPROVED:

_____

Vanessa L. Lougheed, Ph.D., Chair

_____

Elizabeth Walsh, Ph.D.

_____

Lixin Jin, Ph.D.

_____

Aaron Velasco, Ph.D.

.

_____
Benjamin C. Flores, Ph.D.
Dean of the Graduate School

Dedication

I dedicate this thesis to my family: my sisters Laura, Veronica and Miriam, to my brothers Danny, Eddie, and Mike, and to my loving mother Hortencia. I also dedicate this work to Zoe, Zia, Yamileth, and Yahir; all I do, I do for you.

RECENT CHANGES IN THE GROWTH AND NUTRIENT LIMITATION OF

BENTHIC AND PELAGIC ALGAE IN ARCTIC TUNDRA PONDS.

by

CHRISTINA HERNANDEZ, B.S.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science

Environmental Science Program

THE UNIVERSITY OF TEXAS AT EL PASO

December 2012

Acknowledgements

I would like to thank my advisor, Dr. Vanessa L. Lougheed; you have really encouraged me and brought me out of my shell as a scientist but also as a person. I want to thank you V, for believing in me even when I didn't believe in myself. I thank you for friendship and love.

I would also like to thank my lab mates: Mariana Vargas, Christian Andresen, Nickole Miller, Frankie Reyes, Gabriela Contreras, Ector Martell, and Bianca Luong. Your help and collaborations are greatly appreciated and I have learned so much from all of you. Thanks to my friends, Sandra Villarreal and Fernanda De La Cerda, for lending your ears and your hearts on countless occasions.

I would also like to thank my committee: Dr. Elizabeth Walsh, Dr. Lixin Jin, and Dr. Aaron Velasco; you are all great examples of scientists I admire and look up to. I want to thank Dr. Craig Tweedie, John Hobbie, Vera Alexander and the National Science Foundation (ARC-0909502), leading the way for aspiring minds.

Abstract

Arctic ponds are a dominant feature in Barrow, Alaska. Ponds may function as carbon sinks during the growing season from production of photosynthetic organisms like algae; however, little work has been done on this topic. Environmental changes have been occurring in the Arctic stemming from climate change and human perturbations. The focus of this study was to assess a historically studied region for changes in algal primary production that may have been produced due to increases in temperature over the past 40 years and increased human development. Additionally, this study aimed to determine nutrient limitation of algal production in the pelagic and benthic zone in order to attain a better understanding of algal primary production differences among zones. In order to do so, several methods were employed including nutrient diffusing substrate experiments, bottle incubations, and mesocosms. Compared to 40 years ago, increases in phytoplankton biomass were observed in the historically studied region. Periphyton was not significantly higher than the 1970s; however, benthic algae were more productive in sites located closer to the village of Barrow suggesting influences from anthropogenic activity. Since a human impact gradient effect on algal production at the historic site was not observed, other nutrients sources such as permafrost thaw are a likely contributor to algal growth. Nutrient limitation status of phytoplankton has changed since the original study in the historical region from phosphorus (P) limitation to nitrogen (N) or NP co-limitation. Contributions of phosphorus from deglaciation, permafrost thaw, and other sediment interactions may potentially be causing nitrogen limitation. Comparisons of pelagic and benthic experiments revealed differences among these zones with NP limitation in the pelagic zone and N limitation in the benthic zone, reflecting contrasting nutrient limitation status within the same pond. Mesocosm experiments provided support for NP limitation in the water column but, due to experimental limitations, there were no significant findings in the benthos. These findings reflect the changes occurring in Arctic ponds in light of warming temperatures and human perturbations. Further studies are needed in order to fully understand recent and future expected changes in primary production and nutrient cycling in both the pelagic and benthic zones.

Table of Contents

List of Tables

List of Figures

Introduction

The Arctic is especially sensitive to climate change due to polar amplification (Smol et al. 2005). A multitude of changes have been observed in Arctic freshwater ecosystems. Smol and Douglas (2007) have shown that arctic ponds are changing in size and conductance due to evaporation/precipitation ratios. Studies have also reported warmer temperatures (Lougheed et al. 2011), increased nitrogen and phosphorus levels in the water column (Lougheed et al. 2011), increases in algal primary production (Michuletti et al. 2005), and taxonomic shifts in algae (Smol et al. 2005) in Arctic freshwaters. Increased concentrations of nutrients in arctic aquatic systems have been observed or predicted, and these may be attributed to increased evaporation caused by higher temperatures (Schindler and Smol 2006) or thawing of permafrost (Hobbie et al. 1999; Frey and Smith 2005; Frey and McClelland 2009). Together with nutrient enrichment, warming has the potential to greatly increase Arctic aquatic production (Flanagan et al. 2003).

Experimental manipulations have indicated that increased nutrients may potentially lead to increased primary production and other food web effects in arctic streams and lakes (e.g. Peterson et al. 1993; Hobbie et al. 1999, Slavik et al. 2004; Bensted et al. 2005; Lienesh et al. 2005; O'Brien et al. 2005). A long-term study of an Arctic stream found important short and long term changes in stream function with continuous phosphorus enrichment since 1983 (Slavik et al. 2004). Short term responses included increases in periphytic production, insect densities, and fish growth. After 4 years of manipulation however, this study found there was a decrease in grazing pressures due to the replacement of periphyton by moss (Peterson et al. 1985). Moss production increased tenfold which in turn had impacts on NH_4 uptake, primary production, habitat structure, insect population densities and species composition suggesting strong implications to nitrogen cycling and food web composition. This study also found moss species were replaced with mosses typical to environments with high nutrients, temperature, and light (Slavik et al. 2004).

Given the importance of freshwater environments in the Arctic, notably on the Alaskan Arctic Coastal Plain where more than 20% of the land is covered by thaw lakes, and 25-50% is marked by thaw basins filled with ponds (Hinkel et al. 2003; Frohn et al. 2005), understanding productivity of Arctic

tundra ponds is important as it may be a key contributor to carbon cycling, especially in light of the current shifting climate regimes.

Nutrient limitation status experiments are insightful indicators of ecosystem function and processes. Original thinking allowed one limiting nutrient at any given time (Liebig 1842); when that limiting nutrient is added, primary production increases until a point is reached when production is limited by the next limiting nutrient. Advancements in research through the past several decades have allowed for an increasing understanding of nutrient limitation and interpretation of categorization and status. Freshwater systems are typically nitrogen (N) limited, phosphorus (P) limited, or nitrogen-phosphorus (NP) co-limited. NP co-limitation may be the result of oscillations in nutrient limitation by N and P over time, in which changes in the supply of one nutrient may cause limitation to shift to the other nutrient (Allegier et al. 2010).

Meta-analyses by Francoeur (2001), Elser et al. (2007) and Harpole et al. (2011) have found a predominance of NP co-limitation of benthic and pelagic algae in freshwater ecosystems. However, there is a limited understanding of algal nutrient limitation in Arctic environments where elevated rates of warming and changes in nutrient loads could have drastic consequences for primary producers. Levine and Whalen (2001) determined NP co-limitation was predominant for phytoplankton in 54 lakes in the Arctic Foothills region of Alaska. In Alaskan boreal wetlands, mesocosm experiments by Wyatt et al. (2010) showed co-limitation of benthic algae by N in combination with P and/or Silica (Si). Symons et al. (2012), Ogbebo et al. (2009), and Bowden et al. (1992) also found NP co-limitation present in phytoplankton arctic and subarctic lake communities. While these more recent studies indicate that NP limitation is occurring in water bodies in Arctic, subarctic and boreal freshwaters, older studies indicated that P limitation was present. In an Alaskan tundra stream, Peterson et al. (1983) found evidence suggesting P limitation of periphyton. Similarly, in a whole-pond fertilization experiment, Alexander et al. (1980) found that phosphorus limited algal growth in an Arctic tundra pond. Recent studies examining TN:TP ratios, however, do suggest the continuing presence of P limitation in lakes of the Canadian Arctic (Keatley et al. 2007). In addition, Colorado alpine lakes impacted by N-deposition also experienced greater P-limitation (Elser et al. 2009). Application of further nutrient limitation

experiments in Arctic tundra ponds can provide important insight into changes in nutrient cycling and primary production in an environment that is warming faster than any other location on the planet.

While many studies aim to categorize the nutrient limitation status of a freshwater ecosystem, most studies focus on algal nutrient limitation of a single growth form, either benthic or pelagic algae. However, the few studies that address both benthic and pelagic nutrient enrichments found distinct differences among these habitats. Vadeboncoeur et al. (2001) found increased nutrients in Michigan lakes produced increases in phytoplankton and epixylon (wood-associated) but decreases in epipelon (sediment-associated). Studies by Turner et al. (1994) revealed that while phytoplankton photosynthesis was limited by phosphorus, benthic algae growing on rocks were limited by carbon. Working in a high Arctic lake, Bonilla et al. (2005) found that benthic algae mats showed no responses to nutrient enrichments of carbon, phosphorus and nitrogen, while phytoplankton showed strong responses to enrichments, suggesting benthic mats are nutrient sufficient. Differences may exist between the nutrient requirements of algae in the two zones, as different species within the same ecosystems have different nutrient requirements. Furthermore, while algae in the benthic zone interact with nutrients released from the sediment surface, phytoplankton and algae growing on hard surfaces draw their nutrients from the water column (Vadeboncoeur et al. 2001). Differences in environmental factors influence production efficiencies as phytoplankton are greatly affected by a thin highly variable boundary layer leading to more nutrient limitation while periphyton grow slower due to light limitations but have access to nutrients sources in sediments. Access to the sediment nutrient pool in addition to their relatively slow growth rate, make sediment-associated algae less likely to be nutrient limited than phytoplankton (Sand-Jensen and Borum 1991).

In the early 1970s, Barrow, Alaska hosted a detailed ecological survey, the International Biological Program (IBP) which thoroughly described the physical, chemical, and biological processes occurring in the arctic tundra ponds in the region (Hobbie 1980). While these ponds are frozen throughout the majority of the year, they thaw during the growing season (June-September) allowing primary producers to potentially act as carbon sinks by fixing carbon through photosynthesis. From 1971 to 1973, both observational and experimental data were collected, and in these studies two

discoveries were made regarding algal primary production: 1) with warming temperatures algal growth would be stimulated and 2) phosphorus was the limiting nutrient for phytoplankton growth (Alexander et al. 1980). More recently, Lougheed et al. (2011) found that pond temperatures have warmed by 2ºC and water column nutrients concentrations have increased significantly over the past 40 years. These two studies, one historical and one modern, form the foundation of this study. We first examine whether phytoplankton and periphyton biomass in IBP ponds has changed with warming and increased nutrients over the past 40 years, and determine whether any changes could be due to the expansion of the village of Barrow towards the IBP ponds, as Schindler and Smol (2006) suggest synergistic effects of climate change and human perturbations can have significant effects of Arctic freshwaters. Secondly, we utilize several methods to determine the nutrient limitation status of both phytoplankton and periphyton in these historically P limited ponds (Alexander et al. 1980), to determine changes over time and differences among algal growth forms.

Methods

Site Description

The IBP study sites were established in the late 1960s near the village of Barrow, Alaska, the northernmost human settlement in the United States. The aquatic portion of the IBP consisted of a series of small and shallow ponds within low-centered ice wedge polygons within a large thaw lake basin. The entire region is underlain by continuous permafrost, with the active layer thawing during the warm summer months. Water in the ponds is comprised of snowmelt and water flows between ponds only during spring; throughout the rest of the year there is no above or below-ground water flow (Hobbie, 1980).

Throughout the past 40 years, the population of Barrow has steadily increased and has been expanding towards the IBP sites, which were formerly isolated in the landscape. In order to assess the potential impacts of urbanization on the ecology of the IBP ponds, the IBP study sites (shown in yellow in Figure 1), were compared to the more heavily "impacted" ponds located within the village of Barrow (BRW; shown in red in Figure 1), and more isolated "reference" sites located in the Barrow Environmental Observatory (BEO) (shown in green in Figure 1). The BEO is a protected area set aside by the village of Barrow for the purposes of scientific research. Sites were chosen based on proximity to roads and human habitats. Barrow sites (Boxer, Ahmagoak, Utiqtuq, Kignak, Ahma 2, Boxer 2, and Airport; Table 1) are located within the village of Barrow and directly adjacent to roads and houses. IBP sites (IBP B, C, D, E, J, and X; Table 1) were historically more than 0.5 km from any roadways or houses; however, they are now less than 0.25 km from Kaleak Road. Pond sites in the BEO (ITEX-N, ITEX-S, WL02, WL03, and PRP; Table 1) are not near public main roads or homes; the nearest road is more than 1 km away.

Historical comparisons utilizing weekly data included data from ponds IBP B, C, and E due to availability of detailed current and historic data for those ponds (Alexander et al. 1980; Hobbie 1980; Table 1). Samples collected on a weekly basis over the summers in both the 1970s and 2009-2011 are included in the historical comparisons of algal biomass. Additional ponds (IBP D, J, and X), which were

sampled regularly, but less frequently in the 1970s, were included in the analyses of chlorophyll-nutrient relationships. In order to assess any influence from urbanization, a regional study was conducted on all IBP, BEO and BRW ponds in Table 1; data collected every 3 weeks during the summers of 2010-11 was utilized in these analyses. For logistical reasons, some sites in BRW that were sampled in 2010 were replaced with different but similar sites in 2011 (see Table 1). Nutrient limitation studies were conducted in four ponds in the IBP (B, C, D, and J; Table 1), and three ponds in BRW (Boxer, Ahmagoak and Utiqtuq; Table 1) and the BEO (ITEX-N, ITEX-S, and WL02; Table 1).

Figure 1: Location of IBP, Reference (BEO), and Impacted (BRW) pond sites in Barrow, Alaska.

Table 1: Location of ponds used in 3 different studies in 2010 and 2011. See methods section for description of studies

Site Name	Coordinates	Historical Comparison	Regional Comparison	Nutrient limitation study
IBP (Potentially Impacted)				
IBP B	71°17'41.82" N, 156°42'09.52" W	X	X	X
IBP C	71°17'40.63" N, 156°42'07.48" W	X	X	X
IBP D	71°17'41.29" N, 156°42'00.54" W	X	X	X
IBP E	71°17'39.24" N, 156°42'06.30" W	X	X	
IBP J	71°17'37.21" N, 156°42'04.93" W	X	X	X
IBP X	71°17'45.28" N, 156°42'00.30" W	X		
BEO (Reference)				
ITEX-N	71°18'59.53" N, 156°35'22.88" W		X	X
ITEX-S	71°18'44.16" N, 156°35'27.58" W		X	X
WL02	71°16'47.02" N, 156°37'8.01" W		X	X
WL03	71°16'56.33" N, 156°36'58.28" W		X	
PRP	71°17'15.05" N, 156°37'26.75" W		X	
Barrow (BRW; Impacted)				
BOXER	71°18'12.58" N, 156°45'09.25" W		X	X
Ahmagoak (AHMA)	71°18'13.04" N, 156°44'29.29" W		X	X
Utiqtuq (UTIQ)	71°18'07.02" N, 156°43'20.94" W		X	X
Kignak (KIGN) [*2010 only]	71°18'09.30" N, 156°43'07.29" W		X	
AHMA 2[*2011 only]	71°18'14.91" N, 156°44'33.49" W		X	
BOXER 2[*2011 only]	71°18'12.42" N, 156°45'12.12" W		X	
Airport[*2010 only]	71°16'58.03" N, 156°46'46.87" W		X	

Field Procedures

Historical and Regional Comparisons

During the summers of 2010 and 2011, from mid-June to mid-August, phytoplankton and periphyton samples were collected from ponds in the IBP, BEO, and Barrow regions. Samples were collected weekly at IBP and every 3 weeks at BEO and Barrow sites. Samples were also collected at IBP ponds weekly from July to August 2009, and only once in August 2008. Phytoplankton samples were collected by filtering up to 1 L of pond water through GF/C filters. Filters were stored in test tubes, wrapped in foil and kept frozen until analysis. Periphyton samples were collected from pond sediment surfaces at three haphazard locations in each pond using a spatula and an inverted petri dish. All three periphyton samples were combined into one composite sample. Algae were separated from the sediment by rinsing with distilled water, pouring off the supernatant solution and repeating until the surface water ran clear. A subsample consisting of 9 (2011) or 13 mL (2010) of the solution containing algae was stored in a test tube, wrapped in foil and frozen until analysis. At all sites, samples for water chemistry were collected from an open-water location, filtered as required, and stored frozen in acid-washed bottles until analysis.

Nutrient Limitation Experiments

In 1970 and 71, Alexander et al. (1980) completed a whole pond nutrient addition experiment whereby they added phosphorus to IBP Pond D, with IBP Pond C acting as a control pond (i.e. ambient conditions). We present analyses of these two years of data.

Nutrient diffusing substrates (NDS) and bottle nutrient incubations, which expose natural algal communities to known quantities of nitrogen (N) and phosphorus (P), were utilized to determine benthic and pelagic algae nutrient limitation, respectively, during the summers of 2010 and 2011. In both July and August, nutrient diffusing substrates were incubated *in situ* by deploying 60 mL vials of 2% Bacto agar solution containing concentrations of 0.5M KH_2PO_4 (P), 0.5M $NaNO_3$ (N) or both (N+P) and control vials (C) contained agar only (Fairchild et al. 1985). All vials were covered by 20μm Nitex

filters, attached to a plexiglass frame, placed on bottoms of 10 ponds representing all 3 regions (Table 1), and incubated for 21-22 days. Four replicates of each treatment were deployed at each site. Most sites had NDS incubations on 4 separate dates (i.e. July 2010, August 2010, July 2011, August 2011); however, for logistical reasons, one to two sites per region are missing data on one date only (IBP D on July 2010, ITEX-N on July 2010, WL02 on July 2010, AHMA on August 2010, UTIQ on July 2010). After incubations, filters were removed from vials, stored in test tubes, wrapped in foil and frozen until analysis.

To examine the impact of nutrient enrichment in the pelagic zone, water was collected from the water column in all three regions during July and August 2011, filtered through an 80 μm mesh net to remove large invertebrates, dispensed into 500 mL clear bottles, and attached to a PVC frame that suspended the bottles 20 cm below the water surface. Four replicates of each treatment were deployed at each site. All bottles were incubated in a single pond to ensure similar ambient conditions. The enrichment treatments consisted of 400 μg/L KH_2PO_4 (P),400 μg/L NH_4NO_3 (N), a combination of both 400 μg/L N & 400 μg/L P (NP), and a control (C) treatments. Because phytoplankton take less time to react to nutrient enrichments (Sand-Jensen et al. 1991), incubations lasted only approximately 96 hours after which water was filtered through GF/C filters. Filters were stored in test tubes, wrapped with foil, and frozen until analysis.

A third nutrient limitation experiment was used in order to determine concurrent benthic and pelagic nutrient limitation *in situ*. Mesocosms were constructed with clear plastic sides and aluminum collars pushed into the sediment; the upper edge of the mesocosm was held upright with a PVC frame (Figure 2). Four groups of mesocosms were installed into IBP 19 (156° 42'09.89" W, 71° 17'36.71" N), with one replicate of each treatment (i.e. N, NP, P, and C) in each group (total of 16 mesocosms). The average depth of each mesocosm was 23.6 cm, which reflected the average depth of the ponds, and the average volume was approximately 0.05 m^3. Nutrient concentrations in each mesocosm were elevated over a period of one week using KH_2PO_4 (P), NH_4NO_3 (N), a combination of both N & P (NP), and a control (C). Incubations of mesocosms lasted approximately 3 weeks in August 2011. Final concentrations of nutrients in the mesocosms were elevated to greater than 100 times ambient (TP), 170

times ambient (NO_3) and 235 times (NH_3). This was a preliminary experiment that will be repeated by others in 2012 using more realistic nutrient levels.

Figure 2: Mesocosms used in synchronized nutrient limitation experiment in August 2011.

Lab Procedures

In the lab, chlorophyll-a was extracted in 90% acetone for 24 hours in the freezer. Absorbance of the extractant was measured with a Genesis 10UV spectrophotometer. Samples were corrected for turbidity and phaeopigments by acidification (Likens and Wetzel 1991). Concentrations were calculated on a volumetric basis for phytoplankton ($\mu g/L$) and by area sampled for periphyton ($\mu g/cm^2$). In order to allow comparisons to data from the 1970's, the concentration of phytoplankton chlorophyll-a was also calculated using Strickland and Parson's (1968) formula, which was used in the original IBP study (Alexander et al. 1980). To allow comparison with historical data, periphyton concentrations were also calculated using the Lorenzen (1967) method (Alexander et al. 1980) and expressed on the basis of dry sediment ($\mu g/g$).

Nutrient chemistry (e.g. NH_3, NO_3, SRP, TP, TDP, Si, and DOC) was analyzed as described in Lougheed et al. (2011). These samples were analyzed by other students as part a related study. In 2010-

11, water temperature at IBP pond C was logged continuously using HOBO® loggers. Nutrient data from the 1970s were primarily extracted from Barsdate and Prentki (1973).

Statistical Analysis

To allow comparisons among 1970s and 2008-11, the data were first grouped by year and averaged according to the week of year. The averages of the historic data (1970-73) and the current data (2008-11) were then compared using a paired t-test using JMP statistical software.

The effect of nutrient limitation on algal productivity was evaluated by comparing increases in chlorophyll biomass relative to the control and by calculating percent increase in algal biomass relative to the controls. Analysis of Variance (ANOVA) followed by Tukey Kramer HSD statistical tests were used to determine significant differences among treatments using JMP statistical software.

Results

Historical Comparisons

Significantly greater amounts of phytoplankton chlorophyll-a biomass were observed during the growing seasons of 2008-11 compared to the 1970's (paired t-test; p= 0.0022; Figure 3) in IBP tundra ponds. However, periphyton was significantly higher in 1972 than in 2011 (paired t-test, p= < 0.0001; Figure 4); although, there was substantial variability among periphyton samples in the 1970s. Significantly higher maximum weekly temperatures were observed in 2010-11 as compared to 1970 and 1972 (paired t-test, p=0.005; Figure 5).

Figure 3: Mean (+/- 1 standard error (SE)) phytoplankton chlorophyll-a biomass in IBP ponds B, C, and E for 1970-1973 vs 2008-2010.

Figure 4: Mean (+/- 1 SE) periphyton chlorophyll-a biomass in IBP ponds B, C, and E in 1972 vs 2011.

Figure 5: Average maximum temperatures in IBP Pond C throughout weeks of year for 1970, 1972 and 2010-11.

Figure 6. Linear regressions of log chlorophyll-a phytoplankton biomass vs. log total phosphorus in IBP ponds during 1970s (y= -0.84 + 0.65logCHL-a, R^2= 0.33, p=0.11) and 2000s (p>0.10).

Figure 7. Linear regressions of log chlorophyll-a phytoplankton biomass vs. log ammonia in IBP ponds during 1970s (p>0.10) and 2000s (y= -0.03 + 0.11logCHL-a, R^2= 0.23, p= 0.04)

Relationships between phytoplankton biomass and nutrients varied depending on the years sampled. In the 1970s, phytoplankton chlorophyll-a biomass was marginally and positively related to total phosphorus (TP) in the water column (p= 0.11; Figure 6), but showed no relationship with NH_3. Forty years later, phytoplankton biomass was significantly dependent on NH_3 (p= 0.04; Figure 7) and not significantly influenced by total phosphorus. There were no significant relationships observed between phytoplankton biomass and NO_3 or soluble reactive phosphorus (SRP) in either the 1970s or present time. There was insufficient data to create similar relationships for periphyton.

Regional Comparisons

In 2010 and 2011, Barrow was the most productive region with respect to periphyton, but not phytoplankton (Figures 8 and 9). Periphyton was significantly higher in Barrow ponds in both 2010 ($p<0.0001$; Figure 8) and 2011 ($p= 0.0201$; Figure 9). While phytoplankton tended to be higher in Barrow ponds, this difference was not significant in either year. In 2010, BEO ponds had significantly greater phytoplankton biomass than the IBP ponds ($p= 0.0194$; Figure 8).

Figure 8: Mean (1 +/- SE)) phytoplankton (left) and periphyton (right) chlorophyll-a biomass in IBP, BEO, and Barrow ponds during 2010.

Figure 9: Mean (1 +/- SE)) phytoplankton (left) and periphyton (right) chlorophyll-a biomass in IBP, BEO, and Barrow ponds during 2011.

Most nutrients, including total phosphorus (TP), soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), silica (Si), and dissolved organic carbon (DOC) were significantly higher in the Barrow ponds as compared to the BEO and IBP ponds (Table 2). Ammonia (NH_3) was significantly lower in the IBP ponds. When 2010 and 2011 data were combined, periphyton biomass remained significantly higher in Barrow ponds, while there was no difference in phytoplankton biomass among the three regions.

Table 2. Mean chemical and biological characteristics of sampled ponds in IBP, BEO, and BRW regions in 2010 and 2011 (± standard error). Asterisks (*) indicate a significant difference from all other regions (ANOVA, Tukey HSD, $p<0.05$).

Site Name	N	CHL-a (µg/L)	CHL-a (µg/cm²)	NH$_3$ (µg/L)	NO$_3$ (µg/L)	TP (µg/L)	SRP (µg/L)	TDP (µg/L)	Si (µg/L)	DOC (mg/L)
IBP	47	1.01 ± 0.20	2.55 ± 0.35	31.47 ± 4.62*	8.42 ± 13.97	33.69 ± 3.86	5.65 ± 0.76	27.36 ± 4.08	97.42 ± 13.97	18.83 ± 0.69
BEO	40	1.45 ± 0.17	3.15 ± 0.53	57.98 ± 12.07	10.58 ± 1.25	34.61 ± 4.92	7.15 ± 0.91	27.30 ± 3.80	119.22 ± 21.87	22.69 ± 1.29
Barrow	41	1.69 ± 20.37	13.2 ± 2.86*	67.18 ± 6.85	12.71 ± 0.95	80.73 ± 6.91*	13.62 ± 1.90*	62.22 ± 7.12*	534.29 ± 87.02*	29.42 ± 1.23*

Nutrient Limitation

Figure 10. Mean (+/- 1SE) NH_3 (left) and NO_3 (right) in IBP C (ambient) and D (enriched) during the 1970 and 1971 growing season. Asterisks (*) indicate significant differences among treatments in the same year ($p<0.05$); crosses ($\dagger$) indicate marginally significant differences among treatments in the same year ($p<0.10$).

Figure 11. Mean (+/- 1SE) SRP (left) and TP (right) in IBP C (ambient) and D (enriched) during the 1970 and 1971 growing season. Asterisks (*) indicate significant differences among treatments in the same year ($p<0.05$); crosses ($\dagger$) indicate marginally significant differences among treatments in the same year ($p<0.10$).

Figure 12. Mean (+/- 1SE) phytoplankton chlorophyll-a biomass in IBP C (ambient) and D (enriched) during the 1970 and 1971 growing season. Asterisks (*) indicate significant differences among treatments in the same year (p<0.05); crosses ($\dagger$) indicate marginally significant differences among treatments in the same year (p<0.10).

Nutrient limitation experiments in the 1970's consisted of a whole pond nutrient enrichment of IBP pond D with phosphorus during 1970 and 1971; IBP pond C was used a reference pond (Alexander 1980). Analyses of these historical data indicate, as expected, higher concentrations of SRP in the enriched pond in both 1970 and 1971 (p<0.05) and TP in 1970 (p<0.05; Figure 11). Concentrations of NH_3 were marginally higher in the enriched pond in 1970 (p<0.10); NO_3 was significantly higher in 1970 and 1971 (p<0.05) (Figure 10). Additionally, phytoplankton biomass was significantly higher in the enriched pond in 1970 (p<0.05) and marginally higher in 1971 (p<0.10) (Figure 12).

Current pelagic experiments showed more occurrences of N limitation (45%) compared to benthic experiments (25%). NP co-limitation was equally frequent, with 25% of observations, in both pelagic and benthic experiments (Table 3 and 4; Figures13 and 14). The absence of significant nutrient limitation was frequently observed in the benthic environment (50%) (Figure 14); P-limitation was only observed at one site (WL02) in the pelagic experiment (Table 3).

We were unable to examine year-to-year differences in phytoplankton nutrient limitation since these experiments were only conducted in 2011; there tended to be no intra-annual trends in limitation

(Table 3). Very little intra-annual variation in the type of nutrient limitation was observed for periphyton, with either N or NP limitation occurring with the same frequency in either July or August; however, nutrient limitation in the benthic zone was more common in 2010 as compared to 2011 (Table 4).

When summarized by region, NP co-limitation dominated pelagic nutrient limitation experiments in all regions (Figure 15A). N limitation dominated benthic nutrient limitation experiments in IBP and BRW regions, whereas NP limitation occurred in the BEO (Figure 15B). Additionally, nutrient inhibition was observed in P treatments in IBP regions for both benthic and pelagic experiments and in BRW benthic experiments.

Table 3: Phytoplankton nutrient limitation by region and pond as determined by pelagic nutrient limitation experiments. Categories of nutrient limitation include: nitrogen limitation (N), phosphorus limitation (P) and NP co-limitation (NP). Significant increases of nutrient additions relative to controls are indicated by an asterisk (*) (ANOVA, Tukey HSD, p<0.05).

Region	Pond	Date	N	NP	P	Nutrient Limitation Status
IBP	B	Jul-11				
IBP	B	Aug-11	*	*		N
IBP	C	Jul-11	*	*		N
IBP	C	Aug-11	*	*		N
IBP	D	Jul-11				
IBP	D	Aug-11	*	*		N
IBP	J	Jul-11				
IBP	J	Aug-11		*		NP
BEO	ITEX-N	Jul-11	*	*		N
BEO	ITEX-N	Aug-11		*		NP
BEO	ITEX-S	Jul-11	*	*		N
BEO	ITEX-S	Aug-11		*		NP
BEO	WL02	Jul-11		*	*	P
BEO	WL02	Aug-11		*	*	P
BRW	AHMA	Jul-11	*	*		N
BRW	AHMA	Aug-11				
BRW	BOXER	Jul-11		*		NP
BRW	BOXER	Aug-11	*	*		N
BRW	UTIQ	Jul-11	*	*		N
BRW	UTIQ	Aug-11		*		NP

Table 4: Periphyton nutrient limitation by region and pond as determined benthic nutrient limitation experiments. Categories of nutrient limitation include: nitrogen limitation (N), phosphorus limitation (P) and NP co-limitation (NP). Significant increases of nutrient additions relative to controls are indicated by an asterisk (*) (ANOVA, Tukey HSD, $p < 0.05$).

Region	Pond	Date	N	NP	P	Nutrient Limitation Status
IBP	B	Jul-10		*		NP
IBP	B	Aug-10	*	*		N
IBP	B	Jul-11				
IBP	B	Aug-11				
IBP	C	Jul-10		*		NP
IBP	C	Aug-10		*		NP
IBP	C	Jul-11				
IBP	C	Aug-11				
IBP	D	Aug-10				
IBP	D	Jul-11	*	*		N
IBP	D	Aug-11				
IBP	J	Jul-10		*		NP
IBP	J	Aug-10	*	*		N
IBP	J	Jul-11				
IBP	J	Aug-11				
BEO	ITEX-N	Aug-10		*		NP
BEO	ITEX-N	Jul-11				
BEO	ITEX-N	Aug-11				
BEO	ITEX-S	Jul-10		*		NP
BEO	ITEX-S	Aug-10				
BEO	ITEX-S	Jul-11				
BEO	ITEX-S	Aug-11		*		NP
BEO	WL02	Aug-10		*		NP
BEO	WL02	Jul-11				
BEO	WL02	Aug-11	*	*	*	NP
BRW	AHMA	Jul-10	*	*		N
BRW	AHMA	Jul-11		*		NP
BRW	AHMA	Aug-11				
BRW	BOXER	Jul-10	*	*		N
BRW	BOXER	Aug-10	*	*		N
BRW	BOXER	Jul-11	*	*		N
BRW	BOXER	Aug-11				
BRW	UTIQ	Aug-10	*	*		N
BRW	UTIQ	Jul-11				
BRW	UTIQ	Aug-11	*	*		N

Figure 13: Frequency distribution for categories of nutrient limitation of phytoplankton production in 20 pelagic nutrient limitation experiments in the IBP, BEO, and BRW regions of Barrow, Alaska.

Figure 14: Frequency distribution for categories of nutrient limitation of periphyton production in 35 benthic nutrient limitation experiments in the IBP, BEO, and BRW regions of Barrow, Alaska.

A) Pelagic Zones   **B)** Benthic Zones

Figure 15: Mean (+/- 1SE) **a) phytoplankton biomass** in benthic nutrient limitation experiments and **b) periphyton biomass** in pelagic nutrient limitation experiments in IBP, BEO, and BRW regions. All sites in the region were combined in this analysis. Letters show differences among treatments.

During the third week of the mesocosm experiment, experiments showed phytoplankton chlorophyll-a biomass significantly increased in NP mesocosms suggesting NP co-limitation in the water column (p=value 0.0057; Figure 16). There were no significant differences in the biomass of periphyton among mesocosms in 2011; a longer term experiment will be completed in 2012 to elucidate the effect of nutrient additions on the benthic zone.

Figure 16: Mean (+/- 1SE) phytoplankton biomass for mesocosms and pond in synchronized experiments August 19th, 2011. Letters represent statistical differences between means.

Discussion

Alexander et al. (1980) postulated that warming temperatures in the Arctic and phosphorus enrichment of IBP tundra ponds could stimulate algal growth. Indeed, we observed increased phytoplankton biomass in the IBP ponds in 2009-11 as compared to the 1970s; this could be associated with synergistic effects of both increased nutrient levels and warmer temperatures in the region (Hobbie et al. 1999; Flanagan et al. 2003; Lougheed et al. 2011).

Conversely, periphyton showed no significant increase in biomass over the past 40 years, suggesting that different factors are acting to limit the growth of pelagic and benthic algae in these ponds. Vadeboncoeur et al. (2001) observed that increases in phytoplankton biomass caused decreases in benthic algal biomass through a reduction in water clarity; indicating that increases in nutrients can indirectly lead to modification of the light environment. However, this is not likely a driving factor in these nutrient-poor ponds. Periphyton grows more slowly than phytoplankton (Sand-Jensen and Borum, 1991) and have greater access to the sediment nutrient pool, which may allow for benthic stability and nutrient efficiency; in fact, on more that 50% of occasions, periphyton growth was not obviously enhanced by either nitrogen or phosphorus additions.

Any changes in nutrient ratios and sources in arctic ponds may produce changes in algal populations within the pelagic and benthic zones. A study by Vargas (2011) discovered the introduction of many new genera of periphyton into IBP ponds since the 1970s. These new taxa could be responding to elevated nutrient levels and warming in unique ways not observed in the 1970s. Studies are currently being conducted to understand the influence of nutrient limitation on algal community composition.

One hypothesis for the elevated phytoplankton biomass in IBP ponds was the encroachment of the village of Barrow nearer to the ponds over the past 40 years; however, we found no clear indications of this effect. Ponds within the village of Barrow showed significantly higher periphyton biomass, which suggests an influence of urban nutrient pollution on algal growth. Similarly, Schindler et al. (1974) found increases in algal blooms in the Canadian Arctic due to sewage inputs and Peterson et al. (1983) showed increased periphyton production in Arctic streams with continuous additions of phosphorus over

several years. However, IBP ponds showed similar algal biomass to that of more isolated BEO sites suggesting that IBP ponds remain un-impacted from the encroaching village. While ponds in all three regions are relatively a short distance (<6 km) from each other, there were large differences in nutrient chemistry between all three regions, especially BRW ponds. Schindler (1998) explains that even lakes or bodies of water directly adjacent to each other can vary greatly in water quality, community structure and eco-system functions. In particular, while IBP ponds are only meters apart, algal communities did not always respond to nutrient enrichment in a similar fashion. For example, in August 2010, benthos in IBP pond B were N limited, while IBP pond C was NP limited and pond D showed no nutrient limitation. Similarly, phytoplankton in IBP pond D and C were N limited in August 2011, while IBP J was NP limited. Slight differences in physical and environmental factors may be producing contrasting scenarios within ponds of close proximity, while differences in algal communities may lead to species-specific responses to nutrient enrichment. These differences among ponds in close proximity to one another provide evidence that multiple ponds need to be examined in order to accurately describe trends in algal nutrient limitation in these tundra ponds.

In the 1970s, phosphorus limited phytoplankton growth in the IBP ponds (Alexander 1980). Similarly, in other parts of the Alaskan tundra, phosphorus was also found to be limiting to periphytic algae (Peterson et al. 1983), and this P-limitation was maintained over a 16-yr period (Slavik et al. 2004). However in 2010 and 2011, our IBP pelagic experiments showed significantly higher phytoplankton biomass in N and NP treatments, as compared to the control and P treatments, suggesting a change from P limitation to N or NP co-limitation of phytoplankton in IBP ponds. NP co-limitation has also been recently observed in other Arctic phytoplankton communities (Ogbebo et al. 2009, Levine and Whalen 2001) and in large meta-analyses of freshwater ecosystems (Francoeur, 2001; Elser et al. 2007). A combination of N and NP co-limitation, as observed in the IBP ponds, was also observed for phytoplankton in alpine lake enclosure experiments (Nydick et al. 2004). Phytoplankton biomass in the 1970s was strongly positively correlated with total phosphorus loads in the IBP but is now shown to be strongly positively correlated with ammonia. This also lends support for changes in limiting nutrients in the water column to N.

Benthic algae tended to be N-limited in the IBP ponds. This is a unique observation for Arctic periphyton, which have either been observed to be P-limited (Peterson et al. 1983; Slavik et al. 2004) or NP co-limited (Bowden et al. 1992). In a review of terrestrial studies in the tundra, Elser et al. (2007) observed greater response to added N than P and suggested that phosphorus may be abundant in the sediment in tundra systems.

Permafrost degradation has been predicted to continue over the next century (Lawrence and Slater 2005), which could likely cause changes in biogeochemistry of freshwaters in the Arctic (Frey and Smith 2005; Lawrence and Slater 2005). For example, Reyes and Lougheed (in prep) have shown that thawing permafrost can release nutrients into tundra ponds, which could in turn stimulate algal growth. In fact, Lougheed et al. (2011) found that nitrogen, phosphorus and phytoplankton biomass had increased in the water column of IBP ponds over the past 40 years. Thawing of permafrost could potentially be reintroducing previously frozen stores of phosphorus or nitrogen to the sediment surface, which are quickly taken up by periphyton; these changing quantities of N and P may have led to oscillations of both N and P limitation.

Atmospheric deposition of nitrogen in the Arctic has been increasing in recent history (Mayewski et al. 1986); and N-deposition has been shown to affect nutrient limitation of phytoplankton in alpine lakes (Elser et al. 2009; Murphy et al. 2010). Despite the likelihood of N-deposition in the Barrow area (Jeffe et al. 1991), both periphyton and phytoplankton seem to be consistently limited by N, indicating that quantities of N deposition in these tundra ponds were not sufficient to overcome N-limitation.

On a regional level, we saw similar trends. Overall, N or NP co-limitation occurred with a frequency of 70% in all pelagic experiments, while 50% of benthic experiments exhibited N or NP co-limitation. Similar to Symons et al. (2012), we also observed multiple occasions (20% for phytoplankton, 50% for periphyton) where no nutrient limitation was observed. Phytoplankton were more often nutrient limited than periphyton, perhaps because in shallow oligotrophic systems they are less efficient at capturing nutrients and because they are not closely associated with the abundant sediment nutrient pool as are periphyton (Hansonn 1992; Sand-Jensen and Borum 1991).

Organisms at higher trophic levels can have strong effects on algal primary productivity. While, ponds studied in this region are fishless, they have substantial invertebrate communities, which are primarily composed of chironomids, cladocerans, anostracans and copepods (Butler et al. 1980; Lougheed et al. 2011). Differences in nutrient demands, nutrient recycling and grazing by invertebrate grazers may have implications for algal nutrient limitation and growth. Grazers may act to both control algal biomass and alter taxonomic composition of algae (e.g. Hillebrand and Kahlert, 2001), while also providing extra nutrients to algae leading to increases in algal biomass (Rober et al. 2011). Vadeboncoeur et al. (2003) found that in fishless oligotrophic lakes, a positive correlation existed between high density grazers and periphyton biomass as grazing allowed more light to penetrate the water column allowing increases in growth. Some zooplankton groups, like copepods, have high N demands and their increasing abundance may place substantial pressures on N reservoirs and have been linked to N limitation in Arctic phytoplankton (Johnson 2009; Johnson and Luecke 2012).

Increases in nutrients can likewise exert controls on zooplankton taxonomic composition; a whole-lake nutrient addition to an Arctic lake resulting in increased phytoplankton biomass also led to increased *Daphnia* abundance (O'Brien et al. 2005). Conversely, a long-term nutrient enrichment study found that after 4 years, pressures from grazing plateaued and were less significant (Slavik et al. 2004); while an enclosure study in an alpine lake found that zooplankton abundances were not changed by increased phytoplankton biomass (Nydick et al. 2004). Clearly, algal communities will respond differently to further nutrient enrichments in arctic ponds, depending on the synergistic effects of grazing by invertebrates. Additional mesocosm manipulations of multiple trophic levels would provide insightful information about the impact of grazing on periphyton and phytoplankton communities.

Across the region, Barrow ponds were most similar to IBP ponds, with NP limitation in the pelagic zone and N limitation of benthic algae. The BEO was unique in that benthic algae appeared to be NP limited at these sites. This was largely due to one of the BEO sites (WL02), which was the only pond that showed P limitation of periphyton throughout the summer. The BEO is a protected region that remains pristine and relatively isolated from human activities such as ATV and snow machine traffic; however, during the growing season it experiences heavy faunal activity by migratory birds mating,

nesting, and grazing. Studies by Van Geest et al. (2007) have shown that goose droppings can significantly contribute nutrients to freshwater ponds. Similarly, in the BEO, bird droppings may be contributing significant amounts of N therefore causing P to become limited at some point during the summer. Elevated ammonia levels in the BEO ponds as compared to the IBP ponds suggests that fecal matter may be contributing to nutrient limitation by P in these ponds. This is important and may play an even greater role in the future as studies have shown increases in bird populations, like geese, in the Arctic (Prop et al. 1998, Jeffries and Rockwell 2002) and their potential impacts on grazing of terrestrial plants, soil stability, and increases in fecal concentrations.

Variations in physical and environmental variables between years may lead to differences in nutrient limitation status among ponds. Less nutrient limitation in the benthic zone took place in IBP ponds in 2011 than in 2010. Changes in light, temperature, and precipitation from one year to the next may account for these differences; however, there were no apparent differences in precipitation, temperature and solar radiation among years. Therefore, a long-term nutrient limitation program would potentially allow for facilitated identification of long term responses as was the case for Slavik et al. (2004), who examined nutrient limitation over a 16 year study. Our benthic nutrient limitation experiments were only conducted during two consecutive years. Alternately, low levels of nutrient limitation in benthic experiments could also indicate nutrient efficiency of periphyton in IBP ponds caused by favorable conditions. Studies by Symons et al. (2012) reported nutrient efficient status (no nutrient limitation) dominated enrichment experiments in Canadian freshwater phytoplankton. This could potentially be attributed to higher irradiance levels or higher temperatures in that year. Further research in IBP, BEO, and Barrow ponds is required to understand equilibrium thresholds between nutrient efficiency and nutrient limitation of algal communities with special regard to physical and environmental factors.

Autotrophic organisms require different relative amounts of nutrients, therefore periphyton and phytoplankton could be limited by different nutrients within the same pond. For example, some species of Cyanobacteria, which are able to fix nitrogen from the atmosphere, dominate during periods of low nutrients and nitrogen limitation. Vargas (2011) found that Cyanophyta have decreased in relative

abundance in the IBP periphyton since the 1970s, and that they currently are more common in IBP ponds earlier in the summer. Furthermore, diatoms (Bacillariophyceae) are much more abundant now than they were 40 years ago (Vargas, 2011). Other studies have also found shifts in algal community structure upon nutrient addition, most notably the addition of N. Nydick et al. (2004) observed pronounced taxonomic changes with N additions on hard substrates while similarly, Wyatt et al (2010) reported taxonomic changes with N treatments in combinations with other nutrients (P or P + Si). Nitrogen additions have also indicated that some algae may retain N and C up to 2 years, thus exerting great control over N export downstream in arctic stream ecosystems (Peterson et al. 1997). More studies are currently being conducted to examine the influence of algal taxonomic composition on nutrient limitation in the IBP.

The phosphorus rich sediment interface could potentially be providing a fresh supply of P to the benthic zone but becoming limited in the water column. Hobbie (1980) found that shallow Arctic ponds have an abundance of iron in sediments, which readily bind with phosphorus. Phosphorus bound to the sediment resulting in decreased P availability in the water column. This scenario however is unlikely as shallow ponds are usually well mixed by wind and sediments are often re-suspended (Hobbie et al. 1999, Whalen et al. 2008).

Another potential explanation for differences in nutrient limitation status within the same systems could lie in the methodologies used. Differences in nutrient limitation status may have been produced by differences in experimental design of benthic and pelagic nutrient limitation experiments. It is worth noting, that our periphyton were grown on artificial mesh substrates; Nydick et al. (2004) observed that periphyton on tiles were NP- or N-limited, while periphyton on sediment showed no response. Further analytical studies could be completed using different nutrient limitation indices like those used by Elser et al. (2007), Allegier et al. (2011), Keatley et al. (2007), Antoniades et al. (2003) and Symons et al. (2012) to determine how analytical methods affect the identification efficiency of nutrient limitation status. For example, studies by Keatley et al. (2007) and Antoniades et al. (2003) used chemical ratios of N and P (N:P) to determine limiting nutrients. While these studies identified N limitation in the Canadian Arctic, others studies suggest that N:P ratios are poor indicators of nutrient

limitation status (Symons et al. 2012). Symons et al. (2012) found only a small fraction of their nutrient limitation experiments were supported with a corresponding nutrient limitation ratio.

Inhibitions of algal growth with phosphorus addition were observed in the both pelagic and benthic experiments of the IBP and BRW regions. As mentioned by Thompson et al. (2008) and Hobbie et al. (1999), phosphorus supply in sediments is rich in arctic ponds, and Andresen (unpublished data) found similarly high phosphorus concentrations in superficial sediments of IBP and BRW ponds. Further additions of phosphorus in nutrient enrichment experiments may have surpassed nutrient optimums leading to toxic effects to algae (Harpole et al. 2011). Our study, as well as many others, have shown that N and P are equally limiting in these freshwater systems; however, Allegier et al. (2010) suggested the type of co-limitation most often occurring in arctic freshwater systems is antagonistic co-limitation, in which the additive responses of N and P treatments together greatly exceed NP treatment responses. Our study suggests a more synergistic effect (in which NP treatment responses exceed added N and P treatment responses), which may occur due to oscillation between two nutrients in oligotrophic systems (Allegier et al. 2010; Arrigo 2005), as opposed to antagonistic co-limitation, which usually occurs due to a third limiting factor (i.e. macronutrient or light). Synergistic co-limitation in the IBP, BEO, and BRW regions suggests supplies of nutrients to the water column throughout the growing season are small and constant. This does not seem likely in the Barrow region, however, where nutrient levels are significantly greater than the other regions and susceptible to greater anthropogenic pollution. Further studies are needed to determine how various concentrations of each nutrient will influence types of co-limitation in co-limited systems since these nutrients are not likely introduced into arctic ponds at equal or constant rates. Understanding these different types of co-limitations will help predict responses to further nutrient increments and give insight to interactions of limiting nutrient and factors (Allegier et al. 2010).

Whole pond nutrient enrichment experiments in the 1970s showed phosphorus additions increased phytoplankton production while also increasing SRP, TP, NH_3 and NO_3 in 1970 and SRP, and NO_3 in 1971. Alexander suggested that increases in NO_3 may have stemmed from increased N-fixation (Alexander 1980). Even though current studies did not allow for whole pond nutrient enrichments,

mesocosms confirmed that phytoplankton in the IBP ponds were limited by N and P. There was no significant nutrient limitation in the benthos in the mesocosms. These differences could have been caused by several limitations of the mesocosm experiments. Nydick et al. (2004) also found that benthic algae on sediment were not stimulated by nutrient additions; however, algae grown on tiles responded to nutrient additions. In comparison to Wyatt et al. (2010) and Vadeboncoeur et al. (2001), who provided periphyton substrates to colonize on, our methods for sampling periphyton in the mesocosms consisted of direct collection of sediment. The addition of artificial substrates in subsequent mesocosm experiments may enhance the ability of periphyton to colonize quickly. It will be of great importance to choose an appropriate substrate material as this may also have a profound effect on the overall productivity of periphyton; studies have found differences in algal production between sediment of various types (clays, sands, etc), wood, and rocks (Vadeboncoeur et al. 2003). Differences in algal responses may also have been observed because of the short duration of the experiment and the tendency for benthic algae communities to take longer to colonize than pelagic algae communities. Finally, rapid growth of phytoplankton may also have led to inhibition of periphyton growth due to decreased light as suggested by Vadeboncoeur et al. (2001). Additional mesocosm experiments using light level manipulations may provide valuable insight to increased turbidities from phytoplankton/periphyton interactions. Finally, additional mesocosm experiments could also manipulate other nutrients and environmental factors. Wyatt et al. (2010) provided Si nutrient enrichments and Greenwood and Lowe (2006) manipulated pH to determine changes in algal composition in their experiments. Vargas (2011) observed that diatoms had increased in abundance in IBP ponds over the past 40 years; diatoms require Si to build their outer membranes. Further nutrient limitation experiments with added Si would provide valuable additional insight concerning biogeochemistry of IBP ponds influences on algal species composition.

In the 1970s, nutrient limitation experiments in the IBP were conducted by nutrient additions into a whole pond (Alexander 1980). Whole system experiments pose obstacles regarding costs, recovery and lingering of added nutrients, and reduced levels of replication (Schindler 1998); for those reasons our nutrient limitation experiments were scaled down to mesocosm scale. Schindler (1998) suggests that

in doing so, new obstacles are presented such as poor mixing, longer incubations periods, and decreases in algae due to isolation. Additionally, oligotrophic systems respond slower to nutrient additions (Chapin et al. 1986) and miniscule increases in algal production could potentially go unnoticed due to their low statistical significance (Francoeur 2001). Further mesocosm studies are needed to assess the representativeness of mesocosms and to better determine nutrient enrichment effects on periphyton. A long term nutrient limitation program would allow a thorough assessment of the short and long-term ecosystem responses and changes to continuous nutrient enrichments.

Studies using radon and phosphorus tracers have been used to determine P inputs in a eutrophic lake and suggested period of maximum influx of P coincided with period of maximum lake photosynthetic production (Imboden and Emerson 1978). Gibson et al. (2005) reviewed methods using isotopic tracers in hydrologic systems and determined much progress has been made in this field. Methods involving the use of O isotopes and carbonate organic matter fractions may be used to determine hydrological interactions in lakes (Gibson et al. 2005). Studies using isotopic tracers could more accurately address the sources of added nutrients in arctic ponds as well as residential time. For example, Johnson (2009) used isotopes to reveal that zooplankton can provide most, if not all, N or P requirements for phytoplankton primary production in an Alaskan lake; N remained about 16 days in the mixed layer. It would be beneficial to reproduce similar isotopic studies in order to better understand nutrient cycling within these ponds as continued increments of released nutrients from permafrost are predicted to continue and will have long term effects to aquatic production.

Conclusion

The IBP region has endured many changes throughout the past 40 years and continued warming temperatures, changes in nutrient availability and urbanization will continue to impact algal communities and thus may have important implications for carbon cycling. Increased temperatures significantly stimulated phytoplankton production in this region, although effects on periphyton are still unclear, with additional studies required to thoroughly assess warming effects. The findings in the nutrient limitation experiments suggest an obvious shift in nutrient limitation has taken place in the IBP. Our studies indicate that N, or N in combination with P, are more significantly limiting than 40 years ago. This falls in accordance with other freshwater studies in the Arctic. Increased releases of P from thawing permafrost as well as nutrient pollution from urbanization may have influenced this change. Differences in nutrient limitation exist between the benthic and pelagic zones; these differences may exist due to the different environmental conditions and nutrient efficiency factors between phytoplankton and periphyton. While many current nutrient limitation studies provide insight into nutrient limitation status of freshwater ecosystems, many do not consider perspectives from both benthic and pelagic zones; this may yield erroneous or incomplete conclusions regarding whole ecosystem effects. Further mesocosm studies are needed to effectively represent whole systems in order to determine concurrent nutrient limitation in pelagic and benthic zones *in situ*. These studies are crucial to fully understand nutrient limitation in the Arctic and the implications of changes in algal production to carbon budgets.

References

Alexander V., Stanley D.W., Daley R.J., McRoy C.P., 1980. Primary producers. In limnology of tundra ponds, Barrow, Alaska, ed. J.E. Hobbie, 514. Stroudsburg, PA: Dowden, Hutchinson and Ross.

Allegier J.E., Rosemond A.D., Layman C.A., 2011. The frequency and magnitude of non-additive responses to multiple nutrient enrichment. *J. Applied Ecology.* 48: 96-101.

Antoniades D., Douglas M.S.V., Smol J.P., 2003. Comparative physical and chemical limnology of two Canadian high arctic regions: Alert (Ellesmere Island, NU) and Mould Bay (Prince Patrick Island, NWT). Archiv Fur Hydrobiologie. 158: 485-516.

Antoniades D., Douglas M.S.V., Smol J.P., 2003. The physical and chemical limnology of 24 ponds and one lake from Isachsen, Ellef Ringnes Island, Canadian High Arctic. *International Review of Hydrobiology.* 88: 519-538.

Arrigo K.R., 2005. Marine microorganisms and global nutrient cycles. *Nature*, 437, 349–355.

Barsdate R. J. and Prentki R. T., 1973. Nutrient metabolism and water chemistry in lakes and ponds of the Arctic Coastal Tundra. *U.S. Tundra Biome Data Report* 73-27. 163 pp. U.S. Arctic Research Program, U. S. International Biological Program, U. S. Tundra Biome Hanover, NH.

Benstead J.P., Deegan L.A., Peterson B.J., Huryn A.D., Bowden W.B., Suberkropp K., Buzby K.M., Green A.C., Vacca J.A., 2005. Responses of a beaded Arctic stream to short-term N and P fertilization. *Freshwater Biology.* 50: 277-290.

Bernhardt E.S., and Likens G.E., 2004. Controls in periphyton biomass in heterotrophic streams. *Freshwater Biology.* 49: 14-27.

Bowden W.B., Peterson B.J., Finlay J.C., Tucker J., 1992. Epilithic chlorophyll-a, photosynthesis, and respiration in control and fertilized reaches of a tundra stream. *Hydrobiologica.* 240: 121-131.

Brenton J., Vallieres C., and Laurion I., 2009. Limnological properties of permafrost thaw ponds in northeastern Canada. *Can. J. Fish. Aquat. Sci.* 66: 1635-1648.

Brutemark A., Rengefors K., Anderson N.J., 2005. An experimental investigation of phytoplankton nutrient limitation in two contrasting low Arctic lakes. *Polar Biology.* DOI 10.1007/s00300-005-0079-0.

Bonilla S., Villeneuve V., and Vincent W.F., 2005.. Benthic and planktonic algal communities in a high arctic lake: pigment structure and contrasting responses to nutrient enrichment. *Journal of Phycology.* 41: 120-130.

Butler M.G., Miller M.C., and Mozley S.C., 1980. Macrobenthos. In Limnology of tundra ponds, Barrow, Alaska, ed. J.E. Hobbie. 297-330. Stroudsburg, PA: Dowden, Hutchinson and Ross.Chapin III F.S., Vitousek P.M., Van Cleve K., 1986. The nature of nutrient limitation in plant communities. *The American Naturalist.* 127: 48-58.

Cole J.J., Pace M.L., Carpenter S.R., and Kitchell J.F., 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnol. Oceanogr.* 45(8): 1718-1730.

Deegan L.A., Golden H., Harvey C.J., and Peterson B.J., 1999. The influence of environmental variability on the growth of age 0 and adult Arctic grayling. *Trans. Am. Fish. Soc.* 128: 1163-1175.

Deegan L.A., Peterson B.J., Golden H., McIvor C.C., Miller M.C., 1997. Effects of fish density and river fertilization on algal standing stocks, invertebrate communities, and fish production in an arctic river. *Canadian Journal of Fisheries and Aquatic Sciences.* 54: 269-283.

Dugan A.H., Lamoureux S.F., Lewis T., Lafreniere M., 2012. The impact of permafrost disturbances and sediment loading on the limnological characteristics of two high arctic lakes. *Permafrost and Periglacial Processes.* Published online in Wiley Online Library (wileyonlinelibrary.com): ppp.1735.

Elser J.J., Bracken M.E.S., Cleland E.E., Gruner D.S., Harpole W.S., Hillebrand H., Ngai J.T.,

Seabloom E.W., Shurin J.B., Smith J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters.* 10: 1135-1142.

Elser J.J., Kyle M., Steger L., Nydick K.R., Baron J.S., 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. *Ecology.* 90: 3062-3073.

Fairchild W.G., Lowe R.L., Richardson W.B., 1985. Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. Ecology 66: 465-472.

Flanagan K.M, McCauley E., Wrona F., Prowse T., 2003. Climate change: the potential for latitudinal effects on algal biomass in aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences.* 60: 635-639.

Francoeur S.N., 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *J.N. Am. Benthol. Soc.* 20 (3): 358-368.

Frey K.E., and McClelland J.W., 2009. Impacts of permafrost degradation on Arctic river biogeochemistry. *Hydrol. Process.* 23: 169-182.

Frey K.E., and Smith L.C, 2005. Amplified carbon release from vast West Siberian peatlands by 2100. *Geophysical Research Letters.* 32, L09401

Gibson J.J., Edwards T.W.D., Birks S.J., St. Amour N.A., Buhay W.M., McEachern P., Wolfe B.B., Peters D.L., 2005. Progress in isotope tracer hydrology in Canada. *Hydrological Processes.* 19: 303-327.

Greenwood J.L., and Lowe R.L., 2006. The effects of pH on a periphyton community in an acidic wetland, USA. *Hyrobiologia.* 561:71-82.

Hansonn L.A., 1992. Factors regulating periphytic algal biomass. *Limnol.Oceanogr.* 37: 322-328.

Harpole W.S., Ngai J.T., Cleland E.E., Seabloom E.W., Borer E.T., Bracken M.E.S., Elser J.J., Gruner D.S., Hillebrand H., Shurin J.B., Smith J.E., 2011. Nutrient co-limitation of primary producer communities. *Ecology letters.* 14: 452-462.

Hillebrand H., and Kahlert M., 2001. Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichi-ometry in habitats of different productivity. *Limnol. Oceanogr.* 46: 1881-1898.

Hinkel K.M., Eisner W.R., Bockheim J.G., Nelson F.E., Peterson K.M., and Dai X., 2003. Spatial extent, age, and carbon stocks in drained thaw lake basins on the Barrow Peninsula, Alaska. *Arctic, Antarctic, and Alpine Research* 35: 291–300.

Hobbie J.E. 1980. *Limnology of Tundra Ponds, Barrow, Alaska.* (Dowden, Hutchinson and Ross, Stroudsburg, PA), Vol. US/IBP Synthesis Series.

Hobbie J.E., Peterson B.J., Bettez N., Deegan L., O'Brien W.J., Kling G.W., Kipphut G.W., Bowden W.B., and Hershey A.E., 1999. Impact of global change on the biogeochemistry and ecology of an Arctic freshwater system. *Polar Research.* 18(2) 207-214.

Imboden D.M., Emerson S., 1978. Natural radon and phosphorus as limnologic tracers; horizontal and vertical eddy diffusion in Greifensee. *Limnol. Oceanogr.* 23: 77-90.

Jeffries R.L., and Rockwell R.F., 2002. Foraging geese, vegetation loss and soil degradation in an Arctic salt marsh. *Applied Vegetation Science.* 5: 7-16.

Johnson C.R. "Consumer-driven nutrient recycling in Arctic Alaskan lakes: Controls, importance for primary productivity, and influence on nutrient limitation". Dissertation. Utah State University. 2009. Web.

Johnson C.R., Luecke C., 2012. Copepod dominance contributes to phytoplankton nitrogen deficiency in lakes during periods of low precipitation. *J.Plankton Res.* Available online at: http://plankt.oxfordjournals.org/content/early/2012/02/24/plankt.fbs009.full.pdf+html.

Keatley B.E., Douglas M.S.V., Smol J.P., 2007. Physical and chemical limnological characteristics of lakes and ponds across environmental gradients on Melville Island, Nunavut/NWT, High Arctic Canada. *Fundamental and Applied Limnology.* 168: 355-376.

Lawrence D.M. and Slater A.G. 2005. A projection of severe near-surface degradation during the 21st century. *Geophys. Res. Lett.* 32, L24401.

Lienesch P.W., McDonald M.E., Hershey A.E., O'Brien W.J., Bettez N.D., 2005. Effects of a whole-lake, experimental fertilization on lake trout in a small oligotrophic arctic lake. *Hydrobiologia*. 548: 51-66.

Lorenzen C.J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnology and Oceanography*. 12: 343-346.

Lougheed V.L., Butler M.G., McEwen D.C., and Hobbie J.E., 2011. Changes in tundra pond limnology: re-sampling Alaskan ponds after 40 years. *Ambio*. 40: 589-599.

Lougheed V.L., Crosbie B., Chow-Fraser P., 1998. Predictions on the effect of common carp (Cyprinus carpio) exclusion on water quality zooplankton, and submergent macrophytes in a Great Lakes wetland. Can. J. Fish. Aquat. Sci. 55:1189-1197.

Lee H., Schuur E.A.G., Vogel J.G., 2010. Soil CO2 production in upland tundra where permafrost is thawing. *J. Geophys. Res.* 115: G01009.

Levine M.A., Whaeln S.C., 2001. Nutrient limitation of phytoplankton production in Alaskan Arctic foothill lakes. *Hydrobiologia*. 455: 189-201.

Liebig J., 1842. Animal Chemistry, or Organic Chemistry in its Application to Physiology and Pathology. Johnson Reprint Corporation, New York, USA.

Mayewski P.A., W.B. Lyons, M.J. Spencer, M. Twickler, W.Dansgaard, B. Koci, C.I. Davidson, and R.E. Honrath. 1986. Sulfate and nitrate concentrations from a south Greenland ice core. Science 232: 975–977.Michelutti N., Wolfe A.P., Vinebrooke R.D., and Rivard B. 2005. Recent primary production increases in arctic lakes. *Geophysical Research Letters*. 32: 1–4.

Michelutti N., Wolfe A.P., Vinebrooke R.D., and Rivard B. 2005. Recent primary production increases in arctic lakes. *Geophysical Research Letters*. 32: 1–4.

Murphy C.A., Thompson P.L., Vinebrooke R.D., 2010. Assessing the sensitivity of alpine lakes and ponds to nitrogen deposition in the Canadian Rocky Mountains. *Hydrobiologia*. 648: 83-90..

Nydick K.R., Lafrancois B.M., Baron J.S., Johnson B.M., 2004. Nitrogen regulation of algal biomass, productivity , and composition in shallow mountain lakes, Snowy Range, Wyoming, USA. *Canadian Journal of Fisheries and Aquatic Sciences*. 61: 1256-1268

O'Brien W.J., Barfield M., Bettez N., Hershey A.E., Hobbie J.E., Kipphut G., Kling G., Miller M.C., 2005. Long-term response and recovery to nutrient addition of a partitioned lake. Freshwater Biology. 50: 731-741.

Ogbebo F.E., Evans M.S., Waiser M.J., Tumber V.P., Keating J.J., 2009. Nutrient limitation of phytoplankton growth in Arctic lakes of the lower Mackenzie River Basin, northern Canada. *Candian Journal of Fisheries and Aquatic Sciences*. 66: 247-260.

Osterkamp T.E., Jorgenson M.T., Schuur E.A.G., Shur Y.L., Kanevskiy M.Z., Vogel J.G., and Tumskoy V.E., 2009. Physical and ecological changes associated with warming permafrost and thermokarst in interior Alaska. *Permafrost and Periglac. Process.* 20: 235-256.

Paerl H.W., Pinckney J.L. and Steppe T.F.,2000. Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. *Environmental Microbiology*. 2(1):11-26.

Peterson B.J., Bahr M., Kling G.W., 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. *Canadian Journal of Fisheries and Aquatic Sciences.* 54: 2361-2367.

Peterson B.J., Fry B., Deegan L., 1993. The trophic significance of epilithic algal production in a fertilized tundra river ecosystem. *Limnology and Oceanography*. 38: 872-878. Moller B., Ford T.E., Hershey A., Hiltner A., Kipphut G., Lock M.A., Fiebig D.M., Mckinley V., Miller M.C., Vestal J.R., Ventullo R., Volk G., 1993. Biological responses of a tundra river to fertilization. *Ecology*. 74: 653-672.

Peterson B.J., Hobbie J.E., Hershey A.E., Lock M.A., Ford T.E., Vestal J.R., McKinley V.L., Hullar M.A., Miller M.C., Ventullo R.M., and Volk S.S., 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. *Science.* 229: 1383-1386.

Peterson B.J., Hobbie J.E., Corliss T.L., 1983. A continuous flow periphyton bioassay: test of nutrient limitation on a tundra stream. *Limnology and Oceanography.* 28: 583-591.

Prop J., Black J.M., Shimmings P., Owens M., 1998. The spring range of barnacle geese Branta leucopsis in relation to changes in land management and climate. *Biological Conservation.* 86: 339-346.

Reyes F.R. and V.L. Lougheed. (In prep) Potential influence of permafrost thaw on Arctic tundra pond Biogeochemistry.

Rober A.R., Wyatt K.H., Stevenson R.J., 2011. Regulation of algal structure and function by nutrients and grazing in a boreal wetland. *Journal of the North American Benthological Society.* 30: 787-796.

Sand-Jensen K., and Borum J., 1991. Interactions among phytoplankton, periphyton and macrophytes in temperate freshwaters and estuaries. *Aquat. Bot.* 41: 137-75.

Sand-Jensen K, Riis T., Markager S., and Vincent W.F., 1999. Slow growth and decomposition of mosses in Arctic lakes. *Can. J. Fish. Aquat. Sci.* 56: 388-393.

Schindler D.W., Kalff J., Welch H.E., Brunskill G.J., Kling H. and Kritsch N. 1974. Eutrophication in the high Arctic: Meretta Lake, Cornwallis Island (758 N. lat.). *J. Fish. Res. Board Can.* 31, 647–662.

Schindler D.W., 1998. Replication versus realism: the need for ecosystem scale experiments. *Ecosystems.* 1: 323-334.

Schindler D.W., and J.P. Smol. 2006. Cumulative effects of climate warming and other human activities on freshwaters of Arctic and subarctic North America. *Ambio* 35: 160–168.

Slavik K., Peterson B.J., Deegan L.A., Bowden W.B., Hershey A.E., and Hobbie J.E., 2004. Long-term responses of the Kuparuk river ecosystem to phosphorus fertilization. *Ecology.* 84: 939-954.

Smol J.P., Wolfe A.P., Birks H.J.,Douglas M.S.V., Jones V.J., Korhola A., Pienitz R., Rühland K., Sorvari S., Antoniades D., Brooks S.J., Fallu M., Hughes M., Keatley B.E., Laing T.E., Michelutti N., Nazarova L., Nyman M., Paterson A.M., Perren B., Quinlan R., Rautio M., Saulnier-Talbot, Siitonen S., Solovieva N., and Weckström J., 2005. Climate-driven regime shifts in the biological communities of Arctic lakes. *PNAS.* 102(12) 4397-4402.

Smol, J.P., and Douglas M.S.V. 2007. Crossing the final ecological threshold in high Arctic ponds. Proceedings of the National Academy of Sciences of the United States of America 104: 12395–12397.

Strickland J.D.H., and Parsons T. R., 1968. A manual for sea water analysis. *Bull. Fish. Res. Bd. Canada*, 167: 311.

Symons C.C., Arnott S.E., Sweetman J.N., 2012. Nutrient limitation of phytoplankton communities in Subarctic lakes and ponds in Wapusk National Park, Canada. *Polar Biology*, 35:481-489.

Tanks J.L., Dodds W.K., 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology.* 48: 1031-1049.

Thompson P.L., St-Jaques M.C., Vinebrooke R.D., 2008. Impacts of climate warming and nitrogen deposition on alpine plankton in lake and pond habitats: an in vitro experiment. *Arctic, Antarctic and Alpine Research.* 40: 192-198.

Turner M.A., Howell E.T., Robinson G.G.C., Campbell P., Hecky R.E., and Schindler E.U., 1994. Roles of nutrients in controlling growth of epilithon in oligotrophic lakes of low alkalinity. *Can. J. Fish. Aquat. Sci.* 51: 2784-2793.

Vadeboncoeur Y., Lodge D.M., and Carpenter S.R., 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology.* 82: 1065-

1077.

Vadeboncoeur Y., Jeppesen E., Zanden M.J.V., Schierup H., Christoffersen K., Lodge D.M., 2003. From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. *Limnol. Oceanogr*. 48(4): 1408-1418.

Van Geest G.J., Hessen D.O., Spierenburg P., Dahl-Hansen G.A.P., Christensen G., Faerovig P.J., Brehm M., Loonen M.J.J.E., Van Donk E., 2007. Goose-mediated nutrient enrichment and planktonic grazer control in arctic freshwater ponds. *Oecologia*. 153: 653-662.

Vargas M. "Causes and consequences of algal community structure of arctic tundra ponds from Barrow, Alaska". Thesis. University of Texas El Paso. 2011. Web.

Vincent W.F. 2000. Cyanobacterial dominance in the polar regions. *In Potts, W*. [Ed.] *Cyanobacteria: Their Diversity in Time and Space*. Kluwer Academic Publishers, Dordrecht, pp. 321-328.

Vogel J., Schuur, E.A.G., Trucco, C., and Lee H., 2009. Response of CO_2 exchange in a tussock tundra ecosystem to permafrost thaw and thermokarst development. *J. Geophys. Res.* 114: G04018.

Whalen S.C., Chalfant B.A., Fischer E.N, 2008. Epipelic and pelagic primary production in Alaskan Arctic lakes of varying depth. *Hydrobiologia*. 614: 243-257.

Wetzel, R.G., and G.E. Likens., 1991. Limnological Analyses. 2nd. Ed. Springer-Verlag. 391p.

Wyatt K.H., Stevenson R.J., and Turetsky M.R., 2010. The importance of nutrient co-limitation in regulating algal community composition, productivity and algal-derived DOC in an oligotrophic marsh in interior Alaska. *Freshwater Biology*. 55: 1845-1860.

Vita

Christina Hernandez completed her undergraduate degree in Environmental Sciences focusing in hydro-science from the University of Texas at El Paso. During this time she also participated in various internships at various universities throughout New Mexico such as the New Mexico Institute of Mining and Technology, University of New Mexico and New Mexico Highlands University conducting research related to surficial hydrology. After completion of her Bachelor's degree, Hernandez collaborated with Dr. Lougheed to complete her Master's Degree in Environmental Science with research focusing on the limnology of arctic ponds and spent several summers in Alaska. She currently works for the El Paso Independent School District tutoring remedial high school students in biology and for a local environmental firm aiding in desert development restoration projects. In the future, she plans to attain a doctorate degree and to travel abroad to aid in improving water quality for underdeveloped countries.

Permanent address: 724 Phil Hansen Dr.

                    El Paso, Texas 79835

This thesis was typed by Christina Hernandez