Recent Changes In The Zooplankton Communities Of Arctic Tundra Ponds In Response To Warmer Temperatures And Nutrient Enrichment

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RECENT CHANGES IN THE ZOOPLANKTON COMMUNITIES OF ARCTIC TUNDRA PONDS IN RESPONSE TO WARMER TEMPERATURES AND NUTRIENT ENRICHMENT

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Dedication

This dissertation is dedicated to all my incredible family for all the love and support.
RECENT CHANGES IN THE ZOOPLANKTON COMMUNITIES OF ARCTIC TUNDRA PONDS IN RESPONSE TO WARMER TEMPERATURES AND NUTRIENT ENRICHMENT

by

MARIANA VARGAS MEDRANO, B.S., M.S.

DISSEPTION

Presented to the Faculty of the Graduate School of
The University of Texas at El Paso
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of the Requirements
for the Degree of

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Acknowledgments

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This work was inspired, in part, by the pioneering limnology research at the International Biological Program (IBP) site in Utqiaġvik (Barrow), AK by Raymond Stross, John Hobbie, Vera Alexander, Dick Prentki and others in Barrow, AK during the early 1970s.

We thank Christian Andresen, Gabriela Contreras, Christina Hernandez, Nickole Miller, and Francisco Reyes for assistance in the field and/or laboratory. Also undergraduate students Victoria Rodriguez, Marisela Montelongo, Marisol Garcia and Jacob Bilbo for their assistance in the lab. I also want to thank all lab mates for their friendship and company you were a beautiful family in the lab. Thank you to my committee who always supported me. I greatly appreciate the tremendous support of my advisor, Dr. Vanessa Lougheed words are not sufficient to explain my gratitude.
Abstract

Warming is altering Arctic freshwater ecosystems, enriching the water column, increasing primary productivity and shifting the composition, dominance and the timing of emergence and reproduction of zooplankton communities. Historic data from arctic tundra ponds near Utqiaġvik, AK have given us valuable insight into changes that have occurred in recent times. A change of dominance from cyclopoid copepods to *Daphnia middendorffiana* has occurred over a 40 year period. *Daphnia* are more common, abundant and larger, which may be explained by increased primary productivity stimulated by nutrient release from permafrost thaw. Copepods and fairy shrimp emerged earlier from the overwintering states, which may be due to warmer spring temperatures. These trends are supported by both lab experiments and regional surveys of enriched ponds in urban and thermokarst areas, which confirm that warming and enrichment are a driver of change in tundra pond zooplankton communities.

In nutrient addition experiments, nutrient additions and the addition of permafrost sediment affected the dominance of algal groups and the growth and reproduction of *Daphnia*. In water sourced from a tundra pond, phosphorus clearly enhanced Bacillariophyceae density while nitrogen enhanced Cyanophyta abundance. Relative dominance of *Daphnia* increased due to the presence of algae enriched by phosphorus, and especially that co-enriched from thawing permafrost in our experiments. This further supports previous work showing that arctic *Daphnia* depend on benthic algae for their nutrition.

Enrichment by human activities, but particularly thermokarsting, are causing substantial changes to the tundra pond environment and their associated zooplankton communities. In a survey of ponds along a gradient including thermokarst, urban ponds, and reference sites, nutrients increased algal biomass and total abundance of zooplankton especially, *Daphnia middendorffiana* and cyclopoid copepods. This space-for-time substitution lends support to
nutrient enrichment as the driver of increased *Daphnia* biomass over time. Some new taxonomic records for the region were observed: *Bosmina longirostris* and harpacticoid copepods. Both urban enrichment and thermokarsting will have important implications for both primary and secondary producers with future warming.

Both historic changes in tundra ponds, as well as observed drivers in thermokarst and urban ponds, can give us an idea of the future changes due to warming and enrichment in the other ponds in the region. These changes at the level of the zooplankton may have important impacts on other components of the Arctic food web, including algae, as well as upper trophic levels that depend on zooplankton for food. Given that unprecedented warming is occurring, and likely continue into the future, these unique ecosystems must continue to be observed to predict, understand and model future alterations to Arctic ecosystems.
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Chapter 1: General introduction

Warming and Arctic tundra ponds

The Arctic region is experiencing a period of unprecedented warming (Callaghan et al., 2004). In the northernmost part of the US, near Utqiaġvik, AK, air temperatures have risen 2°C since the 1950s (Andresen & Lougheed, 2015). Nearby Arctic freshwater ecosystems, such as tundra ponds, are also warming (Lougheed et al., 2011; McEwen & Butler, 2018). In particular, early spring and later summer appear to be 2°C warmer in ponds as compared to the 1970s (Lougheed et al., 2011). This can have dramatic effects on the structure and function of these small, but abundant aquatic ecosystems.

For example, paleolimnological techniques have recorded dramatic changes in diatom communities of high Arctic lakes and ponds over the last 150 years (Douglas et al., 1994; Smol et al., 2005), which has been linked to increased primary production and changes in algal and invertebrate communities (Smol et al., 2005; Keatley, Douglas & Smol, 2008). Warmer temperatures in Arctic aquatic ecosystems have also been associated with an increase in primary producers (Michelutti et al., 2015), increased nutrients in the water column (Lougheed et al., 2011), changes in nutrient limitation status of algae (Lougheed et al., 2015), changes in periphyton community composition (Douglas et al., 1994; Vargas Medrano, 2011), increased thaw depth (Andresen & Lougheed, 2015); and increased methane efflux (Andresen, Tweedie & Lougheed, 2018). Changes also were observed in the loss of pond area (Smol & Douglas, 2007) and in plant cover (Andresen & Lougheed, 2015). However, few studies have focused on changes in invertebrate communities, particularly zooplankton, as a result of warmer temperatures and the possible drivers of these changes.

What is known about Arctic zooplankton

Surveys across the Arctic have found that zooplankton community composition can be structured by temperature. Warmer ponds tend to be associated with higher species richness (Herbert & Hann, 1986; Novichkova & Azovsky, 2017); however, responses differ among taxa. For example, cladocerans such as small-bodied chydrorids and larger Daphnia may be positively affected by warming (Korhola, 1999; Jose, 2009; Walseng et al., 2018; Klobucar, Gaeta & Budy,
2018). On the contrary, many calanoid copepods tend to do better in lower temperatures (Herbert & Hann, 1986; Jose, 2009; Novichkova & Azovsky, 2017). In general, Arctic ponds have a low number of species but, compared with other Arctic regions, Alaska has high zooplankton species richness with 54 crustaceans reported (Rautio et al., 2008).

Because growing seasons are relatively short in the Arctic, freshwater biotic communities in ponds and lakes in the Arctic are likely adapted to respond quickly to nutrients (Douglas et al., 1994). As such, zooplankton abundances in northern populations tend to be positively affected by increases in nutrient concentrations (O’Brien et al., 2005; Sweetman & Smol, 2006; Van Geest et al., 2007b), food availability (Yurista & O’Brien, 2001), as well as particulate and dissolved organic carbon (Chételat & Amyot, 2009). With observed increases in water column nutrient concentrations attributed to permafrost thaw (Reyes & Lougheed, 2015), which will likely increase with warming, this will have important implications for zooplankton communities in the future.

Arctic ponds are extreme environments for zooplankton, given their short open water seasons, the high likelihood of freezing to the bottom each winter, as well as relatively high levels of ultraviolet radiation. Adaptations to complete their life cycles quickly, overwinter freezing conditions, and others to protect their cells from UV radiation, are thus important physiological adaptations of Arctic zooplankton (Rautio & Tartarotti, 2010).

Because they freeze to the bottom each winter, fish are largely excluded from tundra ponds unless a dispersal route, including overland flow during spring flooding, is opened. In the absence of fish, zooplankton in tundra ponds may become larger and more abundant than communities in nearby lakes. While Arctic ponds generally have low species diversity of zooplankton, they can reach relatively high abundances compared to other freshwater environments, largely because of the absence of fish (Rautio et al., 2011a). The absence of fish also allows large-bodied zooplankton to dominate some Arctic zooplankton communities (Christoffersen et al., 2008; Jeppesen et al., 2017). In addition to the presence of fish, predation and competition within the zooplankton (Stross, Miller & Daley, 1980; Yurista & O’Brien, 2001; Dzialowski et al., 2004; O’Brien & Luecke, 2011) can determine the species composition in ponds.
Benthic algae are often most abundant in these shallow ecosystems as compared to phytoplankton (Alexander et al., 1980; Rautio & Vincent, 2006; Cazzanelli et al., 2012). Benthic algae are an important food source for Daphnia middendorfiana and other zooplankters, such as the fairy shrimp Branchinecta paludosa, in Arctic lakes and ponds, and may contribute substantially to secondary production in high arctic ponds (Rautio & Vincent, 2006; Rautio, Bonilla & Vincent, 2009; Cazzanelli et al., 2012). However, benthic algae alone may not promote survival of Daphnia (Mariash et al., 2014). Zooplankton in Arctic lake ecosystems may also rely on terrestrial-derived carbon as a food source (Rautio et al., 2011b); however, others found that terrestrial carbon was a minor contributor to the diet the Arctic taxon D. middendorffiana. (Cazzanelli et al., 2012). There appears to be a lack of research on the role of allochthonous carbon in Arctic pond food webs, notably the impact on zooplankton communities (Rautio et al., 2011a).

Additional factors that are changing in a warming climate that may structure zooplankton communities include: the expansion of aquatic plants (Andresen & Lougheed, 2015), which may provide zooplankton with protection from predation (Herbert & Hann, 1986). Second, zooplankton from temperature regions with the capacity to expand into northern regions may be more effective competitors for food (Yurista, 1999). Finally, changes in food quality in the future may affect Daphnia growth (Przytulska et al., 2015).

Research in the Arctic is still not enough to understand all the unique processes occurring in this distinctive region, especially as it relates to zooplankton. We must continue to invest in research to better understand the effects of warming on freshwater organisms in order to contribute to global models. Zooplankton and algal communities are changing and the following projects intend to explain how they are changing as a response to warming temperatures.

The International Biological Program (IBP)

Research completed nearly forty years ago at the International Biological Program (IBP) site in Utqiagvik (formerly Barrow) Alaska, is a rare example of detailed historical data on Arctic aquatic ecosystems. The data collected at the IBP site has been summarized in an invaluable and detailed volume titled “Limnology of Tundra Ponds, Barrow, Alaska” (Hobbie, 1980), as well as numerous reports. This study included a detailed analysis of the zooplankton
communities in several tundra ponds (Stross et al., 1980), which have not been resampled since those times. These ponds and their historic data represent a valuable and untapped resource suitable for studying the impact of global change on zooplankton and other environmental factors in the region.

IBP zooplankton taxa in the 1970s were dominated by crustaceans; the most common species were cyclopoid copepods (Cyclops vernalis and Cyclops magnus), Daphnia middendorffiana and Daphnia pulex. Some others such as Branchinecta paludosa, Polyartemiella hazeni, Heterocope septentrionalis, calanoid copepods (Diaptomus glacialis and Diaptomus bacillifer) were rare if they were present (Stross et al., 1980). All taxa appeared at different times during the growing season and they mostly reached the adult stage during the last week of June. During the first days of July, copepods started to increase in density. In late July to mid-August, abundances were reduced to approximately one individual of zooplankton per liter in IBP pond C (Stross et al., 1980).

Goals and objectives

This study addresses the overarching question: “How have zooplankton communities in tundra ponds responded to warming and nutrient enrichment?” Specifically, this study aims to:

1. Capitalize on the historic IBP data and, by comparing these to modern samples, determine the drivers of any changes in zooplankton communities in Arctic tundra ponds through time.
2. Using experiments, understand how arctic algal communities change in response to nutrient addition, and the cascading impacts of these changes on zooplankton, specifically Daphnia.
3. Further understand enrichment effects on arctic tundra pond zooplankton, by studying zooplankton communities in tundra ponds along a gradient from protected areas, to thermokarst ponds, and within the growing community of Utqiagvik, AK.
Study site description

The Arctic Coastal Plain (ACP) is a large expanse of Arctic tundra with low relief located adjacent to the Chukchi and Beaufort Seas of the Arctic Ocean. Regionally, soils remain continuously frozen in permafrost with only the active layer thawing during the summer (Hinkel et al., 2003; Frohn, Hinkel & Eisner, 2005). Nearly than one-half of the land cover of the ACP is composed of thaw lakes or drained thaw lake basins (Frohn et al., 2005), many containing numerous ponds (Hinkel et al., 2003). Across the Arctic permafrost lowlands, 17% of the landscape is covered by ponds and lakes (Muster et al., 2017). Lake basins enlarge due to thawing permafrost and thermal erosion of lake margins; whereas, drainage commonly occurs due to the erosion of ice wedges, streams or coastal margins. This often leaves large lake basins that re-colonize with vegetation and hold dozens to thousands of Arctic tundra ponds. Low-centered polygons delimit these small shallow ponds that are formed as permafrost aggrades and ice wedges grow and push up networks of small ridges (Hobbie, 1980).

Tundra ponds are typically small (50 m across), shallow (<0.5 m deep) ponds at the center of ice wedge polygons, surrounded by wet tundra within a large thaw lake basin. Tundra ponds are formed through the seasonal thaw of the active layer within low-centered polygons. They typically freeze to the bottom each winter and thaw for only a few months from June to September (Stross et al., 1980), thus organisms have only a few months to grow and reproduce (Stross et al., 1980). Water flows between the ponds for only a few days each spring during snowmelt, with little above- or belowground flow for the rest of the summer, except in the rare event of exceptionally heavy rains. During the summer, most water from the ponds is lost through evaporation that is largely balanced by precipitation.

This project included four study regions: the International Biological Program (IBP), the Barrow Environmental Observatory (BEO), thermokarst ponds (TK), and ponds within the village of Utqiaġvik (UTQ), formerly Barrow, Alaska. Maps of these sites are available elsewhere (Lougheed et al. n.d.; Lougheed et al., 2011, 2015)

International Biological Program (IBP)

The IBP area was established south of Utqiaġvik in the 1970s in order to extensively study the ecology of tundra ecosystems. From 1970-73, studies in the tundra ponds focused mainly on fluxes of carbon, nitrogen and phosphorus through the ecosystem, as well as detailed
ecological studies about algae and zooplankton (Hobbie, 1980). In this project, we are rescuing invaluable zooplankton community data from these studies.

**Barrow Environmental Observatory (BEO)**

The Barrow Environmental Observatory (BEO) is a protected area established in 1992 for research purposes regarding the long term evaluation of climate change effects on tundra ecosystems. This area will serve not only as a reference site for this study, but also as a baseline for future studies as the future of the unprotected IBP sites is uncertain.

**Thermokarst ponds**

Thermokarst (TK) ponds are typically formed in discrete locations among polygon troughs and caused by abrupt thawing of ice-rich permafrost, and subsequent land subsidence and slumping. Recent and rapid expansion of thermokarst ponds (or troughs) in permafrost regions have been associated with warming (Liljedahl et al., 2016), as well as human development (Raynolds et al., 2014). These ponds are generally rich in organic matter and dissolved organic carbon (Rautio et al., 2011a).

**Utqiagvik ponds**

Utqiagvik ponds are located within the village of Barrow, in proximity to private residences, commercial properties and associated roads. These ponds are thus enriched by human activities and are considered more impacted than IBP and BEO ponds. While some of these may be true tundra ponds, most of them were likely formed by ponding of water after construction of roads, which are generally constructed by piling of sand and gravel to raise the road above the surrounding land.

**Structure of this Dissertation**

The dissertation presented in this manuscript is composed of a total of four chapters, where three chapters (2-4) are composed of distinct datasets (Table 1). The author of this dissertation (Mariana Vargas Medrano) is the lead author and analyst of each chapter presented in this document.
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Chapter 2: Changes in zooplankton communities of Arctic tundra ponds after 40 years of warming

2.1 Introduction

The climate near Utqiaġvik, formerly Barrow, Alaska has been warming steadily for at least the past 50 years (Lougheed et al., 2015). Similarly, the temperature within tundra ponds has increased over the same period (McEwen & Butler, 2018), with warmer water temperatures most pronounced at the beginning and end of the open water season (Lougheed et al., 2011), leading to a potential lengthening of the growing season. Future projections suggest that Arctic lakes will experience later freeze-ups and earlier break-ups (Prowse et al., 2011), a pattern that we can assume will extend to tundra ponds, and will have important consequences for the growth and reproduction of organisms inhabiting these ponds.

Previous studies have noted critical changes that have occurred in tundra ponds and similar habitats over the past half century including, increased nutrient and algal concentrations (Lougheed et al., 2011), expansion of aquatic vegetation (Andresen et al., 2017), increased wetland plant biomass (Andresen et al., 2017), earlier plant greening dates (Andresen et al., 2018), changes in algal nutrient limitation (Lougheed et al., 2015), and increased methane flux (Andresen et al., 2017). However, very few studies have examined changes over time in consumers, such as zooplankton, in arctic tundra freshwaters due in part to the scarcity of historic, quantitative data.

Observed changes in temperature and other factors, such as primary production, in tundra ponds have the potential to have major impacts on the zooplankton. Zooplankton species richness in ponds across the Arctic appears to be driven by summer temperatures, with warmer sites having greater diversity (Herbert & Hann, 1986; Novichkova & Azovsky, 2017). Species composition may also change with warming, as zooplankton taxa have the potential to respond differently to changes in temperature. For example, several studies of tundra ponds and lakes found that small bodied cladocerans, such as chydorids, were positively associated with warmer water temperatures (Korhola, 1999; Jose, 2009), as were Daphnia (Walseng et al., 2018; Klobucar et al., 2018), whereas copepods tended to do better than cladoderans at cooler temperatures (Herbert & Hann, 1986; Jose, 2009; Novichkova & Azovsky, 2017). Additional factors that may change in response to warming, such as algal production, benthic algae biomass
(Rautio & Vincent, 2006; Rautio et al., 2009; Cazzanelli et al., 2012), macrophyte habitat availability (Herbert & Hann, 1986) and dissolved organic carbon (DOC) (Chételat & Amyot, 2009) have been also identified as important to determine zooplankton species composition in arctic water bodies. Finally, competition and predation are also likely structuring variables in tundra ponds (Stross et al., 1980; Dzialowski et al., 2004; O’Brien & Luecke, 2011), with fishless ponds having relatively high zooplankton abundances (Rautio et al., 2011a).

More than 40 years ago, a detailed research program was initiated in Arctic ecosystem ecology near Barrow (now Utqiaġvik), AK. The International Biological Program (IBP) was established in the 1970s. IBP researchers performed observational and manipulation experiments within tundra ecosystems to better understand the ecology of extreme environments, including freshwater ecosystems (Hobbie, 1980). Of note here are the detailed quantitative surveys of zooplankton abundance performed during the summers of 1971 through 1973 (Stross, 1974; Stross et al., 1980). Historic data from Arctic zooplankton communities are rare, and these detailed historic studies from the IBP have the potential to help us understand the cascading effects of warming temperatures in these unique and sensitive ecosystems. The goal of this study was to capitalize on the historic IBP data and, by comparing these to modern samples, determine the drivers of any changes in zooplankton communities in Arctic tundra ponds through time.
2.2 METHODS

Modern sampling design

Zooplankton were sampled weekly from IBP ponds B, C, D, E and J from 2010, 2011 and 2012; Pond X was sampled weekly in 2011 and 2012. Site locations can be found in Lougheed et al. (2011). The sampling period typically ran from early June to mid-August. Five 2L samples of water were collected from haphazardly selected open water regions in each pond, but within 1m from the edge of macrophytes. These five samples were combined into composite zooplankton samples of 10 L, filtered through a 64 µm filter and preserved with 4% sugar formalin. In the lab, samples were rinsed with water, mixed thoroughly and individual subsamples of 5 to 10 ml were counted under a Leica MZ125 light stereoscope. Subsamples of at least 100 organisms, including at least 20 large organisms (>300 mm mean length) were identified; the whole sample was also surveyed for large and rare organisms (Lougheed & Chow-Fraser, 1998). We recorded the length of representative individuals of all taxa; length data for *Daphnia* are presented here. Anostraca and Cladocera were identified to the lowest possible taxonomic level based on published sources (Dodson & Frey; Pennak, 1978; Thorph & Covich, 2001). Copepods were largely identified to order or family only.

Historic data

Historically, zooplankton samples ranged from 8 to 12 L total volume collected using composite samples similar to modern methods and filtered with 64 µm plankton net. IBP ponds B, C, D, E, and X were sampled weekly to bi-weekly in 1971-1973. Data were extracted from Stross, 1974. For statistical comparisons among decades, including t-tests and NMS analysis, we excluded data from pond E because of an oil spill experiment (Mozley & Butler, 1978; Barsdate et al., 1980) that artificially reduced zooplankton abundances.

Environmental data

Concurrent with zooplankton samples, water samples were collected for analysis of related environmental parameters. These data were collected and analyzed as part of related studies (Miller et al., 1980; Prentki et al., 1980; Lougheed et al., 2011, 2015).
Algal chlorophyll-a (CHLa) was determined as described in Lougheed et al. (2015). In brief, concentrations were calculated on a volumetric basis for phytoplankton (μg/L) and by area sampled for periphyton (μg/cm²). Data from the 1970s were obtained from historical sources (Barsdate & Prentki, 1973), as well as original lab notebooks (Alexander V., pers. comm). In order to allow a comparison of modern and historical data, the concentration of total phytoplankton chlorophyll-a (CHLa) was calculated using the formula of Strickland & Parsons (1972). Periphyton CHL-a, which was determined on a per dry weight basis in the 1970s, was converted to areal concentrations based on a regression between the 2 methods completed for IBP ponds in 2011 and 2012 (n=176, r²=0.8611, p<0.0001). Periphyton concentrations were corrected for turbidity and phaeopigments by acidification (Wetzel & Likens, 2000).

Nutrient chemistry was analyzed as described previously (Lougheed et al. 2011; Reyes & Lougheed, 2015). In brief, total phosphorus (TP) and total dissolved phosphorus (TDP) were determined by the ascorbic acid method following persulphate digestion; soluble reactive phosphorus (SRP) by the ascorbic acid method; NO₃-N by cadmium reduction; total ammonia (measured as NH₃-N) by the salicylate method; silica (Si) by the heteropoly blue method. Total nitrogen (TN) was analyzed with a Lachat IL 550 TOC/TN analyzer. Total alkalinity was also determined using standard methods (APHA, 1998) on a Mettler Toledo G20 autotitrator. Nutrient data from the 1970s were obtained from historical sources (Barsdate & Prentki, 1973). Non-purgeable dissolved organic carbon (DOC) data from the 1970s were compiled from historic sources (Barsdate & Prentki, 1973). For modern samples, DOC was measured on a Lachat IL-550 and was converted to non-purgeable DOC based on a conversion from samples run concurrently on a Shimadzu TOC-V instrument (Lougheed et al. n.d.).

In the 2000s, a YSI 556 multiparameter probe was used to measure temperature, pH and specific conductance. In the 1970s, specific conductance was collected weekly in 1970, which is the year prior to our study. We used these data assuming annual trends in conductivity remained relatively constant through time. Water temperature data collection from the 1970s is described elsewhere (Lougheed et al., 2011). pH data from the 1970s are described in Hobbie (1980).

In 2010, percent cover of open water, as well as the two dominant aquatic plants (Carex aquatilis, Arctophila fulva), was visually estimated on a weekly basis. These data are not
available from the 1970s; however, we have shown that biomass (Andresen et al., 2017) of both these aquatic plants was significantly lower in the 1970s.

**Data analysis**

To compare zooplankton densities among decades, data for each decade were averaged by week of the year, then a paired t-test was performed, with data from the decades matched by week of the year. To determine whether zooplankton taxa were reaching peak abundances at different times among the 2 decades studied, we identified the Julian day, for each site and year, where the greatest abundance was observed for each taxa. These Julian days were then compared among the 2 decades using a Wilcoxon rank test since the data could not be normalized.

A nonmetric multidimensional scaling (NMS) analysis was performed to describe the structure in the zooplankton data, combining both historic and modern data. Weekly average data for each time period were used in this analysis. NMS analysis identifies axes that describe biologically meaningful, multivariate gradients in the community data (McCune and Grace 2002). NMS analysis was performed using a Bray-Curtis distance measure in PC-ORD (version 5). We performed correlations between NMS axis scores and the available environmental variables, as described above, to explain the arrangement and drivers of zooplankton among sites and through time. Only those environmental parameters that showed a significant correlation to an NMS axis are presented; all others were omitted.

**2.3 Results**

In the 1970s, dominant taxa in the IBP ponds were cyclopoid copepods (*Cyclops* spp) and *Daphnia middendorffiana*, with lesser numbers of Diaptomid calanoid copepods (*Arctodiaptomus bacillifer*, *Diaptomus glacialis*), *Heterocope septentrionalis*, *Branchinecta paludosa* and *Polyartemia hazi* (Stross, 1974; Stross et al., 1980). Rarer taxa, for which quantities were not recorded, included *Chydorus sphaericus* and *Eurycercus lamellatus* (Dodson, 1979) (Table 2).
Table 2. Taxa for this study and others reported within Arctic ponds. Single asterisk (*) indicates that quantitative data for cyclopoid copepods were grouped together as *Cyclops* spp (Stross, 1974). Double asterisk (***) indicates that species were reported but not enumerated. Empty cells indicate the taxon was not reported in that study.

<table>
<thead>
<tr>
<th>Taxon (This study)</th>
<th>Stross (1974, 1980)</th>
<th>Dodson (1979)</th>
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</thead>
<tbody>
<tr>
<td><strong>Anostraca</strong></td>
<td></td>
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<tr>
<td><em>Branchinecta paludosa</em></td>
<td><em>Branchinecta paludosa</em></td>
<td><em>Branchinecta paludosa</em></td>
</tr>
<tr>
<td><em>Polyartemiella hazen</em></td>
<td><em>Polyartemiella hazen</em></td>
<td><em>Polyartemiella hazen</em></td>
</tr>
<tr>
<td><strong>Cladocera</strong></td>
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<tr>
<td><em>Chydorus sphaericus</em></td>
<td>-</td>
<td><em>Chydorus sphaericus</em></td>
</tr>
<tr>
<td><em>Daphnia middendorffiana</em></td>
<td><em>Daphnia middendorffiana</em></td>
<td><em>Daphnia middendorffiana</em></td>
</tr>
<tr>
<td><em>Eurycercus lamellatus</em></td>
<td>-</td>
<td><em>Eurycercus lamellatus</em></td>
</tr>
<tr>
<td><strong>Copepoda</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Heterocope septentrionalis</em></td>
<td><em>Heterocope septentrionalis</em></td>
<td><em>Heterocope septentrionalis</em></td>
</tr>
<tr>
<td><em>Arctodiaptomus bacillifer</em></td>
<td><em>Diaptomus glacialis</em></td>
<td><em>Arctodiaptomus bacillifer</em></td>
</tr>
<tr>
<td><strong>Nordodiaptomus alaskaensis</strong></td>
<td><strong>Nordodiaptomus alaskaensis</strong></td>
<td><strong>Nordodiaptomus alaskaensis</strong></td>
</tr>
<tr>
<td><em>Cyclopoid copepods</em></td>
<td><em>Acanthocyclops vernalis</em></td>
<td><em>Megacyclops magnus</em></td>
</tr>
</tbody>
</table>

Although the same taxa remain dominant in the ponds 40 years later, the relative dominance of these taxa has changed. For example, in the 1970s cyclopoid copepods were the most common zooplankter, representing an average of 39% of the abundance across all sites and dates changing to a 23% on the 2010s. On the contrary, *D. middendorffiana* was the most common taxa in the 2010s, forming 37% of the total abundance with a 13% in the 1970s.

Average abundance of zooplankton clearly showed that pond E in the 1970s had substantially lower abundance as compared to all other sites, likely due to oil spill experiments performed at that time (Figure 1, bottom-left). Consequently, zooplankton data from pond E were excluded from all subsequent statistical analyses. Three cladocerans for which we don’t have quantitative data from the 1970s, *C. sphaericus* (0.44 (±0.73)/L), *Eurycercus lamellatus* (0.35 (±0.53)/L), and *Eurytemora canadensis* (0.61 (±0.62)/L), were represented in Figure 1 as others. It is worth noticing that, in recent times, *Chydorus sphaericus* was more abundant than some taxa in the 1970s (i.e. *B. paludosa*), which may represent a change in dominant taxa over time; however, without numerical data on *C. sphaericus* from the 1970s, we cannot confirm this.
To compare statistically among decades, data were averaged by week of the year, then a paired t-test was performed, with data from the two different decades matched by week of the year. Three taxa appeared to significantly differ in abundances now as compared to the 1970s (p<0.05) (Figure 2). *Daphnia middendorffiana* increased dramatically in abundance from an average of 3.41 (±4.92/L (SE)) in the 1970s to 6.94 (±13.30)/L in more modern samples (paired t-test, p=0.01). Conversely, calanoid copepods decreased in abundance from 5.30 (±11.62)/L in the 1970s to 2.81 (±3.96)/L during the 2010s (paired t-test, p=0.007) as well as cyclopoid copepods decreased in abundance from 3.91 (±12.02)/L in the 1970s to 2.20 (±3.67)/L during the 2010s (paired t-test, p=0.03). The average abundances of other taxa did not differ among decades. For example: *Heterocope septentrionalis* in the 1970s was 0.82 (±1.85)/L in the 1970s and 0.90 (±1.24)/L in the 2010s; *Polyartemiella hazeni* was 0.47(±0.98)/L as compared with
0.28 (±0.27)/L more recently; and *Branchinecta paludosa* in the 1970s had a abundance of 0.19 (±0.27)/L with an increase of 0.46(±0.65)/L (paired t-test, p=0.08) in the 2010s. In modern times, *Chydorus sphaericus* was found at two IBP sites with an average of 0.44 (±0.73)/L (not shown); this abundance is more than fairyshrimps (*Polyartemiella hazeni* and *Branchinecta paludosa*) together with an average of 0.37(±0.50)/L (Figure 12).
Figure 2. Comparison of the 1970s and 2010s average zooplankton abundances of 6 taxa by the week of the year. Red triangles represent the averaged zooplankton of the 2010s and the black circles represent the averaged zooplankton of the 1970s. Differences were analyzed using a paired t-test (P<0.05). Data from pond E in the 1970s were excluded from these plots.

Figure 3. Comparison of the relationships among average length (mm) and brood size for *Daphnia middendorffiana* in the 1970s (IBP-C; Stross 1974) and 2010s (all IBP sites).

*Daphnia* in the IBP ponds were not only more abundant in the 2010s, but were also much longer for any given brood size, with average length ranging from 3 to 4mm in recent times, as compared to less than 3mm in length historically (Figure 3). There were several individuals with 9 or 10 eggs in recent times, more than reported in the historical data; however, these represented a very small number of individuals and were not included in the graph.

While there were only three statistically observable differences in species abundance among decades, it is apparent that some taxa may have peak abundances earlier in the season now than they did 40 years previously. When examining the average Julian Days at which taxa reach peak abundances among decades, three taxa tended to peak at significantly different times of year (paired t-test, p<0.10) (Figure 4). *B. paludosa* reached its highest abundances 3 weeks earlier in recent years as compared with historic values (p=0.003). In the 1970s peak abundances
were observed in early-July, whereas the peak abundances in the 2010s occurred just after snow melt in early-June. Cyclopoid copepods also peaked earlier in June than it had historically, having a peak abundance 10 days earlier than that observed in the 1970s (p=0.03). Finally, although only marginally significant (p=0.08), calanoid copepods also appeared to reach peak abundance 11 days earlier in June (Figure 4).

Figure 4. Average Julian days where the highest zooplankton densities for each taxon were observed in the 1970s and 2010s. Data from pond E in the 1970s were excluded. Bars indicated standard error. P-values were from Wilcoxon tests.

An NMS ordination including both 1970s and 2010s average weekly zooplankton densities from 4 (1970s; excluded E) or 5 (2010s) ponds resulted in a 2-dimensional solution
with a final stress of 12.69 (Figure 5). Both axes were statistically significant (p<0.01).

While there was considerable overlap among the eras, 1970s data were found significantly lower (to the left) along axis 1 than were 2010s data (Figure 5). *Daphnia middendorffiana*, which were found in mid-summer and in significantly higher numbers in the 2010s (Figure 2), were strongly positively correlated to this axis (r=0.59, p<0.001), while all other taxa, which tended to peak earlier in the season, showed a significant negative correlation to this axis. This first axis corresponded to an environmental gradient contrasting cooler temperatures, lower nutrients (DOC, TN, Si), higher periphyton but lower phytoplankton, and lower alkalinity on the left (negative) side of the axis, to higher nutrients, temperatures and phytoplankton chlorophyll-a values to the right of the axis (Figure 5).

Axis 2 appeared to be related positively related to pH and nitrate concentrations, and had no association with temperature (Table 3). All taxa showed a positive correlation with this axis. Comparison of NMS 2 scores among decades did not show a significant difference (Figure 6); however, 1970s NMS 2 scores were marginally higher than those in the 2010s (p=0.078). However, NMS 2 did have a negative relationship with the percent cover of *Arctophila fulva* (measured only in 2010)(Table 3). In fact, we noted that sites IBP-X (triangles) and IBP-D (inverted triangles) tended to dominate the bottom half of the bi-plot, or the negative end of axis 2. These were the only sites where the plant *A. fulva* occurred relatively abundantly; there was a significantly greater cover, on average, at these 2 sites as compared to the other 3 sites over the course of the season (ANOVA, Tukey HSD, p<0.0001). Higher cover of *A. fulva* appeared to be negatively associated with the abundances of most zooplankton taxa.
Figure 5. Non-metric multi-dimensional scaling (NMS) analysis of weekly averaged mid-August zooplankton densities from 5 ponds near Utqiaġvik, Alaska in the 1970s and 2010s, including sites and species scores. Solid circles are the 1970s data; open circles are the 2010s data. Species codes: *D. middendorffiana* (DAPMID), cyclopoid copepods (CYCCOP), *B. paludosa* (BRAPAL), *H. septentrionalis* (HETSEP), other (diaptomid) calanoid copepods (CALCOP), *P. hazeni* (POLHAZ).
Figure 6. Comparison of NMS 1 and 2 scores among the 1970s and 2010s data. Red lines represent the averaged NMS scores of the 2010s data and the black lines represent the 1970s. Differences were analyzed using a paired t-test (p<0.05).

Table 3. Correlations of NMS axis scores with environmental variables. Environmental variables were log transformed, as needed. Environmental data collected only in 2010s is indicated by ~. Uncorrected p-value were reported as *<0.05, **<0.01, ***<0.001.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>NMS2</th>
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<tbody>
<tr>
<td>Week of the year</td>
<td>0.37***</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.37**</td>
<td></td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>0.36***</td>
<td></td>
</tr>
<tr>
<td>TN (ppm)</td>
<td>0.36**</td>
<td></td>
</tr>
<tr>
<td>Specific Conductance (mS/cm)</td>
<td>0.44***</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>0.34**</td>
<td></td>
</tr>
<tr>
<td>Si (ppm)</td>
<td>0.30***</td>
<td></td>
</tr>
<tr>
<td>Total Phytoplankton CHLa (µg/L)</td>
<td>0.23*</td>
<td></td>
</tr>
<tr>
<td>Periphyton CHLa (µg/cm²)</td>
<td>-0.29*</td>
<td></td>
</tr>
<tr>
<td>NO3</td>
<td>0.36***</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.22*</td>
<td></td>
</tr>
<tr>
<td>%Cover A. fulva ~</td>
<td>-0.35*</td>
<td></td>
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</tbody>
</table>
2.4 DISCUSSION

While the dominant taxa within the zooplankton community in the IBP ponds have remained largely unchanged over time, at least 1 new zooplankton species has been observed. Numerically, zooplankton composition was dominated by cyclopoid copepods in the 1970s and more recently has changed to a dominance of *D. middendorffiana*. Several species are emerging earlier. The primary drivers of zooplankton abundances appeared to be increases in temperature and algal food availability.

Zooplankton composition in the IBP ponds is not unlike that observed by others in sites throughout the Arctic (Rautio & Vincent, 2006; Abramova *et al.*, 2017), and includes the presence large microzooplankton such as *Daphnia middendorffiana*, *Polyartemiella* & *Branchinecta*, calanoids, as well as the occurrence of smaller *Chydorus sphaericus*. However, unlike other studies in the Arctic (Bégin & Vincent, 2017; Abramova *et al.*, 2017), we found relatively low density and diversity of rotifers. Dominance by daphnids, which we generally found, may prevent the proliferation of rotifers (Christoffersen *et al.*, 2008).

Similar to 40 years prior (Stross *et al.*, 1980), *D. middendorffiana* remained the dominant grazing zooplankton in the IBP ponds; however, it now appears to be more common, more abundant and larger than reported historically. This increase may be associated with warmer temperatures and higher nutrient levels, resulting in greater algal food availability (Lougheed *et al.*, 2011), likely associated with nutrient release from permafrost thaw (Reyes & Lougheed, 2015). Lending further support to these as possible drivers, we have also observed that the most nutrient and algal rich ponds in Uqtiaġvik, urban ponds and thermokarst ponds, have even greater numbers of *D. middendorffiana*, reaching densities as high as 143 individuals/L (Chapter 4). Other researchers have similarly found that growth and reproduction of *Daphnia middendorffiana*, an abundant and common Arctic herbivore, can be associated with increased productivity in the form of dissolved (DOC) and particulate carbon (Chételat & Amyot, 2009), increased algal chlorophyll-a (Yurista & O’Brien, 2001), number of edible algal cells (Stross *et al.*, 1980), benthic algal mats (Mariash *et al.*, 2014) and warmer temperatures. Other *Daphnia* species have also been associated with increased productivity (Luoto, Brooks & Salonen, 2014; Luoto, Oksman & Ojala, 2016) and temperatures (Walseng *et al.*, 2018) in Arctic ponds and lakes. However, it is worth noting that there is a likely limit to the positive effect of temperature...
on *D. middendorffiana*. In experiments with *D. middendorffiana* in alpine ponds, which were warmed from 8 to 14°C, a decline in the population was observed (Thompson & Vinebrooke, 2008). An additional potential future limiting factor could be the observed increases in pCO$_2$ in tundra ponds (Lougheed *et al.* n.d), as elevated levels of pCO$_2$ may make *Daphnia* more vulnerable to predation (Weiss *et al.*, 2018). Finally, it is worth noting that the greater abundances of large-bodied grazers in these ponds in modern times likely had the ability to rapidly consume the available phytoplankton. Increased abundance of *Daphnia* may play an important role in controlling algal biomass in tundra ponds (Van Geest *et al.*, 2007a).

One additional possible explanation for the increased *Daphnia* abundance and size is a change in food availability. Benthic algae are an important food source for *Daphnia middendorffiana* and other zooplankters, such as the fairy shrimp *B. paludosa*, in Arctic freshwaters, and may contribute substantially to secondary production in high arctic ponds (Rautio & Vincent, 2006; Rautio *et al.*, 2009; Cazzanelli *et al.*, 2012). We have documented a dramatic change in the benthic algae community over the past 40 years in IBP Pond B (Vargas Medrano, 2011). In particular, in mid-July, when *Daphnia* have their peak abundance, we found an increased biomass of diatoms in the benthos in 2010 as compared to 1972, when the community was historically dominated by cyanophytes and chlorophytes (Vargas Medrano, 2011). In previous studies, *Daphnia* who fed on diatoms benefited from their high EPA (eicosapentaenoic acid) content to protect them from UVR (Zellmer *et al.*, 2004). In a related study, we found that *Daphnia* provided with an enriched algal food source, as well as benthic structure in the form of thawed permafrost, had significantly enhanced growth and reproduction (Chapter 3).

The increased abundance and size of *Daphnia* in these ponds would likely have resulted in a much higher difference among decades if we had considered biomass of the zooplankton taxa; however, biomass calculations were not performed. Larger *Daphnia* in this study appeared to put more energy into increasing their size than into egg production, perhaps due to predation pressure. *Daphnia* size can be related to predation mechanisms, smaller *Daphnia* species are more vulnerable to predation (Dodson, 1979; O’Brien, 2001). Furthermore, while *Daphnia* size tends to decline with warming related to latitude (Gillooly & Dodson, 2000), recently observe warming in tundra ponds may also have affected Daphnia size distributions.
Finally, the large numbers of *Daphnia* was likely due in part to the fishless nature of these ponds. Zooplankton in tundra ponds can reach relatively high abundances because they are often fishless (Rautio *et al.*, 2011a); the presence of fish can rapidly diminish the presence of large-bodied zooplankton (Lauridsen *et al.*, 2001). Large invertebrate predators can also play a role; several studies have suggested that the carnivorous copepod *Heterocope septentrionalis* determined the species of *Daphnia* present in Arctic freshwaters by promoting the dominance of *D. middendorffiana* over the smaller *D. pulex* (Dzialowski *et al.*, 2004; O’Brien & Luecke, 2011). *Heterocope* also fed on immature *Daphnia* (Stross *et al.*, 1980). Stross *et al.* (1980) stated that all zooplankters serve as a food source for other organisms at different stages of their life cycle. While IBP ponds are generally fishless, we surprisingly observed in mid-August 2012 the appearance of fish in IBP-D, perhaps due to overland travel during spring flows or attachment of propagules to birds. There are, however, insufficient data to describe the role of the occasional occurrence of fish in these ponds.

Zooplankton in Arctic lake ecosystems may also rely on terrestrially-derived carbon for their nutrition (Rautio *et al.*, 2011a); however, others found that terrestrial carbon was a minor contributor to the diet the Arctic taxon *D. middendorffiana* (Cazzanelli *et al.*, 2012). There appears to be a lack of research on the role of allochthonous carbon in Arctic pond food webs, notably the impact on zooplankton communities (Rautio *et al.*, 2011a). This is an area of research worth pursuing given the significant increase in DOC observed in the IBP ponds over the past 40 years (Lougheed *et al.* n.d.).

While others have noted increased zooplankton richness with warming (Herbert & Hann, 1986; Novichkova & Azovsky, 2017), we are unable to say that this has occurred. In a previous study, we also found very little change in the macroinvertebrate taxa from IBP ponds, where only 2 new taxa appeared over the same 40 year period (Lougheed *et al.*, 2011). However, *Chydorus* appears to be substantially more abundant than it may have been previously. Although its presence was noted in the 1970s (Dodson, 1979), it was not considered abundant (Stross *et al.*, 1980). Chydorid species have often been associated with macrophyte or benthic habitats (Lougheed & Chow-Fraser, 2002), and lack of suitable habitat may partly explain the absence of many chydorids from some sparsely vegetated high Arctic ponds (Herbert & Hann, 1986). Recent increases in vegetation biomass and cover in the tundra ponds near Utqiaġvik (Andresen & Lougheed, 2015; Andresen *et al.*, 2018) may have promoted greater dominance of chydorid
species in these ponds. *Chydorus* may also be associated with eutrophication (Vijverberg & Boersma, 1997; Tönnö et al., 2016). In addition, several studies of tundra ponds and lakes found that small bodied cladocerans, such as chydorids, were positively associated with warmer water temperatures (Korhola, 1999; Jose, 2009), whereas calanoid copepods preferred cooler temperature (Jose, 2009) or low arctic sites (Herbert & Hann, 1986). Recent molecular work has emphasized that, although *Chydorus sphaericus* was largely considered a cosmopolitan species, it may well consist of several cryptic regional lineages. Greater work may be required to determine which specific lineage is increasing in abundance in the region (Belyaeva & Taylor, 2009).

Warmer temperatures may also help explain why calanoid copepods were less dense now than in the 1970s, and are also reaching maximum abundance earlier that in the 1970s. Most calanoids overwinter as resting eggs, and take a relatively long time to reach sexual maturity. As such, they tend to be rarer in Arctic environments, where shorter growing seasons may prevent all but the fastest growing taxa to reach sexual maturity before freeze-up (Herbert & Hann, 1986). Spring is not occurring earlier but temperatures are higher and zooplankton might develop more quickly as a response to warming.

The date of maximum abundance of several taxa, cyclopoid copepods, calanoid copepods, and *Branchinecta paludosa*, changed over the 40 year period. All three are peaking in abundance 10-21 days earlier in the season now than in the 1970s, which represents an advancement of approximately 0.5 days/y. This is not an unreasonable number considering that freshwater plankton communities in general, including phytoplankton and zooplankton, have been observed to have advancing maximum abundance dates by an average of 1.5 days per year (Vadadi-Fülöp & Hufnagel, 2014). Although cyclopoid copepods are peaking earlier than observed in the 1970s, their abundance is reduced. Explanations for this could include competition for food between cyclopoid copepods, their nauplii and *Daphnia* (Vanni, 1986), which have increased substantially in number.

The density of zooplankton is determined by the individuals that overwinter (Stross et al., 1980). Zooplankton have various strategies for overwintering and cues for spring emergence likely depend, at least in part, on temperature. As such, day of maximum abundance depends on latitude, with species emerging later at higher latitudes (Gillooly & Dodson, 2000). While snow
appears to be melting earlier in Utquiaġvik (Stone et al., 2002), McEwen & Butler (2018) modeled that the IBP ponds were not thawing significantly earlier, when data were compared from 1973 to 2012. However, they did find that temperatures at the start of the season were significantly higher (Lougheed et al., 2011; McEwen & Butler, 2018). Cyclopoid copepods, in particular, are highly adapted to the extreme environments in the Arctic, as they can produce eggs throughout the growing season, and overwinter as encysted pre-adult copepodids, which allows for rapid population growth immediately after ice-out (Stross, 1974; Abramova et al., 2017). As such, warmer spring temperatures have been associated with earlier emergence of cyclopoid taxa (Gerten & Adrian, 2002), as we observed in this study.

2.5 Conclusion

The warming of the IBP tundra ponds has impacted the zooplankton communities in various ways, with increased abundance and size of *Daphnia*, but fewer calanoid copepods. Several taxa are also showing peak maximum abundance earlier in the season. These changes can likely be attributed to warmer temperatures, which relate to temperature tolerances, as well as warmer temperatures promoting permafrost thaw, the release of nutrients into the water column, and subsequent increases in the availability of algal food. More studies are needed to continue with the understanding of these important changes in ecosystems functioning.
Chapter 3. The impact of nutrient addition and permafrost thaw on Arctic phytoplankton and its cascading effects on Daphnia

3.1 INTRODUCTION

The Arctic region is experiencing a period of unprecedented warming (Callaghan et al., 2004), increasing at twice the rate in comparison with lower latitudes (McBean et al., 2005). In the northernmost part of the US, near Utquiaġvik, AK, air temperatures have risen 2°C since the 1950s (Andresen & Lougheed, 2015). Arctic freshwater systems are also warming (Houghton, Jenkins & Ephraums, 1990; Hansen, Nielsen & Levinsen, 1999; Yurista, 1999; Rothrock, Zhang & Yu, 2003; Smol et al., 2005; Lougheed et al., 2011) with a multitude of resulting effects on freshwater ecosystems, including an increase in primary production (Michelutti et al., 2005); decrease in cold stenotherms (algae, benthic macroinvertebrates, and fish) (Lindholm et al., 2015); loss of pond area due to evaporation (Smol & Douglas, 2007); permafrost degradation and plant encroachment (Andresen & Lougheed, 2015); an increase of nutrients in the water column (Lougheed et al., 2011), likely due to release of nutrients from permafrost (Reyes & Lougheed, 2015); changes in algal communities (Douglas et al., 1994; Vargas Medrano, 2011); changes in the primary nutrient limiting algal growth (Lougheed et al., 2015); increase in methane efflux (Andresen & Lougheed, 2015); increase of snow depth; and decrease in snow cover extent and duration (Bring et al., 2016). However, the implications of warming and these consequent changes have rarely been examined for anything other than primary producers, with very little research completed on changes in primary consumers, such as zooplankton.

Lougheed et al., (2011) suggested that nutrient enrichment is occurring in Arctic freshwaters, with tundra ponds having changed physically, chemically, and biologically since the 1970s. In ponds, warming temperatures are leading to permafrost thaw, adding more nutrients to the water (Reyes & Lougheed, 2015), which has important implications for algal nutrient limitation (Lougheed et al., 2015). In addition to permafrost thaw, nutrient concentrations may be increasing due to anthropogenic inputs (Schindler, 1974; Douglas & Smol, 2000; Antoniades et al., 2011) or atmospheric N or P deposition (Elser et al., 2009). Atmospheric deposition of nitrogen in the Arctic has been increasing in recent history (Mayewski et al., 1986), particularly in the Utquiaġvik area (Jaffe, 1992).
Recent studies have shown that algal communities in tundra ponds near Utqiaġvik, AK have changed from one limited by phosphorus in the 1970s (Alexander et al., 1980) to one experiencing NP co-limitation (Lougheed et al., 2015). The mechanism for this change may be the addition of nutrients from permafrost thaw (Reyes & Lougheed, 2015), as well as competition for bioavailable N with an expanding macrophyte community (Andresen & Lougheed, 2015). Additional changes to algal communities include an increase in algal biomass (Lougheed et al., 2011), as well as changes in the periphyton communities (Vargas Medrano, 2011), over the past 40 years. Other studies have similarly found a change in algal communities in the Arctic (Alexander et al., 1980; Smol et al., 2005; Rautio et al., 2011a; Rühland et al., 2015). The potential for further future changes with warming has important implications for invertebrates that depend on algal food.

Northern populations of zooplankton, including Daphnia spp, have been shown to increase in abundance with enrichment by phosphorus (O’Brien et al., 2005; Sweetman & Smol, 2006; Van Geest et al., 2007a), dissolved organic carbon (DOC) (Chételat & Amyot, 2009), and at greater food concentrations (Yurista & O’Brien, 2001). Consequently, several authors have suggested that increases in productivity with warming may expand the number and distribution of Daphnia in the north (Chételat & Amyot, 2009; Sweetman, Rühland & Smol, 2010). However, other authors have shown that elevated temperatures may reduce assimilation rates (Yurista, 1999) or body fatty acid composition (Przytulska et al., 2015) of arctic Daphnia. Nonetheless, rates of primary production are generally low in arctic lakes (Markager, Vincent & Tang, 1999), perhaps due in part to top-down control by grazers (Hessen et al., 2004; Rautio & Vincent, 2006; Van Geest et al., 2007b).

Nutrient enrichment from thawing permafrost could have important implications for these organisms, as Arctic zooplankton, including D. middendorffiana, have been found to graze preferentially on benthic substrates (Rautio & Vincent, 2006; Cazzanelli et al., 2012). Lougheed et al. (2015) suggested that zooplankton preferentially grazing on P-rich benthic algae may have accounted for reduced algal growth on in situ P-enriched nutrient diffusing substrates in tundra ponds. This is particularly relevant, as benthic algae may contribute substantially more to total algal primary production as compared to phytoplankton (Alexander et al., 1980; Rautio & Vincent, 2006; Cazzanelli et al., 2012).
The goal of this study was to understand how arctic algal communities change in response to nutrient addition, and the cascading impacts of these changes on herbivorous zooplankton.

3.1 METHODS

Algal culture

Water was collected from Utqiaġvik, Alaska pond IBP B, shipped to UTEP and stored in a dark cold room at 15°C for no more than a year. The primary stock algal stock cultures was then cultivated in a 5 L aquarium with spring water and constant light at 15°C. Into 5 L glass aquaria, we added 500 mL of water from the primary stock algal cultures and 4 L of spring water. These individual algal cultures were enriched with either: N (nitrogen), P (phosphorus), or N+P (nitrogen and phosphorus). Nutrient stock solutions were prepared as follows: The N stock solution (10,000 ppm) was prepared with 7.1 g of NH₄NO₃ into 250ml of deionized water. The second P stock solution (10,000 ppm) was prepared with 11 g of KH₂PO₄ added to 250 ml of deionized water. Both stock solutions were kept them in the refrigerator at 4°C.

Nutrients were added to the algal cultures every four days in order to result in a significant increase in concentration relative to the controls. In order to maintain nutrient levels, water samples were collected prior to adding nutrients and analyzed spectrophotometrically for total phosphorus (TP), nitrate-nitrogen (NO₃-N), and ammonia (NH₃-N) using standard methods (American Public Health Association, 1998). TP was determined by ascorbic acid method followed by persulfate digestion; NO₃-N by cadmium reduction; NH₃ by the salicylate method. We also recorded temperature and pH in all treatments. 10 ml of sterilized pond water was added to all treatments and controls to maintain micronutrient levels. Cultures were enriched for 11 days to allow the algae to respond to nutrient enrichment (Van Geest et al., 2007a) prior to the next step in the experiment.

To simulate moderate regional warming, we incubated all experiments in a cold room at 15°C, which is at the low end of the maximum July-August temperature observed from 2010-2013 (14-20°C). Algae cultures were maintained with 24 hours of light, like those conditions in Utqiaġvik during the summer.
Zooplankton culture

*Daphnia middendorfiana* is a dominant herbivorous zooplankton in Arctic tundra ponds (Chapter 4). In this experiment, we use *Daphnia pulex*, which can be found in Arctic trough pools (Edmondson, 1955; Stross *et al.*, 1980), but is not generally present in tundra polygon ponds (O’Brien & Luecke, 1988). They are also easily available from commercial sources. These invertebrates produce a low number of infertile males, produce more than one generation per season and are capable of controlling algal biomass under nutrient-rich conditions (Steiner, 2002). *Daphnia pulex* were acquired from the Carolina Biological Supply Company; therefore, these are not clones from Arctic environments, as used in other studies (Luecke & O’Brien, 1983; Wolf & Hobaek, 1986; Weider & Hebert, 1987; Hessen *et al.*, 1999). To simulate Utqiagvik conditions, the cultures were kept at constant light exposure (24 hours). We fed them with the control algal stocks on a daily bases for 30 days until there were enough juveniles to perform the experiments.

Based on multiple authors work, the following experimental conditions were developed: (1) constant temperature and light, as well as enough food to avoid ephippia formation (Stross, 1969; Thompson & Vinebrooke, 2008); (2) experimental time period of at least 34 days so all individuals may reproduce at least once (Stross, 1969; Stross *et al.*, 1980; Holzapfel & Vinebrooke, 2005; Thompson & Vinebrooke, 2008); (3) addition of benthic substrates to improve *Daphnia* growth rates (Rautio & Vincent, 2006; Cazzanelli *et al.*, 2012).

**Nutrient enrichment experiments with *Daphnia***

Enriched algae were divided into 6 replicate 500mL beakers prelabeled as the control, or the three treatments (N, P, N+P). At the beginning of the experiment, we added 10 *Daphnia* juveniles, all of them of the same size, to each beaker. Enriched algae were added every three days to feed *Daphnia*.

The experiment was divided into two parts due to space, time and resources needed: experiments with algal cultures in water only were conducted separately from those with permafrost added. Thus, a second series of experiments, in which 100 grams thawed permafrost soil was dispensed into the beakers with identical nutrient amendments, was also conducted. All experiments were placed in a cold room at 15°C and constant light.
For *Daphnia* reproduction and survival rates, we monitored the experiments every three days. We identified and counted the total number of *Daphnia* and total number of *Daphnia* offspring in each beaker and treatment. Algae were identified as Bacillariophyceae, Chlorophyta or Cyanophyta only. To characterize the algal groups, algae samples were collected, counted and identified every 3 days. The experiment continued for 32 days following the *Daphnia* addition to allow all of them to grow and reproduce at least once. Finally, at the end of the experiment phytoplankton and benthic chlorophyll-a concentrations were determined for each treatment, as relevant.

### 3.3 Results

**Nutrient concentrations**

All nutrient enrichments were maintained at a significantly higher concentration relative to the controls (Table 4; paired t-test, Bonferonni corrected p<0.05); however, it is worth noting that dissolved inorganic nitrogen (DIN) in the P-only treatments was consistently lower than the control concentrations. Phosphorus concentrations were always higher in the permafrost treatments relative to the water only treatments (paired t-test, Bonferonni corrected, p<0.01); however, DIN concentrations were not significantly different among experiment types.

Table 4. Total phosphorus (TP) and dissolved inorganic nitrogen (DIN; NO$_3$ + NH$_4$) concentrations in nutrient addition experiments averaged over all days of the experiments (n=12) in water and permafrost (PF) treatments. The asterisk indicates a significant increase relative to the relevant control (water only or permafrost added) (paired t-test, Bonferonni corrected p<0.05). The ~ indicates a significant decrease relative to the control.

<table>
<thead>
<tr>
<th></th>
<th>TP (ppb)</th>
<th>DIN (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>17.7 ± 6.1</td>
<td>703.9 ± 328.4</td>
</tr>
<tr>
<td>P</td>
<td>973.5 ± 300*</td>
<td>254.9 ± 356.5~</td>
</tr>
<tr>
<td>N</td>
<td>18.9 ± 7.2</td>
<td>3398.1 ± 1146.7*</td>
</tr>
<tr>
<td>N+P</td>
<td>937.1 ± 285.8*</td>
<td>1908.6 ± 894.0*</td>
</tr>
<tr>
<td>C_PF</td>
<td>52.8 ± 18.8</td>
<td>365.6 ± 226.5</td>
</tr>
<tr>
<td>P_PF</td>
<td>1488 ± 515.5*</td>
<td>152 ± 149.3~</td>
</tr>
<tr>
<td>N_PF</td>
<td>41.9 ± 6.1</td>
<td>1799 ± 1488.3*</td>
</tr>
<tr>
<td>N+P_PF</td>
<td>1628.9 ± 400*</td>
<td>1085.8 ± 1291.0</td>
</tr>
</tbody>
</table>
Algal stocks

The algal stocks enriched with nutrients largely differed among treatments with and without phosphorus. Algal stocks were created with water only, without permafrost added; however, as the 2 experiment types (water and permafrost) a at different times, results for both water only stocks and permafrost stocks are presented. Chlorophyta did not show any statistically significant differences among any nutrient treatments during either water or permafrost experiments; however, the densities of Chlorophyta were relatively low (<6/L on average). On the contrary, Bacillariophyceae and Cyanophyta tended to have much higher densities. Bacillariophyceae showed a statistical difference among treatments (Tukey-Kramer HSD, p<0.05; Figure 7); where P and N+P treatments had statistically higher diatom numbers than the C and N treatments during both water and permafrost experiments (Figure 7). Conversely, Cyanophyta were more abundant in the C and N treatments, during permafrost experiments only (Tukey-Kramer HSD, p<0.05; Figure 7). In summary, cultures enriched with N only tended to have less Bacillariophyta and more Cyanophyta, while cultures enriched with P, regardless of N status, had more Bacillariophyta and less Cyanophyta.
Figure 7. Averaged algal counts in the algal stocks, during both water and permafrost treatments, for each different nutrient addition (N, P, and N+P) and the control (C) for Chlorophyta, Bacillariophyceae and Cyanophyta algal groups. The letter above bars indicates statistically significant difference calculated by Tukey-Kramer HSD (p<0.05) and the bars indicate standard error. Statistical comparisons are done within (not among) each experiment type (i.e. water only, permafrost added).
Nutrient enrichment experiments with \textit{Daphnia}

Figure 8. Average of \textit{Daphnia} (A) and offspring (B) densities for all nutrient addition treatments. Solid lines represent treatments with water only (no sediment). Dashed lines represent the treatments with permafrost additions. Error bars indicate standard errors. PF means permafrost experiments.

Clearly, permafrost additions enhanced \textit{Daphnia} densities (Figure 8), which was more remarkable in P and N+P additions with permafrost added where an increase in offspring near Day 25 (Figure 8b) led to many more adults observed after Day 30 (Figure 8a). In the water only
treatment, P and N+P experiments also lead to higher *Daphnia* abundances. C and N treatments had the lowest densities of *Daphnia* and offspring, but with permafrost additions, these two treatments were similar to P and N+P with no permafrost added (Figure 8). Repeated measure ANOVA (RM-ANOVA) indicated an effect of treatment and time on *Daphnia* and offspring densities as a response to the enrichment of water by nutrient additions and permafrost. There was also an interaction effect of time and treatment (Table 5).

Table 5. Repeated Measures ANOVA results of *Daphnia* and offspring densities. The Greenhouse-Grier correction was used due to p<0.05 in Mauchly’s sphericity test.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daphnia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>11.49</td>
<td>17.75</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time</td>
<td>22.89</td>
<td>4.43</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * Treatment</td>
<td>11.49</td>
<td>17.75</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>Offspring</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>11.74</td>
<td>11.09</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time</td>
<td>23.12</td>
<td>2.77</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * Treatment</td>
<td>11.74</td>
<td>11.09</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

In order to illustrate the effect of treatment, mean densities among treatments were compared. Mean density of *Daphnia* and their offspring showed a significant response to nutrient additions. Overall, significantly greater abundances were observed in the N+P and P treatments, while treatments with N only were not different from the control (Tukey-Kramer HSD test, p<0.05; Figure 9).
Figure 9. Mean densities of *Daphnia* and their offspring among experiments in water and with permafrost added (Tukey-Kramer HSD, p<0.05). Statistical comparisons are done within (not among) each experiment type (i.e. water only, permafrost added).

Overall, the number of *Daphnia* and offspring in the permafrost addition treatments was significantly greater than those in the water only (no permafrost added) experiments. The sole exception was the phosphorus treatment, where there was no statistical difference in offspring numbers among the permafrost addition and water only treatment (Table 66).
Table 6. Student t-test was calculated to compare experimental means of *Daphnia* abundances for each nutrient addition treatment among the water only and permafrost experiment types. The _PF means permafrost experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daphnia</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>C vs C PF</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>N vs N PF</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>P vs P PF</td>
<td>p&lt;0.05</td>
<td>No significant difference</td>
</tr>
<tr>
<td>N+P vs N+P PF</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

**Algae in experimental treatments**

Percent loss of algae from the treatments, as compared to the initial nutrient enriched stocks, indicated that the lowest percent loss tended to be in the water only experiments, most notably in the control and N treatments. The highest percent loss occurred with nutrient addition in addition to permafrost. Interestingly, Bacillariophyceae in the P and N+P permafrost treatments decreased more than 40% suggesting consumption of these algae by *Daphnia*. Conversely, diatoms increased in abundance in the control beakers, with greater loss of Cyanophyta and Chlorophyta (Figure 10). On the contrary, some algal groups increased in density relative to the stocks (negative bars); however, there appeared to be no consistent trend here among treatment or experiment effects. In terms of Chl-a in water algal stocks the highest concentration occurred in P and N+P treatments (Figure 11) in both algae stocks and *Daphnia* experiments.
Figure 10. Percent loss of algae relative to the stock solution for water and permafrost experiments. Bars above zero represent a percentage lost relative to stocks, while bars below zero represents an increase of algal cells per liter in treatments as compared with stocks.

Figure 11. Phytoplankton Chl-α concentrations for algal stocks and Daphnia experiments with water only. Bars represent the standard error for each treatment averages. Tukey-Kramer HSD test (p<0.05) was performed to water treatments and is represented with the letters. Comparisons among algal stocks were not analyzed statistically due to low numbers of data.
On average, average total phosphorus concentrations across treatments and experiments appeared to play an important role in the growth and reproduction of *Daphnia*. Mean number of offspring was significantly related to TP concentrations ($r^2=0.80$, $p=0.0026$; Figure 12), as were total number of *Daphnia* ($r^2=0.55$, $p=0.0353$; not shown). Similarly, percent loss of diatoms increased with TP (Figure 12). In fact, when the unusual gain in diatoms abundance in the permafrost control is excluded, there is a highly significant effect of *Daphnia* offspring numbers on percent loss of diatoms ($r^2=0.91$, $p=0.0008$; not shown).

![Figure 12. Relationship between total phosphorus concentrations and *Daphnia* offspring numbers (left) and percent loss of diatoms (right).](image)

### 3.4 Discussion

This experiment showed the substantial impact that nutrient-enriched algae can have on *Daphnia* growth and reproduction. More specific to the Arctic, the presence of arctic-sourced algae enriched by phosphorus, and especially that co-enriched from thawing permafrost, greatly enhanced the abundance of *Daphnia* observed in our experiments. These algae cultures tended to have a greater relative amount of Bacillariophyceae relative to Cyanophytes.

#### Algal stocks and nutrient limitation

The initial algal stocks in both experiments were dominated by Bacillariophyceae and Cyanophyta, followed by Chlorophyta. This composition is not unlike that seen in natural arctic
phytoplankton and benthic communities. For example, periphyton composition is often mainly Bacillariophyceae and Cyanophyta (Douglas & Smol, 1995; Vincent & Hobbie, 2000; Michelutti, Douglas M.S.V. & Smol, 2003; Vargas Medrano, 2011), whereas, phytoplankton may be dominated by Cyanophyta, Bacillariophyceae, Chlorophyta and Chrysophyceae (Sheath, 1986). Furthermore, paleolimnological studies suggest that diatoms (Bacillariophyceae) are increasing in Arctic regions due to warming effects causing a transition from benthic to more planktonic species (Rühland, Priesnitz & Smol, 2003).

Algal nutrient limitation was observed in the algal stocks for both Bacillariophyceae and Cyanophyta. Bacillariophyceae experienced sequential P limitation, with both P and N+P additions leading to higher biomass. Siliceous algae, such Bacillariophyceae tend to be good competitors for P, as compared to non-siliceous algae (Grover, 1989) and fertilization with H$_3$PO$_4$ has been shown to increase in diatom biomass in the Arctic (Miller, DeOliveira & Gibeau, 1992). On the contrary, Cyanophyta appeared N limited under some circumstances, and this was more unexpected. Generally, we expect that P addition would lead to an increase in nitrogen-fixing cyanophytes (Prentki et al., 1980), who are able to fix nitrogen from the atmosphere (Schindler, 1977; Schindler & Smol, 2006).

In Utqiaġvik AK, algae were historically considered P limited (Alexander et al., 1973), changing in recent years to an NP co-limitation (Lougheed et al., 2015). Benthic and phytoplankton algae limitation were described by Lougheed et al. (2015), with algal nutrient limitation varying depending on the time of year and location. According to our findings, which appeared to indicate P-limitation, the type of limitation might be related to the dominant algal group. For example, over the course of the growing season, periphyton algal densities in tundra ponds varied from a dominance of Cyanophyta at the beginning and end of the season, while Bacillariophyta and Chlorophyta were more abundant in mid-season (Vargas Medrano, 2011).

Interestingly, the addition of permafrost cores to the algal stocks did not have as substantial an impact on algal biomass as we expected. Permafrost thaw can rapidly release macro- and micronutrients into the water column (Reyes & Lougheed, 2015). However, this appeared to reduce overall counts of Bacillariophyceae, and have a modest effect on nutrient limitation of Cyanophyta. It is important to note, however, that we did not enumerate periphyton algal communities, which may have varied among treatments.
**Nutrient enrichment experiments with *Daphnia***

*Daphnia* abundances and offspring production were enhanced by the presence of algae enriched by phosphorus, and especially that co-enriched from thawing permafrost. Increased *Daphnia* abundances have also been associated with enrichment of Arctic ponds in other studies (Van Geest *et al.*, 2007a; Luoto *et al.*, 2014). These large herbivorous cladocerans require high levels of P-rich ribosomal RNA molecules (Elser *et al.*, 2000) and tend to sequester more P relative to N (Woods *et al.*, 2003; Johnson & Luecke, 2012). Urabe, Clasen & Sterner (1997) found that *Daphnia* growth was negatively affected by P-limited algae in poor P conditions, likely because of insufficient P-content. Interestingly, when we compare among permafrost and water only treatments we found that the abundance of offspring produced did not differ with the phosphorus addition. Whereas, for all other nutrient treatments, *Daphnia* and offspring abundances were lower in water treatments than permafrost. Phosphorus enhanced *Daphnia* abundance and offspring.

Notably, the concentration of phosphorus in the P and N+P with permafrost treatments was highly elevated relative to the water only P and N+P treatments (Figure 8). The additional phosphorus in the water likely came from the permafrost thaw and/or zooplankton excretion. In Arctic ponds, *Daphnia* recycles secretes high percentages of phosphorus, which fertilizes the algae (Prentki *et al.*, 1980; Stross *et al.*, 1980). We observed similar trends in permafrost experiments with P and N+P additions, where *Daphnia* grazed almost all algae available.

Contrary to the idea that *Daphnia* are water column filter feeders (Chisholm, Stross & Nobbs, 1975), *Daphnia middendorfiana* and other grazers are known to graze directly on benthic substrates in arctic systems (Rautio & Vincent, 2006; Cazzanelli *et al.*, 2012). As such, Lougheed *et al.* (2015), suggested that zooplankton in arctic tundra ponds preferentially grazed on P-rich benthic algae on *in situ* P-enriched nutrient diffusing substrates. The composition of the algae on the benthos may have played an important role in determining the results of this experiment. We observed that the *Daphnia* had a very interesting behavior where they fed on the bottom in both the water and permafrost experiments. In particular, in the permafrost sediment, which was composed of large organic debris, young daphnids swam through the interstitial spaces to swim. Detritus may be a big part of some zooplankton diets (Wright, 1959).
In the control and nitrogen treatments, Cyanophyta were the dominant algal group and *Daphnia* growth and reproduction was very low as compared with the other treatments. Cyanophyta, which are often filamentous or mucilaginous algae, have low nutritional quality and can have a negative effect on *Daphnia* nutrition (Przytulska *et al.*, 2015), clogging the filtration apparatus (Hawkins & Lampert, 1989). There is also a secondary effect of nutrient limitation: According to (Brett & Muller-Navarra, 1997), digestibility and the biochemical quality of algae may be reduced. This reduces the transference of energy and nutrients to the other trophic levels, having a bigger effect on aquatic food webs.

There were filamentous cyanobacteria in our experiments that were not observed in natural pond systems. These algae formed mats, primarily at the bottom of the C and N water only experiments. Filamentous cyanobacteria are inedible to *Daphnia* and can obstruct swimming by these zooplankters. Regardless of this issue, which could be due to either contamination of the stocks or appearance of an algae we had not previously observed, we think our results were very similar to the ponds composition and other authors’ findings.

Although not examined here, warming may also have important implications for *Daphnia* grazing and community changes. Increased temperatures enhance the feeding rates of many filter-feeding zooplankters (McKee & Ebert, 1996) and also accelerate *Daphnia* growth. Under warming conditions, *Daphnia* have also been shown to feed on all the algae in mesocosm experiments causing their extinction (Beisner, McCauley & Wrona, 1997). Interestingly, phytoplankton after these types of experiments may be decomposed of grazing-resistant species (Strecker, Cobb & Vinebrooke, 2004).

### 3.6 Conclusion

In conclusion, nutrient additions and the addition of permafrost sediment affected the dominance of algal groups and the growth and reproduction of *Daphnia*. In water sourced from a tundra pond, phosphorus clearly enhanced Bacillariophyceae density while nitrogen enhanced Cyanophyta abundance. Relative dominance of *Daphnia* increased due to the presence of algae enriched by phosphorus, and especially that co-enriched from thawing permafrost in our experiments. These results demonstrate a clear implication of permafrost thaw and nutrient release, from either permafrost our urban inputs, on the food webs in Arctic tundra ponds.
Additional studies in the Arctic need to observe the effects of continuous warming on zooplankton communities, paying attention in egg abundance, size and rare species. Nutrient enrichment studies in the Arctic, performed at different temperatures, with native algal communities and other species of zooplankton, are also needed to better understand the unique responses of species to warming and its effects in these sensitive environments.
Chapter 4: What is the impact of urban development and thermokarsting on arctic tundra pond zooplankton communities?

4.1 ABSTRACT

Human development and warming in the Arctic are increasingly putting new pressures on the abundant freshwater ecosystems in the region. In particular, recent and rapid expansion of thermokarst ponds in permafrost regions have been associated with these stresses. Our goal was to examine the relative impacts of pond enrichment by urban encroachment and thermokarsting, compared to relatively unimpacted regions, on the environmental conditions and zooplankton communities of tundra ponds near Utqiagvik, AK. Both urban ponds and thermokarst ponds were found to have substantially different environmental conditions, as well as distinctive zooplankton communities relative to reference sites. Thermokarst ponds presented an environment rich in DOC and nutrients (N, P and Si) and phytoplankton biomass. Enrichment by nutrients and DOC in thermokarst ponds led to significantly greater zooplankton abundance, composed largely of Daphnia middendorffiana and cyclopoid copepods. Urban ponds had moderate nutrient levels, with elevated benthic algal biomass. These ponds had intermediate numbers of Daphnia, as well as significantly higher numbers of diatomids and chydorids, as compared to one or both reference regions. The two reference regions, BEO and IBP, had consistently lower nutrient concentrations, algal levels and zooplankton abundances. Finally, harpacticoids and Bosmina longirostris were new records for the zooplankton in the region. Further collections are required to determine the drivers for these new observations. These results identify the large potential relative contributions of urban inputs and thermokarsting to enrichment of Arctic aquatic ecosystems and point to thermokarsting, which will likely increase with warming, as a major contributor to change in these systems.

4.2 INTRODUCTION

Increasingly, human development and warming in the Arctic are putting new pressures on abundant arctic freshwater ecosystems. Recent and rapid expansion of thermokarst ponds (or troughs) in permafrost regions have been associated with warming (Liljedahl et al., 2016), as well as human development (Raynolds et al., 2014). This can have implications for hydrology
(Liljedahl et al., 2016), landscape carbon flux (Lara et al., 2015), and nutrient cycling (Colombo et al., 2018; Koch et al., 2018). Human activities also contribute directly to polluting freshwater ecosystems via urban inputs of nutrients, as well as inputs of metals and other contaminants from mining activities and oil extraction (Schindler & Smol, 2006). Pollutants such as pesticides from agricultural activities and chemicals from industries, which are not common in Alaska, move from other sites in the earth to these cold regions via the global distillation process (Hurrel, 2003). All these pollutants may have substantial effects on aquatic communities in the Arctic.

Tundra ponds are characterized as nutrient poor systems but recently an increase in nutrient concentrations has been observed (Wrona et al., 2006; Lougheed et al., 2011). Nutrient enrichment to these ponds may come from the waste products of seabirds (Van Geest et al., 2007a; Stewart et al., 2012), from atmospheric deposition, and from permafrost thaw (Reyes & Lougheed, 2015), many of which are enhanced by warmer temperatures (Luoto et al., 2014). Warming may also increase the rate of human development in the Arctic and consequent waste water releases (Schindler & Smol, 2006). However, records of human impact, land use and nutrient loading and their ecological impacts have not been well documented for Arctic freshwater ecosystems. In particular, scientists are concerned about the effects of increases in nutrient inputs, combined with warming, on freshwater organisms including zooplankton, algae and benthos (Antoniades et al., 2011; Stewart et al., 2012, 2014).

In temperate regions, zooplankton community composition has been shown to vary along environmental gradients, such as the transition from urban and agricultural to forested land-use (Lougheed & Chow-Fraser, 2002; Lougheed, Parker & Stevenson, 2007; Lougheed et al., 2008). Factors such as turbidity, nutrient concentrations and macrophyte cover are often cited as key driving factors in zooplankton community structure (Lougheed & Chow-Fraser, 1998, 2002; Lougheed et al., 2008). Within the Arctic, nutrient gradients also appear to play an important role in several surveys done over large geographic areas. Because growing seasons are relatively short in the Arctic, freshwater biotic communities in ponds and lakes in the Arctic are likely adapted to respond quickly to nutrients (Douglas & Smol, 1994). In high Arctic lakes, the abundance of *Daphnia middendorffiana*, an abundant and common herbivore, has been associated with increased productivity in the form of dissolved (DOC) and particulate carbon (Chételat & Amyot, 2009). Similarly, cladoceran zooplankton in lakes along the Canadian Arctic treeline, ranging from the Arctic to the more productive boreal region, were structured in by
DOC, temperature and total phosphorus (TP) (Sweetman et al., 2010). In northern Siberia thermokarst lakes, temperature, water depth, silica, and sulfate were important drivers (Frolova et al., 2014). However, few studies have appeared to look at the impact of urban development, especially as compared to thermokarsting, on the Arctic on zooplankton communities.

Recently, we found that ponds near the arctic city of Utqiaġvik, AK had significantly higher levels of nutrients, as well as planktonic and benthic chlorophyll-as concentrations, as compared to nearby tundra ponds in protected areas (Lougheed et al., 2015). We have also observed elevated DOC and other nutrients in thermokarst ponds (Lougheed et al. submitted), which are typically formed by abrupt thawing of ice-rich permafrost, and subsequent land subsidence and slumping. To further understand enrichment effects on arctic tundra pond zooplankton, we are analyzing zooplankton communities along this gradient from protected areas, to thermokarst ponds, and within the growing community of Utqiaġvik, AK.

4.3 Methods

Zooplankton were sampled in mid-August 2010-2013 from 4 main regions: the International Biological Program (IBP) sites (historical reference sites), the Barrow Environmental Observatory (BEO) (protected from development), Utqiaġvik (UTQ; urban sites), and thermokarst ponds (TK; nutrient-rich permafrost thaw ponds). The six IBP ponds were near to the village of Utqiaġvik, but at least 200-m from any roads and with only few scattered nearby homes. IBP ponds X, B, E, D, J, and C were established as research sites in the 1970s and revisited for this study and others (Lougheed et al., 2015). Another 6 ponds were sampled from two areas within the BEO and are distant from any development: WL-3, ITEX-S, ITEX-N, ITEX-N2, WL-18, and WL-20. Ponds within these two reference regions (BEO, IBP) could be considered true tundra ponds, which are those formed through the seasonal thaw of the active layer within low-centered polygons. The ponds within the village of Utqiaġvik were 7 ponds that were named after their adjacent roadways: Boxer, Boxer-2, Ahmagoak, Ahmagoak-2, Utiqtuq, Yugit and Kignak. Housing density varied from 19 – 11 buildings/block (0.02 km$^2$) near these ponds. The first 4 ponds were in older developments, with higher housing density and commercial developments, while the latter 3 were in newer, less dense housing developments. While some of these may be true tundra ponds, most of them were likely formed by ponding of
water after construction of roads, which are generally constructed by piling of sand and gravel to raise the road above the surrounding land. Finally, 5 thermokarst ponds within the BEO, at least 250m from any roadways (TK1, TK2, TK3, TK5, and TK6) were included to examine the impact of accelerated warming in the absence of other human impacts. Thermokarst ponds are typically formed among polygon troughs and caused by abrupt thawing of ice-rich permafrost, and subsequent land subsidence and slumping.

Half the sites were sampled in all four years, this included all the IBP sites, and some of the BEO and urban sites. One-quarter of the sites were sampled three times; this included the remaining BEO and urban sites. Finally, the thermokarst ponds were visited in 2012 and 2013 only. Site locations are mapped in Lougheed et al., 2015 and Lougheed et al., (submitted).

Five 2 L samples of water were collected from haphazardly selected open water regions in each pond and combined into composite zooplankton samples of 10 L, filtered through a 64 µm filter and preserved with 4% sugar formalin. In the laboratory, samples were rinsed with DI water, mixed thoroughly and individual subsamples of 5 to 10 ml were counted under a LEICA MZ125 light stereoscope. Subsamples of at least 100 organisms, including at least 20 large organisms (>3 mm mean length) were identified; the whole sample was also surveyed for large and rare organisms (Lougheed & Chow-Fraser, 1998). Anostraca and Cladocera were identified to the lowest possible taxonomic level based on published sources (Dodson & Frey; Pennak, 1978; Thorp & Covich, 2001). Copepods were largely identified to order or family only.

Environmental data

Concurrent with zooplankton samples, water samples were collected for analysis of related environmental parameters. These data were collected and analyzed as part of related studies (Lougheed et al., 2011, 2015). Algal chlorophyll-a (CHLa) was determined as described in (Lougheed et al., 2015). Concentrations were calculated on a volumetric basis for phytoplankton (µg L⁻¹) and by area sampled for periphyton (µg cm⁻²). Chlorophyll-a concentrations were corrected for turbidity and phaeopigments by acidification (Wetzel & Likens, 2000). Nutrient chemistry was analysed as described previously (Lougheed et al., 2011, 2015). In brief, total phosphorus (TP) was determined by the ascorbic acid method following persulphate digestion and silica (Si) by the heteropoly blue method. Total alkalinity was also
determined using standard methods (APHA, 1998) on a Mettler Toledo G20 autotitrator.
Dissolved organic carbon (DOC) and total nitrogen (TN) were measured on a Lachat IL-550;
DOC was converted to non-purgeable DOC, based on a conversion from samples run
concurrently on a Shimadzu TOC-V instrument (Lougheed et al. n.d.). A YSI 556 multiprobe
was used to measure temperature, pH and specific conductance. Percent open water was a
qualitative estimate made by consensus among 2-3 people sampling the site on any given date.

Data analysis

A nonmetric multidimensional scaling (NMS) analysis was performed to describe
structure in the zooplankton data. All taxa that occurred in greater than 5% of samples were
included; thus, the relatively rare rotifers and gastropods were excluded. NMS analysis identifies
axes that describe biologically meaningful, multivariate gradients in the community data
(McCune & Grace 2002). NMS analysis was performed using a Bray-Curtis distance measure in
PC-ORD (version 7). We performed correlations between NDS axis scores and the available
environment variables, as described above, to explain the arrangement and drivers of
zooplankton among sites and regions. Only those environmental parameters that showed a
significant correlation to an NMS axis are presented; all others are omitted.
4.4 RESULTS

In general, the thermokarst ponds had the highest levels of phytoplankton chlorophyll-a, nutrients (TN, TP), and color (DOC, light extinction) (Error! Reference source not found.7). Thermokarst ponds also had moderately high levels of periphyton; however, it is worth noting that periphyton was only measured in 1 year at these ponds (n=5 for periphyton). The urban ponds in Utqiaġvik had the next highest levels of nutrients and DOC, together with the highest levels of periphyton chlorophyll-a. They also had significantly higher levels of specific conductance, alkalinity and silica. Finally, the ponds in the BEO and IBP largely had the lowest levels of nutrients, DOC and chlorophyll-a. BEO ponds tended to have slightly higher phytoplankton chlorophyll-a as compared to the IBP ponds.

Table 7. Comparison of environmental variables (Mean (SD). Letters represent statistically significant difference according to a Tukey-Kramer HSD (p<0.05). Data were log-transformed in order to comply with assumptions of the normal distribution.

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>BEO (n=21)</th>
<th>IBP (n=24)</th>
<th>Utqiaġvik (n=23)</th>
<th>Thermokarst (n=10) (*n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>9.20 (1.89) b</td>
<td>8.42 (2.94) ab</td>
<td>7.45 (2.31) a</td>
<td>9.09 (2.46) ab</td>
</tr>
<tr>
<td>Specific Conductance (µS cm⁻¹)</td>
<td>0.351 (0.235) b</td>
<td>0.156 (0.019) c</td>
<td>0.789 (0.215) a</td>
<td>0.207 (0.054) c</td>
</tr>
<tr>
<td>Light extinction (cm⁻¹)</td>
<td>0.050 (0.02) a</td>
<td>0.037(0.02) a</td>
<td>0.038 (0.01) a</td>
<td>0.147 (0.06) b</td>
</tr>
<tr>
<td>pH</td>
<td>5.94 (0.76) b</td>
<td>6.24 (0.54) b</td>
<td>7.00 (0.52) a</td>
<td>5.10 (0.30) c</td>
</tr>
<tr>
<td>Alkalinity (mg L⁻¹)</td>
<td>6.06 (4.02) c</td>
<td>15.44 (3.04) b</td>
<td>244.17 (92.09) a</td>
<td>9.25 (4.52) bc</td>
</tr>
<tr>
<td>DOC</td>
<td>19.72 (6.90) c</td>
<td>17.92 (3.35) c</td>
<td>27.53 (6.97) b</td>
<td>84.57 (44.72) a</td>
</tr>
<tr>
<td>TP (µg L⁻¹)</td>
<td>30.87 (18.4) c</td>
<td>20.90 (12.03) c</td>
<td>94.81 (115.6) b</td>
<td>176.73 (103.9) a</td>
</tr>
<tr>
<td>TN (mg L⁻¹)</td>
<td>1.44 (0.5) bc</td>
<td>1.15 (0.44) c</td>
<td>1.97 (0.72) b</td>
<td>5.81 (5.14) a</td>
</tr>
<tr>
<td>Si (µg L⁻¹)</td>
<td>151.1 (176.1) b</td>
<td>94.5 (85.2) b</td>
<td>696.9 (571.1) a</td>
<td>373.6 (241.6) a</td>
</tr>
<tr>
<td>Phytoplankton CHL-a (µg L⁻¹)</td>
<td>3.51 (5.54) ab</td>
<td>1.11 (0.73) b</td>
<td>1.35 (1.06) b</td>
<td>5.89 (6.18) a</td>
</tr>
<tr>
<td>Periphyton (µg cm⁻²)</td>
<td>2.42 (3.11) b</td>
<td>2.87 (1.69) b</td>
<td>14.76 (11.91) a</td>
<td>6.70 (6.25) ab *</td>
</tr>
</tbody>
</table>

Zooplankton mean abundances were clearly highest in the thermokarst ponds as compared with Utqiaġvik, IBP and BEO ponds (Error! Reference source not found.3). While, the reference sites (BEO and IBP) zooplankton abundances were statistically similar, the protected area (BEO) was no different from the urban impacted ponds (Tukey-Kramer HSD, p<0.05).
Figure 13. Average mid-August mean total zooplankton abundance in BEO, IBP, Thermokarst, and Utqiaġvik ponds. Error bars indicate standard deviations and the letters above bars indicate the statistically significant difference calculated by Tukey-Kramer HSD (p<0.05).

A total of 12 different zooplankton taxa were found during this survey: *Bosmina longirostris*, *Branchinecta paludosa*, *Chydorus sphaericus*, *Daphnia middendorffiana*, *Eurycercus lamellatus*, cyclopoid copepods, diaptomid copepods, *Heterocope septentrionalis*, harpacticoid copepods, nauplii (copepod larvae) and small numbers of the rotifers *Notholca accuminata*, and *Trichocerca* sp.

Two taxa occurred in almost all ponds and on all sampling dates: *Daphnia middendorffiana* (93% of all samples) and cyclopoid copepods (91%). The next three most common taxa were diaptomid copepods (68%), *Heterocope* (60%) and *Chydorus* (50%). On average, the most common taxa also tended to be the most abundant. For example, cyclopoid copepods and *Daphnia* averaged 34 to 38 individuals per liter, respectively, while *Chydorus* and diaptomid copepods were the next more abundant with an average of 10 individuals per liter.

In thermokarst ponds, there were significantly more cyclopoid copepods and *Daphnia*, while urban Utqiaġvik ponds contained significantly more *Chydorus* and diaptomid copepods (Table 8). These urban ponds also tended to have abundances of cyclopoid copepods and *Daphnia* that were intermediate between the high densities in thermokarst ponds, and the
significantly lower densities in the IBP and BEO sites. Reference sites tended to have significantly lower densities of most taxa. *Bosmina* and harpacticoid copepods, while low in average abundance, were new taxonomic records for the region. Both were absent from urban ponds.

Proportionally, in thermokarst ponds, *Daphnia* and cyclopoid copepods contributed equally to nearly all (96%) of the total abundance (*Error! Reference source not found.*). The urban ponds, also had a large proportion of *Daphnia* (35%), with greater representation by *Chydrorus* (24%) and diaptomids (20%). IBP ponds were dominated proportionally by three main taxa: *Daphnia* (36%), cyclopoid copepods (27%) and diaptomids (22%). Finally, the BEO ponds had a relatively large proportion of cyclopoid copepods (30%), with fairly equal representation by 4 additional taxa: *Bosmina* (15%), *Daphnia* (12%), diaptomids (15%) and copepod nauplii (16%).
Figure 14. Mean mid-August zooplankton proportional abundances for taxa found in BEO, IBP, thermokarst (TK), and Utqiagvik (UTQ) ponds during 2010-2013. The “Other” category included the less abundant taxa: rotifers, harpacticoid copepods, gastropods, *Eurycercus lamellatus* and *Branchinecta paludosa*.
Table 8. Comparison of species abundances in the four regions (Mean (SD) as compared to historical species lists. For modern samples, letters represent the statistically significant differences according to a Tukey-Kramer HSD (p<0.05). Taxa with mean abundances <0.02 individual per liter are excluded (rotifers, gastropods, *Branchinecta paludosa*); there were no differences among regions noted for these taxa. S=(Stross et al., 1980); D=(Dodson, 1979)

<table>
<thead>
<tr>
<th>Taxon (This study)</th>
<th>Previous studies</th>
<th>BEO</th>
<th>IBP</th>
<th>Utqiagvik</th>
<th>TK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladocera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bosmina longirostris</em></td>
<td>-</td>
<td>2.95 (8.85)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0.21 (0.46)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Chydrorus sphaericus</em></td>
<td>D</td>
<td>0.97 (2.14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18 (0.45)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.64(20.24)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7(3.89)&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Daphnia middendorffi ana</em></td>
<td>D, S</td>
<td>2.41(6.29)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66(1.83)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.99(29.94)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.27(44.92)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Eury cercus lamellatus</em></td>
<td>D</td>
<td>0.05(0.09)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.03(0.09)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19(0.35)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09 (0.28)&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Copepoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>……<em>Heterocope septentrionalis</em></td>
<td>D, S</td>
<td>0.86 (0.88)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41(0.97)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.09 (2.57)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89(2.41)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>……<em>Cyclopoid copepods</em></td>
<td>D, S</td>
<td>5.93 (6.13)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.25(1.26)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.98(9.11)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.1(59.63)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>……<em>Diaptomid copepods</em></td>
<td>D, S</td>
<td>3.04(5.43)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.0(1.56)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.21(12.77)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03 (0.09)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>……<em>Harpacticoid copepods</em></td>
<td>-</td>
<td>0.07 (0.21)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04 (0.13)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15 (0.21)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 15. NMS plot based on average mid-August zooplankton (Ind L$^{-1}$) from IBP-reference, BEO-reference, Thermokarst (TK) and Urban (Utqiagvik) ponds, which were subdivided into those found in areas of low vs. high housing density. Species codes are DAPMID (Daphnia middendorffiana), CYCL (cyclopoid copepods), CHYSPH (Chydomus sphaericus), EURSP (Eurycercus lamellatus), DIAP (Diaptomus copepods), BOSLON (Bosmina longirostris), HETSEP (Heterocope septentrionalis), HARP (harpacticoid copepods) and BRAPAL (Branchinecta paludosa).

An NMS ordination of mid-August zooplankton densities averaged per IBP, BEO, TK, and Utqiagvik pond resulted in a 3-dimensional solution with final stress of 7.73. All 3 axes were statistically significant (p<0.04); however, axis 1 and 3 described more meaningful environmental gradients and are presented here (Table 99).

In general, the NMS contrasted nutrient and CHLa-rich thermokarst and urban sites of higher housing density, which were largely found in the upper-left quadrant, with IBP reference sites in the lower right. *Daphnia*, cyclopoids and chyadorids (*C. sphaericus*, *E. lamellatus*) were
associated with these nutrient-rich sites. Higher concentrations of periphyton CHLα were also associated with NMS 3 (Figure 15).

*Bosmina* and diaptomids each occurred in their own quadrants of the plot, associated with moderate enrichment levels. *Bosmina* and WL20, co-occurred in the bottom left of the plot. This species was most abundant at this site and relatively rare at all others. WL20 was relatively deep and cold compared to other BEO sites: 7°C compared to the regional average of 8°C. Diaptomids dominated several urban sites, as well as WL18, found in the upper right quadrant which was associated with higher pH and more open water (>75%); these sites were also often cold (<7.5°C) relative other sites. There was, however, no correlation of temperature with the axes scores (Figure 15).

**Table 9.** Correlations of NMS axes with environmental variables. Environmental variables were log transformed, as needed, in order to comply with normality. Uncorrected p-values are reported as ¯<0.10, *<0.05, **<0.01, ***<0.001.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NMS1</th>
<th>NMS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN (ppm)</td>
<td>-0.80***</td>
<td>0.70***</td>
</tr>
<tr>
<td>TP (ppb)</td>
<td>-0.71***</td>
<td>0.76***</td>
</tr>
<tr>
<td>DOC</td>
<td>-0.77***</td>
<td>0.61**</td>
</tr>
<tr>
<td>Si (ppb)</td>
<td>-0.50*</td>
<td>0.68***</td>
</tr>
<tr>
<td>Phytoplankton CHLα (µg L⁻¹)</td>
<td>-0.41*</td>
<td>0.59**</td>
</tr>
<tr>
<td>Light extinction</td>
<td>-0.65***</td>
<td>0.45*</td>
</tr>
<tr>
<td>% Open water</td>
<td>0.49*</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.42*</td>
<td></td>
</tr>
<tr>
<td>TSS (mg L⁻¹)</td>
<td>-0.65*</td>
<td>0.66*</td>
</tr>
<tr>
<td>Periphyton CHLα (µg/cm²)</td>
<td></td>
<td>0.36~</td>
</tr>
</tbody>
</table>

**4.5 DISCUSSION**

This study shows that human development and thermokarsting in the Arctic are having important impacts on zooplankton communities. Both urban ponds and thermokarst ponds were found to have substantially different environmental conditions, as well as distinctive zooplankton communities relative to reference sites. Nutrient enrichment, regardless of source, increased the algal biomass and the total abundance of zooplankton, especially *Daphnia middendorffiana.*
Other differences in zooplankton appeared to be unique to either thermokarst or urban enrichment.

Thermokarst ponds presented an environment rich in DOC and nutrients (N, P and Si), which likely contributed to abundant phytoplankton biomass. Urban ponds had moderate nutrient levels, with elevated benthic algal biomass. The two reference regions, BEO and IBP, had consistently lower nutrient and algal levels. These results identify the potential relative contributions of urban pollution (Schindler & Smol, 2006) and thermokarsting (Colombo et al., 2018; Koch et al., 2018) to enrichment of arctic aquatic ecosystems and point to thermokarsting, which will likely increase with warming (Liljedahl et al., 2016) as a major contributor to change in these systems.

Other studies in the Arctic have similarly found that nutrients and productivity were a primary driver in structuring zooplankton communities (Chételat & Amyot, 2009; Sweetman et al., 2010; Frolova et al., 2014). However, few studies have appeared to look at the impact of urban development in the Arctic on zooplankton communities or its impact relative to thermokarst related nutrient enrichment. In our study, enriched thermokarst ponds had the highest abundances of zooplankton as compared with reference sites (BEO and IBP) and urban ponds (UTQ); however, thermokarst ponds had a relatively low level of diversity dominated by 2 primary taxa: *Daphnia middendorffiana* and cyclopoid copepods. Harpacticoid copepods were somewhat more abundant at the TK sites, and appeared to be a new observation for the region. The presence of a moss layer at the bottom of the TK ponds (Alexander et al., 1980; Osterkamp et al., 2009) is relatively unique among tundra ponds in the region and may account for this difference. However, more research would be needed to confirm this relationship, since harpacticoids were also observed in low numbers at reference sites. On the other hand, urban ponds, especially those in the most urbanized areas, had a greater abundance of diaptomidaceae and chyadorids.

As found in previous studies, zooplankton communities in Utqiagvik tundra ponds had relatively few species as compared with other regions (Stross et al., 1980). In this study, 11 different zooplankton taxa were found; 5 taxa were found in 80% or more of samples (*Chydorus, Daphnia, Diaptomus, Heterocope*, diaptomids, cyclopoids), with the remaining taxa, including rotifers, relatively rare and found in low densities. With this newer regional survey, most species
found in this project were reported previously by Stross et al., (1980) and Dodson, (1979); however, harpacticoids and Bosmina may be a new record for the region. Notably, we have recently observed a change in the density of historically rarer species as well as some dominant taxa in recent years as compared with 1970s study, as explained in more detail in Chapter 2.

*Daphnia middendorffiana* was significantly more abundant in both enriched pond types: TK and UTQ ponds. TK enrichment, with the highest levels of both nutrients and phytoplankton CHLa, dramatically increased the number of daphnids even more than the urban enriched sites, which had the next highest concentration of nutrients, as well as substantial periphyton CHLa. Algae enriched with phosphorus have been shown to improve the growth and reproduction of *Daphnia* (Urabe et al., 1997), perhaps because of the necessity of their P-rich ribosomal RNA-molecules (Elser et al., 2000). Large numbers of *Daphnia* are able to control algal biomass through their grazing (Van Geest et al., 2007a). Benthic algae can also be an important food source for *Daphnia middendorffiana* and other zooplankters in Arctic lakes and ponds, and may contribute substantially to secondary production (Rautio & Vincent, 2006; Rautio et al., 2009; Cazzanelli et al., 2012). However, at high levels of DOC, *Daphnia* in arctic ponds may switch to a diet rich in terrestrial organic matter via the microbial loop (Mariash et al., 2018). Thus, a diversity of food sources likely contributed to the abundance of *Daphnia* in these ponds.

The large numbers of *Daphnia* was likely due in part to the fishless nature of these ponds. While arctic ponds generally have low species diversity of zooplankton, they can reach relatively high abundances compared to other freshwater environments, largely because of the absence of fish (Rautio et al., 2011a). The absence of fish also allows large-bodied zooplankton, such as *Daphnia*, to dominate some arctic zooplankton communities (Christoffersen et al., 2008; Jeppesen et al., 2017). Several studies have suggested that the carnivorous copepod *Heterocope septentrionalis* also observed here, can determine the species of *Daphnia* present in arctic freshwaters by promoting the dominance of *D. middendorffiana* over the smaller *D. pulex* (Dzialowski et al., 2004; O’Brien & Luecke, 2011).

The occurrence of very high DOC and, in the case of the TK ponds, higher light extinction coefficients may have reduced the necessity of these zooplankton to spend energy on UVR (ultraviolet radiation) protection mechanisms. Arctic *Daphnia* species often become pigmented with photoprotective melanin as a protective response to UV radiation (*e.g.*, (Herbert
Anecdotally, we observed that *Daphnia* in the TK ponds were less pigmented than those often found at other pond sites.

Cyclopoid copepods were also found in high density in the nutrient-rich thermokarst ponds, as well as moderate density in the urban-enriched systems. Cyclopoids are highly adapted to the extreme environments in the Arctic, as they can produce eggs throughout the growing season, and overwinter as encysted copepodids, which allows for rapid population growth immediately after ice-out (Stross, 1974; Abramova *et al*., 2017). As such, warmer spring temperatures have been associated with earlier emergence of cyclopoid taxa (Gerten & Adrian, 2002). However, cyclopoids, particularly younger stages including nauplii, are susceptible to UVR damage (Rautio & Tartarotti, 2010); thus, their relatively high abundance in the TK ponds may be a response to the protective effects of elevated DOC. It is worth noting that greater taxonomic resolution, beyond that used in this study, may reveal interesting trends in cyclopoid populations.

In this study *C. sphaericus* appeared to be an indicator of urban impacts. While it appeared in low numbers at sites throughout the region, its average density was significantly higher in urban ponds. Ponds in the areas of highest housing density in Utqiagvik had the highest maximum abundances of *Chydorus sphaericus*: Boxer-2 (87.3 L⁻¹), Boxer (33.3 L⁻¹), AHMA (28.2 L⁻¹) and AHMA-2 (20.6 L⁻¹). These ponds were not only nutrient-rich, but also tended to have floating blooms of algae on their surfaces. *C. sphaericus* can often be found in eutrophic waters (Gannon & Stemberger, 1978; Hofmann, 1998). In paleolimnological studies of lakes, its presence in cores has been used as indicators of eutrophication (Szeroczyńska, 1991, 1998). *C. sphaericus* is able to use detritus as a food source to grow and reproduce (Vijverberg & Boersma, 1997), and also often co-exists with cyanobacterial blooms, which can provide a major food source (Tõnno *et al*., 2016). The lower number of *Chydorus* in the more highly enriched thermokarst ponds may be due to the absence of these algae. Additionally, like other taxa in this study, *Chydorus sphaericus* can be inhibited by UVR (Cabrera, López & Tartarotti, 1997). The 4 ponds where *Chydorus* was located had DOC levels (29.6 ± 6.6 ppm) intermediate to the thermokarst and reference sites (p<0.05), which may have served as alternative protection against UV damage.
Chydorids, in general, may be associated with aquatic vegetation (Lougheed & Chow-Fraser, 2002) and lack of suitable habitat may partly explain the absence of many chydorids from some sparsely vegetated high arctic ponds (Herbert & Hann, 1986). Recent increases in vegetation biomass and cover in the tundra ponds near Utqiaġvik (Andresen & Lougheed, 2015; Andresen et al., 2018) may have promoted greater dominance of chydorid species in some ponds.

*Bosmina longirostris* was generally rare but numerically abundant in pond WL20 every year, with an average abundance of 21 individuals L\(^{-1}\). It was also found at low densities (<1 L\(^{-1}\)) on 3 dates in the thermokarst ponds. While not previously reported in the tundra ponds near Barrow (Dodson, 1979; Herbert & Hann, 1986), they have been noted in other studies in the Arctic (Herbert & Hann, 1986; Abramova et al., 2017). Their appearance elsewhere has been associated with eutrophic conditions (McNaught, 1975) and the presence of toxic cyanobacteria (Xiaodong et al., 2016). In polar regions, a recent increase in *Bosmina* (*Eubosmina*) spp in the sediment record of 32 subarctic lakes was attributed to climate warming and an increase in sediment organic matter (Leppänen, Siitonen & Weckström, 2017). Others have noted the absence of *Bosmina* from clearer arctic waters due to high UVR in these systems (Rautio & Tartarotti, 2010). Additional surveys in the region may be needed to determine the factors promoting the appearance of this taxa. Their absence from the urban ponds suggest nutrient enrichment is not the sole factor determining their distribution.

Calanoid copepods tend to be rarer in arctic environments, where shorter growing seasons may prevent all but the fastest growing taxa from reaching sexual maturity before freeze-up (Herbert & Hann, 1986). In this study, they appeared to be largely associated with cooler, often urban, ponds with more open water. Several studies have found that calanoid copepods preferred cooler temperatures (Jose, 2009). In a related study (Chapter 1), we found they had decreased in abundance through time in the IBP ponds. However, it appears that moderate inputs of nutrients may promote the presence of diaptomids in arctic ponds. Identification of the specific species responding to this enrichment should be an avenue for future study.

Finally, it is important to note that proximity of sites may have influenced the similarity of taxa among ponds. For example, the IBP ponds were in relatively close proximity to each other, ranging from 27 m – 270 m apart. Conversely, the TK and UTQ ponds were separated by
a maximum of more than 1.3 km, and sites in the BEO were greater than 4 km distant at their greatest. Resting eggs or organisms can move to other ponds by overland flow when ice thaws or can be transported by birds (Taylor et al., 2016).

4.6 Conclusion

In conclusion, human development and warming in the Arctic are having considerable impacts on small freshwater ecosystems. Enrichment by human activities, but particularly thermokarsting, are causing substantial changes to the tundra pond environment and their associated zooplankton communities. These changes in both total abundance and composition at the level of the zooplankton may have important impacts on the other components of the Arctic food web. Arctic Daphnia, in particular, are able to control algal biomass (Van Geest et al., 2007a) and also may form an important part of the diet of some waterfowl (Dodson & Egger, 1980). Future warming and development in these regions has the potential to drastically alter the tundra pond environment.
Chapter 5: General discussion

Key research findings

How and why have zooplankton communities in Arctic tundra ponds changed through time? (Chapter 2)

Over a 40 year period, from the early 1970s to the early 2010s, we recorded a change in the dominant zooplankton species in tundra ponds and earlier dates of peak abundance for others. Species composition was similar to that reported in the 1970s (Dodson, 1979; Stross et al., 1980); however, *Chydorus* appears to be a new record for the IBP ponds. Rotifers were very rare, their abundance likely inhibited by high *Daphnia* abundance (Christoffersen et al., 2008). The community in the 1970s was dominated by cyclopoid copepods, but more recently, we observed that *Daphnia middendorffiana* dominated the ponds. These *Daphnia* also appeared to be larger than those observed 40 years prior. These changes are likely attributable to warmer temperatures, increased food availability and changes in competition and predation. These large, abundant *Daphnia* may be capable of controlling algal biomass in the ponds (Van Geest et al., 2007a). However, this large change in *Daphnia* raises the question about any upper limit of zooplankton abundance. *D. middendorffiana* likely have upper temperature limits (Thompson & Vinebrooke, 2008) and may be more vulnerable to predation by observed elevated pCO$_2$ levels (Weiss et al., 2018). Finally, warmer spring temperatures may promote earlier emergence of cyclopoids after overwintering (Gerten & Adrian, 2002).

How do arctic algal communities change in response to nutrient addition, and what are the cascading impacts of these changes on zooplankton, specifically *Daphnia*? (Chapter 3)

Experiments clearly showed the impact of nutrient enrichment on algae and the cascading effect on *Daphnia* density and reproduction. The abundance of Bacillariophyceae in phosphorus enriched experiments or co-enriched by permafrost likely contributed to increased abundance of *Daphnia*. Diatom abundances in these experiments reflects observations in the nature, including a substantial increase in diatoms in the periphyton since the 1970s (Vargas Medrano, 2011). Paleolimnological studies also found that Bacillariophyceae are increasing
because the warmer conditions (Rühland et al., 2003). Permafrost appeared to be very important for algae and zooplankton nutrition. The interstitial spaces in the arctic sediment likely supported benthic algae growth (Rautio & Vincent, 2006).

In Utqiaġvik, AK, the algae were historically P limited (Alexander et al., 1973) and changed to NP co-limitation recently (Lougheed et al., 2015). Lougheed et al. 2015 described benthic limitation which varies during the growing season and at different locations; this likely changed in part because the algal dominant groups also changed. In our study, Bacillariophyceae experienced sequential P limitation, with both P and N+P additions leading to higher biomass. Siliceous algae, such Bacillariophyceae tend to be good competitors for P, as compared to non-siliceous algae (Grover, 1989) and fertilization with H₃PO₄ has been shown to increase in diatom biomass in the Arctic (Miller et al., 1992). Cyanophyta appeared N limited under some circumstances, contrary to our expectation that P addition would lead to an increase in nitrogen-fixing cyanophytes (Prentki et al., 1980), who are able to fix nitrogen from the atmosphere (Schindler, 1977; Schindler & Smol, 2006).

*Daphnia* increased in density with P or N+P additions, and most notably in presence of permafrost. It is well known that *Daphnia* need high amounts of phosphorus because of their P-rich ribosomal RNA (Elser et al., 2000) and they tend to sequester P (Woods et al., 2003; Johnson & Luecke, 2012). It appears that at least some of the P was provided via permafrost thaw (Reyes & Lougheed, 2015), or via excretion by abundant *Daphnia* (Prentki et al., 1980; Stross et al., 1980), as the concentration of nutrients in these treatments was more elevated than in the water only treatments. In the C and N treatments, *Daphnia* density was lower, while Cyanophyta were abundant. Cyanophyta were mainly mucilaginous or filamentous algae that had low nutritional quality (Przytulska et al., 2015), can clog the filtration apparatus of *Daphnia* (Hawkins & Lampert, 1989), and impede swimming by *Daphnia.*
What are the enrichment effects on arctic tundra pond zooplankton, as examined in tundra ponds along a gradient from protected areas, to thermokarst ponds, and within the growing community of Utqiaġvik, AK? (Chapter 4)

Human development and enrichment by accelerated thaw of thermokarst ponds had substantial effects on zooplankton communities that made them different as compared with reference sites. These impacts included increased algal biomass and zooplankton abundance, especially *Daphnia* biomass, similar to other studies that identified that productivity and nutrients drive zooplankton community composition (Smol *et al.*, 2005; Chételat & Amyot, 2009; Sweetman *et al.*, 2010; Frolova *et al.*, 2014).

In the thermokarst ponds, elevated DOC sourced from terrestrial organic matter may have also contributed to the diet of *Daphnia* (Mariash *et al.*, 2018). Because of the very high DOC levels, these daphnids likely had relatively low melanin secretion as compared with the other sites; melanin was not needed to protect the *Daphnia* from UV radiation (Rautio & Tartarotti, 2010). Cyclopoid copepods were also abundant in TK ponds; these organisms are well adapted to extreme environments (Stross, 1974; Abramova *et al.*, 2017). Finally, harpacticoids were observed in the TK ponds, which may be related to a characteristic layer of moss at the bottom of these ponds (Alexander *et al.*, 1973); this was a new taxonomic observation for the region.

In urban ponds, we found relatively high numbers of *Chydorus sphaericus*, which we identified as a possible indicator of enrichment. These taxa have been used as an indicator of eutrophication in paleolimnological studies (Szeroczyńska, 1991, 1998). Sites with *Chydorus* also tended to have floating booms of algae, which may be found eutrophic waters (Alexander *et al.*, 1980). *Chydorus*, also tend to tolerate cyanobacteria blooms better than *Daphnia* (Tônno *et al.*, 2016) and they feed on detritus to grow and reproduce (Vijverberg & Boersma, 1997). Chydorids tend to be associated with vegetation (Lougeed & Chow-Fraser, 2002) and may have increased in abundance with the observed increase in vegetation in ponds (Andresen & Lougheed, 2015; Andresen *et al.*, 2018).

*Bosmina* also is often associated with eutrophic conditions (McNaught, 1975) however, that may not have been a driver for its presence in this study. Interestingly, it was not previously
found in this region; however, it has been reported in other sites in the Arctic (Herbert & Hann, 1986; Abramova et al., 2017).

**Future research priorities**

Given the substantial changes observed in Arctic ponds, largely due to warming and ensued enrichment, it is highly important to continue with the monitoring of pond ecosystems to observe the effects of continuous warming and development on these sensitive Arctic ecosystems. In particular, both historic ponds and those along enrichment gradients, must continue to be studied to understand current and future change in both environmental conditions and zooplankton communities. It is necessary to keep looking at the rare and new species that may increase further in density. Furthermore, recognizing the importance of studying these organisms at the species level, taxonomic resolution of copepod identifications must be increased in order to detect any possible species-level changes in these communities. Additional work could also focus on analyzing changes in egg abundance and size for all taxa, as well as developing length-biomass formulae to assist in zooplankton community biomass calculations. Finally, future monitoring could examine the effect of the presence of plants (Arctophila fulva, Carex aquatilis) and moss on zooplankton communities in these ponds to understand better how they impact or support zooplankton and algal communities.

Further experiments could be designed to analyze determine how different zooplankton species may respond to temperature and nutrients enrichment in presence and absence of permafrost, plants and mosses. Little work has been done on the role of DOC in structuring these communities, as such additional experiments could address the effect of DOC lability and concentration on algae and zooplankton and how these affect growth, reproduction and coloration of zooplankton taxa.

**Conclusion**

Warming is altering Arctic freshwater ecosystems, enriching the water column, increasing primary productivity and shifting the composition, dominance and the timing of emergence and reproduction of zooplankton communities. Historic data has given us valuable insight into changes that have occurred in recent times. The use of experiments, as well as
sampling enriched ponds in urban and thermokarst areas, confirms that warming and enrichment are a driver of change in tundra pond zooplankton communities. The observed changes in thermokarst and urban ponds can give us an idea of the future changes in the other ponds in the region. These changes at the level of the zooplankton may have important impacts on the other components of the arctic food web, including algae, as well as upper trophic levels that depend on zooplankton for food. Given that unprecedented warming is occurring, and likely continue into the future, these unique ecosystems must continue to be observed to predict, understand and model future alterations to Arctic ecosystems.
Literature cited


Algae. *Limnology and Oceanography* 34, 341–348
345–355. https://doi.org/10.1093/plankt/fbs009


McKee D. & Ebert D. (1996). The interactive effects of temperature, food level and maternal
phenotype on offspring size in Daphnia magna. *Oecologia.* https://doi.org/10.1007/BF00327902


Vita

Mariana Vargas Medrano was born in Juarez, Mexico. The second daughter of Socorro and Javier, she graduated from COBACH #6 high school in Juarez, Mexico, during the spring of 1999 and attended the University of Juarez, Mexico (Universidad Autonoma de Ciudad Juarez). As part of her bachelor’s degree in Biology, she finished an undergraduate thesis on taxonomic study of mushrooms in a Chihuahuan forest, thus contributing to a taxonomic list of fungi in the state of Chihuahua. She was also a member of national Mycology scientific association in Mexico. When she finished her bachelor’s degree she worked at the University of Juarez as a laboratory and profesor’s assistant. Then, she worked in the healthcare industry for two years as a microbiological technician and participated in a FDA product certification. She returned to school in January 2009 to obtain a Master of Science degree from the University of Texas at El Paso (UTEP). While completing her Master’s degree she was an active member of the national SACNAS scientific association and was the treasurer of the UTEP SACNAS student chapter. She also obtained a travel scholarship to attend and perform an oral presentation in the SACNAS national science meeting. She continued her education at UTEP pursuing a Ph.D. in Ecology and Evolutionary Biology with an award for two years. She attended the ITEX conference in 2012 and the Ecological society of America (ESA) in 2013 for poster presentations. During her Masters and Ph.D. she taught or assisted professors in a variety of lab courses related with biology (Human anatomy, Topics in the study of life, Biostatistics with R, Arctic research and statistics) for approximately 7 years.

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