UNIVERSITÉ DU QUÉBEC À CHICOUTIMI

INFLUENCE DU DÉGEL DU PERGÉLISOL SUR LA MATIÈRE ORGANIQUE ET LES RÉSEAUX TROPHIQUES DANS LES EAUX DOUCES CIRCUMPOLAIRES NORDIQUES

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A la mémoire de la multitude de daphnies et
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FOREWORD

This thesis covers the work resulting from my doctoral research conducted under the supervision of Dr. Milla Rautio and includes three chapters as well as a general introduction and an overall conclusion. The first chapter explores the increasing dominance of terrigenous organic matter in circumpolar freshwaters in the context of permafrost thaw. This chapter was published in the special issue *Carbon cycling in inland waters: Progress and perspectives* in the scientific journal *Limnology and Oceanography Letters* (February 2018). It was the result of a collaboration between researchers from Canada and Europe that I co-led with Dr. Milla Rautio. I was responsible specifically for designing the study approach, analyzing and interpreting the data, and leading the preparation of the manuscript. This manuscript was particularly well received within the scientific community, being one of the journal's top downloaded papers published between January 2017 and December 2018. The database related to this first chapter was also published in *Nordicana D*. The second chapter investigates the influence of degrading permafrost on the aquatic food web, and more specifically on the carbon and fatty acid composition of zooplankton. The final chapter focuses on the vertical distribution of zooplankton in thermokarst ponds. I am also the first author of these two latter chapters. I was responsible for research planning, fieldwork, laboratory and data analyses, the interpretation of results, and writing of the scientific papers. The chapters have been submitted respectively to *Ecology* (June 2019) and *Limnology and Oceanography* (June 2019). The main conclusions of these three chapters are discussed in a final section that highlights and contextualizes the contributions of this thesis and presents potential avenues for future research.

Below are the corresponding manuscripts and database quoted as published or submitted for publication in the scientific literature:

Wauthy, M., M. Rautio, K. S. Christoffersen, L. Forsström, I. Laurion, H. Mariash, S. Peura, and W. F. Vincent. 2017. Dissolved organic carbon and related environmental data from ponds and lakes in the circumpolar North, v. 1.0 (2002-2016). Nordicana **D31**. doi:10.5885/45520CE-0A48ADE0E2194290

Wauthy, M., M. Rautio, K. S. Christoffersen, L. Forsström, I. Laurion, H. L. Mariash, S. Peura, and W. F. Vincent. 2018. Increasing dominance of terrigenous organic matter in circumpolar freshwaters due to permafrost thaw. Limnol. Oceanogr. Lett. **3**: 186-198. doi:10.1002/lol2.10063

Wauthy, M., and M. Rautio. 2019. Effect of permafrost thaw on planktonic food webs in subarctic ponds. Submitted manuscript.

Wauthy, M., and M. Rautio. 2019. Vertical distribution of zooplankton in steeply stratified subarctic thaw ponds, northern Quebec. Submitted manuscript.

During my PhD, I also contributed to the four following manuscripts:

Peura, S., M. Wauthy, D. Simone, A. Eiler, K. Einarsdóttir, M. Rautio, and S. Bertilsson. 2019. Ontogenic succession of thermokarst thaw ponds is linked to dissolved organic matter quality and microbial degradation potential. Limnol. Oceanogr. Manuscript accepted for publication.

Nevalainen, L., M. Rantala, H. Kivilä, A. Lami, M. Wauthy, M. Rautio, and T. Luoto. 2019. Biogeochemical and photobiological responses of subarctic lakes to UV radiation. Submitted manuscript.

Rautio, M., and others. 2019. Water quality and lipid composition in Arctic freshwaters, Nunavut, Canada. Manuscript.

Grosbois. G., and others. 2019. Fatty acid composition in seston, zooplankton and fish along a DOC gradient at the pan-Arctic scale. Manuscript.

I also presented the results from my research at several national and international conferences and seminars:

Wauthy, M., and M. Rautio. 2019. Zooplankton diel vertical migration in thermokarst ponds, northern Quebec. Poster presentation, GRIL Annual Symposium 2019, March 14th-16th 2019, Orford, QC.

Wauthy, M. 2019. Influence du dégel du pergélisol sur la dynamique du carbone dans les écosystèmes d'eau douce nordiques. Oral presentation, Université de Montréal, February 18th 2019, Montreal, QC.

Wauthy, M., and M. Rautio. 2018. *Daphnia* carbon composition and health in a context of increasing terrigenous inputs due to permafrost thaw. Oral presentation, Sentinel North Annual Meeting, August 27th-30th 2018, Quebec City, QC.

Wauthy, M. 2018. Global warming and thawing permafrost: impacts on carbon cycle in freshwaters. Elevator pitch, Sentinel North International Ph.D. School, July 12th-24th 2018, Baffin Bay, NU.

Wauthy, M., and M. Rautio. 2018. Zooplankton carbon and fatty acid composition in the context of increasing dominance of terrestrial organic matter due to permafrost thaw. Oral presentation, ASLO 2018 Summer Meeting, June 10th-15th 2018, Victoria, BC.

Wauthy, M., and M. Rautio. 2018. Influence du dégel du pergélisol sur la dynamique du carbone dans les écosystèmes d'eau douce nordiques. Oral presentation, 86ème congrès de l'ACFAS, May 7th-11th 2018, Saguenay, QC.

Wauthy, M. 2018. Influence of thawing permafrost on carbon dynamics in circumpolar freshwater ecosystems. Oral presentation, Université du Québec à Chicoutimi, April 12th 2018, Saguenay, QC.

Wauthy, M., and M. Rautio. 2018. Thawing permafrost influence on *Daphnia* carbon composition and health. Oral presentation, GRIL Annual Symposium 2018, March 22th-24th 2018, Orford, QC.

Wauthy, M., and others. 2017. Carbon composition of dissolved organic matter and zooplankton in circumpolar ponds. Oral presentation, Arctic Change 2017, December 11th-15th 2017, Quebec City, QC.

Wauthy, M., and others. 2017. Thawing permafrost induces more allochthony in subarctic ponds. Poster presentation, GRIL Annual Symposium 2017, March 16th-18th 2017, Orford, QC.

Wauthy, M., and others. 2017. Importance of terrestrial organic matter in northern freshwater systems linked to the permafrost thaw. Oral presentation, CEN Workshop 2017, February 16th-17th 2017, Quebec City, QC.

Wauthy, M. 2017. Global warming and thawing permafrost: impacts on carbon cycle in subarctic freshwater systems. Oral presentation, Centre Eau Terre Environnement, INRS, February 15th 2017, Quebec City, QC.

Wauthy, M., and M. Rautio. 2016. Permafrost thaw makes subarctic lakes more terrestrial. Oral presentation, SIL 2016, XXXIII Congress, July 31st-August 5th 2016, Torino, Italy.

Wauthy, M., and M. Rautio. 2015. Zooplankton vertical distribution in strongly stratified subarctic thaw ponds, northern Quebec. Poster presentation, World Water Day 2015: Water and Sustainable Development, March 22th 2015, Saguenay, QC.

Wauthy, M., and M. Rautio. 2015. Influence of thawing permafrost on zooplankton community, northern Quebec. Poster presentation, GRIL 25th Annual Meeting, March 12th-14th 2015, Saint-Hippolyte, QC.

Wauthy, M., and M. Rautio. 2014. Influence of thawing permafrost on zooplankton metabolism. Poster presentation, THAW 2014 - THermokarst Aquatic ecosystems Workshop, March 12th-15th 2014, Quebec City, QC.

Wauthy, M., and M. Rautio. 2014. Zooplankton feeding and carbon assimilation in strongly stratified subarctic thaw ponds, northern Quebec. Poster presentation, GRIL 24th Annual Meeting, February 20th-22th 2014, Saint-Hippolyte, QC.

Finally, I participated in five interviews to present the results of my research to the general public:

Wauthy, M. 2019. Interview by Abigail Eisenstadt. Melting frozen soil is eroding Arctic communities, scientists say. Boston University News Service, February 17th 2019.

Wauthy, M. 2018. Interview by Karla Lant. As arctic permafrost thaws, northernmost lakes brown. Environmental Monitor, March 20th 2018.

Wauthy, M. 2018. Interview by Michelle Pucci. Thawing permafrost causes 'browning' of lakes, upsetting aquatic ecosystems, study says. CBC North, March 13th 2018.

Wauthy, M. 2017. Interview by Louise-Emmanuelle Paris and Alex Royer-Lavallée. Permafrost thaw in the North. ScienceÉnergie, CKIA FM Radio, Quebec City, QC, aired on April 21st 2017.

Wauthy, M. 2017. Interview by Laure Gagnon-Tremblay. Maxime Wauthy étudie le cycle du carbone. Le Quotidien, January 30th 2017.

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ABBREVIATIONS

%Cx	relative abundance of any component x
[Cx]	maximum fluorescence of any component x
^{2}H	deuterium
^{13}C	carbon-13
^{15}N	nitrogen-15
A_{254}	absorbance at 254 nm
a_{320}	absorption coefficient at 320 nm
a_{440}	absorption coefficient at 440 nm
AICc	corrected Akaike information criterion
ANOVA	analysis of variance
C	carbon
CDOM	chromophoric dissolved organic matter
CH_4	methane
Chla	chlorophyll *a*
CO_2	carbon dioxide
Cx	component x
DHA	docosahexaenoic acid
DistLM	distance-based linear models
DOC	dissolved organic carbon
DOM	dissolved organic matter
EEMs	excitation-emission matrices
EFA	essential fatty acids
EPA	eicosapentaenoic acid
FA	fatty acid
FASTAR	fatty acid sources tracking algorithm in R
FDOM	fluorescent dissolved organic matter
Fe	iron
FI	fluorescence index
KWK	Kwakwatanikapistikw River valley
NMDS	nonmetric multidimensional scaling

O_2	oxygen
PARAFAC	parallel analysis factor
PAR	photosynthetically active radiation
PCA	principal component analysis
PERMANOVA	permutational multivariate analysis of variance
PERMDISP	permutational analysis of multivariate dispersions
POC	particulate organic carbon
PUFA	polyunsaturated fatty acid
PUFA:SAFA	polyunsaturated:saturated fatty acid ratio
S	spectral slope
S_{285}	spectral slope for the intervals 275–295 nm
S_{289}	spectral slope for the intervals 279–299 nm
S_{375}	spectral slope for the intervals 350–400 nm
SAFA	saturated fatty acid
SAS	Sasapimakwananisikw River valley
SI	stable isotope
S_R	slope ratio
$SUVA_{254}$	specific ultraviolet absorbance at 254 nm
TN	total nitrogen
TP	total phosphorus
UV	ultraviolet

RÉSUMÉ

Les sols gelés en permanence du paysage circumpolaire constituent l'un des plus grands gisements de carbone organique sur Terre. Le réchauffement climatique et le dégel du pergélisol qu'il entraîne ont accru le risque qu'une grande partie de ce carbone soit libérée dans l'atmosphère sous forme de gaz à effet de serre, notamment sous forme de méthane depuis les nombreux écosystèmes aquatiques présents dans les régions subarctiques et arctiques. Ce scénario a attiré l'attention de la communauté scientifique sur le cycle du carbone dans les écosystèmes circumpolaires. Cependant, les changements induits par ces apports croissants de matière terrestre sur le réservoir de carbone organique et sur le réseau alimentaire des lacs et des étangs nordiques ont été peu étudiés.

Ce projet de thèse vise à explorer l'influence du dégel du pergélisol sur les lacs de haute latitude. Premièrement, nous avons évalué les effets du dégel du pergélisol sur la matière organique dissoute (MOD) par des analyses optiques de 253 étangs couvrant 200 degrés de longitude à travers l'Arctique. Pour un sous-échantillon de dix plans d'eau subarctiques, nous avons également quantifié la contribution terrestre à la MOD en utilisant l'approche des isotopes stables. Dans un second temps, en mesurant la concentration en nutriments et en chlorophylle *a*, et en utilisant des approches basées sur les acides gras et les isotopes stables, nous avons étudié l'influence du dégel du pergélisol sur les producteurs et les consommateurs primaires de la chaîne alimentaire planctonique au sein de huit étangs subarctiques impactés différemment par les apports terrestres venant du bassin versant. Enfin, nous avons exploré la communauté et la biomasse de zooplancton dans huit étangs de dégel subarctiques, ainsi que la manière avec laquelle la stratification thermique et les variables environnementales associées déterminent la distribution verticale du zooplancton dans ces mares arctiques fortement affectées par le dégel du pergélisol.

Les mesures optiques montrent une plus forte proportion de carbone terrestre et une moindre contribution des algues à la MOD dans les eaux touchées par le dégel du pergélisol. La composition de la MOD est largement dominée (moyenne de 93%) par de la matière d'origine terrestre dans les sites influencés par le dégel du pergélisol, tandis que l'influence terrestre est beaucoup moins importante dans les masses d'eau situées dans des bassins hydrographiques non affectés par des processus thermokarstiques (39%). De plus, nos résultats mettent en évidence l'influence positive du dégel du pergélisol sur la teneur en nutriments et l'abondance en algues planctoniques. Cependant, l'impact sur les consommateurs primaires reste très limité, avec une faible contribution de la matière organique terrestre à la biomasse du zooplancton dans les étangs de dégel subarctiques (35% selon l'approche basée sur les isotopes stables, 18% selon celle basée sur les acides gras). Enfin, cette thèse fait état de la prédominance des rotifères dans les mares de thermokarst subarctiques (35 à 93% de la biomasse du zooplancton) et met en évidence la stratification prononcée du zooplancton, laquelle est principalement déterminée par un compris entre l'accès à suffisamment d'oxygène (O_2) et à une diète algale de qualité, ainsi qu'à la pression de prédation exercée par des larves de *Chaoborus*.

Pris collectivement, ces résultats soulignent la forte influence du dégel du pergélisol sur le réservoir de carbone organique des eaux douces septentrionales, avec une tendance à la domination croissante du carbone organique d'origine terrestre pouvant altérer les voies métaboliques et les processus biogéochimiques dans le Nord. Ces impacts peuvent être considérés comme un exemple extrême de brunissement qui va probablement altérer les lacs de haute latitude vers des conditions davantage hétérotrophiques et alimenter leur boucle microbienne. En outre, alors que le dégel du pergélisol stimule la croissance du phytoplancton aux dépens des algues benthiques, ces mêmes producteurs primaires planctoniques semblent être la principale ressource qui supporte le réseau alimentaire dans les étangs de dégel, indépendamment de l'écrasante domination de la matière organique issue du bassin versant. Enfin, le réchauffement et le brunissement à venir des plans d'eau circumpolaires devraient renforcer la stabilité thermique des lacs, augmentant ainsi le risque d'anoxie dans la colonne d'eau, et créant par conséquent une incertitude quant à la réponse future de la communauté zooplanctonique face au réchauffement climatique et au dégel du pergélisol.

Mots clés: pergélisol, étangs de dégel, allochtonie, brunissement, MOD, réseau alimentaire, zooplancton, isotope stable, acide gras, stratification

ABSTRACT

Frozen tundra soils are one of the largest pools of organic carbon in the Earth system. Climate warming and the associated permafrost thaw have increased the risk that a large fraction of this carbon will be released to the atmosphere as greenhouse gases, particularly in the form of methane produced by the numerous aquatic ecosystems found throughout the subarctic and arctic regions. This scenario has drawn the attention of the scientific community to the carbon cycle in circumpolar ecosystems. Some of this carbon released from thawing permafrost is transported into freshwater systems. However, the changes to the carbon pool and the northern freshwater food webs by the increased terrigenous input have not been studied extensively.

This PhD project aims to investigate how permafrost thaw influences northern waterbodies across the Arctic. First, we evaluated the effects of thawing ice-rich permafrost on the dissolved organic matter (DOM) in freshwaters. We used optical analyses of 253 ponds covering 200 degrees of longitude across the circumpolar North. For a subset of 10 waterbodies in subarctic Quebec, we used stable isotopes (SI) to quantify the contribution of terrestrial sources to the DOM pool. Second, using nutrients, chlorophyll *a*, fatty acids (FA), and SI, we investigated the influence of thawing permafrost on primary producers and primary consumers of the planktonic food web of 8 subarctic ponds affected to different degrees by permafrost carbon. Finally, we explored the zooplankton community and biomass in 8 subarctic thaw ponds and determined the extent to which thermal stratification and the associated environmental variables drive the vertical distribution of zooplankton in these arctic freshwaters that are affected by degrading ice-rich permafrost.

The optical measurements reveal a higher proportion of terrestrial carbon and a lower algal contribution to DOM in waters affected by thawing permafrost. DOM composition is dominated largely (mean of 93%) by terrestrial substances at sites influenced by thawing permafrost, whereas the terrestrial influence is much less (39%) in waterbodies located in catchments unaffected by thermokarst processes. Also, our results highlight the stimulating influence of eroding and degrading ice-rich permafrost on nutrients and planktonic algae. However, the effect on consumers remains relatively constrained, with a limited contribution of terrestrial organic matter to the biomass of the filter-feeding zooplankton in subarctic thaw ponds (35% according to SI- and 18% according to FA-based mixing models). Finally, this thesis reports the dominance of rotifers in subarctic thaw ponds (35–93% of the zooplankton biomass), and highlights the pronounced stratification of zooplankton that is driven mostly by a combination of oxygen (O_2), *Chaoborus* predation, phytoplankton, and essential FA supply.

Collectively, these findings emphasize the strong influence of thawing permafrost on the carbon pool of northern freshwaters and a shift toward increased dominance by land-derived organic carbon that may alter metabolic pathways and biogeochemical processes in the North. These impacts may be considered as an extreme example of browning that will likely shift high-latitude freshwaters more toward net heterotrophic conditions and fuel the microbial loop. Furthermore, while permafrost thaw stimulates phytoplankton growing in the water column at the expense of benthic algae, these same planktonic primary producers appear to be the key resource that fuels the food web in thaw ponds, regardless of the overwhelming dominance of terrigenous organic matter. Finally, future warming and browning of circumpolar freshwaters are expected to enhance the thermal stability of thaw ponds. This will increase the risk of anoxia throughout the water column and, as such, add uncertainty as to the future response of arctic and subarctic zooplankton communities to global warming and degrading permafrost.

Keywords: permafrost, thaw ponds, allochthony, browning, DOM, food web, zooplankton, stable isotope, fatty acid, stratification

INTRODUCTION

0.1 Statement of the problem

Frozen tundra soils hold one of the Earth's largest pools of organic carbon. With global warming, permafrost is thawing at an accelerated rate, promoting the formation of thermokarst ponds. These waterbodies have become increasingly abundant in subarctic areas, representing up to 90% of all lakes in some regions (Walter et al. 2006). They act as recipients of the vast amount of carbon stored in permafrost that, due to thawing, is transported to aquatic ecosystems. Originally transparent ponds—characterized by high oxygen (O_2) production by benthic algae and general net autotrophy—they are receiving increasing amounts of terrestrial organic material from the changing watershed with consequences for ecosystem metabolism. The greater input of terrestrial-sourced carbon and nutrients stimulates bacterial production and respiration (Roiha et al. 2016) and decreases light availability for benthic primary production. The net result is an increasing number of circumpolar freshwaters becoming important sources of carbon dioxide (CO_2) and methane (CH_4) to the atmosphere (Laurion et al. 2010).

In this scenario, global warming and the related permafrost thaw fuel further natural emissions of greenhouse gases and, therefore, reinforce this greenhouse gas–permafrost thaw positive feedback loop (Fig. 0.1). This scenario has focused the attention of the scientific community on the carbon cycle in arctic and subarctic freshwaters and, in particular, on aquatic microbial communities (e.g., Crevecoeur et al. 2015; Roiha et al. 2015; Deshpande et al. 2016) and gas exchange with the atmosphere (e.g., Laurion et al. 2010; Matveev et al. 2016). However, several steps of this feedback loop still need to be investigated and clarified.

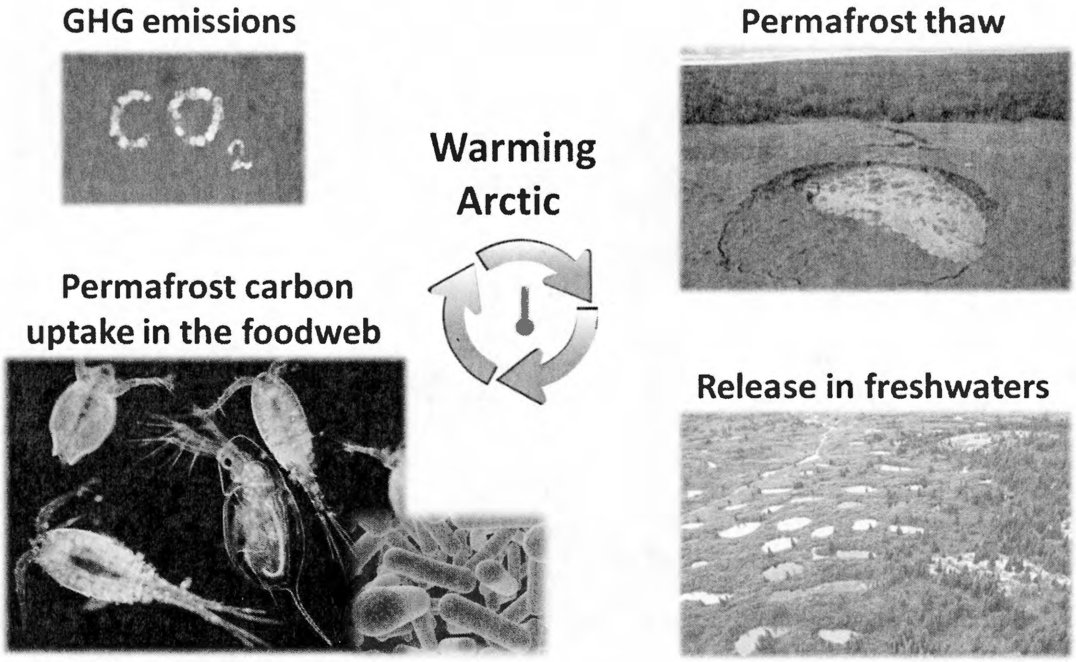

Figure 0.1 Schematic representation of the carbon cycle in northern freshwaters in the context of climate change and global warming.

The combined effect of the increased input of humic compounds from the degrading permafrost and the associated modifications in pond metabolism contribute to altering the composition of the aquatic carbon pool. Nonetheless, the extent and rate of this change remain unknown despite shallow ponds being the dominant freshwater ecosystem in the circumpolar North and representing an integral component of the carbon cycle at high latitudes.

Furthermore, although heterotrophic bacteria are responsible for the main turnover of terrigenous carbon in natural waters (Roiha et al. 2016; Wurzbacher et al. 2017), allochthonous inputs from degrading permafrost are also expected to have a marked influence on internal lake processes and the pelagic food web, from phytoplankton at the base of the trophic chain to zooplankton at the higher levels. Given the absence of fish in most arctic and subarctic ponds, zooplankton can attain a very high biomass in these waterbodies (Rautio and Vincent 2006). Furthermore, permafrost thaw and the resulting terrestrial inputs from the degrading watershed induce a strong thermal stratification in thermokarst freshwaters (Breton et al. 2009). Consequently, deeper thaw ponds are transformed into novel environments for arctic waterbodies. The installation of a steep thermal stratification restructures both the physicochemical environment and the biological communities of these systems. Yet, despite these striking transformations of circumpolar freshwaters due to permafrost thaw, the repercussions on higher trophic levels have only been addressed superficially.

The aim of this project was to investigate the consequences of permafrost thaw on the dissolved organic matter (DOM) pool and the planktonic food webs in circumpolar waterbodies as well as survey the zooplankton community and its biomass in subarctic thaw ponds to isolate the main variables controlling the vertical distribution of zooplankton throughout the water column. How great is the terrestrial imprint on northern freshwater ecosystems in degrading ice-rich permafrost catchments? What is the impact of thawing permafrost on the carbon pool within circumpolar ponds? How much of this newly available terrestrial carbon do zooplankton assimilate in thaw ponds? Is it the high-quality algal diet that mainly drives the distribution of zooplankton within the water column of thermokarst waterbodies? Finding answers to these questions will improve our understanding of arctic freshwaters in the context of permafrost thaw and clarify how degrading permafrost affects the ecology of planktonic organisms.

0.2 State of the science

0.2.1 Permafrost in the Arctic

van Everdingen (1998) defined permafrost as a « ground (soil or rock and included ice and organic material) that remains at or below 0 °C for at least two consecutive years ». Permafrost in the northern hemisphere covers approximately 23×10^6 km^2, representing almost a quarter of the total hemispheric land area. Nearly 70% of this multiannual frozen soil is spread between 45°N and 67°N (Zhang et al. 1999). In Canada, up to 50% of the territory is covered by permafrost (Smith and Riseborough 2002). Permafrost can be divided into four categories based on coverage (Fig. 0.2): continuous (> 90% of the territory is underlain by permafrost), discontinuous (50–90%), sporadic (10–50%), and isolated (< 10%) (Brown et al. 1998).

Permafrost plays a major role in the global carbon cycle, as it stores vast quantities of organic carbon beneath its active layer (Schuur et al. 2015). Frozen soils hold twice as much carbon as the atmosphere and represent one of the largest pools of carbon in the Earth system (Zimov et al. 2006). This organic carbon comprises the remnants of animals and plants accumulated and stored in the permafrost, possibly over thousands of years (Schuur et al. 2015).

In the context of climate change, however, permafrost is subject to heightened summer thaw, and its southern limit is migrating northward on a yearly basis (Halsey et al. 1995). For example, along the eastern coast of Hudson Bay, northern Quebec, around 80% of the surface that was underlain by permafrost in 1957 thawed over the following 50 years (Payette et al. 2004). Given the projections of increasing temperatures in the future, especially at high latitudes (ACIA 2005), permafrost thaw is expected to accelerate and thus release vast amounts of currently captive carbon into the numerous arctic and subarctic freshwaters (Vincent et al. 2013). The thawing of the permafrost will therefore lead to a considerable increase in greenhouse gas emissions by northern freshwaters and intensify, via a positive feedback loop, the effects of global warming (Schuur et al. 2009).

Source: International Permafrost Association, 1998.
Circumpolar Active-Layer Permafrost System (CAPS), version 1.0.

Figure 0.2 Permafrost distribution in the circumpolar Arctic (Brown et al. 1998).

0.2.2 Permafrost thaw and thermokarst freshwaters

Permafrost thaw creates new waterbodies across northern landscapes. Produced by the erosion and collapse of ice-rich frozen soil (Vonk et al. 2015), these thermokarst lakes and ponds, hereafter referred to as thaw ponds, are presently becoming particularly numerous and now represent one of the most abundant freshwater ecosystem types in northern regions (Pienitz et al. 2009). Collectively, they cover an estimated 250,000 to 380,000 km^2 (Grosse et al. 2013); this accounts for approximately 25% of the total area covered by lakes and ponds in the Arctic (1.4×10^6 km^2; Muster et al. 2017).

Despite the highly variable conditions that produce thaw ponds (Bouchard et al. 2017), the main processes of their formation depend on the specific permafrost zone (Fig. 0.3). In continuous permafrost regions, thaw ponds are formed within melted ice wedges and above depressed polygons (Fig. 0.3a–d; Fig. 0.4a) (Czudek and Demek 1970). In subarctic regions, where permafrost is discontinuous or sporadic, thaw ponds result from the collapse of small mounds of frozen soil, which are composed mostly of organic (palsa) or inorganic (lithalsa) matter (Fig. 0.3e–h; Fig. 0.4b) (Seppälä 2006; Calmels et al. 2008). Through successive periods of erosion and thaw, these waterbodies can expand vertically and horizontally and eventually merge. In some parts of the Arctic landscape, these ponds are disappearing by drainage, evaporation, or infilling (Fig. 0.3), whereas in other regions they are expanding in size and number (Vincent et al. 2017). In subarctic Quebec, these thaw ponds are relatively persistent (Smith et al. 2005) as impermeable silt below the thawing permafrost prevents their drainage (Bhiry et al. 2011).

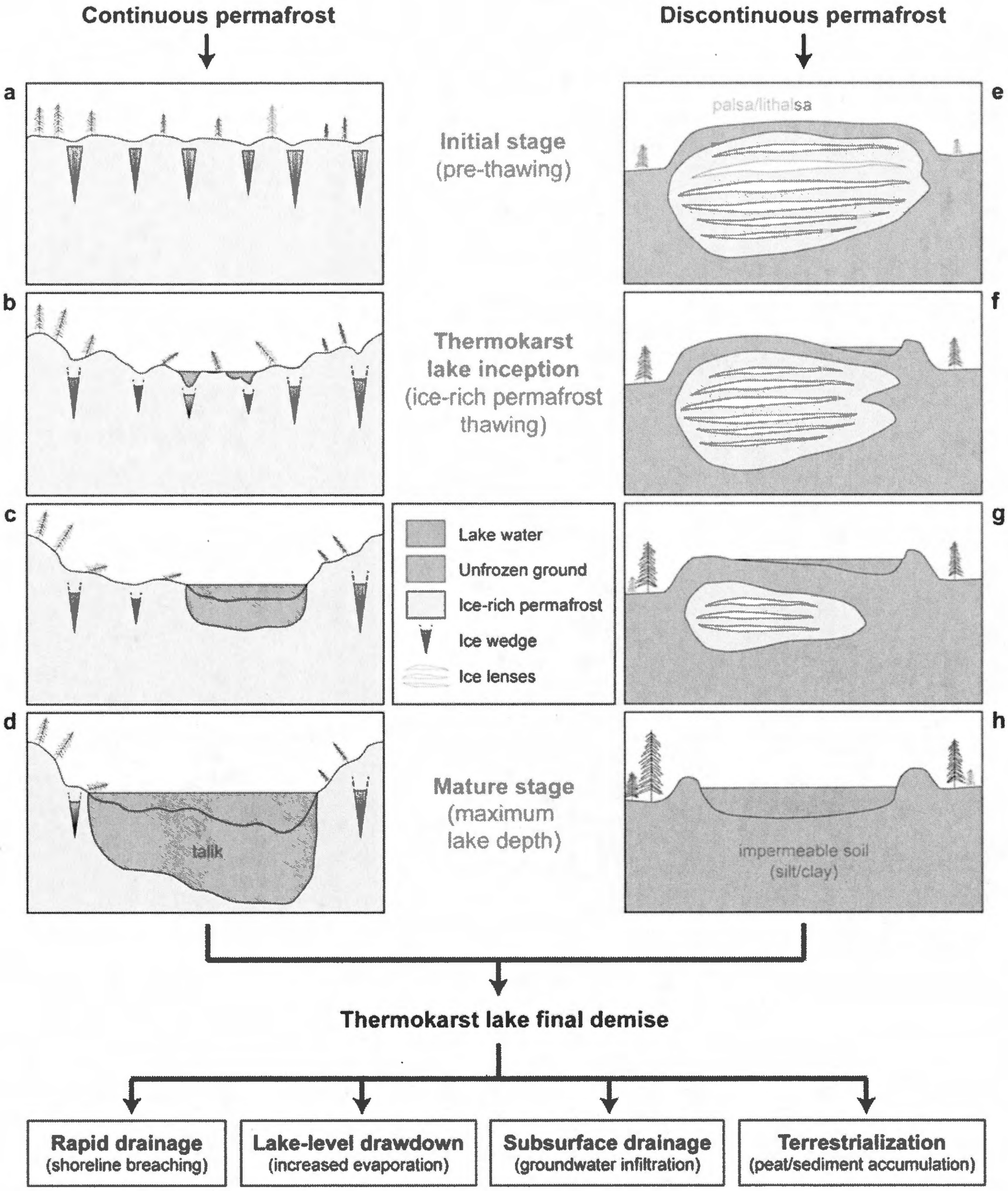

Figure 0.3 Thaw pond formation and evolution in (a–d) continuous and (e–h) discontinuous permafrost regions (Bouchard et al. 2017).

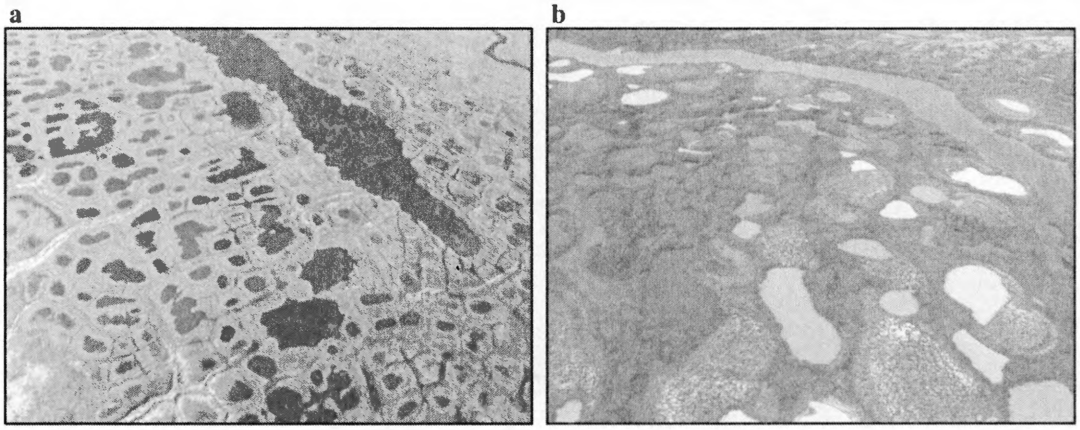

Figure 0.4 Typical thaw ponds in (a) continuous and (b) discontinuous or sporadic permafrost zones. (a) Polygonal landscape on Bylot Island, Nunavut, with ice-wedge trough ponds. (b) Thaw ponds displaying a wide range of color within areas of remaining palsa and lithalsa in the BGR valley close to Umiujaq, northern Quebec (Photos: Isabelle Laurion).

Most thaw ponds are relatively small and shallow (Vonk et al. 2015); for example, thermokarst freshwaters sampled in northern Quebec and Nunavut had surface areas of 81 to 605 m^2 and maximum depths of 0.8 to 3.3 m (Breton et al. 2009; Laurion et al. 2010; Crevecoeur et al. 2015). However, much larger (up to 37.4 km^2 in Alaska) and deeper (up to 22 m deep in the Yukon Coastal Plain) thermokarst lakes have been recorded (West and Plug 2008; Arp et al. 2011).

Thermokarst waterbodies act as recipients of the large amounts of eroded permafrost material transported to aquatic ecosystems (Schuur et al. 2008). However, the nature of the surroundings and the state of landscape degradation vary considerably, leading to variable amounts and forms of organic and inorganic matter input into the waters. Due to this heterogeneity, thaw ponds encompass a wide variety of hydrological, physicochemical, and limnological properties (Vonk et al. 2015). Among other things, this variability is expressed in the color of the waters, which can vary greatly between thaw ponds, even those separated by only a few meters (Fig. 0.4b). Watanabe et al. (2011) demonstrated that these variations were due in large part to the combined concentrations of DOM, originating from degrading permafrost, and suspended non-algal particulate material, originating from the surrounding soils that are often rich in clay and silt. This high content of dissolved and particulate matter strongly attenuates light penetration through the water column. These limited light conditions reduce benthic and, therefore, overall primary production (Vadeboncoeur et al. 2008). The high absorption of solar radiation by the DOM and suspended particles also warms the thermokarst surface waters, while the bottom waters remain cooled by the underlying permafrost (Vonk et al. 2015). Consequently, despite their shallow depth, many thaw ponds display stable stratification during the entire summer period, with no mixing between the epilimnion and the hypolimnion (Breton et al. 2009; Laurion et al. 2010). During the long ice-covered period, this thermal stratification is even stronger in thaw ponds that are deep enough to avoid freezing to the bottom (Deshpande et al. 2015).

The variable inputs from the eroding permafrost also strongly affect the concentration of organic and inorganic matter in thermokarst waterbodies. The concentration of dissolved organic carbon (DOC) is expected to increase with the continuing permafrost thaw (Vonk et al. 2015) and appears to be correlated directly with the amount of vegetation in the watershed (Pienitz et al. 1997). Although some studies have highlighted a decrease in nutrient concentrations in shallow lakes affected by permafrost slumping (Thompson et al. 2012), most thaw ponds have higher total phosphorus (TP) and total nitrogen (TN) concentrations (Bowden et al. 2008; Abbott et al. 2014) relative to non-thaw ponds. Furthermore, Roiha et al. (2015) emphasized the higher concentrations of nutrients in bottom waters compared to the surface, recording bottom–water TP values of 198 to 432 $\mu g\ L^{-1}$ and TN values of 267 to 496 $\mu g\ L^{-1}$.

In addition to the critical influence on the physicochemical aspects of aquatic systems, inputs from degrading permafrost alter markedly the biological properties of thermokarst freshwaters (Vonk et al. 2015). Chlorophyll *a* (Chla) concentrations vary considerably between thaw ponds but generally display values more typical of mesotrophic or eutrophic systems (Rossi et al. 2013). In addition, as with nutrients, the deeper and darker thermokarst waters contain higher values of Chla (Roiha et al. 2015). Thaw ponds also display an intense microbial activity. Roiha et al. (2015) and Deshpande et al. (2016) observed high bacterial growth and the dominance of bacterioplankton in the production of organic biomass within thermokarst waterbodies, during both ice-free and ice-covered periods. The abundance of bacterioplankton in thaw ponds is also comparable to that of eutrophic lakes in temperate regions (Breton et al. 2009).

Given the steep thermal stratification and the high rate of bacterial metabolism (Deshpande et al. 2016), thaw ponds display hypoxia, especially in their bottom waters (Deshpande et al. 2015). In general, this O_2 depletion is greater in winter; many thermokarst freshwaters experience anoxia in the entire water column during the prolonged ice-covered period (Vonk et al. 2015). The mixing of the water column occurs only in spring and autumn for a few days; the result is sometimes an incomplete mixing, and the hypolimnion can remain completely isolated from the upper water layers for an extended period of time (Burn 2003; Laurion et al. 2010; Deshpande et al. 2015). Thaw ponds are also known as hotspots of substantial emissions of greenhouse gases to the atmosphere (Walter et al. 2006; Laurion et al. 2010; Abnizova et al. 2012; Negandhi et al. 2013; Sepulveda-Jauregui et al. 2015). At the bottom of thermokarst waterbodies in northern Quebec, for example, Matveev et al. (2016) recorded CO_2 and CH_4 concentrations up to 5613 μmol L^{-1} and 552 μmol L^{-1}, respectively.

Although in general thaw ponds encompass a variety of transparencies and trophic conditions, given the increasing carbon inputs from thawing permafrost soils, they are likely to become more turbid, eutrophic, and heterotrophic compared to the typical crystal-clear, oligotrophic waters of high-latitude lakes not affected by permafrost thaw (Vonk et al. 2015).

0.2.3 Dissolved organic matter

Dissolved organic matter (DOM) refers to the complex mixture of aromatic and aliphatic hydrocarbon structures that are associated with various functional groups such as ketones, carboxyls, and hydroxyls. Dissolved in the water (< 0.45 µm), DOM represents the largest pool of organic carbon in freshwater systems. This organic carbon derives from the degradation of organic matter of terrestrial or aquatic origin (Wetzel 2001; Tranvik et al. 2009). Thus, DOM is often classified according to its source. The portion of DOM produced by autotrophic organisms and macrophytes within freshwater systems is referred to as autochthonous DOM, whereas the portion derived from the degradation of terrestrial organic matter, such as soil leachates, leaf litter, and plant residues, is designated as allochthonous DOM (Curtis 1998). Therefore, determining the origin of the DOM provides a practical framework for investigating and visualizing the connection between the aquatic environment and its surrounding watershed.

Use of optical properties is an effective means of investigating the dynamics and characteristics of DOM. A significant portion of DOM, the chromophoric (or more simply colored) dissolved organic matter (CDOM) can absorb light (Kowalczuk et al. 2003). Moreover, a subset of the larger CDOM pool can re-emit light by fluorescence and is referred to as the fluorescent dissolved organic matter (FDOM) (Cory and Kaplan 2012). Thus, via spectrophotometric and spectrofluorometric measurements of DOM, we can estimate its likely origin and identify its nature. For this purpose, several indices have been developed to assess the concentration (Blough and Del

Vecchio 2002), origin (McKnight et al. 2001; Loiselle et al. 2009), aromaticity (Weishaar et al. 2003), and molecular weight (Helms et al. 2008) of DOM. Furthermore, Stedmon and Bro (2008) developed a multivariate modeling technique—parallel analysis factor (PARAFAC)—to decompose the excitation-emission matrices (EEMs) obtained for each sample into a number of molecule families (or components) that share the same well-established fluorescence profile. From this, we can estimate the relative contributions of these families to the total fluorescence.

0.2.4 Allochthony in food webs

In the classical view of lake food webs, aquatic autotrophic organisms are considered as the only producers of the trophic chain (Wetzel 1983). However, multiple studies of freshwater systems have demonstrated that higher trophic levels, such as zooplankton and fish, can feed on other food sources (Wallace et al. 1997; Pace et al. 2004; Rautio and Vincent 2006; Cole et al. 2011). These alternative food sources arrive essentially as DOC and particulate organic carbon (POC) from the surrounding terrestrial environment (Wetzel 1995). This idea of carbon flux between aquatic and terrestrial ecosystems and the possible assimilation of terrestrial organic matter by higher trophic levels once again highlighted the importance of distinguishing aquatic organic carbon—pelagic and benthic photosynthetic primary producers (autochthonous source)—from terrestrial carbon (allochthonous source) (Wetzel 1995).

Autochthonous and allochthonous sources differ in their carbon quality (i.e., the ease by which the carbon is assimilated by organisms) and quantity (Curtis 1998). Most of the time, terrestrial carbon is available in high quantities, but it is more recalcitrant than autochthonous carbon (Sondergaard and Middelboe 1995; Brett et al. 2012). Furthermore, autochthonous materials are more energy-rich and promote a greater biomass, abundance, and production of higher trophic levels than does allochthonous carbon (Brett et al. 2009). Most decomposition of terrestrial DOC and POC is carried out by planktonic bacteria. Thus, allochthonous carbon enters into the aquatic food web essentially via the microbial loop (Daniel et al. 2005); however, the share of terrestrial matter transferred to higher trophic levels remains debated (Brett et al. 2017; Tanentzap et al. 2017). Several studies have estimated the contribution of allochthonous matter to zooplankton and fish at 20% to 70% (Pace et al. 2004; Solomon et al. 2011; Karlsson et al. 2012); other authors have found a very limited role for terrestrial carbon in zooplankton biomass and secondary production (Brett et al. 2009; Brett et al. 2012; Galloway et al. 2014).

As mentioned above, input of allochthonous material from the degrading watershed into thaw ponds can be high and induce strong turbidity (Vonk et al. 2015). This light limitation can severely restrain autochthonous primary production (Kimmel and Groeger 1984). Therefore, the assimilation of terrestrial matter by grazers and higher trophic levels could be substantial in thermokarst systems compared to waterbodies less affected by allochthonous input.

There exist several approaches for investigating the carbon origin and organic matter fluxes in aquatic food webs. Initial investigations of food assimilation relied on gut content extraction and analysis; this method, however, is extremely tedious, time-consuming, and difficult to apply to microorganisms. As well, gut content reveals ingestion and not assimilation; these can be sensibly different (Schindler and Lubetkin 2004). More recently, stable isotope (SI) and fatty acid (FA) analyses coupled to mixing models have offered a means of determining the contribution of various carbon sources to the trophic chain (Wilkinson et al. 2013; Galloway et al. 2014; Taipale et al. 2016; Grosbois et al. 2017a).

Stable isotope (SI) analysis is the most widely used technique for studying aquatic food webs and watershed–lake interactions. The concept behind SI analysis is that the ratio between the abundance of two stable isotopes of a same element (e.g., $^{13}C/^{12}C$), weighted by a standard, is characteristic of each food source and studied consumer. Thus, each source of organic matter has a specific SI ratio (hereafter referred to as a signature), and the proportions of these food sources that make up consumer tissues can therefore be traced (Fry 2006). Isotopic ratios are expressed as:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000\,, \qquad \text{(eq. 1)}$$

where R is the ratio of the less abundant isotope over the more common isotope measured in the sample or in an international standard (Fry 2006). Owing to the carbon-based compounds that characterize organic matter molecules, the relatively high abundance of ^{13}C in organisms and the environment, and the good preservation of the ratio $^{13}C/^{12}C$ in the food chain, $\delta^{13}C$ is the most commonly used SI in trophic studies. It is combined mainly with $\delta^{15}N$ (Fry 2006). However, algal and terrestrial end-members have the tendency to overlap with these two SI, making their interpretation difficult. To overcome this problem, $\delta^{13}C$ can be combined with $\delta^{2}H$; this combination separates clearly aquatic and terrestrial matter (Doucett et al. 2007).

Fatty acids (FA) are a family of molecules that play a vital role in the survival, growth, and reproduction of most animals, including zooplankton (Wenzel et al. 2012). However, some FA cannot be synthesized de novo by consumers; therefore, these essential fatty acids (EFA) need to be assimilated into the organism's diet (Arts et al. 2009). Most EFA are polyunsaturated fatty acids (PUFA) and more specifically omega-3 PUFA. These FA are almost entirely produced by algal primary producers, confirming the required presence of phytoplankton in zooplankton diet (Taipale et al. 2014). Some metabolically stable FA are synthesized exclusively by other sources; for example, the saturated fatty acids (SAFA) C20:0 and C22:0 are specific to terrestrial plants, and the SAFA C15:0 is specific to bacterial production (Taipale et al. 2015). Therefore, FA are excellent biomarkers for tracing carbon fluxes through the aquatic food web (Napolitano 1999).

Bayesian mixing models represent a powerful mathematical approach designed to untangle and quantify the contribution of different food sources to the consumer (Phillips and Gregg 2003). Mixing models have been run widely on SI (Parnell et al. 2013). The restricted number of end-members (Fry 2013) and the overlapping of SI signatures inside seston (Taipale et al. 2008) can limit the power of this approach; however, Bayesian mixing models can remain robust when there are limited numbers of sources (Semmens et al. 2013). Given the great number of available end-members and the difference in FA content between algal and non-algal matter, FA-based mixing models circumvent these limitations (Galloway et al. 2014). Combining SI- and FA-based approaches can therefore provide a more thorough understanding of the organic matter fluxes inside aquatic food webs (Perga et al. 2006).

0.2.5 Zooplankton in arctic and subarctic thaw ponds

Most high-latitude ponds are fishless given their shallow depth coupled with the risk of freezing solid to the bottom in winter and the lack of connectivity to deeper freshwaters (Rautio et al. 2011a). Due to this absence of fish, zooplankton communities play a key role in the aquatic trophic chain of small and shallow arctic waterbodies. Zooplankton are often at the top of the food web (Hansson et al. 1993) and display a larger size and greater abundance compared with other circumpolar northern lakes (Rautio and Vincent 2006).

Despite the importance of zooplankton in arctic and subarctic shallow waterbodies, our knowledge of their communities remains limited and sporadic (e.g., Rautio et al. 2011a). This lack of knowledge is particularly evident for thaw ponds, as very few studies have investigated the higher trophic levels of fishless waterbodies affected by thawing permafrost. Nigamatzyanova and Frolova (2016) described the community structure of zooplankton in thermokarst lakes of the Lena River delta in eastern Siberia; they recorded a higher diversity and abundance of Rotifera compared to Copepoda or Cladocera. Bégin and Vincent (2017) observed similar patterns in subarctic Quebec and also highlighted the higher zooplankton abundance and biomass of thaw ponds relative to freshwaters not affected by degrading permafrost. Bégin and Vincent (2017) also identified the potential top-down control applied by the larval phantom midge *Chaoborus* on rotifers.

0.3 Thesis objectives, hypotheses, and structure

The overall objective of this thesis is to better understand the influence of permafrost thaw on the carbon pools and food webs in northern circumpolar freshwaters. This study also aims to fill knowledge gaps related to the carbon cycle in subarctic and arctic thaw ponds. This general goal can be broken down into a series of sub-objectives, each representing one of the three chapters in this thesis (referred to by their roman numeral). Each chapter is written in the form of a scientific article.

I. Investigate the influence of permafrost thaw on the concentration and composition of DOM in circumpolar northern freshwaters. We hypothesized that DOM concentrations and carbon compounds of terrestrial origin are higher in ponds draining thawing permafrost soils than in waterbodies not affected by thermokarst processes.

II. Explore the influence of thawing permafrost on planktonic food webs in subarctic ponds, with a special focus on zooplankton feeding. We expected an increase in DOM and nutrient concentrations as well as greater light absorption in thaw ponds relative to non-thaw ponds. We also expected these conditions to affect the phytoplanktonic community. We further hypothesized that, relative to non-thaw ponds, zooplankton record a higher prevalence of terrestrial compounds in their biomass and a poorer PUFA composition within ponds affected directly by permafrost thaw. This pattern would lead to a reduction in the health of primary consumers in the thaw ponds compared with zooplankton in non-thaw ponds.

III. Investigate the community and biomass of zooplankton in subarctic thaw ponds and determine the main variables that control the vertical distribution of zooplankton within the water column. Given the anticipated strong thermal structure of the water column and hypoxic conditions of the hypolimnion in most sampled thaw freshwaters, we hypothesized a pronounced stratification of zooplankton. This stratification would be the result of a compromise between obtaining a good-quality algal diet, ensuring O_2 accessibility, and avoiding top-down predation.

0.4 Methodological approach and study sites

To answer the research questions of this project, we selected various approaches that we performed within multiple regions of the Arctic.

Chapter I investigated the effect of permafrost thaw on the concentration and composition of DOM using a suite of chemical, biological, optical, and stable isotopic measurements. The optical properties of CDOM were examined by spectrophotometric and fluorometric analyses combined with PARAFAC; this combination allowed us to explore qualitatively the terrigenous inputs to thaw ponds from the degrading watershed. Furthermore, using a dual-isotope Bayesian mixing approach ($\delta^{13}C$ and $\delta^{2}H$), we quantified the contribution of terrestrial, benthic, phytoplanktonic, and macrophytic sources to the DOM pool. Although this approach is commonly used for investigating food webs (e.g., Wilkinson et al. 2013), to our knowledge its application to determining DOM sources is very uncommon and innovative.

Chapter II focused on the influence of thawing permafrost on planktonic food webs. In addition to characterizing the DOM, CDOM, and nutrients available for primary producers in the context of a watershed experiencing permafrost thaw, we also evaluated the effect of degrading permafrost on the FA composition of seston and zooplankton body mass. We also used SI- and FA-based mixing models to estimate how permafrost thaw affected zooplankton feeding. Although mentioned as a very interesting and innovative approach for studying ecosystem over extended periods of time (Perga et al. 2006), it remains quite rare in ecological research to combine these two historically separate techniques—SI and FA analyses—and merge the complementary information obtained from each method.

Chapter III explored zooplankton community and biomass in thaw ponds and the factors controlling their vertical distribution throughout the water column. To do so, we studied the zooplankton populations of several subarctic thaw ponds. We also recorded thermal stratification and the associated environmental variables of O_2, phytoplankton biomass, EFA, and the abundance of both bacteria and the larval phantom midge *Chaoborus*.

In total, we sampled 256 ponds distributed in 14 circumpolar regions across the Arctic (Fig. 1.1). The sample sites cover almost 200 degrees of longitude and 30 degrees of latitude (from Alaska to the Canadian high arctic islands, northern Quebec, Greenland, Scandinavia, and Russia) and encompass a wide range of temperatures (mean annual temperatures from -2.6 °C to -18 °C), vegetation types (from spruce or birch forest and shrub tundra to polar desert), and permafrost cover (from sporadic to continuous coverage).

Table 0.1 Summary of the study areas, regions, sites, ponds, measured parameters, and the numerical and statistical methods used in the three chapters of the thesis. See the text for abbreviations.

	Chapter I	**Chapter II**	**Chapter III**
Study area	Circumpolar Arctic	Subarctic Quebec	Subarctic Quebec
Regions	14	1	1
Sites	17	2	2
Sampled ponds	253	8	8
Main focus	DOM	Food web	Zooplankton
Variables			
Temperature			•
O_2			•
PAR			•
pH	•		
TP	•	•	
TN	•		
Fe	•		
Chla	•	•	•
DOC	•	•	
CDOM	•	•	
PARAFAC	•		
SI	•	•	
FA	•	•	•
Phytoplankton			•
Bacteria			•
Chaoborus			•
Zooplankton			•
Mixing models	SI	SI, FA	
Ordination methods	PCA	NMDS	
Statistical methods	Kruskal-Wallis, PERMANOVA	ANOVA, Kruskal-Wallis, PERMANOVA	ANOVA, DistLM

More details regarding the parameters and the methods used in this thesis are presented in the summary Table 0.1 and in the Methods sections of the following chapters. Additional information concerning the sampled regions and sites is available in Table 1.S1.

CHAPTER I

IMPORTANCE OF TERRESTRIAL ORGANIC MATTER IN NORTHERN FRESHWATER SYSTEMS LINKED TO THE PERMAFROST THAW

PUBLISHED RESEARCH ARTICLE

Title: Increasing dominance of terrigenous organic matter in circumpolar freshwaters due to permafrost thaw

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Author contribution statement: MW and MR co-led the study, which was based on a research question formulated by MR. MW and MR designed the approach and all authors contributed data. MW conducted the statistical analyses and interpretation. MW led the manuscript preparation and all authors contributed to the text.

Running head: Increasing allochthony in arctic freshwaters

Keywords: DOM, northern circumpolar ponds, PARAFAC, permafrost thaw, stable isotopes, terrestrial carbon

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1.1 Scientific significance statement

Frozen tundra soils are one of the largest pools of organic carbon in the Earth system. Climate warming and associated permafrost thawing have increased the risk that a large fraction of this carbon will be released to the atmosphere as greenhouse gases, particularly in the form of methane produced by the many aquatic ecosystems present throughout the subarctic and arctic regions. However, the changes induced in the carbon pool of northern waterbodies by these increasing terrigenous inputs have been little studied. Our synthesis of data for ponds at diverse locations across the circumpolar North reveals the strong influence of thawing permafrost on northern freshwaters, with a shift toward increasing dominance by land-derived organic carbon that may alter metabolic pathways and aquatic food webs.

1.2 Abstract

Climate change and permafrost thaw are unlocking the vast storage of organic carbon held in northern frozen soils. Here we evaluated the effects of thawing ice-rich permafrost on dissolved organic matter (DOM) in freshwaters by optical analysis of 253 ponds across the circumpolar North. For a subset of waters in subarctic Quebec, we also quantified the contribution of terrestrial sources to the DOM pool by stable isotopes. The optical measurements showed a higher proportion of terrestrial carbon and a lower algal contribution to DOM in waters affected by thawing permafrost. DOM composition was largely dominated (mean of 93%) by terrestrial substances at sites influenced by thawing permafrost, while the terrestrial influence was much less in waterbodies located on bedrock (36%) or with tundra soils unaffected by thermokarst processes (42%) in the catchment. Our results demonstrate a strong terrestrial imprint on freshwater ecosystems in degrading ice-rich permafrost

catchments, and the likely shift toward increasing dominance of land-derived organic carbon in waters with ongoing permafrost thaw.

1.3 Introduction

Northern permafrost regions contain one of the largest pools of carbon in the Earth system (Schuur et al. 2015), and increased attention is now focused on how these massive carbon stocks may be mobilised and converted to greenhouse gases with ongoing climate change. One trajectory for this conversion is via microbial metabolism in the lakes and ponds that receive dissolved and particulate organic carbon from eroding permafrost soils. Many of these waterbodies are created by thermokarst (erosion and collapse of ice-rich permafrost), resulting in thaw or thermokarst lakes and ponds (Vonk et al. 2015), hereafter referred to as thaw ponds. In some parts of the Arctic landscape these ponds are disappearing by drainage, evaporation or infilling, while in other northern regions they are becoming larger and more numerous (Vincent et al. 2017). These waters represent one of the most abundant freshwater ecosystem types in northern regions, and collectively they have an estimated total surface area in the range 250,000 to 380,000 km^2 (Grosse et al. 2013); this accounts for approximately 25% of the estimated total area covered by lakes and ponds in the Arctic (1.4×10^6 km^2; Muster et al. 2017). They encompass a variety of transparencies and trophic conditions, and are likely to become more turbid and heterotrophic with increasing carbon inputs from thawing permafrost soils (Vonk et al. 2015). Thaw ponds are also known to be hotspots in the landscape for strong emissions of greenhouse gases to the atmosphere (Abnizova et al. 2012; Negandhi et al. 2013; Sepulveda-Jauregui et al. 2015; Matveev et al. 2016).

Although the effect of increasing export of terrestrial organic compounds from the catchment and shifts in aquatic metabolism are known to change the carbon pool composition and the proportion of carbon of terrestrial origin (which we refer to as allochthony) in temperate freshwaters (Solomon et al. 2015), little is known about these effects in circumpolar ponds, despite the abundance of these ecosystems across the northern landscape (Muster et al. 2017) and their key role in carbon cycling (Abnizova et al. 2012). In this study, we investigated the impact of permafrost thaw on the concentration and composition of dissolved organic matter (DOM) using a suite of chemical, biological, optical, and stable isotopic ($\delta^{13}C$ and $\delta^{2}H$) measurements. We hypothesized that DOM concentrations are higher in ponds that have emerged due to thawing permafrost soils than in waterbodies not affected by thermokarst processes. We further hypothesized that terrestrial carbon compounds are most abundant in ponds draining thawing soils, while ponds in regions with rock outcrops and non-degrading permafrost soils are relatively more enriched in autochthonous DOM, the fraction originating from aquatic primary production. Specifically, we predicted that these relative proportions of terrestrial matter in the DOM pool of different pond types are expressed in the DOM $\delta^{13}C$ and $\delta^{2}H$ isotopic composition.

1.4 Methods

1.4.1 Study regions

During the summer periods from 2002 to 2016, we sampled a total of 253 ponds distributed in 14 circumpolar regions (Fig. 1.1), for a total of 356 samples, including from a subset of ponds (55) that were sampled more than one time during the 15 years. The regions span over a wide geographic area, covering around 200 degrees of longitude (from Alaska to Russia) and 30 degrees of latitude (from Subarctic to High Arctic), and encompassing a large range of temperatures (mean annual from -2.6 °C to -18 °C), vegetation types (from spruce or birch forest and shrub tundra to polar

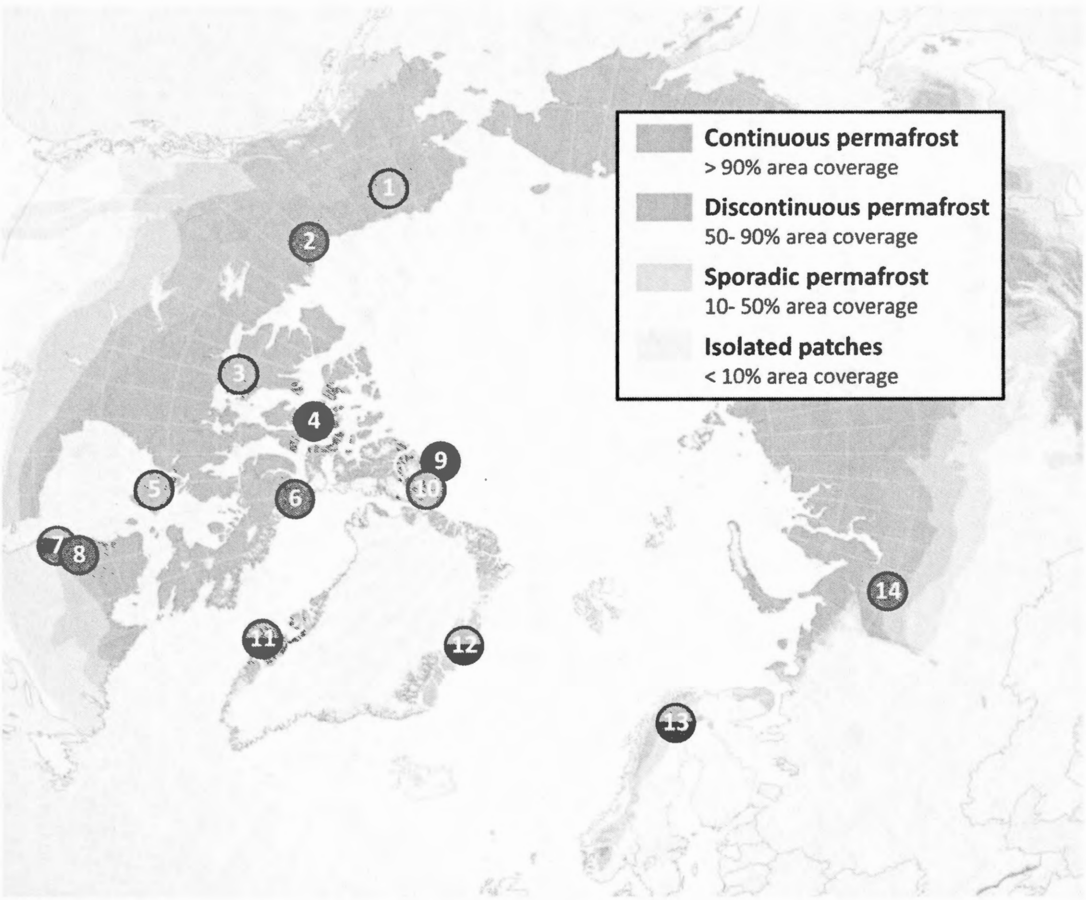

Figure 1.1 Location of the 14 regions sampled in the north circumpolar permafrost zone. Circle colors indicate the types of ponds in the region: blue for bedrock, green for tundra and red for thaw. 1 = Toolik, 2 = Mackenzie Delta, 3 = Cambridge Bay, 4 = Resolute Bay, 5 = Coral Harbour, 6 = Bylot Island, 7 = Kuujjuarapik, 8 = Umiujaq, 9 = Ward Hunt, 10 = Hazen, 11 = Kangerlussuaq. 12 = Zackenberg, 13 = Kilpisjärvi, 14 = Seida. Source of the permafrost map: Brown et al. (1998).

desert), and permafrost cover (from sporadic to continuous coverage; further details in Supporting information Table 1.S1). We divided the ponds into three categories according to their catchment characteristics and exposure to permafrost thaw, following Rautio et al. (2011a): (1) bedrock ponds (21 ponds), characterized by rocky surroundings and with little terrestrial vegetation, and no effect of thawing permafrost soils; (2) tundra ponds (88 ponds), not directly formed or significantly impacted by degrading permafrost soils, but surrounded by grass-, shrub- or forest-tundra vegetation; and (3) thaw ponds (144 ponds), which are thermokarst waterbodies formed by thawing and collapse of ice-rich permafrost (Vonk et al. 2015). Photographs of the ponds in each category are shown in Supporting information Fig. 1.S1. The sampled ponds were smaller than 10 ha, with the exception of 17 larger but shallow (< 3.5 m) waterbodies.

1.4.2 Chemical and biological analyses

We collected surface water samples to measure pH, total phosphorus (TP), total nitrogen (TN) and total dissolved iron (Fe) concentrations, and filtered subsamples through pre-rinsed cellulose acetate filters (0.2 µm) in order to analyze dissolved organic carbon concentrations (DOC) and perform optical analyses on the chromophoric DOM (CDOM; see below). This filter pore size removes small-size inorganic soil particles common in thaw ponds (Watanabe et al. 2011), and was chosen to be consistent with earlier studies on circumpolar ponds (Breton et al. 2009; Laurion et al. 2010; Roiha et al. 2015). The samples were stored in acid-washed and combusted glass vials at 4 °C in the dark, and DOC quantification was carried out using a carbon analyzer (TOC-5000A or TOC-VCPH, Shimadzu, Kyoto, Japan). Seston in the surface water was filtered onto GF/F glass fiber filters to determine phytoplankton chlorophyll *a* (Chla) concentrations, as in Nusch (1980).

1.4.3 Optical analyses

CDOM absorbance was measured between 250 and 800 nm using a UV-visible Cary 100 (Agilent, Santa Clara, California), Cary 300 (Agilent, Santa Clara, California) or LAMBDA 650 (PerkinElmer, Waltham, Massachusetts) spectrophotometer, depending on sample origin. After subtracting the blank spectrum, we applied a nullpoint adjustment, using the mean value from 750 to 800 nm, and report CDOM as the absorption coefficient at 320 (a_{320}) and 440 nm (a_{440}) according to the equation:

$$a_\lambda = 2.303 \times A_\lambda / L, \qquad \text{(eq. 1.1)}$$

where a_λ is the absorption coefficient (m^{-1}) at wavelength λ, A_λ the absorbance corrected at wavelength λ and L the path length of the cuvette (m) (Blough and Del Vecchio 2002). The specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) was determined from DOC normalized A_{254} as an index of aromaticity and the relative proportion of terrestrial versus algal carbon sources in DOM (Weishaar et al. 2003). Iron can complex humic substances and increase DOM absorbance in elevated concentrations, inducing an overestimation of $SUVA_{254}$ (Xiao et al. 2013), and we therefore applied the following equation when the Fe concentration was higher than 2 mg L^{-1} (Poulin et al. 2014):

$$A_{254\,\text{corrected}} = A_{254\,\text{measured}} - (0.0653 \times [\text{Fe}]) \qquad \text{(eq. 1.2)}$$

The Fe concentration was only higher than 2 mg L^{-1} in some ponds of Bylot Island (region 6), but this variable was not available for the regions of Zackenberg (region 12) and Seida (region 14). Therefore, the interpretation of the optical indices should be made with caution, especially in systems influenced by active permafrost erosion and containing high iron concentrations. We also determined spectral slopes (S) following Loiselle et al. (2009) for the intervals 279-299 (S_{289}, named by center wavelength), 275-295 (S_{285}) and 350-400 nm (S_{375}), and performed the regression calculations using SciLab v 5.5.2. (Scilab Enterprises 2015). We used S_{289} to estimate the importance of fulvic and humic acids related to algal production (Loiselle et al. 2009), and the slope ratio (S_R) S_{285}/S_{375} was calculated as an index of CDOM molecular weight (Helms et al. 2008).

For a subsample of 100 ponds, we also recorded fluorescence intensity on a Cary Eclipse spectrofluorometer (Agilent, Santa Clara, California) across the excitation waveband from 250-450 nm (10 nm increments) and emission waveband of 300-560 nm (2 nm increments) in order to construct excitation-emission matrices (EEMs). We calculated the fluorescence index (FI) as the ratio of fluorescence emission intensities at 450 nm and 500 nm at the excitation wavelength of 370 nm to investigate the origin of fulvic acids (McKnight et al. 2001). To identify and quantify the main DOM components, we ran a parallel analysis factor (PARAFAC) model on 129 samples from 95 ponds in MATLAB v R2013a (MathWorks, Natick, Massachusetts), as in Murphy et al. (2013). We corrected EEMs for Raman and Rayleigh scattering and inner filter effects, and standardized the fluorescence to Raman units using the FDOMcorr 1.4 toolbox (Murphy et al. 2010). The model was performed on corrected EEMs and validated by split-half analysis (Supporting information Fig. 1.S2) using the drEEM toolbox from Murphy et al. (2013). For each sample, we summed the maximum fluorescence [Cx] of the different components x to determine the total fluorescence (F_T) and calculated the relative abundance of any component x, according to the following equation:

$$\%\mathrm{Cx} = ([\mathrm{Cx}]/\ F_\mathrm{T}) \times 100 \qquad \text{(eq. 1.3)}$$

To identify the components of the model, we compared the excitation and emission spectra to published components from more than 70 papers available in the OpenFluor database, following Murphy et al. (2014).

1.4.4 Stable isotope analyses

To quantify the relative contribution of terrestrial carbon among pond types, we carried out stable isotope (SI) analyses on surface-water DOM and its potential sources in a subsample of 10 ponds in the vicinity of Kuujjuarapik, subarctic Quebec (region 7 in Fig. 1.1). Given the presence of the three pond types in this same region sharing similar environmental conditions, Kuujjuarapik was a convenient place to perform such analyses. In addition to collecting soils surrounding ponds, DOM, benthic bulk material and seston were sampled in bedrock and tundra waterbodies for $\delta^{13}C$ and $\delta^{2}H$ analyses. Thaw ponds in Kuujjuarapik are located in a peatland with abundant semi-aquatic macrophytes. Because of their high turbidity, no light reaches the bottom in these ponds (as measured with an underwater radiometer; Li-Cor Biosciences, Lincoln, Nebraska), and strong thermal stratification limits the exchange between bottom and surface waters (Matveev et al. 2016). Therefore, we considered the contribution of benthic material to surface-water DOM to be negligible and took the wetland macrophytes into account as a potential source to DOM in these thaw ponds. DOM samples for stable isotopes were collected and stored as indicated in section 2.2. Soil samples were collected from the top layer (0-5 cm) close to the ponds. For bedrock and tundra ponds, this layer represents the shallow soils around the ponds, while for the thaw ponds, this was surface soils from the organic-rich palsas that are collapsing into the ponds (see Fig. 7 in Vincent et al. 2017). In order to remove the carbonate, we applied an acid fumigation to the soil samples during 96h prior the $\delta^{13}C$ analyses, as described in Ramnarine et al. (2011). Decaying submerged macrophytes (*Carex* sp.) were sampled from the edge of thaw ponds. In bedrock and tundra ponds, we sampled the surface of submerged rocks by scraping with a spatula to collect the epibenthic material. All samples were freeze-dried before SI analyses. DOM samples were analyzed for $\delta^{13}C$ using an Aurora 1030W TOC Analyzer (O.I. Corporation, College Station, Texas) coupled to a Finnigan DELTA plus Advantage MS (Thermo Fisher Scientific, Waltham, Massachusetts) in the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Ontario). The $\delta^{13}C$ analyses on soil and macrophyte samples were carried out using a FLASH 2000 OEA interfaced with a Delta V Plus MS (Thermo Fisher Scientific, Waltham, Massachusetts) in the RIVE Research Center (Université du Québec à Trois-Rivières, Trois-Rivières, Quebec). All $\delta^{2}H$ analyses were performed in the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, Arizona) as in

Doucett et al. (2007), using a CONFLO II coupled to a Delta Plus XL MS (Thermo Fisher Scientific, Waltham, Massachusetts). To determine the phytoplankton $\delta^{13}C$ signature, we used specific algal fatty acids (FA) extracted from bulk seston as a proxy, performing SI analyses on 16:1n7, 18:2n6, 18:3n3 and 20:5n3 fractions (Taipale et al. 2015; Grosbois et al. 2017a; Grosbois et al. 2017b). The FA were transmethylated according to a protocol adapted from Lepage and Roy (1984). The $\delta^{13}C$ analyses on FA were carried out in the Stable Isotope Laboratory of Memorial University (Memorial University of Newfoundland, St. John's, Newfoundland and Labrador), using a 6890N GS (Agilent, Santa Clara, California) linked to a Delta V Plus MS (Thermo Fisher Scientific, Waltham, Massachusetts). We estimated the phytoplankton $\delta^{2}H$ signature from filtered water as in Grosbois et al. (2017a). As for the phytoplankton, we extracted the FA from the benthic bulk material and performed $\delta^{13}C$ analyses on the 14:0, 16:0 and 18:0 fractions, which are saturated FA specific to most organisms (Napolitano 1999), and were thus considered representative of the $\delta^{13}C$ isotopic composition of the benthic organic matter.

1.4.5 Mixing model

We considered three potential sources contributing to DOM in each pond type: soils, phytoplankton, and benthic bulk for bedrock and tundra ponds, and soils, phytoplankton, and macrophytes for thaw ponds. We performed a dual Bayesian mixing model adapted from Wilkinson et al. (2014), using $\delta^{13}C$ and $\delta^{2}H$ as end-members. The model was run in R v 3.3.2. (R Development Core Team 2018).

1.4.6 Statistical analyses

To determine how the chemical, biological and optical properties were individually influenced by permafrost thaw, the data were analyzed by Kruskal-Wallis rank tests, with pair-wise comparisons using a post hoc test (Bonferroni). We carried out principal component analyses (PCAs) and permutational multivariate analyses of variance (PERMANOVAs) on $\log_{10}(x+1)$-transformed data to illustrate and test the influence of pond type on optical properties. The data were centered and standardized before applying the PCAs. In the PERMANOVAs, Euclidean distance was used as the dissimilarity index, and the number of permutations was fixed at 999. Pair-wise comparisons were performed using Bonferroni correction to identify differences among the types of ponds. The multivariate homogeneity of group dispersions was verified by performing a permutational analysis of multivariate dispersions (PERMDISP). All statistical analyses were performed on R v 3.3.2. (R Development Core Team 2018). The data from the 55 ponds that were sampled more than once but in different years were considered independent based on the assumption that in these rapidly changing landscapes, the biological, chemical and optical properties of the ponds can vary greatly from one sampling year to another.

1.5 Results

1.5.1 Chemical, biological and optical properties

The overall data set (archived in Wauthy et al. 2017) showed a strong effect of permafrost thaw (Fig. 1.2), with highly significant differences between thaw and non-thaw ponds for all variables, except for pH and %C4 ($P < 0.01$, Kruskal-Wallis by rank test). Chla, TP and TN had highest values in thaw ponds, indicating a more enriched trophic state. There were wide variations in DOC concentration, from 1.0 to 116.8 mg L^{-1}, with highest values in the thaw ponds. CDOM followed the same trend, as indicated by a_{320} and a_{440} values. $SUVA_{254}$ was also higher in thaw ponds, suggesting a larger proportion of terrestrial DOM in these ponds as compared to bedrock and tundra ponds. Consistent with this interpretation, S_{289}, S_R and FI values were lowest in thaw ponds, suggesting smaller amounts of carbon derived from aquatic primary production, higher DOM molecular weights and a terrestrial origin for fulvic acids, respectively.

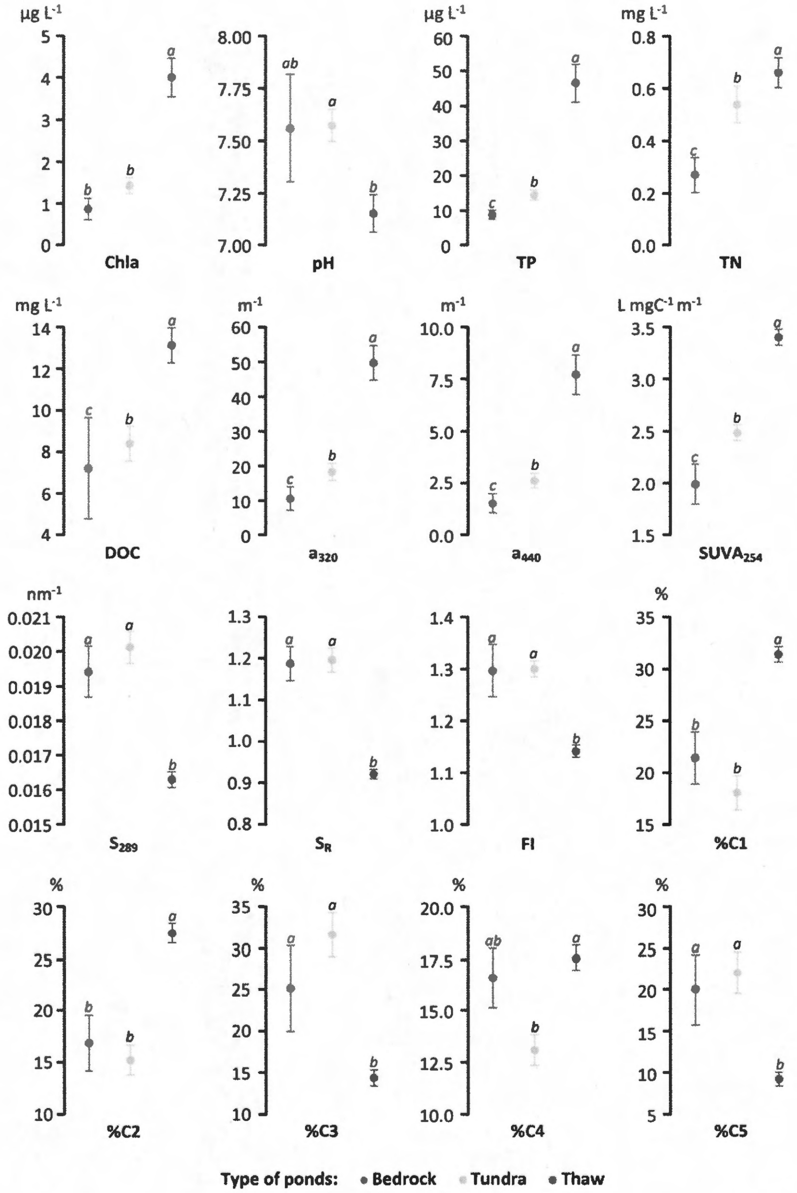

Figure 1.2 Scatter plots of the mean (+SE) values for biological, chemical and optical variables in the different pond types. Abbreviations are defined in the text. Different letters above error bars indicate significant differences between ponds types ($P < 0.05$, Bonferroni post hoc test).

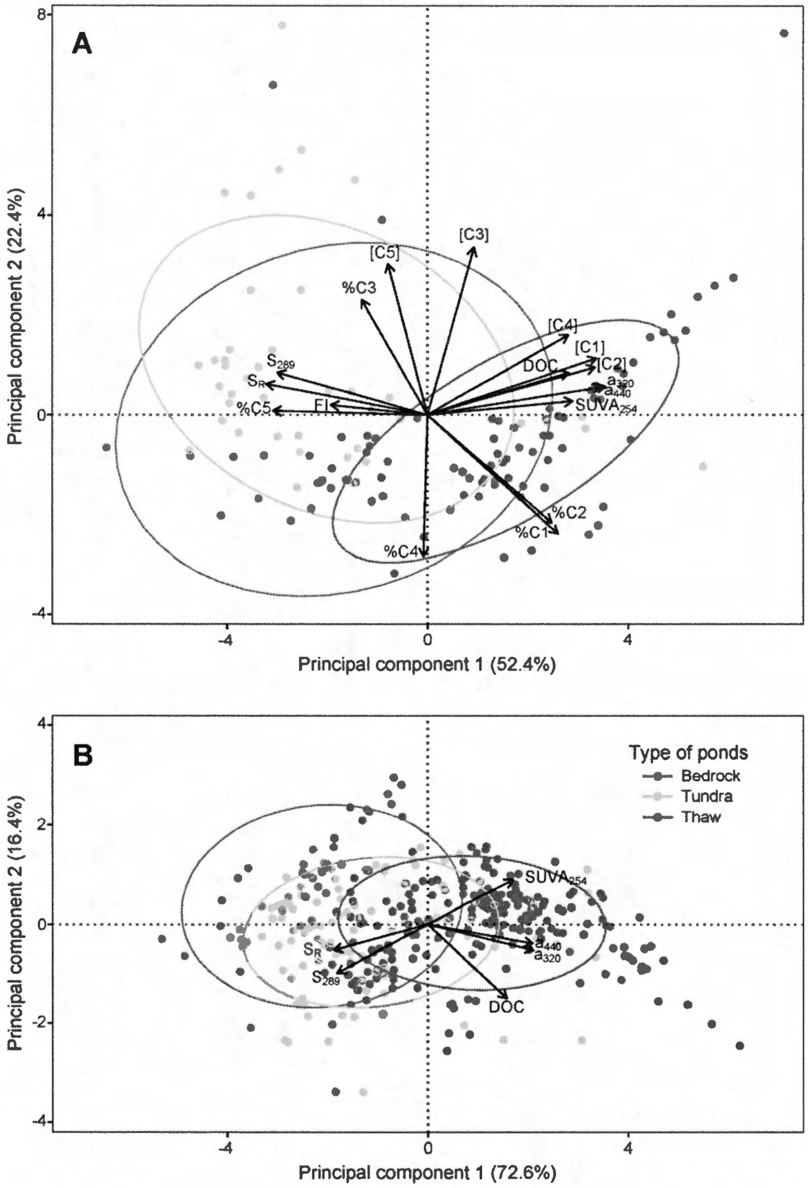

Figure 1.3 Principal component analyses of DOM optical variables across bedrock, tundra and thaw ponds, for spectral and fluorescence indices, and PARAFAC components in 95 ponds (A), and for only spectrophotometric indices, including all 253 ponds (B). Arrows indicate the loadings of the different variables. Ellipses group each pond type (ellipse probability = 0.68). Abbreviations are defined in the text.

The PARAFAC model identified five fluorescence components (Supporting information Fig. 1.S3), of which four shared fluorescence characteristics with humic materials from terrestrial (C1-C3) and microbial origin (C4). The last component (C5) presented spectra similar to amino acids or proteins, and was attributed to algal production (Stedmon and Markager 2005) (more details in Supporting information Table 1.S2). The percentage of terrestrial humic components (%C1-%C3) showed different patterns among the pond types (Fig. 1.2): while %C1 and %C2 were significantly higher in thaw ponds, %C3 was greater in the ponds not affected by permafrost thaw. Although terrestrial humic components dominated in all pond types, thaw ponds showed higher proportions of C1-C3 (73.2 ± 10.2%) than bedrock (63.4 ± 19.7%) and tundra ponds (64.9 ± 15.5%). Moreover, thaw ponds had the highest mean value for %C4 (17.6 ± 5.1%), further indicating the presence of humic-dominated microbial material in these ponds. However, the proportion was not different from bedrock ponds (16.6 ± 5.6%). Finally, the proportion of DOM associated with algal production was small in the thaw ponds, as indicated by its significantly lower %C5 (9.2 ± 7.0%).

The two PCAs showed the tendency of pond types to group according to optical properties (Fig. 1.3). Figure 1.3A is for the PCA performed on the subsample of 95 ponds for which we had both spectrophotometric and fluorometric properties; the first two components explained 74.8% of the total variability (PC1 52.4 and PC2 22.4%). We observed a significant effect of catchment type (PERMANOVA, $F_{2,126} = 19.1$, $P = 0.001$), with the three pond types forming three distinct clusters. However, only the thaw pond cluster was significantly different from the bedrock and tundra groups (pair-wise PERMANOVA comparisons, P-adjusted < 0.05). We applied the second PCA on the complete dataset, but only for DOC and DOM spectrophotometric proxies; the first two components explained 89.0% of the variance (PC1 72.6 and PC2 16.4%) (Fig. 1.3B). The effect of catchment type was again significant (PERMANOVA, $F_{2,353} = 65.52$, $P = 0.001$) and the three pond types formed three significantly different clusters (pair-wise PERMANOVA comparisons, *P*-adjusted < 0.05). Bedrock and tundra ponds were mainly defined by their positive correlation with S_R, S_{289} and %C5, but were also positively correlated to FI, %C3 and [C5]. On the other hand, the thaw pond clustering was mostly defined by positive correlation with fluorescence components [C1], [C2], %C1 and %C2, and by CDOM (a_{320} and a_{440}), with further positive correlations with [C4], DOC, $SUVA_{254}$.

1.5.2 Source contributions to DOM

The isotopic signatures of DOM showed similar $\delta^{13}C$ values between the different pond types in the Kuujjuarapik region, within the range -29.5 to -27.3‰ (Fig. 1.4). However, the DOM deuterium signature $\delta^{2}H$ was more depleted in thaw ponds (-153.9 ± 11.0‰) compared to other ponds (-131.2 ± 8.2‰). Similarly, the phytoplankton $\delta^{13}C$ signature was more negative in thaw ponds than in non-thaw ponds (-37.1 ± 2.5‰ vs. -30.5 ± 0.8‰). According to its location in biplots (Fig. 1.4A-B), DOM in bedrock and tundra ponds fitted well within the polygon of source end-members, and appeared to be mainly composed of benthic and terrestrial materials. The DOM in thaw ponds was positioned at the limit of the polygon, with closest affinity to terrestrial sources (Fig. 1.4C).

The mixing model showed a large contribution of benthic (median of 55%) and terrestrial (median of 22%) material to the DOM of bedrock ponds, although the range of the 95% highest contribution probability showed high variabilities (0-97% and 0-99%, respectively) (Fig. 1.5A). The terrestrial sources were more substantial in tundra ponds (median of 42%), but the benthic material remained the principal contributor to DOM (median of 47%) (Fig. 1.5B). However, the range was also high for the two main sources (2-90% for the benthic source, and 2-82% for the terrestrial source). Phytoplankton was a significant but minor source of DOM in both pond types not affected by thawing permafrost, with a median of 11% and 6% in bedrock and tundra ponds, respectively. In the thaw ponds, there was a major shift to a higher proportion of carbon from terrestrial origin for DOM, with terrestrial sources contributing 96% (median, Fig. 1.5C) and negligible contributions from phytoplankton and macrophytes (medians less than 2%).

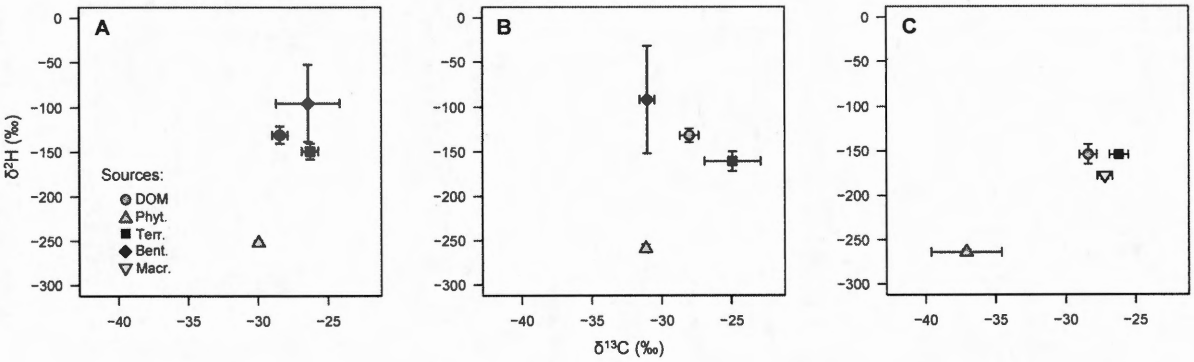

Figure 1.4 Distribution of $\delta^{13}C$ and $\delta^{2}H$ DOM signatures inside a polygon of the potential sources (+ SD) in Kuujjuarapik ponds (region 7) with (A) bedrock catchment, (B) tundra catchment unaffected by thermokarstic processes, and (C) thawing permafrost catchment. The sources are phytoplankton (Phyt.), terrestrial organic matter (Terr.), benthic organic matter (Bent.) and macrophytes (Macr.).

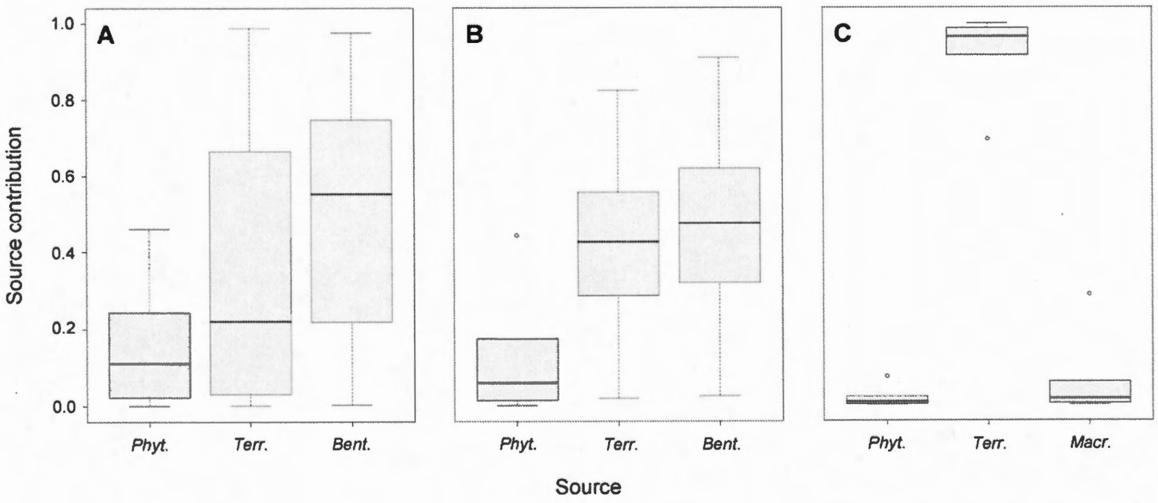

Figure 1.5 Source contributions to DOM in Kuujjuarapik ponds (region 7) with (A) bedrock catchment, (B) tundra catchment unaffected by thermokarstic processes, and (C) thawing permafrost catchment, based on a dual isotope ($\delta^{13}C$ and $\delta^{2}H$) Bayesian mixing model. The sources are phytoplankton (Phyt.), terrestrial organic matter (Terr.), benthic organic matter (Bent.) and macrophytes (Macr.). Whiskers and boxes show the distribution of 95% and 50% highest densities of contribution probabilities, respectively, with the median value indicated by the line within each box.

1.6 Discussion

1.6.1 Nutrients and primary producers

The studied 253 ponds spanned a wide gradient of environmental conditions, from transparent oligotrophic waterbodies in areas not affected by thawing permafrost, to humic- and nutrient-rich ponds exposed to thermal erosion (Fig. 1.2). Nutrients in bedrock and tundra ponds were typical of oligotrophic freshwater systems, and comparable to values usually found in clear-water high-latitude ponds (Rautio et al. 2011a). In thaw ponds, they showed values more characteristic of mesotrophic and eutrophic systems, and likely originated from the eroding catchment (Larsen et al. 2017). Following the low nutrient concentrations in the water column, Chla values in bedrock and tundra ponds indicated low phytoplankton biomass. Consequently, most primary production in clear-water ponds is produced by the benthic mat and biofilm communities, with phytoplankton often representing less than 2% of the total photosynthetic biomass in these systems (Bonilla et al. 2005; Rautio et al. 2011a). In thaw ponds, more elevated Chla values suggested a higher planktonic primary production, more probably supported by the higher nutrient concentrations (Vonk et al. 2015). However, the higher CDOM concentrations and suspended solids in thaw ponds efficiently attenuate the solar radiation (Watanabe et al. 2011), limiting benthic and therefore overall primary production (Vadeboncoeur et al. 2008). Increased terrestrial DOM in circumpolar surface waters could therefore lead to a considerable decrease in the light availability for photosynthesis, resulting in a shift toward a heterotrophic production-based food web as has been documented in experimental conditions (Forsström et al. 2015), and a high production of CO_2 and CH_4 (Roiha et al. 2015).

1.6.2 DOC and CDOM properties

Highest DOC and CDOM values were observed in the thaw ponds, with several CDOM proxies indicating higher terrestrial inputs from the catchment (Fig. 1.2). Similar accumulation of DOM has also been reported previously at circumpolar sites with high terrestrial inputs (Vonk et al. 2013; Abbott et al. 2014; Roiha et al. 2015). The elevated values of a_{320} and a_{440} in thaw ponds indicate high concentrations of CDOM, inducing more light attenuation in the water column. Consistent with earlier studies, S_R, S_{289} and $SUVA_{254}$ indicated fresher aromatic compounds of higher molecular weight and a large proportion of terrestrial versus algal carbon sources in thaw ponds (e.g., Roiha et al. 2015). This high degree of DOM allochthony in thaw ponds was also supported by low values of FI, an indicator of large inputs of fulvic acids from terrestrial sources and a function of carbon storage in the catchment (Rantala et al. 2016).

The association of [C1] and [C2] with $SUVA_{254}$ and other CDOM proxies in ponds influenced by thawing permafrost also indicates strong DOM allochthony in these waters (Fig. 1.3A), with high inputs of DOM from the catchment. C3 also had fluorescence characteristics of humic materials from a terrestrial origin, but showed an opposite association with C1 and C2. This suggests that C3 could be the product of biological transformation of C1 and C2 in the water column (Jørgensen et al. 2011). C4 matched well with humic materials of microbial origin, and therefore can be linked to the degradation of both algal and terrestrial sources. Finally, the amino acid-or protein-like algal component C5 showed higher values in bedrock and tundra ponds, and negative relationships with $SUVA_{254}$ and thawing permafrost, indicating a greater algal origin of DOM in non-thaw ponds. This algal signature likely reflects the benthic primary production in these ponds, given its dominant contribution to overall algal biomass in clear-water circumpolar ponds (Rautio et al. 2011a).

It is important to note that we focused on thermokarst ponds, with permafrost thaw and degradation along pond banks, as is commonly found across the North. There are other modes of permafrost thaw that can have different consequences for surface-water DOM concentrations and composition. For instance, in certain hydrological conditions, catchment-scale permafrost thaw via active layer thickening or permafrost loss can reduce DOC concentrations or $SUVA_{254}$ values (Cory et al. 2013; O'Donnell et al. 2014). The highly variable organic carbon content of thawing permafrost soils (Vincent et al. 2017) may also influence the DOM properties in the receiving waterbodies. In this study, we did not measure the organic carbon content in the watershed, but the existing information from the study regions (Bouchard et al. 2015; Vincent et al. 2017) as well as our observations of the bank morphology and benthic substrates indicate that the studied thaw ponds were predominantly located in organic-rich sites. Therefore, substantial impacts of permafrost thaw on pond DOM were expected.

Thawing of ice-rich permafrost appears to have a strong effect on the ratio of allochthonous to autochthonous DOM in surface waters, resulting from direct inputs of allochthonous DOM from eroding permafrost soils, and from its effect on DOM age (O'Donnell et al. 2014), in situ transformations and respiration (Laurion and Mladenov 2013; Cory et al. 2014). In particular, the level of bacterial and photochemical transformation of terrigenous DOM is likely to vary between the turbid and bacteria-rich thaw ponds as compared to clear and oligotrophic waters of non-thaw ponds, with important consequences on DOM allochthony. Moreover, drivers that are independent of permafrost thaw could potentially explain part of the differences observed between thaw and non-thaw ponds. These drivers include the composition, size and slopes of the catchment (Olefeldt et al. 2014; Vonk et al. 2015), influence of ground water and precipitation (Olefeldt et al. 2013), water retention time (Catalán et al. 2016) and temperature (Porcal et al. 2015). However, our sampling covered a great variety of environments, mitigating the influence of these other factors as drivers of the observed DOM differences among pond types. Furthermore, although some of these other factors likely differed considerably between bedrock and tundra ponds, the overall water chemistry as well as the DOM concentrations and optical characteristics differed less between these two pond types than for the thaw ponds. Additionally, the DOM sourcing by stable isotope analysis for ponds in the Kuujjuarapik region indicated that these other drivers were less likely relative to allochthony. Overall, our results point to the importance of thawing permafrost for the biogeochemistry of circumpolar surface waters.

1.6.3 DOM contribution quantification

In the subarctic region of Kuujjuarapik (region 7), the SI data and the mixing model supported the optical analyses and confirmed a high terrestrial DOM input in thaw ponds (Fig. 1.5). However, terrestrial sources were also important in non-thaw ponds, particularly in tundra ponds where soils and benthic material contributed equally to DOM. This proportional influence of terrestrial inputs likely reflects the limitation of autochthonous sources under oligotrophic nutrient conditions, as well as the large perimeter length per unit area of these small waterbodies that would favor interactions with the surrounding tundra soils.

The DOM contribution by phytoplankton was low in bedrock and tundra ponds of the region, as to be expected given the low algal biomass and primary production in the water column compared to the benthos in these ponds (Rautio and Vincent 2006), and supporting studies that have shown a low contribution of phytoplankton to the food webs of circumpolar ponds (Cazzanelli et al. 2012; Mariash et al. 2014). In the thaw ponds, phytoplankton biomass was higher, possibly from more enriched nutrient conditions, but nevertheless its contribution to DOM was insignificant and overwhelmed by the terrestrial signal.

The $\delta^{13}C$ signatures of phytoplankton were more depleted in the thaw ponds versus non-thaw ponds (Fig. 1.4), and an explanation of this may lie in their elevated concentrations of methane (Matveev et al. 2016). Due to the fractionation during methanogenesis, methane has more negative $\delta^{13}C$ values and can be metabolized by methanotrophic bacteria, which then enter the food web (Bastviken et al. 2003). Mixotrophic algae constitute a major fraction of the total phytoplankton community growing in the thaw ponds of the region (Bégin and Vincent 2017) and could rely on the methanotrophic bacteria as an energy source, explaining the more depleted $\delta^{13}C$ signature of the phytoplankton. Given the extreme shallowness (< 1 m) and the absence of stratification in bedrock and tundra ponds, the benthic source was an important contributor to DOM, likely resulting from the resuspension of decomposed benthic materials (Evans 1994) and by diffusion of benthic carbon exudates to the water column (Rautio et al. 2011a; Rodríguez et al. 2013).

In the thaw ponds, the DOM isotopic signature was close to the outside of the polygon defined by our three selected sources (Fig. 1.4C). One explanation could be that we missed a source contributing to DOM. Since the studied thaw ponds are located in a peatland, mosses (*Sphagnum* spp.) are abundant in the catchment and could be a candidate for this missing source. However, we sampled *Sphagnum* mosses as a potential end-member, and found that their isotopic signature was similar to macrophytes ($\delta^{13}C$ of -26.6‰ vs. -27.2‰, $\delta^{2}H$ of -180.9‰ vs. -177.8‰); hence *Sphagnum* effect was considered to be included in the macrophyte values in the mixing model. A more plausible explanation may be that labile, ^{13}C-enriched terrestrial matter is rapidly metabolised by bacteria once in the ponds, leaving the more depleted and recalcitrant fraction dissolved in the water column, and causing more negative $\delta^{13}C$ DOM values (Biasi et al. 2005; Bianchi and Canuel 2011).

1.7 Conclusions

Consistent with previous syntheses (Vonk et al. 2015; Wrona et al. 2016), our observations underscore the large variations in limnological properties among northern high-latitude waters. Despite this variability, there was a clear effect of permafrost thaw, resulting in more allochthony, higher DOC concentrations, and DOM with a predominantly land-derived signature. To our knowledge, this is the first time the DOM has been reported in this extent to waterbodies in the circumpolar North. The increasing terrestrial influence on arctic and subarctic ponds results in decreased water column transparency, which in turn will affect biogeochemical processes, energy supply to benthic primary producers, and the balance of autochthonous and allochthonous production. These impacts may be viewed together as an example of 'browning' that is being observed throughout the world with increasing concern (Graneli 2012; Williamson et al. 2015). Because of the erodible and DOM-rich nature of the permafrost landscape, northern browning is an extreme version of this global phenomenon, and may be compounded in the future by increased rainfall and extreme weather events (Vincent et al. 2017). This extreme browning will likely shift northern freshwaters more toward net heterotrophic conditions, increased DOM flocculation and O_2 depletion, and greater production of greenhouse gases across the circumpolar North.

1.8 Acknowledgments

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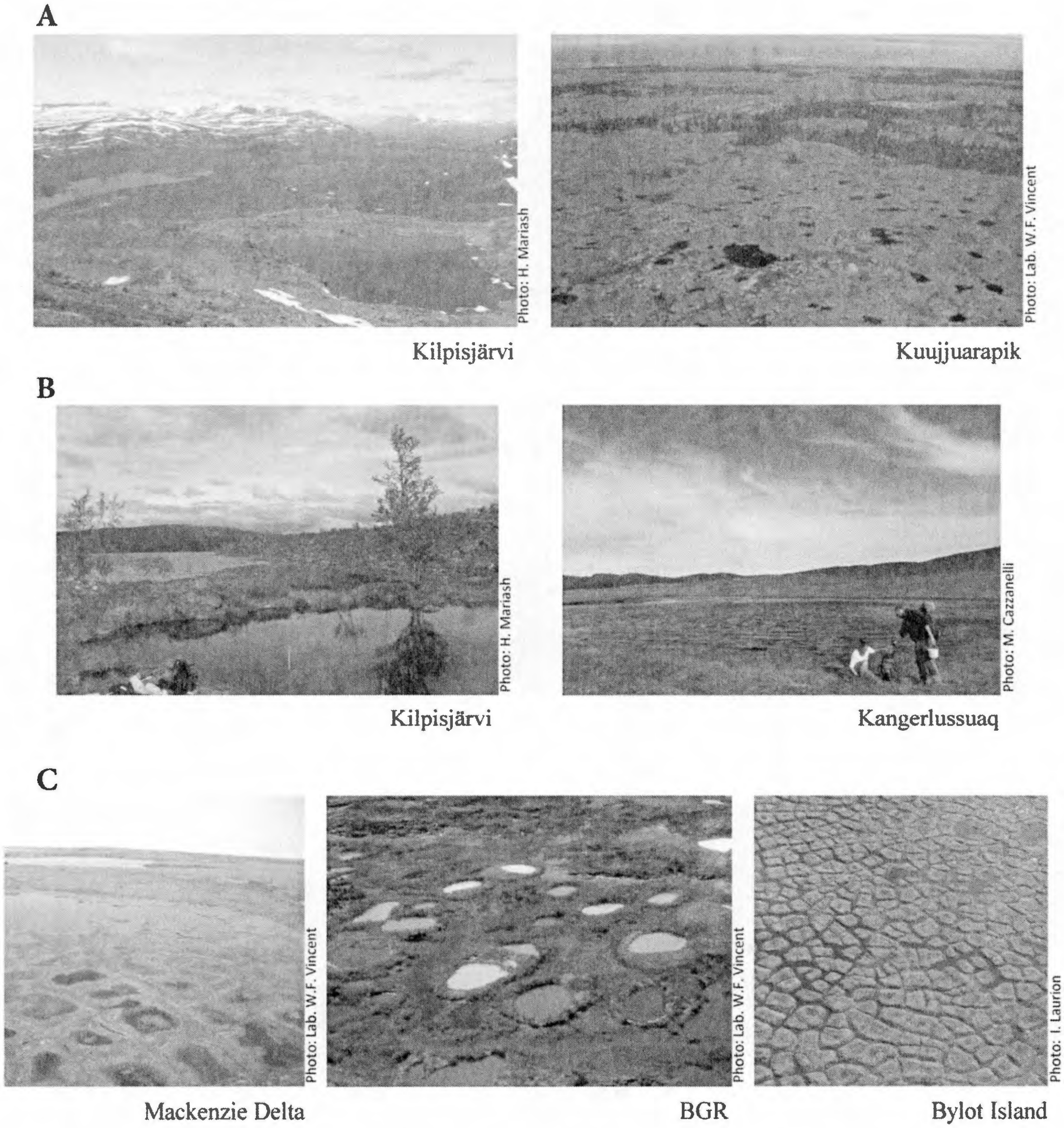
1.9 References

The reference list of each individual chapter has been included in the final reference section at the end of the thesis.

1.10 Supporting information



Kilpisjärvi Kuujjuarapik

Kilpisjärvi Kangerlussuaq

Mackenzie Delta BGR Bylot Island

Figure 1.S1 Photos of studied ponds in (A) bedrock, (B) tundra, and (C) thaw ponds categories.

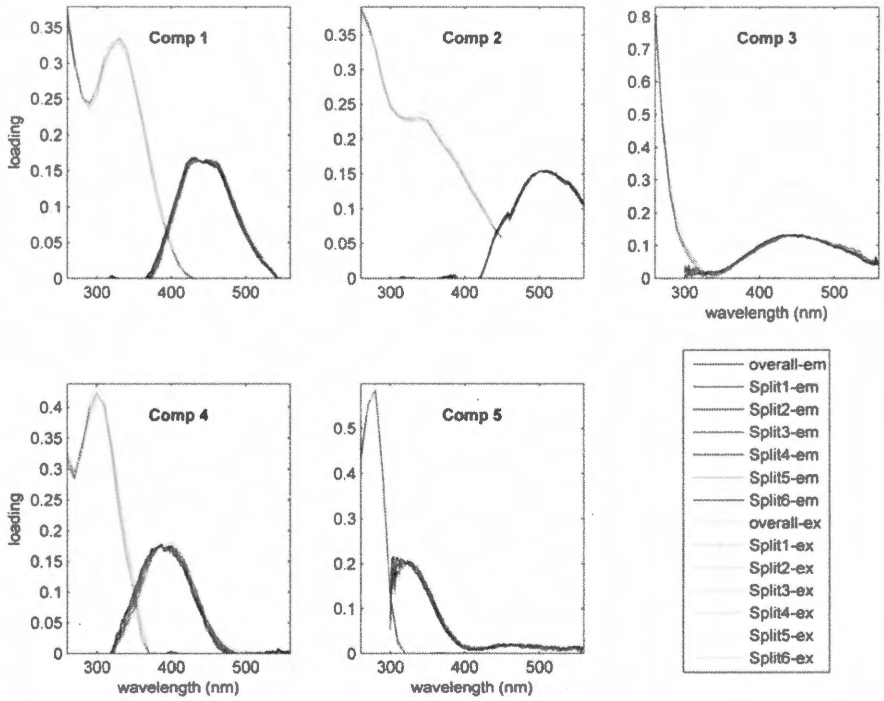


Figure 1.S2 Split-half validation of the 6 splits in the 5 component PARAFAC model.

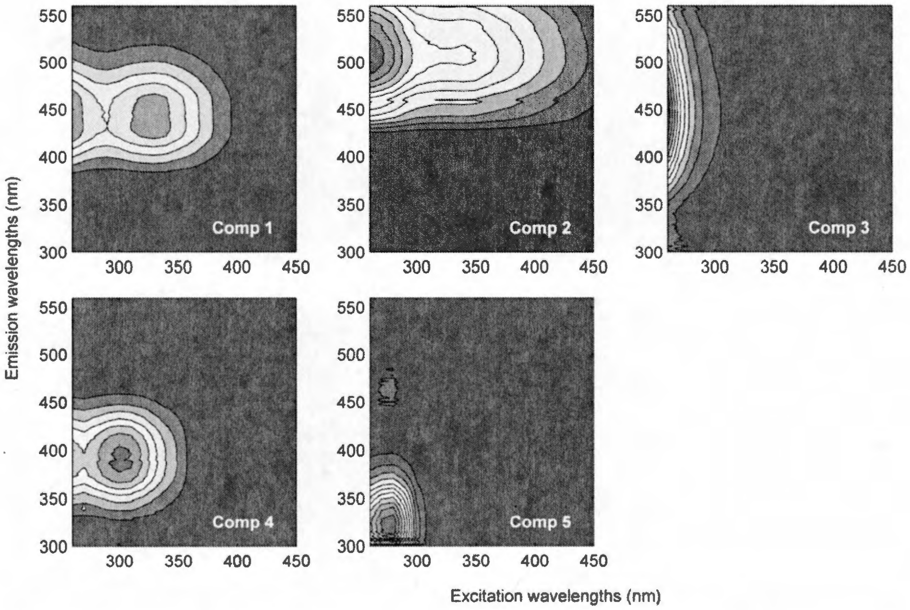


Figure 1.S3 Fluorescence signatures of the 5 components identified by the PARAFAC model.

Table 1.S1 Description of the studied circumpolar regions.

Region (Site)	Ponds studied	Pond type	Coordinates	Site Description	Type of permafrost
Toolik[1,2]	8	tundra	68° 38′ N 149° 36′ W	Shrub tundra	Continuous
McKenzie Delta[3]	7	thaw	69° 42′ N 134° 28′ W	Peatland tundra	Continuous
Cambridge Bay[4]	5	tundra	69° 07′ N 105° 01′ W	Shrub tundra	Continuous
Resolute Bay[5]	4	bedrock	74° 41′ N 94° 49′ W	Desert tundra	Continuous
Coral Harbour[6]	35	tundra	64° 00′ N 82° 05′ W	Shrub tundra	Continuous
Bylot Island[7]	57	thaw	73° 09′ N 79° 59′ W	Peatland tundra	Continuous
Kuujjuarapik					
KW[8]	19	bedrock/tundra	55° 17′ N 77° 44′ W	Rocky shore/Forest tundra	Sporadic
SAS[8]	17	thaw	55° 13′ N 77° 41′ W	Peatland tundra	Sporadic
KWK[9]	34	thaw	55° 20′ N 77° 30′ W	Peatland forest tundra	Sporadic
Umiujaq					
Tasiapik[10]	5	thaw	56° 33′ N 76° 28′ W	Peatland shrub tundra	Discontinuous
BGR[11]	14	thaw	56° 37′ N 76° 13′ W	Peatland shrub tundra	Discontinuous
Ward Hunt[12]	2	bedrock	83° 04′ N 74° 10′ W	Desert tundra	Continuous
Hazen[13]	4	tundra	81° 50′ N 70° 25′ W	Shrub tundra	Continuous
Kangerlussuaq[14]	9	bedrock/tundra	67° 00′ N 50° 40′ W	Shrub tundra	Continuous
Zackenberg[15]	7	bedrock/tundra	74° 30′ N 20° 40′ W	Desert tundra	Continuous
Kilpisjärvi[16]	16	bedrock/tundra	69° 02′ N 20° 50′ E	Birch forest/shrub tundra	Sporadic
Seida[17]	10	thaw	67° 03′ N 62° 56′ E	Peatland tundra	Sporadic

[1] Nelson et al. (1985); [2] Neff and Hooper (2002); [3] Pienitz et al. (1997); [4] Johnson (1962); [5] Rautio and Vincent (2006); [6] Mallory et al. (2006); [7] Fortier and Allard (2004); [8] Arlen-Pouliot and Bhiry (2005); [9] Bouchard et al. (2012); [10] Provencher-Nolet et al. (2014); [11] Calmels and Allard (2004); [12] Villeneuve et al. (2001); [13] Bronwyn et al. (2007); [14] Wimpenny et al. (2010); [15] Christoffersen et al. (2008); [16] Rautio (1998); [17] Sjöberg et al. (2013).

Table 1.S2 Spectral characteristics of the five components validated by PARAFAC modeling, number of matches in *OpenFluor* (minimum similarity of 0.95), assignment source and common origin according literature. Number in brackets refers to the secondary peak of excitation.

Component	Maximum excitation (nm)	Maximum emission (nm)	Number of *OpenFluor* matches[a]	Assignment Source	Common origin[b]
C1	< 260 (330)	432	5	Humic-like	T[18,19,20]
C2	< 260	510	17	Humic-like	T[19,20,21]
C3	< 260	448	8	Humic-like	T[18,19]
C4	300	386	9	Humic-like	M[18,20]
C5	280	314	15	Amino acid- and protein-like	Au[19,21]

[a] *http://www.openfluor.org*; test conducted April 12, 2017. [b] T: terrestrial; M: microbial; Au: autochthonous.
[18] Osburn et al. (2011); [19] Walker et al. (2009); [20] Lambert et al. (2016); [21] Osburn and Stedmon (2011).

CHAPTER II

ZOOPLANKTON CARBON AND FATTY ACID COMPOSITION IN THE CONTEXT OF INCREASING DOMINANCE OF TERRESTRIAL ORGANIC MATTER DUE TO PERMAFROST THAW

RESEARCH ARTICLE

SUBMITTED MANUSCRIPT

Title: Effect of permafrost thaw on planktonic food webs in subarctic ponds

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Author contribution statement: MW led the study, which was based on a research question formulated by MR. MW and MR designed the approach. MW conducted the statistical analyses and interpretation. MW wrote the article with the help of MR.

Running head: Permafrost thaw influence on food webs

Keywords: permafrost thaw, thaw ponds, thermokarst, browning, food web, zooplankton, *Daphnia*, allochthony, terrestrial carbon, stable isotope, fatty acid, mixing model

2.1 Abstract

Frozen tundra soils hold one of the Earth's largest pools of organic carbon. Climate warming and the associated permafrost thaw release a large fraction of this carbon into circumpolar lakes, inducing extreme browning that fuels the heterotrophic microbial food web. How this permafrost carbon affects organisms higher in the food chain remains unknown. Using dissolved organic matter (DOM) optics, total phosphorus (TP), chlorophyll *a* (Chla), fatty acids (FA), and stable isotopes (SI), we investigated the influence of thawing permafrost on primary producers and primary consumers of the planktonic food web. We sampled 4 subarctic thaw ponds that were affected by permafrost carbon and another 4 ponds that were not. Our results highlight the stimulating influence of eroding and degrading ice-rich permafrost on nutrients and planktonic algae. Relative to the non-thaw ponds, the permafrost thaw-influenced freshwaters had higher TP concentrations (15 vs. 32 $\mu g\ L^{-1}$, respectively). This in turn led to a higher Chla (2.7 vs. 6.5 $\mu g\ L^{-1}$) and seston omega-3 FA concentrations (0.3 vs. 0.7 $\mu g\ mg^{-1}$ dry weight) despite significantly reduced light for primary production. Differences between the thaw and non-thaw ponds ceased at the primary consumer level. *Daphnia pulex*, which dominated the crustacean zooplankton community, did not respond to the higher omega-3 availability in the thaw ponds but rather assimilated the high-quality FA equally in all ponds, possibly because their metabolic needs were already saturated. However, the lower quality terrestrial carbon compounds from permafrost ended up in the *D. pulex* body mass, resulting in a median allochthony of 35% and 18% based on SI and FA mixing models, respectively; non-thaw ponds had median allochthony mixing model estimates of 3% (SI) and 8% (FA). Our findings demonstrate that while the effect of permafrost thaw ranges from markedly stimulating planktonic primary producers to an almost negligible effect on zooplankton omega-3 content, the increased input of terrestrial

DOM clearly affects primary producer composition and shifts the feeding of primary consumers toward a lower quality terrestrial diet.

2.2 Introduction

Permafrost is defined as a perennially frozen ground and represents a major constituent of the northern landscape. These frozen regions also hold one of the Earth's largest pools of organic carbon (Schuur et al. 2015). Due to climate warming, these frozen soils are thawing at an accelerated rate. The erosion and collapse of permafrost-rich terrain is forming small and shallow freshwaters (Vonk et al. 2015), hereafter referred to as thaw ponds. Over the past decades, these ponds have become increasingly numerous at northern latitudes and now represent one of the most abundant types of freshwater systems in arctic and subarctic regions (Grosse et al. 2013). They act as recipients of the large amounts of carbon released and transported to aquatic ecosystems from the thawing permafrost (Schuur et al. 2008). A recent study of the influence of degrading permafrost on dissolved organic matter (DOM) composition in northern freshwaters highlighted the stronger terrestrial imprint in thaw ponds, emphasizing a shift toward an increasing dominance of land-derived organic carbon (Wauthy et al. 2018). The increasing terrestrial influence in arctic and subarctic ponds is an example of browning, a global phenomenon that refers to an increase in DOM concentrations and a change in water optical properties, which affect particularly northern freshwaters (Creed et al. 2018; Wauthy et al. 2018). Transparent arctic ponds and lakes—characterized by high O_2 concentrations and benthic primary production (Rautio et al. 2011a) and a general net autotrophy (Bogard et al. 2019)—are being replaced by anoxic turbid ponds that receive increasing amounts of terrestrial organic material from the changing watershed. This shift has consequences for ecosystem metabolism (Deshpande et al. 2015; Vonk et al. 2015). The greater influx of terrestrial carbon and nutrients stimulates bacterial production and respiration (Roiha et al. 2016) and decreases light availability for pelagic (Karlsson et al. 2009) and benthic primary production (Forsström et al. 2013). As a result, an increased number of circumpolar freshwaters have become net heterotrophic systems (Roiha et al. 2015; Creed et al. 2018) as well as significant sources of carbon dioxide and methane emissions to the atmosphere (Walter et al. 2006).

These emissions of greenhouse gases from northern freshwaters have brought attention to the carbon cycle in tundra ecosystems and more precisely to heterotrophic bacteria that are responsible for the main turnover of terrigenous carbon in natural waters (Roiha et al. 2016; Wurzbacher et al. 2017). However, changes in allochthonous DOM are also expected to have a strong effect on internal lake processes and the pelagic food web, from phytoplankton at the base of the trophic chain to higher levels such as zooplankton. Given the absence of fish, zooplankton have a very high biomass in most arctic and subarctic freshwaters (Rautio and Vincent 2006). Phytoplankton are expected to respond to a DOM-controlled decrease in light penetration (Williamson et al. 1996), a greater thermal stability (Houser 2006), and an increase in the total nutrient pool (Solomon et al. 2015). The current assumption is that phytoplankton are stimulated in these initially oligotrophic lakes, but they are affected adversely in dystrophic systems that receive further loading of terrestrial DOM. The influence on zooplankton and higher trophic levels would depend on the quantity and quality of available food (Creed et al. 2018).

Both the production and transfer of high-quality fatty acids (FA) to zooplankton through the food web are good indicators of food quality. Lipids and FA are critical for the survival and fitness of all vertebrates and, quite probably, of almost all invertebrates as well (Napolitano 1999). Therefore, FA can be used as chemical proxies to investigate the health of an ecosystem or its organisms, such as zooplankton. Moreover, FA can also be used as trophic markers to study food webs (Napolitano 1999). The polyunsaturated fatty acids (PUFA) are particularly valuable for metazoans, playing an important role in cellular membrane structure and function. They are also precursors of bioactive compounds and are required for somatic growth, survival, and reproduction (Parrish 2009). A subcategory of PUFA, the omega-3, is essential for metazoans and must be included in their diet (Ahlgren et al. 2009). These omega-3 are also typical for aquatic systems, most of them produced only by phytoplankton (Ahlgren et al. 2009). On the other hand, the saturated fatty acids (SAFA) are considered as low-quality FA that are of less interest for zooplankton, but they can have potentially severe impacts on zooplankton health when abundant in the diet.

In this study, we explored the influence of thawing permafrost on the planktonic food webs of subarctic ponds. We characterized the DOM, light absorbance, and nutrients available for primary producers in the context of permafrost thaw. We also estimated the effect of degrading permafrost on FA composition in the seston and zooplankton body mass. We then used stable isotope (SI) and FA mixing models to estimate how permafrost thaw has affected zooplankton feeding. Given the higher terrestrial imprint of DOM in subarctic freshwaters exposed to degrading permafrost (Wauthy et al. 2018), we hypothesized that permafrost thaw induces an increase in DOM and nutrient concentrations as well as greater light absorption, thereby adversely affecting the phytoplanktonic community in these already dystrophic ponds. We further hypothesized that zooplankton in these thaw ponds assimilate a high proportion of terrestrial compounds into their biomass (i.e., more allochthonous in ponds subjected to permafrost thaw) and that their omega-3 FA concentrations are lower in ponds affected by permafrost degradation, leading to less healthy primary consumers. To remove the confounding effects of interspecies differences, we focused on *Daphnia pulex* (hereafter referred to as *Daphnia*), an abundant and cosmopolitan filter-feeding cladoceran in subarctic lakes (Rautio et al. 2011a; Bégin and Vincent 2017).

2.3 Methods

2.3.1 Study sites

Field work was carried out during August 2014 and 2015 in the vicinity of Kuujjuarapik-Whapmagoostui, Nunavik, Québec (55°17'N, 77°47'W). In this area, permafrost is sporadic and thawing at an accelerated rate. We selected 8 waterbodies distributed across 2 subarctic sites based on their exposure to thawing permafrost: (1) 4 non-thaw ponds, which were not influenced by degrading permafrost soils; and (2) 4 thaw ponds, which were thermokarst freshwaters directly in contact with thawing permafrost. Photographs of sampled ponds and sites are presented in Supporting Information Fig. 2.S1.

The non-thaw ponds are surrounded by grass- and shrub-tundra vegetation that partly covers the rocky substrate watershed. These small (mean surface area 375 m^2) and shallow (mean depth 0.9 m) waterbodies are oligotrophic, unstratified, and transparent (Rautio et al. 2011a). Benthic algae represent more than 99% of the biomass and primary production in these freshwaters (Rautio et al. 2011a). In contrast, the thaw ponds—characterized by a small size (mean surface area 70 m^2) and a depth ranging from 2.4 to 2.9 m—displayed very different properties due to their exposure to degrading permafrost soils. Located in a peatland mainly colonized by *Sphagnum* and the semi-aquatic macrophyte *Carex* (Arlen-Pouliot and Bhiry 2005), thermokarst freshwaters are strongly stratified, eutrophic waterbodies having very turbid water and no light reaching the anoxic bottom (Deshpande et al. 2015).

2.3.2 Sample collection

Water and *Daphnia* were collected from just below the surface using a water sampler (Limnos Ldt., Turku, Finland) and a 50-µm plankton net. The samples were transferred to Nalgene bottles pending subsequent treatments in the laboratory. Lab analyses were performed within 3 h of sample collection. A subsample of zooplankton (3 replicates per pond) was preserved in formaldehyde (4% final concentration) for later identification. Phytoplankton was collected as a seston sample from the surface water and brought to the lab in a 4-L Nalgene bottle for filtration. We collected the upper horizon (0–5 cm) of soils along the pond edge. In the non-permafrost sites, these samples were a thin layer of organic matter on the rock substrate close to the ponds, whereas in the thermokarst system, samples were the surface soils from organic-rich palsas that were collapsing into the ponds. Benthic algae were sampled only in the non-thaw waterbodies. The surface of submerged rocks was scraped with a spatula to collect the epibenthic material. Due to the high turbidity of the thermokarst sites, no light reached the bottom of the thaw ponds (<1% of incoming surface photosynthetically active radiation light left at 1.1 m depth, as measured with an underwater radiometer; Li-Cor Biosciences, Lincoln, Nebraska). The strong stratification was also believed to limit the exchange between the bottom and surface waters (Matveev et al. 2016). Thus, the benthic contribution to the diet of *Daphnia* in thaw ponds was considered insignificant. We instead sampled decaying, submerged *Sphagnum* and macrophytes surrounding the thaw ponds and considered these as potential food sources.

In the lab, surface water was filtered through pre-rinsed cellulose acetate filters (0.2 µm) to analyze dissolved organic carbon concentrations (DOC) and perform optical analyses on chromophoric DOM (CDOM). For total phosphorus (TP) analyses, we also added 67 µL of H_2SO_4 30% to 20 mL of unfiltered surface water. The DOC, CDOM, and TP samples were stored in acid-washed glass vials at 4 °C in the dark. We also filtered surface water on GF/F filters to determine surface chlorophyll *a* (Chla) concentrations. Live *Daphnia* were sorted and picked under binocular microscope at 12× magnification. Three replicates, each with approximately 100 individuals, were prepared for each pond. For phytoplankton analyses, seston samples were passed through a 50-µm sieve to remove zooplankton and then filtered onto pre-combusted and pre-weighted GF/F filters to collect the seston. The filtered water was also kept and stored in acid-washed and combusted glass vials at 4 °C in the dark for deuterium SI analyses. Unless stated otherwise, samples were stored at -80 °C until freeze-drying and further analyses.

2.3.3 Limnological and optical analyses

The quantification of DOC was carried out using an Aurora 1030W TOC Analyzer (O.I. Corporation, College Station, Texas) in the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Ottawa, Ontario). CDOM concentrations were estimated as the absorption coefficient of DOM at 320 (a_{320}) and 440 nm (a_{440}) following Wauthy et al. (2018). The concentration of TP was determined using persulphate digestion and ascorbic acid following the standard methods section 4500-P.E. (APHA 1998). Surface Chla concentrations were calculated according to Nusch (1980).

2.3.4 Fatty acid analyses

Fatty acids from *Daphnia* and their potential diet sources were extracted as per Mariash et al. (2011) using a modified extraction method from Bligh and Dyer (1959). FA were transmethylated according to a protocol adapted from Lepage and Roy (1984) at our Fatty Acid Analytical Laboratory. The resulting FA methyl esters were analyzed by gas chromatography–mass spectrometry (GC-MS) using a 7890A GC (Agilent, Santa Clara, California) coupled to a 5975C MS with triple-axis detector (Agilent, Santa Clara, California) and a J&W DB-23 column (Agilent, Santa Clara, California). Data are expressed in μg FA mg^{-1} dry weight or in percentage of FA (%). Several FA proxies were calculated for *Daphnia* and seston: the amount of PUFA, omega-3, SAFA, and specific algal, terrestrial, and bacterial FA, respectively. In addition, the PUFA:SAFA ratio was used as an index to investigate FA quality

(Napolitano 1999). The lists of identified FA and specific FA biomarkers are presented in Supporting Information Table 2.S1.

2.3.5 Stable isotope analyses

To quantify the relative carbon contribution of both pond types, we carried out SI analyses on *Daphnia* and its potential carbon sources. *Daphnia*, soils, *Sphagnum*, and macrophyte samples were analyzed for $\delta^{13}C$ using a FLASH 2000 OEA interfaced with a Delta V Plus MS (Thermo Fisher Scientific, Waltham, Massachusetts) at the RIVE Research Center (Université du Québec à Trois-Rivières, Trois-Rivières, Québec). Before the $\delta^{13}C$ analyses, soil samples were acid fumigated for 96 h to remove carbonates, as described in Ramnarine et al. (2011). To estimate the phytoplanktonic and benthic algal isotopic signature, we ran $\delta^{13}C$ analyses on the specific algal FA 16:1n7, 18:2n6, 18:3n3, and 20:5n3 extracted from bulk seston and benthos (Taipale et al. 2015; Grosbois et al. 2017a; Grosbois et al. 2017b). The $\delta^{13}C$ analyses on FA were performed in the Stable Isotope Laboratory of Memorial University (Memorial University of Newfoundland, St. John's, Newfoundland and Labrador), using a 6890N GC (Agilent, Santa Clara, California) linked to a Delta V Plus MS (Thermo Fisher Scientific, Waltham, Massachusetts). All $\delta^{2}H$ analyses were done in the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, Arizona) following Doucett et al. (2007), using a CONFLO II coupled to a Delta Plus XL MS (Thermo Fisher Scientific, Waltham, Massachusetts). The $\delta^{2}H$ of phytoplankton were estimated from the deuterium signatures of filtered water using a fractionation distribution as per Wilkinson et al. (2013).

2.3.6 Stable isotope and fatty acid mixing models

Mixing models were run separately for both pond types and for SI and FA. For SI, we ran a dual Bayesian mixing model adapted from Wilkinson et al. (2014) using the measured $\delta^{13}C$ and $\delta^{2}H$ values of different putative *Daphnia* diet sources as end-members. The sources contributing to *Daphnia* were considered based on the presence or absence of degrading permafrost in the watershed: soils, phytoplankton, and benthic algae for non-thaw ponds, and soils, phytoplankton, and macrophytes for thaw ponds. In the thermokarst sites, the isotopic signature of *Sphagnum* was similar to macrophytes (δ^{13}C of -26.6‰ vs. -27.2‰, δ^{2}H of -180.9‰ vs. -177.8‰) and both sources were combined in the mixing model. For FA, we performed the Bayesian mixing model "FA Sources Tracking Algorithm in R" (FASTAR) based on the percentage of 14 FA (see list in Table 2.S1) within each sample, following the method of Galloway et al. (2014). The putative sources for non-thaw ponds were soils, a phytoplankton source that included Chlorophyceae and Cyanophyceae, another phytoplankton source that included Cryptophyceae, and benthic algae. For the thaw systems, we targeted macrophytes and *Sphagnum* instead of benthic algae. The splitting of phytoplankton into different sources was carried out to be consistent with Galloway et al. (2014). We used the algal FA database from Lang et al. (2011) to determine the phytoplankton FA profiles; they were based on the FA profiles of 302 strains of Chlorophyceae and Cyanophyceae and 18 strains of Cryptophyceae. Due to the under-representation or absence of Chrysophyceae, dinoflagellates, and diatoms in the algal FA database, they were not included in the model. For each source group included in the model, we calculated the FA means ± SD (in %). We estimated trophic fractionation from calibration coefficients specific to each FA and its origin (algal versus terrestrial), which were then multiplied with the FA percentage of the respective source. The calibration coefficients were calculated as the ratio between FA profiles (in %) of *Daphnia* feeding on different specific diets (data from Galloway et al. 2014) and FA profiles (in %) of the respective sources measured in this study (data available in Supporting Information Table 2.S2). The algal calibration coefficients were applied to the phytoplanktonic and benthic algal sources, whereas the terrestrial calibration coefficients were applied to soils, macrophytes, and *Sphagnum*. Allochthony was estimated as the median soil contribution (in %) to

Daphnia for both the SI and FA mixing models. All mixing models were run in R v 3.5.1. (R Development Core Team 2018).

2.3.7 Statistical analyses

Limnological and optical variables were analyzed by one-way analyses of variance (ANOVA) or Kruskal-Wallis rank tests when the assumptions of normality and homogeneity of variance were not fulfilled. FA proxies were rank-transformed and analyzed by factorial two-way ANOVA with the pond type (non-thaw or thaw) and material (seston or *Daphnia*) as independent variables, followed by pairwise comparisons using a Tukey post hoc test. All FA profiles of *Daphnia* were combined and ordered according to pond type by nonmetric multidimensional scaling (NMDS) with Wisconsin transformation and a Euclidean index. To test the influence of pond type on *Daphnia* SI and FA, we performed permutational multivariate analyses of variance (PERMANOVA) on normalized or Wisconsin transformed data, respectively, using Euclidean distance as a dissimilarity index. The number of permutations was fixed at 999, and the multivariate homogeneity of group dispersions was verified by performing permutational analyses of multivariate dispersions (PERMDISP). All statistical analyses were run in R v 3.5.1. (R Development Core Team 2018).

2.4 Results

2.4.1 Limnological and optical properties

The limnological and optical parameters measured at the surface water illustrated a strong effect of permafrost thaw (Table 2.1) with significant differences between pond types for all variables, except for TP and Chla ($P < 0.05$, one-way ANOVA or Kruskal-Wallis by rank test). The amount of DOC varied between 4.60 and 20.43 mg L^{-1} with the highest values in ponds impacted by degrading permafrost. The CDOM concentrations followed the same trend, as indicated by the a_{320} and a_{440} values. Although not significant, TP and Chla concentrations were higher in thaw ponds, reflecting a slightly more enriched trophic state.

Table 2.1 Mean limnological properties (± SD) of subarctic non-thaw and thaw ponds at the water surface, including dissolved organic carbon (DOC), absorption coefficient of dissolved organic matter at 320 nm (a_{320}) and 440 nm (a_{440}), total phosphorus (TP), and chlorophyll *a* concentration (Chla).

Pond type	DOC (mg L^{-1})	a_{320} (m^{-1})	a_{440} (m^{-1})	TP (μg L^{-1})	Chla (μg L^{-1})
Non-thaw	9.23 ± 3.58	29.07 ± 10.01	4.40 ± 1.45	14.75 ± 12.91	2.66 ± 2.09
Thaw	16.23 ± 2.82	98.64 ± 10.32	15.64 ± 2.09	31.83 ± 26.62	6.48 ± 6.37

2.4.2 Characteristics of seston and primary consumers

While the FA proxies between seston and *Daphnia* differed markedly, we observed less difference in the FA composition between thaw and non-thaw ponds (Table 2.2). Significant differences were observed only for the terrestrial and bacterial specific FA biomarkers in seston ($P < 0.01$, factorial two-way ANOVA) (Table 2.2). The amount of PUFA, omega-3, algal specific FA, and the PUFA:SAFA ratio were all highest in *Daphnia.* A similar ranking was also observed for the bacterial biomarker FA; however, their amount in seston was significantly lower in non-thaw ponds than thaw ponds (factorial two-way ANOVA, $F_{1,77} = 8.236$, $P = 0.005$). Specific terrestrial FA showed the opposite pattern; we recorded the lowest amounts of terrestrial FA in *Daphnia* and the highest amounts in seston. Non-thaw ponds recorded higher terrestrial FA values than thaw ponds. Moreover, the interaction effect between pond and material type was only significant for the PUFA (factorial two-way ANOVA, $F_{1,77} = 4.543$, $P = 0.036$).

Table 2.2 Mean values (± SD) of polyunsaturated (PUFA), omega-3, specific algal, terrestrial, bacterial, and polyunsaturated:saturated (PUFA:SAFA) fatty acids (FA) in seston and *Daphnia pulex* from non-thaw and thaw ponds. Units are expressed in µg mg^{-1} dry weight.

Material	**Pond type**	**PUFA**	**Omega-3**	**Algal FA**	**Terrestrial FA**	**Bacterial FA**	**PUFA:SAFA**
Seston	Non-thaw	0.50 ± 0.39	0.27 ± 0.34	1.98 ± 1.03	0.16 ± 0.07	0.22 ± 0.11	0.16 ± 0.08
	Thaw	1.08 ± 1.23	0.74 ± 1.13	2.48 ± 1.42	0.08 ± 0.08	0.43 ± 0.27	0.21 ± 0.21
Daphnia pulex	Non-thaw	6.98 ± 2.49	4.60 ± 2.23	7.81 ± 2.75	0.02 ± 0.01	0.54 ± 0.26	1.01 ± 0.24
	Thaw	5.82 ± 1.64	4.32 ± 1.24	6.44 ± 1.63	0.01 ± 0.00	0.60 ± 0.32	1.07 ± 0.41

The isotopic signatures of *Daphnia* highlighted a strong difference (PERMANOVA, $F_{1,10}$ = 9.476, P = 0.004) between the thermokarst and non-thaw ponds (Fig. 2.1a). The $\delta^{13}C$ signature of *Daphnia* was more depleted in thaw ponds (-34.5 ± 1.1‰) than non-thaw ponds (-30.6 ± 1.1‰). SI signatures of *Daphnia*, however, had similar δ^2H values for both pond types, ranging between -148.5‰ and -195.7‰. The NMDS ordination of *Daphnia* FA composition also separated the two pond types (PERMANOVA, $F_{1,19}$ = 5.883, P = 0.001). NMDS associated the thaw-pond *Daphnia* with specific bacterial and algal FA, whereas *Daphnia* within the non-thaw ponds were associated with terrestrial biomarker FA (Fig. 2.1b).

As observed in *Daphnia*, phytoplankton $\delta^{13}C$ was more depleted in thaw ponds than in non-thaw ponds (-35.7 ± 0.2‰ vs. -31.1 ± 0.1‰), whereas δ^2H was similar for both pond types (-263.6 ± 3.4‰ vs. -259.0 ± 4.7‰) (Fig. 2.2). Terrestrial organic matter had similar $\delta^{13}C$ and δ^2H signatures for all ponds (-26.2 ± 0.8‰ vs. -24.9 ± 2.0‰, and -154.4 ± 4.0‰ vs. -160.8 ± 10.9‰, for thaw and non-thaw ponds, respectively).

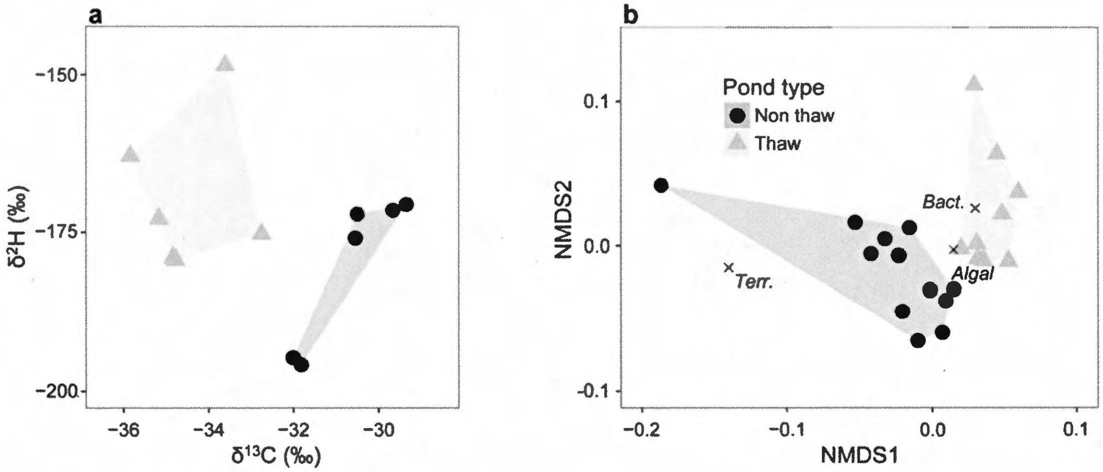

Figure 2.1 *Daphnia* grouping visualization according to pond types and based on their (a) $\delta^{13}C$ and $\delta^{2}H$ signatures and (b) non-metric multidimensional scaling (NMDS) carried out on all identified fatty acids. Crosses indicate the position of specific terrestrial (Terr.), algal (Algal) and bacterial (Bact.) biomarker fatty acids.

2.4.3 Source contributions to primary consumers

Permafrost thaw affected *Daphnia* feeding as indicated by the SI composition of this zooplankton taxon (Fig. 2.2). Based on its location in the biplot (Fig. 2.2a), the *Daphnia* signature in the non-thaw ponds fitted well within the polygon of the source end-members and appeared to be composed mostly of benthic materials. In thaw ponds, however, *Daphnia* was positioned well outside the polygon with $\delta^{13}C$ values closest to phytoplankton and $\delta^{2}H$ closest to terrestrial sources (Fig. 2.2b). Accordingly, the SI mixing models showed a large contribution of benthic (median of 71%) and planktonic (median of 22%) algal material to *Daphnia* in non-thaw ponds, although the range of the 95% highest contribution probability was highly variable (2–99% and 0–89%, for benthic and planktonic material, respectively) (Fig. 2.3a). Terrestrial organic matter constituted a minor source in the non-thaw ponds (median of 3%). In the thaw ponds, phytoplankton was the major contributor to *Daphnia* (median of 57%), but we also observed a major shift to a higher proportion of carbon of terrestrial origin, making up 35% of the *Daphnia* biomass (median, Fig. 2.3b). The contribution from macrophytes was negligible (median of 5%).

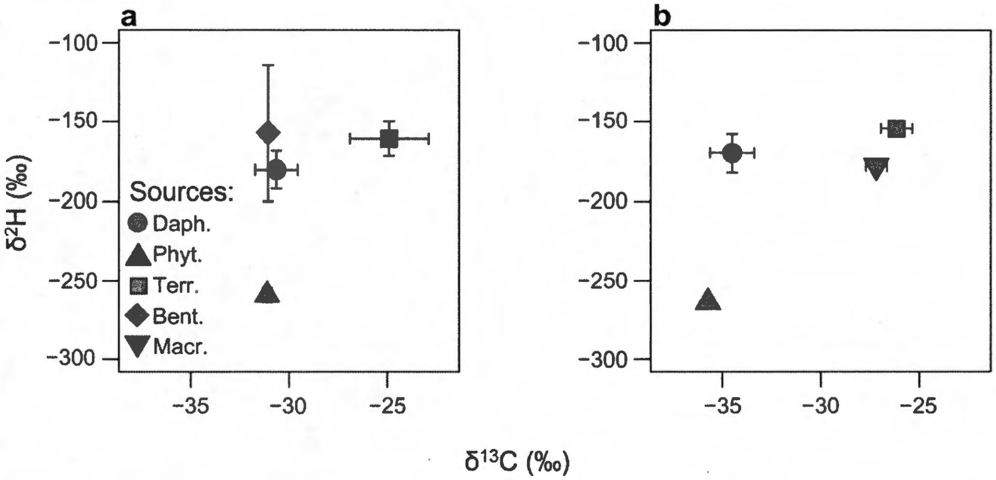

Figure 2.2 Distribution of $\delta^{13}C$ and $\delta^{2}H$ *Daphnia* (Daph.) signatures in relation to the polygon of the potential sources (± SD) for (a) non-thaw ponds and (b) thaw ponds. The sources are phytoplankton (Phyt.), terrestrial organic matter (Terr.), benthic algae (Bent.), and macrophytes (Macr.).

The FA mixing models showed similar patterns for non-thaw and thaw ponds, the FA composition of *Daphnia* being mainly from phytoplankton (median of 78% and 67%, for non-thaw and thaw ponds, respectively), and more precisely from the algal planktonic group encompassing the Chlorophyceae and Cyanophyceae (Fig. 2.3c and 3d). In non-thaw ponds, the other sources had a significant but minor contribution (median less than 10%) (Fig. 2.3c). In the thaw ponds, however, a higher terrestrial contribution was observed (median of 18%), whereas macrophytes and *Sphagnum* were secondary contributors (median of 5% and 2%, respectively) (Fig. 2.3d).

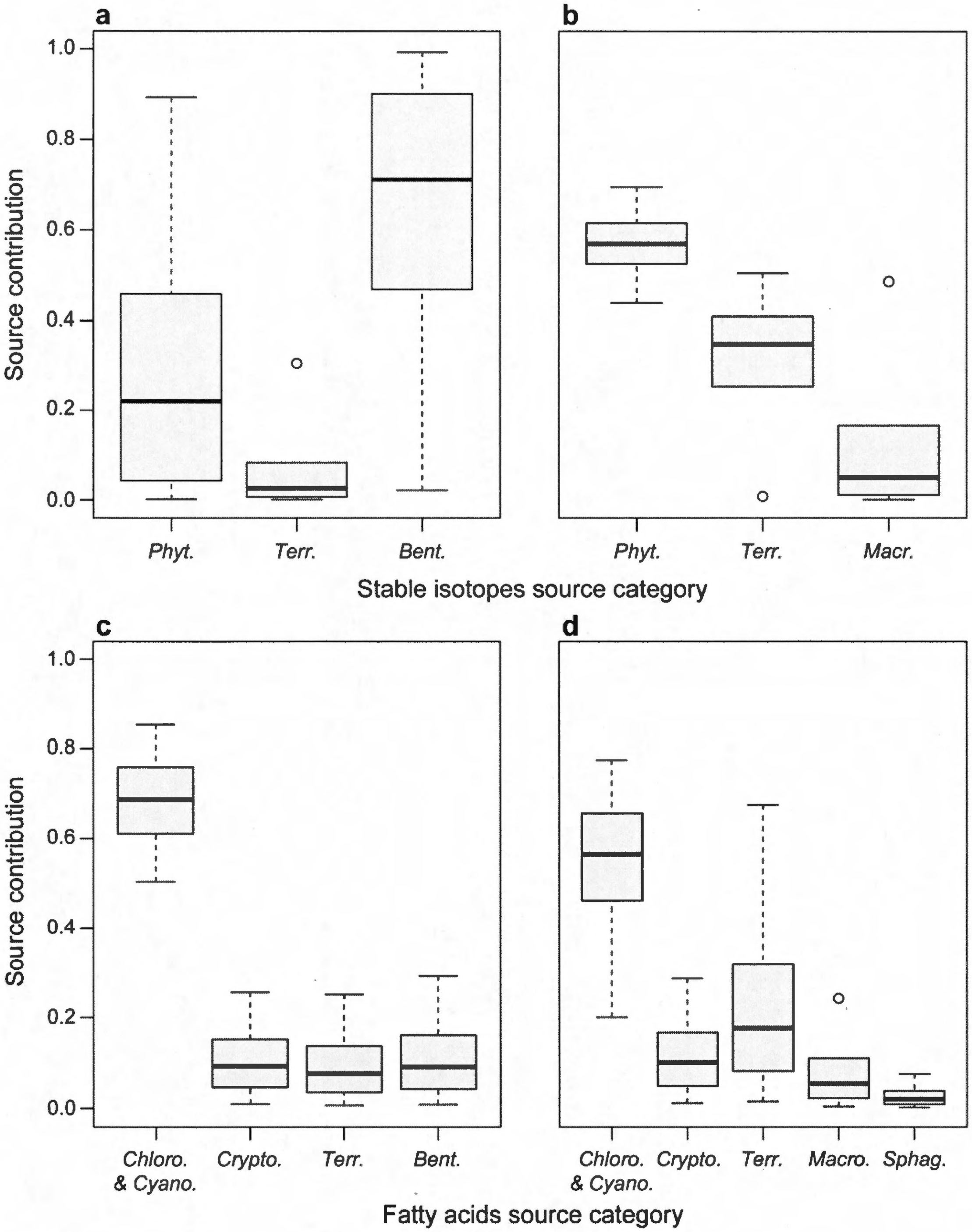
a
b
c
d
Source contribution
1.0
0.8
0.6
0.4
0.2
0.0
Phyt.
Terr.
Bent.
Phyt.
Terr.
Macr.
Stable isotopes source category
Chloro. & Cyano.
Crypto.
Terr.
Bent.
Chloro. & Cyano.
Crypto.
Terr.
Macro.
Sphag.
Fatty acids source category

Figure 2.3 Source contributions to *Daphnia* based on a dual isotope ($\delta^{13}C$ and $\delta^{2}H$) Bayesian mixing model in ponds having (a) a tundra catchment unaffected by thermokarst processes and (b) a thawing permafrost catchment, and on Bayesian mixing models based on a 14 fatty acids in (c) non-thaw and (d) thermokarst ponds, respectively. The sources are phytoplankton (Phyt.), terrestrial organic matter (Terr.), benthic algae (Bent.), macrophytes (Macr.), Chlorophyceae and Cyanophyceae (Chloro. & Cyano.), Cryptophyceae (Crypto.), and *Sphagnum* (Sphag.). Box and whisker plots present the distribution of 50% and 95% highest densities of contribution probabilities, respectively, with the median value indicated by the line within each box.

2.4.4 Stimulating effect of permafrost thaw on algal biomass and zooplankton allochthony

Overall, permafrost thaw altered the chemical properties of the surface water and the different levels of the food chain from those measured in non-thaw ponds (Fig. 2.4). Excepting the amount of omega-3 in zooplankton, which was the same for both pond types (Table 2.2), all other variables were 76% to 1251% higher in the thaw ponds compared to the non-thaw ponds. While light absorption increased up to 247%, the availability of nutrients was also higher in thaw ponds (TP increased by 116%). This TP increase most likely more than compensated for the adverse effect of less light and explained the higher omega-3 content (177% increase) and Chla concentration (144% increase) in seston. When exposed to thawing permafrost and higher CDOM, the median allochthony of *Daphnia* increased by 1251% and 130% according to the SI and FA mixing models, respectively.

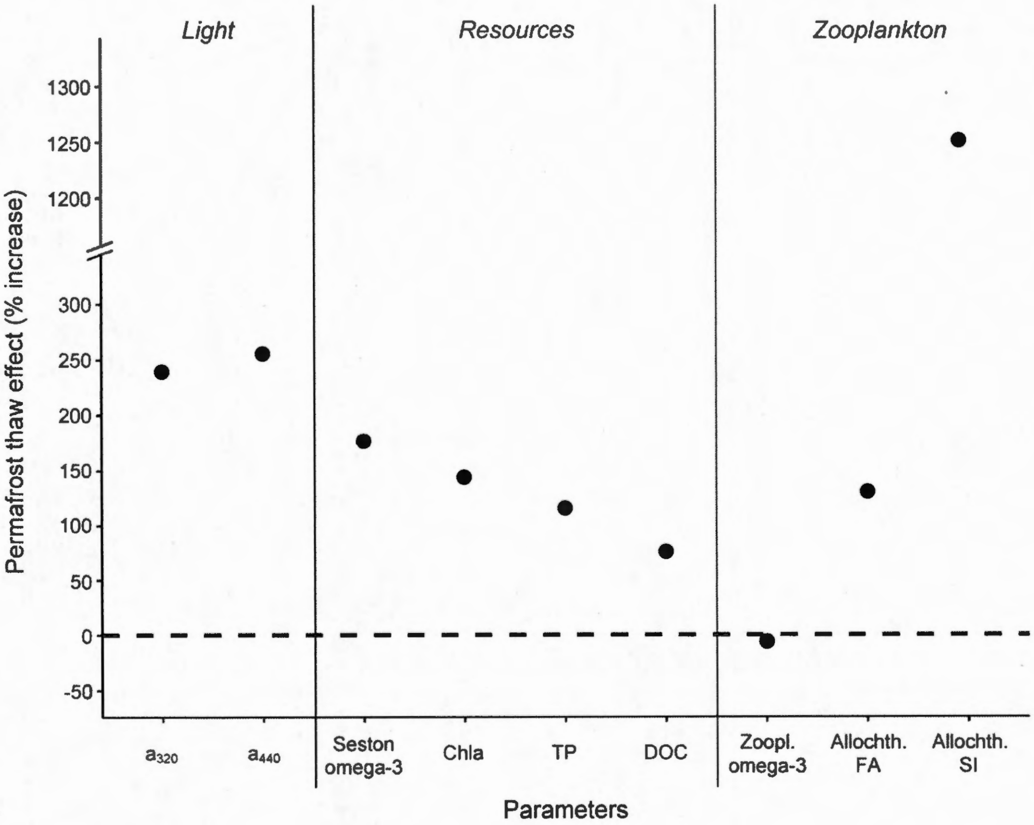

Figure 2.4 Effect of thawing permafrost (expressed in term of percentage of increase) on surface water chemical properties and different levels of the food chain, including the absorption coefficient of dissolved organic matter at 320 nm (a_{320}) and 440 nm (a_{440}), seston fatty acid omega-3 amounts (Seston omega-3), chlorophyll *a* (Chla), total phosphorus (TP) and dissolved organic carbon (DOC) concentrations, zooplankton fatty acid omega-3 amounts (Zoopl. omega-3), and zooplankton allochthony based on fatty acid (Allochth. FA) and stable isotope (Allochth. SI) mixing models.

2.5 Discussion

Our results show clearly that permafrost thaw alters both the physicochemical environment as well as the biomass composition of the planktonic food web in subarctic ponds; however, specific environmental variables and food web compartments were regulated very differently by the increased quantities of terrestrial carbon. In general, the non-living environment was more affected than the organisms. Within the food web, permafrost thaw had a greater effect on primary producers than primary consumers. While our results are in line with other studies indicating that browning influences biogeochemistry (Wauthy et al. 2018) and increases the allochthony of zooplankton (Wilkinson et al. 2013), we also showed that permafrost thaw stimulates rather than suppresses the production of high-quality omega-3 in the food web—within a mean DOC increase from 9 to 16 mg L^{-1}—a pattern opposite to our hypothesis.

2.5.1 Permafrost thaw impact on light, nutrients, and algal biomass

Permafrost thaw induced important changes in the chemical properties of surface waters (Table 2.1, Fig. 2.4). The higher DOC and CDOM concentrations, supporting recent publications on DOM in the context of permafrost thaw (Vonk et al. 2015; Wauthy et al. 2018), strongly decreased light availability for primary producers. In the thaw ponds, only the uppermost meter remained sufficiently illuminated, and the majority of the water column received <1% of the incident PAR. Such high absorption is common in thaw ponds (Watanabe et al. 2011) and can shift primary production from benthic-dominated to pelagic-dominated production (Vadeboncoeur et al. 2008; Forsström et al. 2013). In oligotrophic arctic ponds surrounded by rocky catchments, this shift often leads to an overall decrease in total primary production—and hence lower algal biomass—because nutrient-limited phytoplankton cannot attain the high production levels of benthic algae (Rautio and Vincent 2006). In our study sites, the non-thaw ponds were oligotrophic and mesotrophic freshwaters and comparable to other clear-water arctic ponds (Rautio et al. 2011a). In our studied thaw ponds, the situation was different. Phosphorus concentrations were more typical of mesotrophic and eutrophic systems, with phosphorus likely originating from the degrading watershed, as observed in recent studies (Larsen et al. 2017). Consequently, the Chla concentrations were higher, suggesting a stimulated pelagic primary production from this greater nutrient influx (Vonk et al. 2015), whereas the relatively lower TP concentrations in the non-thaw systems produced Chla values similar to other circumpolar rock basins (Rautio et al. 2011a).

2.5.2 Increased supply of omega-3 fatty acids in thaw ponds

When terrestrial subsides of DOM increase, the food webs may shift from a reliance on autochthonous and autotrophic production to one based on heterotrophic production supported by terrestrial carbon (Rautio et al. 2011b; Mariash et al. 2018). In such conditions, a decline in the production and transfer of high-quality omega-3 is expected due to a reduction in phytoplankton biomass or dilution of the omega-3 by an increase in bacterial biomass (Creed et al. 2018). Whereas previous studies illustrated a high abundance of bacterial biomass in thaw ponds (Roiha et al. 2015), our results here show that the phytoplankton are able to concurrently sustain a high biomass. This may result from the dominance of flagellated (i.e., motile) and mixotrophic species that are known to dominate in thaw ponds (Forsström pers. comm., Roiha et al. 2015). Such organisms compensate for low light by phagotrophy on bacteria, and they can migrate daily to the deeper layers (Jones 1991) where higher nutrient concentrations occur below the thermocline (Roiha et al. 2015). In this environmental setting, Chla, PUFA, omega-3, and specific algal FA had higher concentrations in the seston of the thaw ponds (Table 2.2, Fig. 2.4).

The different phytoplankton SI values between both pond types reveal that the algal communities, or their metabolism, differed; this may also contribute to the differences in seston PUFA concentrations. The seston $\delta^{13}C$ signatures were more depleted in ponds subjected to thawing permafrost (Fig. 2.2). This may result from the high concentration of methane in thaw ponds (Matveev et al. 2016) and its bioaccumulation in mixotrophic algae. Methane has a more negative $\delta^{13}C$ value due to fractionation during methanogenesis; it can be metabolized by methanotrophic bacteria and then enter the food web (Bastviken et al. 2003). The phytoplankton community in the thaw ponds of the region is mainly mixotrophic (Bégin and Vincent 2017) and can rely on these methanotrophic bacteria as an energy source, explaining the more depleted $\delta^{13}C$ signature of phytoplankton and hence *Daphnia*. Algal metabolism based on methanotrophic bacteria cannot, however, explain the higher PUFA, as bacteria lack these essential FA. We propose that the higher concentration of omega-3 in the thaw-pond seston is a combination of a high algal biomass, sustained by the high concentration of nutrients originating from the thawing permafrost, and a community composition dominated by motile mixotrophic algae that are able to access both nutrients and sufficient light.

2.5.3 Fatty acid composition of *Daphnia*

The FA composition of *Daphnia* in the thaw ponds associates zooplankton with specific bacterial FA (Fig. 2.1b). This matches the findings of previous studies that highlight the abundant bacterial communities and high bacterial growth in thaw ponds (e.g., Deshpande et al. 2016). The FA proxies confirmed this association, *Daphnia* being richer in FA biomarkers specific to bacteria in thermokarst freshwaters, although this is not significant (Table 2.2). However, the FA extraction method used in this study prevented us from separating and measuring the concentrations of FA specific to methanotrophic bacteria, such as C16:1n6c or C18:1n6c (Taipale et al. 2015). These specific FA are likely particularly important in thermokarst freshwaters, due to the high methane concentrations of these waterbodies (Matveev et al. 2016). Therefore, the amount of bacterial FA biomarkers may be underestimated in the thaw ponds relative to ponds not influenced by thawing permafrost.

Considering the strong terrestrial imprint on thermokarst systems (Wauthy et al. 2018), the higher amount of specific terrestrial FA in the non-thaw ponds for both

Daphnia and seston was unexpected (Table 2.2). However, most limnological studies involving FA are carried out in boreal, temperate, or tropical regions having forested catchments. Consequently, FA considered as terrestrial, e.g., C20:0 or C22:0, are mostly FA specific to trees. Yet, the tundra landscape is very different, being deprived of most vascular plants and colonized mainly by small herbaceous plants and mosses. Therefore, using these FA as a proxy for terrestrial matter might not fit in the context of permafrost and arctic regions.

The amount of omega-3 in zooplankton was the same in the thaw and non-thaw ponds, despite the higher concentration of omega-3 in the seston of thaw ponds (Table 2.2, Fig. 2.4). This reduction in the transfer of omega-3 through the food chain could be explained by the dilution of these high-quality FA by the higher abundance of *Daphnia* in thaw vs. non-thaw ponds (4.1 vs. 0.4 individuals L^{-1}, respectively), an increase in bacterial biomass, or a shift in algal community composition for the benefit of phytoplanktonic species of lower FA quality. While the latter has been suggested by Creed et al. (2018), the higher PUFA and omega-3 content of the seston in thaw ponds (Table 2.2) does not indicate a presence of a poorer quality algae. It may be that the phytoplankton production of PUFA in both pond types was sufficient to saturate the metabolic needs of *Daphnia*; therefore, the higher PUFA concentration in seston in thaw ponds was not displayed by *Daphnia*. This is supported by the higher content of PUFA, omega-3, algal FA biomarkers, and PUFA:SAFA ratio in *Daphnia* compared to seston (Table 2.2). This pattern emphasizes *Daphnia*'s ability to selectively accumulate and retain higher quality FA. The specific terrestrial FA also highlighted the selective assimilation, showing a reverse pattern with smaller amounts of these low-quality FA in *Daphnia* tissue as in seston.

2.5.4 Allocation of permafrost carbon to *Daphnia* biomass

Although the PUFA content of *Daphnia* was not significantly altered by exposure to permafrost carbon, the overall FA composition—along with the SI composition—

showed that some organic material from the permafrost was assimilated by *Daphnia*, as was indicated by the zooplankton's higher allochthony. We used SI- and FA-based mixing models to quantify the contribution of different organic material sources to *Daphnia*. The SI data and models emphasized a higher terrestrial imprint on *Daphnia* in ponds surrounded by degrading permafrost (Fig. 2.3a and 2.3b). In non-thaw ponds, *Daphnia* were supported mostly by autochthonous production, the model estimating a 94% contribution from benthic and pelagic algae (Fig. 2.3a). As expected, benthic algae contributed much more than phytoplankton in these ponds, given the low algal biomass and primary production in the water column compared to benthos (Rautio and Vincent 2006). Our findings support studies that have also highlighted the low contribution of phytoplankton to the food web of arctic ponds (Cazzanelli et al. 2012; Mariash et al. 2014). Indeed, given the absence of stratification in non-thaw ponds, benthic material can be resuspended (Evans 1994) and diffused (Rautio et al. 2011a; Rodríguez et al. 2013) throughout the entire water column, making it available for primary consumers. In thaw ponds, despite the higher imprint of phytoplankton, autochthonous algae contributed less to *Daphnia*, thereby emphasizing the influence of thawing permafrost on the food web (Fig. 2.3b). This is consistent with Wauthy et al. (2018) who highlighted the shift toward an increasing dominance of terrigenous organic carbon in freshwaters subjected to ongoing permafrost thaw. However, compared to the DOM allochthony in Wauthy et al. (2018), *Daphnia* in this study showed less terrestrial influence, again suggesting their ability to select higher quality food items in the water column.

It needs to be noted, however, that the *Daphnia* isotopic signature in the thaw ponds fell outside of the polygon defined by the 3 selected sources (Fig. 2.2b). Therefore, we must interpret the respective SI mixing models with some caution. One explanation could be that we missed an important food source of *Daphnia* in thaw ponds, although this seems unlikely. A more plausible explanation is that we estimated incorrectly the δ^2H signature of phytoplankton in thaw ponds. The calculation developed by Wilkinson et al. (2013) is for phytoplankton growing in culture conditions that favor autotrophy. In thaw ponds, phytoplankton consist mostly of mixotrophic species (Bégin and Vincent 2017) and thus could be predominantly heterotrophic, feeding on bacteria and DOM. Therefore, in ponds strongly affected by terrestrial inputs, the δ^2H of phytoplankton would be less depleted and closer to the deuterium of DOM (-153.9 ± 11.1‰), which is approximately in the same range as the *Daphnia* δ^2H signature (169.6 ± 12.0‰). Thus, the isotopic signature of *Daphnia* would be much closer to the phytoplankton in thaw ponds, highlighting even more their ability to select higher quality food of autochthonous origin despite the overwhelming abundance of terrestrial organic matter in the water column.

The FA mixing models are consistent with previous studies (e.g., Galloway et al. 2014) and support the SI results, testifying to the importance of algae in the diet of *Daphnia* in both thaw and non-thaw ponds (Fig. 2.3c and 2.3d). Moreover, the significant contribution of phytoplankton of high biochemical quality (Cryptophyceae), despite their relatively low abundance in these freshwaters (L. Forsström pers. comm.), highlighted the ability of filter-feeding zooplankton to select higher quality food present in the water column. The FA-based models displayed similar patterns between non-thaw and thaw ponds; however as for the SI-based models, the FA emphasized the higher terrestrial imprint in those ponds surrounded by degrading permafrost. Nonetheless, this terrestrial contribution remained minor, suggesting no influence of degrading permafrost on the health of filter-feeding Cladocera, *Daphnia* being able to grow and reproduce while relying on poor-quality diets as long as high-quality food was available, even if only available in small quantities (Taipale et al. 2012).

An important assumption of these FA mixing models is that the differences in the FA content of *Daphnia* are driven by diet and the retention of lipids from this diet inside their tissues (Galloway et al. 2014). Other factors, such as temperature (Masclaux et al. 2009), zooplankton growth, and egg production (Vargas et al. 2006; Schneider et al. 2017) can directly influence lipids. Our sampling, however, was carried out over 2 short time windows, and consequently, the effects of different life-history stages on *Daphnia* FA content are assumed to be negligible.

2.6 Conclusion

Thawing permafrost induces rapid changes in northern freshwaters, releasing stocks of organic carbon that end up in aquatic systems and lead to a decrease of water column transparency. This in turn affects biogeochemical processes and the balance of autotrophic and heterotrophic production (Wauthy et al. 2018) and will favor algal species in the plankton at the expense of benthic algae (Karlsson et al. 2009; Forsström et al. 2013). Despite the significant influence of eroding and degrading permafrost on water chemistry, optical properties, and algae at the base of the trophic chain (Fig. 2.4), our results highlight the small impact of this extreme version of browning on higher levels of the food web, as terrestrial organic matter contributes little to the biomass of the filter-feeding *Daphnia* in arctic lakes. Our results show that phytoplankton remain the key resource that fuels the food web in arctic ponds and emphasize the ability of zooplankton to select a higher quality diet. Future work is needed to determine if *Daphnia* selectively forage on phytoplankton, or whether the high-quality FA are retained preferentially in zooplankton tissue.

2.7 Acknowledgments

We are grateful to the Centre for northern studies (CEN) Whapmagoostui-Kuujjuarapik Station and Claude Tremblay for providing us access to transportation and lab facilities. We also thank Alexandre Dubé, Félix Faucher, David-Alexandre Gauthier, Zoe Kroonenburg, Julien Lebrun, Flora Mazoyer, Sophie Robitaille, and Maxime Saunier for their help and cheerfulness in the field, Pierre Carrier-Corbeil for fatty acid analyses, Guillaume Grosbois for his advice regarding stable isotope data analyses, and Murray Hay for his editorial assistance. The funding was provided by the Canada Research Chairs Program, the Canada Foundation for Innovation, and the CEN. The PhD grant of Maxime Wauthy was partly supported by the Merit Scholarship Program for Foreign Students from the Ministère de l'Éducation et de l'Enseignement Supérieur du Québec.

2.8 References

The reference list of each individual chapter has been included in the final reference section at the end of the thesis.

2.9 Supporting information

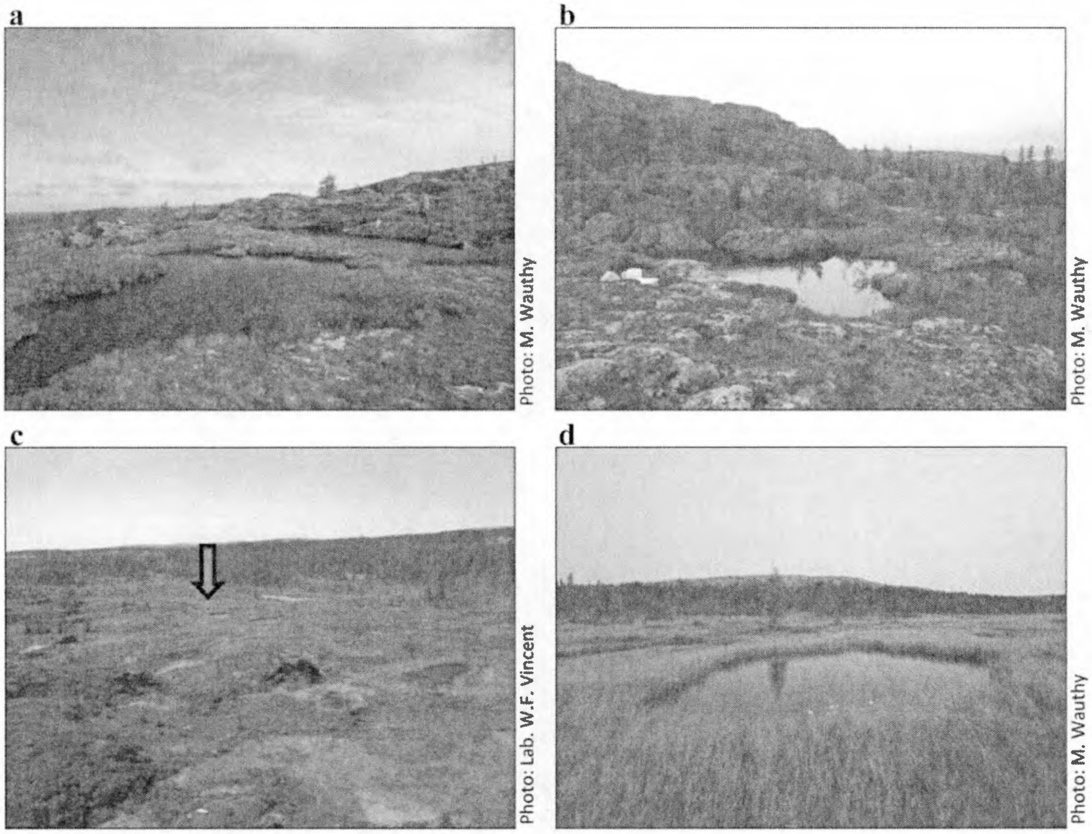

Figure 2.S1 Photos of ponds sampled in the study. (a) and (b): Ponds not influenced by permafrost thaw. (c): Thermokarstic site undergoing accelerated permafrost thaw. (d): Thaw pond affected by degrading permafrost. The pond in (d) is marked by an arrow in (c).

Table 2.S1 List of fatty acids (FA) identified after extraction and analyses. The specific FA biomarkers for algae, terrestrial organic matter and bacteria are specified (Taipale et al. 2015; Grosbois et al. 2017b), as well as FA used in the FASTAR mixing model.

FA name	Algal FA	Terrestrial FA	Bacterial FA	FASTAR
C13:0				
C14:0				●
2-OH C14:0				
3-OH C14:0				
C14:1n5				
C15:0			●	
i-C15:0			●	
a-C15:0			●	
C15:1n5				
C16:0				●
i-C16:0				
2-OH C16:0				
C16:1n7	●			●
C17:0				
i-C17:0			●	
c17:1n7				
C18:0				●
C18:1n7				●
C18:1n9				●
C18:2n6	●			●
C18:3n3	●			●
C18:3n6	●			●
C18:4n3	●			●
C20:0		●		●
C20:1n9	●			
C20:1n11				
C20:2n6	●			
C20:3n3	●			
C20:3n6	●			
C20:4n6	●			●
C20:5n3	●			●
C21:0				
C22:0		●		
C22:1n9				
C22:2n6				
C22:6n3	●			●
C23:0		●		
C24:0		●		
C24:1n9	●			

Table 2.S2 Algal and terrestrial calibration coefficients calculated for each of the 14 fatty acids run in the FASTAR mixing model.

FA name	Algal	Terrestrial
C14:0	3.45	0.34
C16:0	0.77	1.44
C16:1n7	8.89	1.26
C18:0	4.19	1.53
C18:1n7	0.52	87.39
C18:1n9	0.92	0.65
C18:2n6	0.63	0.33
C18:3n3	1.00	0.31
C18:3n6	0.82	5.67
C18:4n3	0.40	2.31
C20:0	2.69	0.00
C20:4n6	29.20	2.48
C20:5n3	6.09	3.40
C22:6n3	1.97	0.70

CHAPTER III

ZOOPLANKTON VERTICAL DISTRIBUTION IN SUBARCTIC FRESHWATERS IN THE CONTEXT OF PERMAFROST THAW

RESEARCH ARTICLE

SUBMITTED MANUSCRIPT

Title: Vertical distribution of zooplankton in steeply stratified subarctic thaw ponds, northern Quebec

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Author contribution statement: MW led the study, which was based on a research question formulated by MR. MW and MR designed the approach. MW conducted the statistical analyses and interpretation. MW wrote the article with the help of MR.

Running head: Zooplankton stratification in thaw ponds

Keywords: permafrost, thaw ponds, thermokarst, zooplankton, rotifers, vertical distribution, stratification, hypoxia, top-down predation, phytoplankton, essential fatty acids, weighted mean depth

3.1 Abstract

Climate change and associated permafrost thaw are creating new shallow waterbodies in vast regions of the circumpolar Arctic and provoking a shift from the dominance of oligotrophic clear-water ponds to a dominance of mesotrophic dark-water ponds. These thaw ponds are characterized by high concentrations of colored dissolved organic matter originating from the degrading watershed, inducing a strong vertical thermal stratification. We investigated the zooplankton community and biomass in 8 subarctic thaw ponds and evaluated the extent to which thermal stratification and associated environmental variables, including oxygen (O_2), phytoplankton biomass, essential fatty acids (EFA), and bacteria and larval phantom midge *Chaoborus* biomass contribute to the vertical distribution of zooplankton in this increasingly common type of arctic freshwater system. The zooplankton community was extremely abundant in all ponds (up to 3548 ind L^{-1}) and dominated mainly by rotifers (35%–93% of the biomass). The shallow epilimnion (0.2 to 1.5 m) was characterized by higher concentrations of dissolved O_2 but low concentrations of seston EFA. Contrariwise, the water column beneath the thermocline was anoxic but rich in algae. According to weighted mean depth, most zooplankton aggregated from 0 to 1 m below the thermocline and their distribution was affected by a combination of O_2, *Chaoborus*, phytoplankton, and EFA that were supplied from opposite directions. Our findings improve our understanding of arctic freshwaters in the context of thawing permafrost and the associated effects on the ecology of planktonic organisms.

3.2 Introduction

Frozen tundra soils represent a major component of circumpolar landscapes. They cover a considerable area of the Arctic (Zhang et al. 1999) and are one of the Earth's largest pools of organic carbon (Schuur et al. 2015). Ongoing climate warming in subarctic regions is causing this ice-rich permafrost to thaw rapidly and favor thermokarst processes that create shallow waterbodies, hereafter referred to as thaw ponds (Vonk et al. 2015). These freshwater systems have increased rapidly in number at high latitudes over the last decades, and these ponds now represent one of the most abundant types of aquatic systems in the North (Grosse et al. 2013). They are strongly influenced by terrestrial inputs and act as recipients for large amounts of carbon and nutrients released from the degrading permafrost. As a result, water transparency and biogeochemical processes become highly altered (Wauthy et al. 2018). The growing influence of incoming terrigenous matter stimulates bacterial production (Deshpande et al. 2016) and respiration (Roehm et al. 2009) in these thaw ponds, making them highly net heterotrophic systems (Deshpande et al. 2015) and major greenhouse gases emitters to the atmosphere (Matveev et al. 2016). While the increasing amount of terrigenous nutrients also promotes pelagic primary producers (Przytulska et al. 2016), the high turbidity from these terrestrial inputs decreases light availability and induces a switch from benthic to pelagic primary production (Forsström et al. 2013). Furthermore, terrestrial inputs potentially reduce the nutritional quality of algae (Creed et al. 2018) and modify the species composition of primary consumers.

Although several studies have highlighted a stimulating influence of permafrost thaw on bacterial communities (e.g., Crevecoeur et al. 2015; Deshpande et al. 2016) and primary producers (e.g., Przytulska et al. 2016), few studies have investigated the higher trophic levels in thaw ponds. Nigamatzyanova and Frolova (2016) described the community structure of zooplankton in the thermokarst lakes of the Lena River delta in eastern Siberia, and they observed a higher diversity and abundance of rotifers compared to copepods or cladocerans. Bégin and Vincent (2017) had similar observations for thaw ponds in subarctic Quebec. They also highlighted that zooplankton abundance and biomass were higher in thaw ponds relative to ponds not affected by degrading permafrost. The dominance of rotifers can be explained by their pelagic feeding mode and their ability to graze very efficaciously on diverse sources of diet such as algae, bacteria, and even particulate organic matter (Arndt 1993). In addition, their particularly short lifespans and high reproductive rates make these *r*-strategy zooplankton particularly well adapted to perturbed environments (Jalal et al. 2005). Previous studies have also highlighted the ability of rotifers to gain an initial advantage when nutrients and phytoplankton biomass increase (Marty et al. 2002; Jalal et al. 2005) and have illustrated the importance of bottom-up controls on these microzooplankton (Yoshida et al. 2003). However, Bégin and Vincent (2017) found no such controls in thaw ponds, but rather they identified the importance of a top-down pressure on rotifers by *Chaoborus* larvae.

In addition to the stimulation of microzooplankton, permafrost thaw and the resulting terrestrial inputs from the degrading watershed induce a strong thermal stratification in thermokarst freshwaters (Breton et al. 2009). Consequently, deeper thaw ponds transform into a new environmental setting for arctic freshwaters. This steep thermal stratification has important implications for both the physicochemical environment and the organisms living in these systems. Thus, the distribution of nutrients through the water column of these thaw ponds is uneven; for example, Roiha et al. (2015) showed that total phosphorus levels were 6.5× higher in bottom waters than at the surface. Chlorophyll *a* (Chla) displayed the same pattern, ranging from 9.5 $\mu g\ L^{-1}$ at the surface to 133.4 $\mu g\ L^{-1}$ in the bottom waters. This suggests the presence of an abundant algal community in the deep hypolimnion of these deeper thermokarst freshwaters (Roiha et al. 2015). Prokaryotes and heterotrophic nanoflagellates may also have a strong vertical gradient (Roiha et al. 2015; Deshpande et al. 2016), and the substantial bacterial production coupled to the steep thermal stratification can induce severe hypoxia in the bottom waters of thaw ponds (Deshpande et al. 2015). The result is a marked impact on the organisms living in thermokarst systems.

Despite these major changes in circumpolar freshwaters due to permafrost thaw, the repercussions on the higher trophic levels have only been addressed superficially. The main objective of this study was to investigate the zooplankton community and biomass in subarctic thaw ponds and determine the main variables controlling their vertical distribution in the water column. Given the anticipated strong thermal structure and hypoxia observed in the hypolimnion of most sampled thaw ponds (Deshpande et al. 2015), we hypothesized a pronounced stratification of zooplankton. This stratification was expected to be the result of a compromise between a good-quality algal diet, oxygen (O_2) accessibility, and top-down predation. To our knowledge, this study is the first to investigate in detail the vertical distribution of zooplankton and the main drivers of this distribution in thaw ponds.

3.3 Methods

3.3.1 Study sites

Field work was conducted in early August 2013 in a pair of thawing permafrost–affected valleys located in an area of sporadic permafrost near the village of Kuujjuarapik-Whapmagoostui, northern Quebec, Canada (55°17'N, 77°47'W). The Kwakwatanikapistikw River valley (KWK) is filled with numerous thaw ponds formed in clay-silt bed depressions created by the thawing of mineral palsas (Bouchard et al. 2011). Their watershed is mainly colonized by dense shrubs (*Salix* spp., *Alnus crispa*, *Myrica gale*) and sparse trees (e.g., *Picea mariana*, *P. glauca*, *Betula glandulosa*), with some patches of mosses (*Sphagnum* spp.) and macrophytes (*Carex* spp.) (Bouchard et al. 2011). The Sasapimakwananisikw River valley (SAS) is a peatland that is covered predominantly by mosses (*Sphagnum* spp.) and macrophytes (*Carex* spp.) and surrounded by thermokarst waterbodies and collapsing organic palsas (Arlen-Pouliot and Bhiry 2005). Most of the thaw ponds in KWK and SAS are small (mean surface area 157 m^2) and shallow (depth 0.6–3.5 m) freshwaters having high nutrient (TP about 55 μg L^{-1}) and dissolved organic matter concentrations (about 13 mg L^{-1}), and a high turbidity (Breton et al. 2009) that severely limits the penetration of light through the water column. All sampled ponds are fishless. We sampled 4 ponds in the KWK (KWK1, KWK2, KWK6, and KWK12) and 4 others in the SAS (SAS2A, SAS2B, SAS2C, and SAS2D). Pictures of the study sites are available in the Supporting Information Fig. 3.S1. Further details about KWK and SAS are provided in Bouchard et al. (2014) and Arlen-Pouliot and Bhiry (2005).

3.3.2 Sample collection

We collected profiles of temperature, dissolved O_2 concentrations, and photosynthetically active radiation through the water column of each sampled pond using a YSI 550A probe (YSI Inc., Yellow Springs, Ohio) and a PUV-2500 profiler radiometer (Biospherical Instruments, San Diego, California). In addition, we collected water for Chla, fatty acids, phytoplankton, and bacteria from 4–5 depths—that included the epi-, meta-, and hypolimnion—using a water sampler (Limnos Ldt., Turku, Finland). Zooplankton and *Chaoborus* phantom midge larvae were sampled from a 6-L water sample (3 replicates per depth) that was concentrated using a water sampler (Limnos Ldt., Turku, Finland) coupled to a 50-μm plankton net. The organisms were preserved in 4% formaldehyde until counting. The sampling took place between 12:00 and 16:00 to standardize the effect of potential diel vertical migration on plankton depth distribution.

In the lab, a 200-mL subsample of water (2 replicates) from each pond was filtered through a GF/F glass-fiber filter (0.7 μm, 47 mm, Whatman plc, Maidstone, United Kingdom) and stored at -80 °C until extraction in 95% ethanol to determine Chla concentration following the protocol of Nusch (1980). For 3 ponds (KWK6, KWK12, and SAS2A), water was also filtered for investigating bacterial and phytoplankton biomass and fatty acid concentrations. Prior to filtration, bacterioplankton were preserved by adding pre-filtered (0.2 µm) glutaraldehyde (2% final concentration) to a 10-mL sample and stored at 4 °C for 12 h. Each sample was then stained with 4',6-diamidino-2-phenylindole (DAPI) (5 µg mL^{-1} final concentration) for 5 min and filtered through a nucleopore black polycarbonate membrane (0.22 µm, 25 mm, Whatman plc, Maidstone, United Kingdom) placed on the top of a GF/F glass-fiber filter (0.7 µm, 25 mm, Whatman plc, Maidstone, United Kingdom). The nucleopore membrane was then mounted on a microscope slide covered with non-fluorescent immersion oil and a cover slip and kept at -20 °C until counting. Phytoplankton were preserved with acid Lugol's solution and stored at 4 °C until counting. Water samples for fatty acids were filtered onto pre-combusted and pre-weighted GF/F glass-fiber filters (0.7 µm, 47 mm, Whatman plc, Maidstone, United Kingdom). We visually inspected the filters and removed zooplankton and larger detritus before storing the filters at -80 °C.

3.3.3 Zooplankton community and biomass

The zooplankton community was identified and enumerated using the Utermöhl sedimentation method (Utermöhl 1958). Counts were made under an Axio Observer.A1 inverted microscope (Carl Zeiss, Oberkochen, Germany) at 100× to 400× magnification. At least 400 specimens were counted for each sample. To estimate the mean zooplankton biomass, we measured the body width and length of 10 individuals per species using microphotographs captured by an Axiocam ERc 5S coupled to the AxioVision Rel. 4.8.2. software (Carl Zeiss, Oberkochen, Germany). Rotifer biovolumes were estimated as per McCauley (1984). A 1:1 ratio was considered between the biovolume (in mm^3) and the wet weight (in mg). This was converted to dry weight using a ratio of 0.1 as in Pace and Orcutt (1981). We calculated the biomass of the cladoceran *Bosmina* spp. and cyclopoid copepod nauplii as according to Rosen (1981), and we used the regression equations of Malley et al. (1989) to estimate the biomass of *Holopedium gibberum* and cyclopoid copepodites

CI–CV. The biomass of the cladoceran *Daphnia pulex* was estimated as in Dumont et al. (1975). In addition to zooplankton, we counted the dipteran larvae *Chaoborus* sp. and measured their body length under a SteREO Discovery.V12 microscope (Carl Zeiss, Oberkochen, Germany). We then calculated their biomass following the regression equation of Mason (1977).

3.3.4 Bacteria and phytoplankton biomass

We analyzed bacterioplankton samples by epifluorescence microscopy using an Axio Observer.A1 inverted microscope (Carl Zeiss, Oberkochen, Germany) at 1000× magnification under UV light. The bacteria biomass was calculated by multiplying the bacterial abundance with the average bacteria cellular carbon content (18.1 fg C cell^{-1}) as in Roiha et al. (2015). We identified and counted phytoplankton following Utermöhl (1958) and calculated biomass from biovolumes as per Forsström et al. (2015). In the following sections, bacteria are considered as a poor-quality food source for zooplankton, whereas a diet composed of phytoplankton is deemed as excellent (Brett et al. 2009).

3.3.5 Fatty acid analyses

Seston fatty acids were extracted as per Mariash et al. (2011) using a redesigned extraction method from Bligh and Dyer (1959) and were transmethylated following an adapted protocol from Lepage and Roy (1984). The resulting fatty acid methyl esters were scanned through a 7890A gas chromatographer (Agilent, Santa Clara, California) coupled to a 5975C mass spectrometer with triple-axis detector (Agilent, Santa Clara, California) and a J&W DB-23 column (Agilent, Santa Clara, California). The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are polyunsaturated omega-3 fatty acids synthesized almost exclusively by algal primary producers, and they are required for the normal growth and reproduction of most heterotrophic organisms (Taipale et al. 2014). As zooplankton are unable to produce

EPA and DHA de novo, they need to obtain these fatty acids from their diet (Parrish 2009). Consequently, in this study, the concentration of EPA and DHA were summed and hereafter designated as essential fatty acids (EFA).

3.3.6 Data analyses

The weighted mean depth relative to the thermocline was calculated for each zooplankton group (Rotifera, Copepoda, Cladocera) in each sampled pond using the following adapted equation of Worthington (1931):

$$Weighted\ mean\ depth = D_{th} - (\sum(d_i B_i l_i) \,/\, \sum(B_i l_i))\ , \qquad \text{(eq. 3.1)}$$

where D_{th} is the starting depth of the thermocline (m) in the sampled pond, d_i is the sample depth (m), and B_i is the biomass of each zooplankton group (µg L^{-1}) measured in d_i. Since the sampling depth intervals were not equal over the water column, l_i was introduced to the equation and calculated as the distance (m) from half the distance to the previous sampling depth (or surface) to half the distance to the next sampling depth (or bottom). The taxa differences in weighted mean depths were tested using a one-way ANOVA, with zooplankton groups as independent variables after a visual verification of normality and homoscedasticity, using R v 3.5.1. (R Development Core Team 2018).

We investigated the relationships between the vertical distribution of zooplankton—total zooplankton, rotifer, and copepod biomass—and the potential explanatory variables of EFA concentration and phytoplankton biomass (algal variables), *Chaoborus* and bacteria biomass (non-algal variables), and dissolved O_2 concentrations (physicochemical variable), in 3 ponds (KWK6, KWK12, SAS2A) using distance-based linear models (DistLM). Due to their absence from KWK6 and KWK12, cladocerans were not included in these analyses. Zooplankton biomass was square-root transformed, and resemblance matrices were generated using Bray-Curtis similarity. Explanatory variables were square-root transformed and normalized. The collinearity between explaining variables was also tested via a draftsman plot, and variables that were highly correlated ($r > |0.65|$) were removed from the analysis. Thus, we excluded several environmental factors that could potentially explain the vertical distribution of zooplankton, such as light, temperature, and Chla. Due to the small numbers of samples (13), we used the corrected Akaike information criterion (AICc) to rank models for all possible combinations of explanatory variables, with lower AICc values and higher AICc weights indicating a better model performance (Burnham and Anderson 2002). DistLM analyses were run using PRIMER v 6.1.16. with the add-on package PERMANOVA+ v 1.0.6. (PRIMER-E Ltd, Plymouth, United Kingdom). The number of permutations was fixed at 9999.

3.4 Results

3.4.1 Limnological properties

Despite their shallow depths, all sampled thaw ponds showed strong physicochemical stratification. Temperatures decreased 7.9 ± 2.1 °C, and dissolved O_2 concentrations dropped 7.7 ± 1.4 mg L^{-1} between the surface and bottom waters (Fig. 3.1). The KWK ponds were thermally homogenous from the surface until 0.5 to 1.5 m depth, whereas in the darker SAS ponds, the thermocline was at a shallower depth (mean of 0.3 m) and was best detected by the combination of temperature and O_2 profiles.

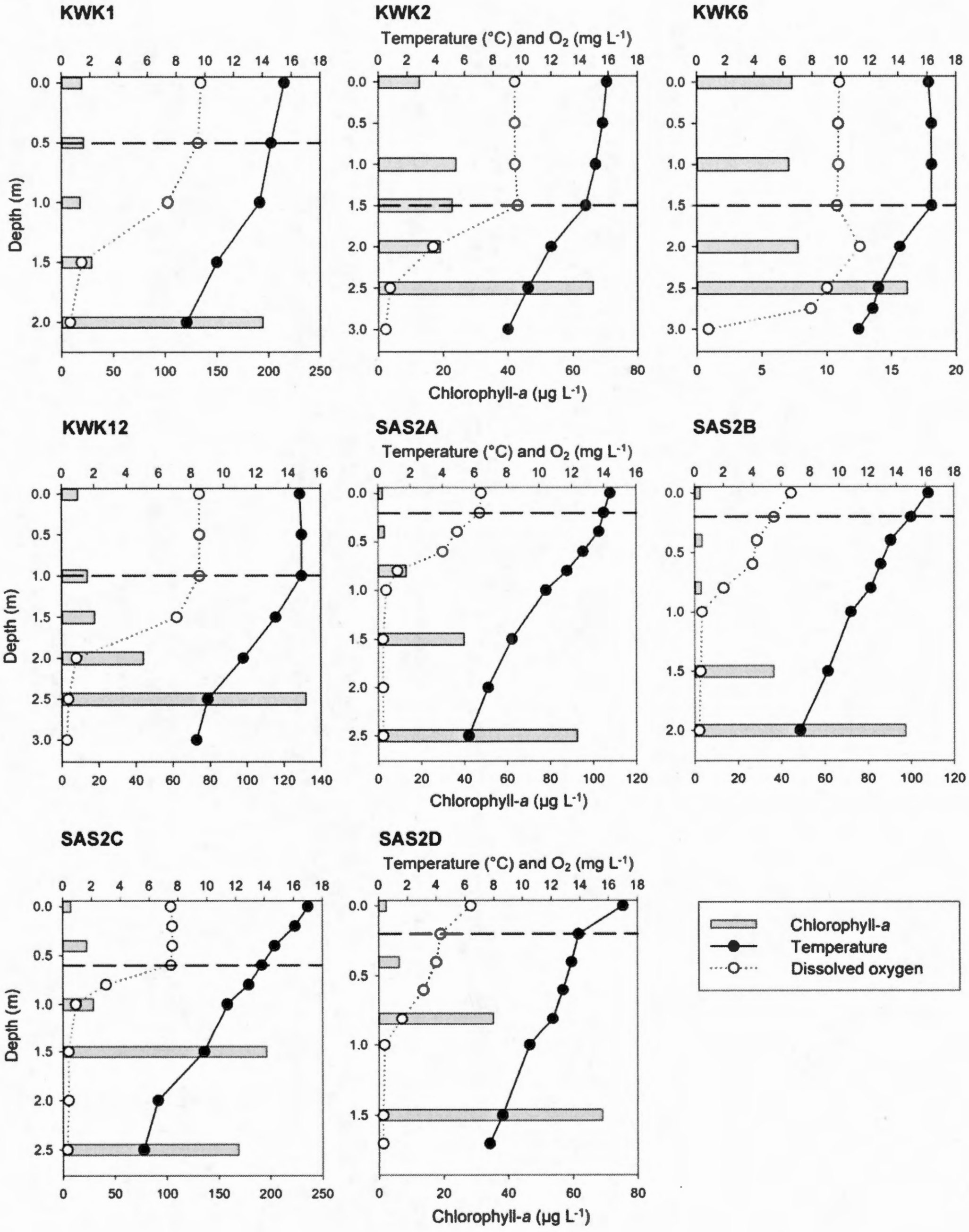

Figure 3.1 Vertical profiles of temperature (°C), dissolved oxygen (O_2) (mg L^{-1}), and chlorophyll *a* (µg L^{-1}) in the sampled KWK and SAS thaw ponds. The dashed line indicates the thermocline depth.

Temperature profiles alone identified temporal thermoclines that typically formed in the dark waters on warm days. In the clearer KWK ponds, the first half of the water column was well oxygenated (7.1–9.9 mg L^{-1}), whereas in the SAS ponds the epilimnion O_2 concentrations ranged between 4.3 and 7.6 mg L^{-1} (Fig. 3.1). All ponds were hypoxic at the bottom (0.4 ± 0.2 mg L^{-1}). Photosynthetically active radiation decreased rapidly with depth in all ponds, especially in the SAS ponds where the 1% surface radiation depth averaged 1.1 m (Supporting Information Fig. 3.S2).

Chla concentrations varied widely among the thaw ponds (Fig. 3.1), both at the surface (2.3 to 19.9 µg L^{-1}) and in deeper water (16.2 to 196.5 µg L^{-1}). In all ponds, Chla concentrations increased with depth and were 2.2× (KWK6) to 45.1× (SAS2A) higher at the bottom than at the surface.

3.4.2 Zooplankton community and biomass

We identified 38 zooplankton taxa from all lakes combined, with 14 to 25 taxa observed per pond (Supporting Information Table 3.S1). Rotifers were the most diverse group with 34 taxa. We observed only 3 Cladocera and 1 Copepoda taxa in all ponds. The total abundance of zooplankton averaged 442 to 3548 ind L^{-1}, and rotifers represented 97.6 ± 1.6% of this total abundance (Supporting Information Table 3.S1).

The average zooplankton biomass per pond was 42 to 529 µg L^{-1}. Rotifers dominated the zooplankton assemblages, representing 34.6 to 92.6% of the total biomass. The exception was pond SAS2B that was dominated by *D. pulex* (Table 3.1). Eight taxa represented > 95% of the rotifer biomass: *Polyarthra* spp. (73.0%), *Keratella testudo* (5.3%), *Trichocerca* spp. (5.0%), *Ascomorpha* spp. (4.0%), *K. cochlearis* (3.4%), *K. tecta* (3.3%), *Asplanchna* spp. (2.6%), and *Kellicottia longispina* (1.7%). In the subclass of Copepoda, we only observed cyclopoida and most specimens were at the nauplii stage. *D. pulex* was the most dominant cladoceran taxon in terms of biomass, except in pond KWK2, where *H. gibberum* was the main species.

Table 3.1 Mean water column biomass (µg L^{-1}) of zooplankton (Rotifera, Copepoda, and Cladocera) in the sampled subarctic thaw ponds.

Taxa	KWK1	KWK2	KWK6	KWK12	SAS2A	SAS2B	SAS2C	SAS2D
Rotifera	**58.34**	**56.34**	**38.74**	**37.92**	**127.77**	**19.89**	**271.32**	**87.82**
Ascomorpha spp.	0.05	4.09	0.20	4.75	0.54	0.12	27.49	0.39
Asplanchna spp.	2.60	3.93	0.70	2.18	2.02	0.00	0.00	0.00
Kellicottia longispina	1.56	1.85	0.35	1.96	1.79	0.00	0.00	0.43
Keratella cochlearis	0.73	1.00	2.96	5.76	0.63	0.06	0.15	0.18
Keratella tecta	2.61	7.31	0.20	2.38	0.00	0.16	2.54	0.10
Keratella testudo	1.15	0.99	0.02	0.00	8.35	1.25	6.61	20.34
Polyarthra spp.	49.05	33.68	33.86	6.89	111.87	17.61	229.76	64.78
Trichocerca spp.	0.19	2.03	0.00	12.42	0.91	0.18	2.95	0.42
Copepoda	**17.50**	**22.09**	**3.10**	**28.29**	**46.43**	**14.42**	**195.95**	**18.96**
Cyclopoida nauplii	15.42	20.52	3.10	28.29	44.87	14.42	189.27	16.80
Cyclopoida copepodites	2.08	1.57	0.00	0.00	1.56	0.00	6.68	2.16
Cladocera	**0.73**	**7.80**	**0.00**	**0.00**	**14.18**	**23.18**	**62.01**	**16.71**
Bosmina sp.	0.00	0.00	0.00	0.00	0.00	0.00	23.48	3.06
Daphnia pulex	0.73	1.48	0.00	0.00	12.63	23.18	38.54	11.47
Holopedium gibberum	0.00	6.32	0.00	0.00	1.55	0.00	0.00	2.18
Total zooplankton	**76.57**	**86.23**	**41.84**	**66.21**	**188.39**	**57.5**	**529.29**	**123.49**

3.4.3 Vertical distribution of zooplankton and the associated variables

The weighted mean depth of each zooplankton group indicated a similar pattern of vertical alignment within the water column (one-way ANOVA, $F_{2,21} = 2.325$, $P = 0.125$), displaying a preference for the meta- and hypolimnion (Fig. 3.2). Overall, rotifers and copepods occupied the first meter below the upper limit of the thermocline in most ponds, whereas cladocerans were situated slightly higher, at the interface between the epilimnion and thermocline. In ponds KWK6 and KWK12, we observed no cladocerans.

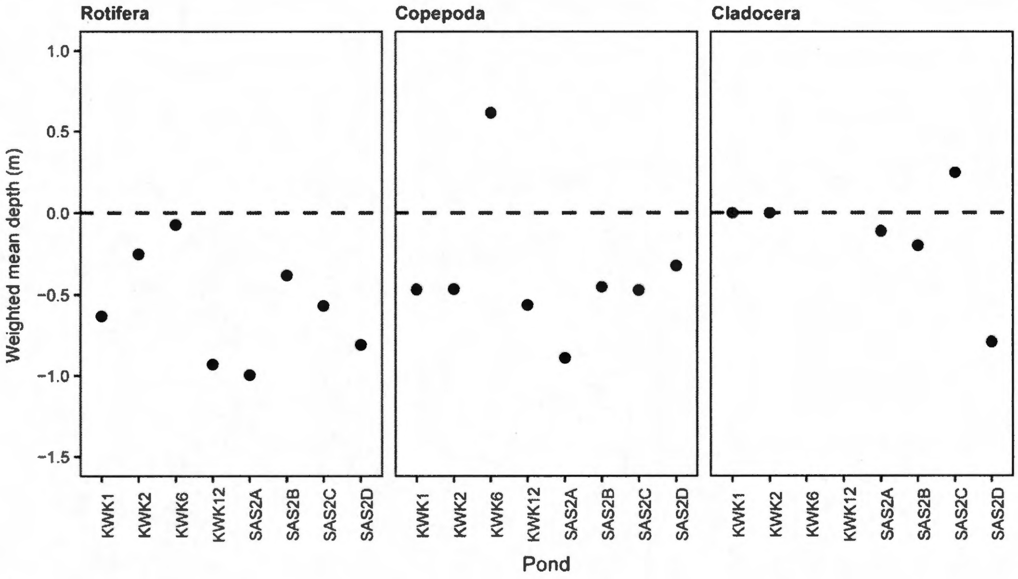

Figure 3.2 Weighted mean depth (m) of Rotifera, Copepoda, and Cladocera biomass relative to the thermocline in the sampled subarctic thaw ponds.

In the 3 ponds (KWK6, KWK12, and SAS2A) where we examined zooplankton distribution in greater detail and associated the zooplankton with the physicochemical and biological variables, we noted specific patterns (Fig. 3.3). In KWK6, copepods were only found in the epilimnion, whereas rotifers were abundant in the epi- and hypolimnion, both layers being well oxygenated in this pond (Fig. 3.1). However, it should be noted that we did not collect zooplankton samples from the thermocline in this pond, the zone where most copepods aggregated in all other ponds (Fig. 3.2). In KWK12, zooplankton were most abundant in the hypolimnion at 2.0 m depth (Fig. 3.3) where O_2 concentrations declined rapidly. In SAS2A, the highest zooplankton biomass was observed in the metalimnion (rotifers up to 407 µg L^{-1} at 1.0 m depth). Cladocerans represented most of the zooplankton biomass (55%) at the oxygenated surface, but they were absent in the anoxic hypolimnion.

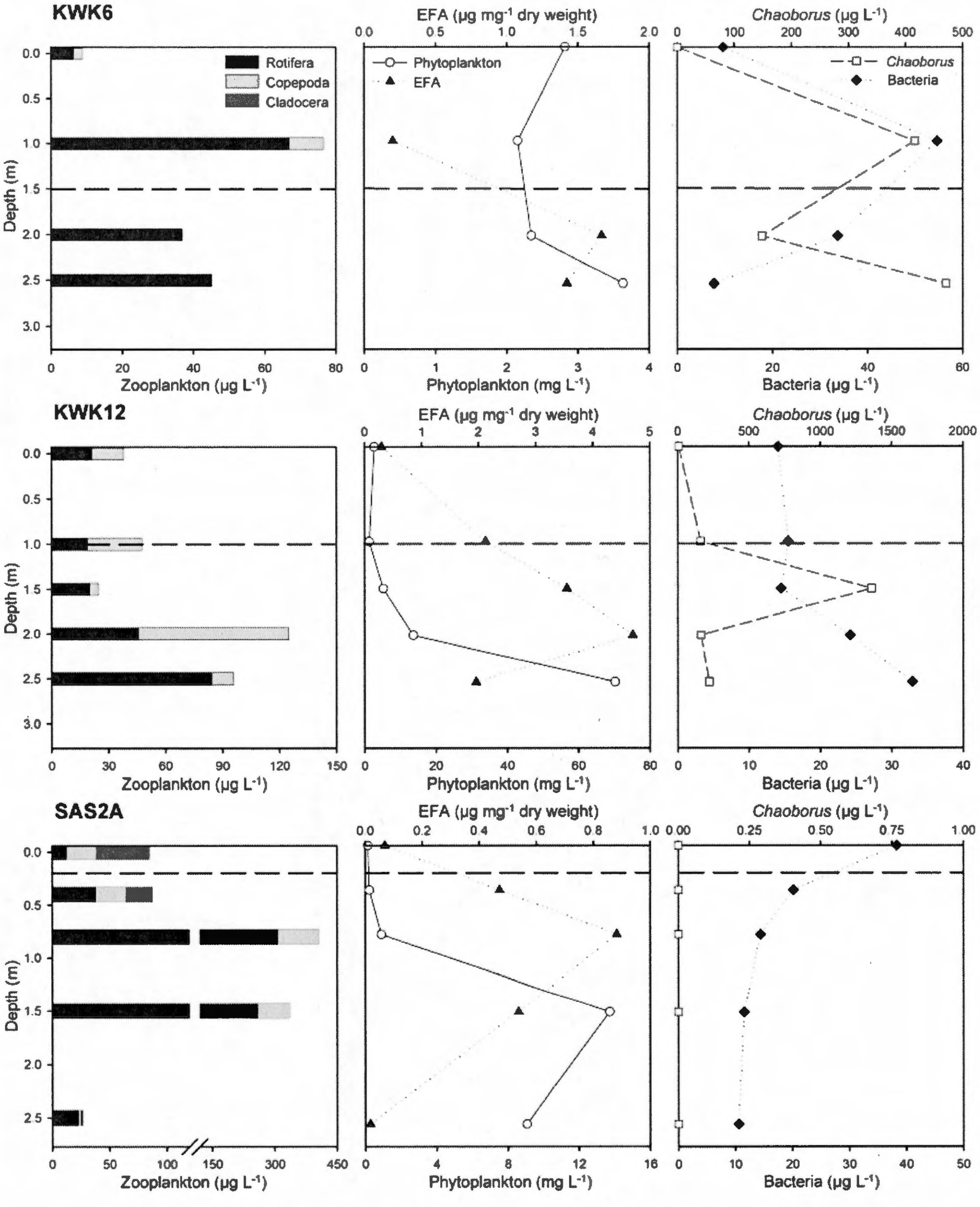

Figure 3.3 Vertical distribution of zooplankton biomass and the associated algal (phytoplankton biomass and concentration of essential fatty acids (EFA)) and non-algal (*Chaoborus* and bacterial biomass) variables in KWK6, KWK12, and SAS2A ponds. The dashed line indicates the thermocline depth.

In these 3 ponds, phytoplankton biomass varied from 0.1 to 70.2 mg L^{-1}. We observed the lowest values in the epilimnion and the highest values in the hypolimnion (Fig. 3.3). EFA had very low concentrations at the surface, then increased rapidly at the thermocline to reach maximum values in the hypolimnion. EFA decreased close to the pond bottom. Bacteria and *Chaoborus* did not present any distinct generalized vertical distribution patterns among the ponds. Each pond had its own distribution profile for these organisms; for example, bacterial abundance peaked at 1.0 m depth in KWK6, close to the bottom at 2.5 m in KWK12, and at the surface in SAS2A. No *Chaoborus* were found in SAS2A.

The vertical distribution of zooplankton had a clear, positive relationship with O_2 concentrations, reflected in the model fit values of $R^2 = 0.29$ for total zooplankton, $R^2 = 0.25$ for rotifers, and $R^2 = 0.16$ for copepods (Table 3.2). Iterating *Chaoborus*, phytoplankton, and EFA into the models improved the fit, at $R^2 = 0.36$ for total zooplankton, 0.28 for rotifers, and 0.29 for copepods, respectively. For total zooplankton, the best model was based on the relative likelihood of O_2 alone (depicted by the AICc weight of 0.25), whereas the second (*Chaoborus*) and third (O_2 + phytoplankton) best models had respective AICc weights of 0.11 and 0.09. The vertical distribution of rotifers was best explained by the models that included O_2, phytoplankton, and O_2 + phytoplankton; however in these models, the O_2-alone model explained the zooplankton distribution 3.4× better than the next model based on phytoplankton biomass (AICc weights of 0.31 and 0.09, respectively) as well as explaining most of the R^2. The other variables had a much smaller effect. For the copepods, O_2 and *Chaoborus* biomass alone constituted the two best models, having similar AICc and AICc weights; however, EFA was an important variable by doubling the R^2 when integrated (Table 3.2). When considered alone, only O_2 was significant (DistLM marginal test, $P < 0.05$), explaining 29% of the overall variation of total zooplankton (Supporting Information Table 3.S2).

Table 3.2 Best models explaining total zooplankton, rotifer, and copepod biomass distribution based on distance-based linear modeling (DistLM). Variables included in the models: CB = *Chaoborus* biomass, O_2 = dissolved oxygen, EFA = essential fatty acids, PB = phytoplankton biomass.

AICc	AICc weight	R^2	RSS	No. variables	Variable selection
Total zooplankton					
78.09	0.25	0.29	3539.4	1	O_2
79.73	0.11	0.19	4015.5	1	CB
80.12	0.09	0.36	3170.5	2	O_2, PB
Rotifera					
81.17	0.31	0.25	4484.8	1	O_2
83.56	0.09	0.10	5390.6	1	PB
84.09	0.07	0.28	4302.3	2	O_2, PB
Copepoda					
91.90	0.17	0.16	10238	1	O_2
92.00	0.16	0.15	10318	1	CB
93.21	0.09	0.29	8678.1	2	CB, EFA

3.5 Discussion

3.5.1 Limnological properties

Consistent with previous studies performed in the region (e.g., Breton et al. 2009; Deshpande et al. 2015), we observed a steep thermal stratification within the pelagic zone of all sampled thaw ponds (Fig. 3.1). Such a strong temperature gradient in shallow freshwaters can be explained be the high concentration of chromophoric dissolved organic matter (Roiha et al. 2015; Wauthy et al. 2018) and total suspended solids (Watanabe et al. 2011; Crevecoeur et al. 2015) that decrease considerably light penetration through the water column due to diffusion and absorption (Supporting Information Fig. 3.S2). Given this strong thermal stratification and light attenuation, gas exchange is limited between the hypolimnion and atmosphere during the summer (Deshpande et al. 2015), thereby favoring O_2 depletion in bottom waters (Fig. 3.1). This hypoxia was more pronounced in the SAS ponds, which can be related to the stronger light attenuation in these ponds (Supporting Information Fig. 3.S2).

Sampled thaw ponds displayed high concentrations of Chla, supporting previous studies carried out on thermokarst freshwaters (Wauthy et al. 2018). Permafrost thaw releases organic matter and nutrients from the eroding watershed, stimulating phytoplankton and pelagic primary production despite the light limitations (Vonk et al. 2015). Regardless of the large inter-pond variability in terms of Chla concentrations, Chla increased with depth in all ponds (Fig. 3.1), suggesting a higher phytoplankton biomass in the aphotic hypolimnion. Nonetheless, Chla and phytoplankton biomass did not have a consistent ratio or even pattern among the sampled ponds, e.g., SAS2A (Fig. 3.3). One explanation could be a slow rate of photooxidation and chemical degradation of photosynthetic pigments—including Chla—in dead phytoplankton cells in the bottom waters due to the absence of light, low temperatures, and anoxic conditions (Vallentyne 1960). Another explanation may be the abundant green-sulphur bacteria that have been recorded in the hypoxic bottom waters of KWK ponds (Rossi et al. 2013; Przytulska et al. 2016). These photosynthetic bacteria synthesize chlorophyll-*b* and bacteriochlorophyll (Olson 1998). These compounds can interfere with algal Chla measurements in the fluorometric method used in this study (Tolstoy and Toth 1980; Coveney 1982). Thus, it is possible that the Chla values in our study overestimate the actual algal biomass in the samples collected from hypoxic or anoxic layers. The fact that the Chla concentrations were lowest in KWK6, the only pond where most of the water column was well oxygenated, supports this explanation.

3.5.2 Zooplankton community and biomass

Subarctic thaw ponds sampled in this study displayed a high zooplankton abundance and biomass, especially for Rotifera (Supporting Information Table 3.S1). The dominance of rotifers compared to planktonic crustaceans is consistent with previous studies from thermokarst sites (Nigamatzyanova and Frolova 2016; Bégin and Vincent 2017). Moreover, despite their small size, rotifers contributed substantially to the overall zooplankton biomass (Table 3.1), corroborating the observations of Bégin and Vincent (2017) and highlighting the rotifer dominance in thermokarst systems, in contrast with arctic (Rublee 1992), subarctic (Pinel-Alloul et al. 1982), and temperate lakes (Herzig 1987) not affected by degrading permafrost. Although the suppression of rotifers by large *Daphnia* populations through exploitative competition for food resources and mechanical interference are well documented (Gilbert 1988), the high biomass displayed by rotifers in our study sites, irrespective of the presence of *Daphnia,* suggests there is no significant influence of cladocerans on rotifer biomass (Table 3.1).

The low abundance and biomass of copepods and cladocerans is probably a characteristic of thaw systems related to the physicochemical and limnological properties of these waterbodies (Breton et al. 2009). However zooplankton, and in particular cladocerans, can migrate horizontally between offshore and littoral zones, especially in small and shallow freshwater systems (Burks et al. 2002). Macrophytes along the shoreline of all our thaw ponds serve as potential refuges for zooplankton from *Chaoborus* predation pressure that can limit cladoceran abundance in the centre of the ponds during the day (Bégin and Vincent 2017). This scenario of horizontal migration, however, is unlikely to explain the complete absence of *Bosmina*, for example, in some ponds and the generally low abundance of copepods and cladocerans in these small fishless ponds.

On average, we identified 17 rotifer taxa per thaw pond (Supporting Information Table 3.S1). This rotifer diversity is 10 taxa higher than that found in a previous study from thaw ponds in the same region (Bégin and Vincent 2017). This difference is likely due to samples collected at different depths; the latter study sampled at the surface where, in our study, we found few rotifers (Fig. 3.3). The 8 rotifer taxa that dominated the thermokarst zooplankton communities are common throughout northern Quebec (Pinel-Alloul et al. 1982; Bégin and Vincent 2017). These taxa can rely on various food sources, including phytoplankton and other species of rotifers, but also bacteria and heterotrophic flagellates (Pourriot 1977; Arndt 1993). However, the taxon *Polyarthra* spp., which represented almost 75% of the rotifer biomass, are selective feeders of larger prey, such as pelagic algae (Pourriot 1977).

In contrast with the rotifers, zooplankton crustaceans displayed a very low diversity in the sampled thaw ponds. We found only 3 members of Cladocera, a diversity much less than that usually observed in ponds and lakes of subarctic Canada (Pinel-Alloul et al. 1982; Paterson et al. 2014). The most dominant cladoceran we observed was *D. pulex*, a cosmopolitan species able to feed on algal (Pennington 1941) and bacterial (Peterson et al. 1978) food sources. For the subclass of Copepoda, our taxonomic resolution was at the order level due to the absence of adult copepods in the samples, limiting a more precise identification. However, all encountered copepod larvae (nauplii) and juveniles (copepodites) were cyclopoids. *Microcyclops* was the only genus of the Cyclopoida order observed in KWK and SAS sites in a previous study (Bégin and Vincent 2017), whereas 11 species of copepods are known to exist in the ponds and lakes of the region (Swadling et al. 2001).

3.5.3 Vertical distribution of zooplankton

The weighted mean depth results indicated that zooplankton, especially rotifers and copepods, stayed preferentially in the metalimnion, in the first meter below the start of the thermocline (Fig. 3.2). In most ponds, the O_2 concentrations declined rapidly at this depth (Fig. 3.1), suggesting zooplankton can tolerate some degree of hypoxia. Several studies have reported the presence of similar or even higher abundances of rotifers in hypoxic layers (e.g., Bērziņš and Pejler 1989; Mikschi 1989), and calanoid nauplii can also tolerate prolonged O_2 depletion (Stalder and Marcus 1997). The slightly higher position of Cladocera in the water column, although not significantly different from the other taxa, suggests less tolerance to low or absent O_2, although they can tolerate hypoxic conditions in some systems (Larsson and Lampert 2012). If O_2 was the only environmental variable controlling the vertical distribution of zooplankton, we would have expected zooplankton to remain in the best-oxygenated waters at the surface or top layers of the epilimnion. However, zooplankton appeared drawn to the depths where Chla, and presumably their prey, was more abundant.

The more detailed analyses of the vertical distribution of zooplankton in KWK6, KWK12, and SAS2A shed light on the environmental variables driving this distribution. In these ponds, the highest zooplankton biomass was in the metalimnion (Fig. 3.3) and based on the DistLM models, dissolved O_2 concentrations emerged as the most important environmental predictor of total zooplankton, rotifer, and copepod distribution (Table 3.2). Given the presence of cladocerans solely at the oxygenated surface (Fig. 3.3), dissolved O_2 would seem to be the main driver of cladoceran vertical distribution, although the low sample numbers prevented us from testing this specifically. The models also indicated that top-down predation applied by *Chaoborus* explained an appreciable proportion of variation. Thus, O_2 concentration and predator avoidance drove much of the vertical distribution of the total zooplankton community. In the absence of fish and adult copepods, *Chaoborus* larvae are expected to be the main predators of zooplankton, both for microzooplankton and cladocerans (Lewis 1977; MacKay et al. 1990). *Chaoborus* biomass varied greatly among ponds, ranging from 0 to 1360 $\mu g\ L^{-1}$. However, as for Cladocera, the abundance of *Chaoborus* can be underestimated; the diel vertical and horizontal migration of larval phantom midges is well documented (Voss and Mumm 1999).

Bégin and Vincent (2017) emphasized the absence of *Chaoborus* in SAS2A samples collected at noon, whereas they observed high numbers in samples collected around midnight. This pattern reflected the trait of *Chaoborus* to hide in the sediments and littoral zones during the day and migrate back to the pelagic zone during the night (Hare and Carter 1986).

The models also included the algal food sources (phytoplankton and EFA) among the best models, reflecting their influence on the vertical distribution of zooplankton. Phytoplankton biomass was highest in the hypolimnion of the thaw ponds (Fig. 3.3), most probably due to the high concentration of nutrients observed at these depths (Roiha et al. 2015). However, the light limitation in deeper waters (Supporting Information Fig. 3.S2) is likely to severely restrict photosynthesis and primary production, thereby promoting the dominance of mixotrophic species in the algal community. Accordingly, most of the phytoplankton taxa in the sampled thaw ponds (*Chlorella* sp., *Chrysochromulina* sp., and *Cryptomonas* sp., data not shown) are well documented as mixotrophic taxa that can act as both producers and consumers of organic carbon, depending on environmental conditions (Jones 1994). Furthermore, certain species of phytoplankton can migrate vertically in the water column (Reynolds 1984), especially in humic and sharply stratified shallow ponds where they can move between the photic zone and nutrient-rich hypolimnetic waters (Jones 1991). Despite the overall high biomass of phytoplankton in deeper waters, the concentration of EFA increased only to a certain depth before decreasing close to the dark bottom. This was particularly evident in the very turbid waters of SAS2A (Fig. 3.3; Supporting Information Fig. 3.S2) and suggests a low quality of algae in the aphotic bottom waters of thaw ponds. The best models did not include bacterial biomass as an explaining variable for any of the taxonomic groups. Although rotifers and cladocerans can feed on bacteria, the higher quality algal diet seemed to be more important in determining the vertical position of zooplankton in the water column.

Our sampling was restricted to the daytime, and zooplankton are known to migrate diurnally throughout the water column (Hutchinson 1967). The patterns of diel vertical migration displayed by rotifers and copepods are, however, known to be rather consistent in these ponds (Bégin and Vincent 2017).

3.6 Conclusion

Climate change and the associated thawing permafrost are modifying northern landscapes and altering drastically hydrological regimes, biogeochemical processes, and biological production in arctic and subarctic lakes and ponds (Wrona et al. 2016; Wauthy et al. 2018). Presently, rotifers appear to benefit from these rapid changes, with subarctic thaw ponds containing a high biomass of these pelagic micrograzers. The strong thermal structure of thermokarst lakes and ponds leads to a pronounced stratification of O_2 that drives the vertical distribution of zooplankton and possibly the overall species composition as well by selecting and organizing species according to their tolerance to hypoxic conditions. Future warming (Wrona et al. 2016) and browning (Wauthy et al. 2018) of circumpolar freshwaters are expected to enhance the thermal stability of thaw ponds and therefore increase the potential of anoxia throughout the water column (Deshpande et al. 2015). This scenario adds much uncertainty in terms of the future response of arctic and subarctic zooplankton communities to climate warming and degrading permafrost.

3.7 Acknowledgments

We are grateful to the Centre for northern studies (CEN) Whapmagoostui-Kuujjuarapik Station and its manager, Claude Tremblay, for providing access to transportation and lab facilities. We also thank Laura Forsström for her help and cheerfulness in the field as well as for the estimates of phytoplankton biovolume, Pierre Carrier-Corbeil for fatty acid analyses, Ginette Méthot for her expertise in zooplankton identification, and Murray Hay for his editorial assistance. The funding was provided by the Canada Research Chairs Program and the CEN. The PhD grant of Maxime Wauthy was also partly supported by the Merit Scholarship Program for Foreign Students from the Ministère de l'Éducation et de l'Enseignement Supérieur du Québec.

3.8 References

The reference list of each individual chapter has been included in the final reference section at the end of the thesis.

3.9 Supporting information

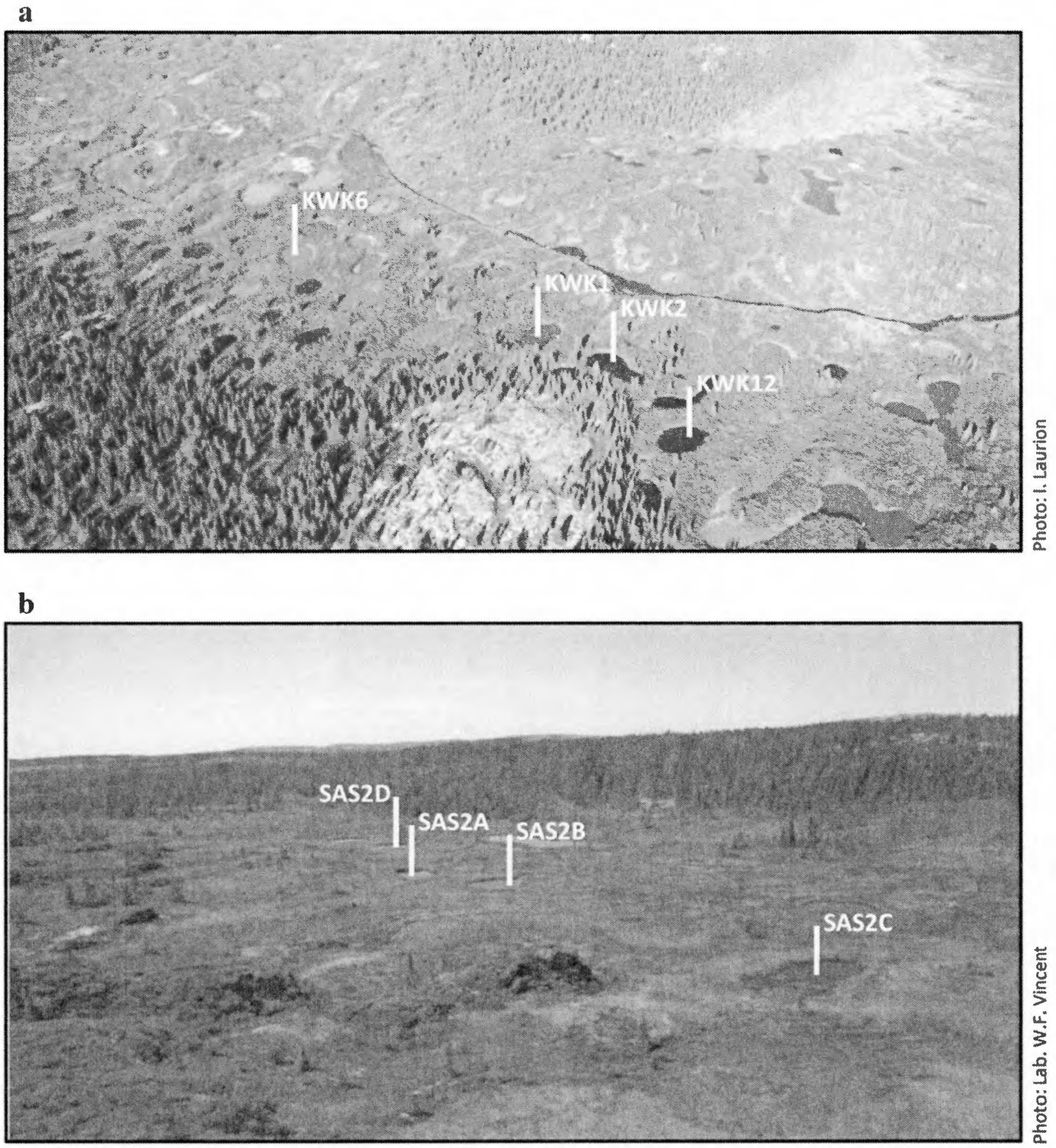

Photo: I. Laurion

Photo: Lab. W.F. Vincent

Figure 3.S1 Oblique aerial photographs showing the sampled ponds in the thermokarst valleys of the (a) Kwakwatanikapistikw (KWK) and (b) Sasapimakwananistikw (SAS) rivers.

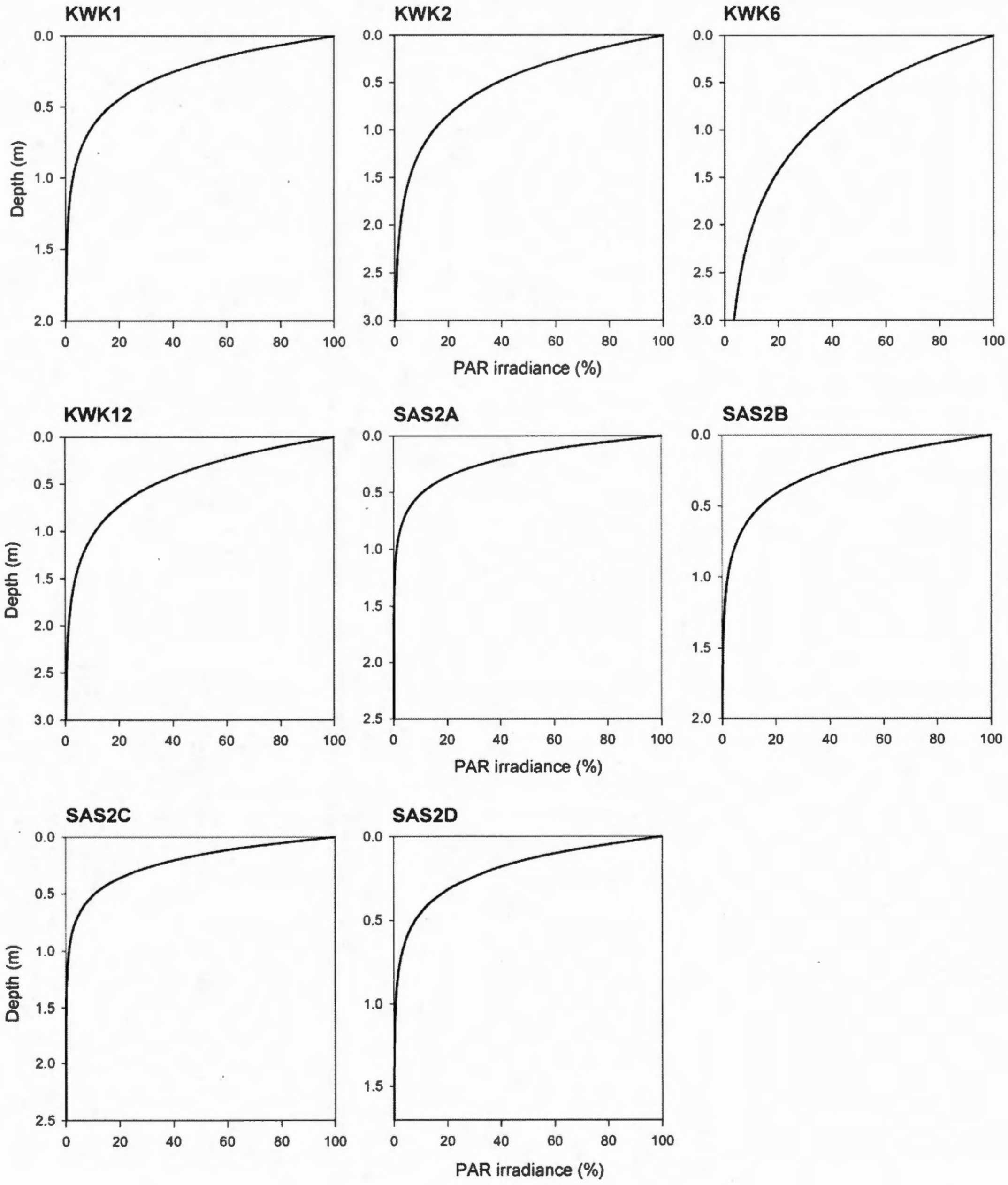

Figure 3.S2 Vertical profile of photosynthetically active radiation (PAR) percentage in sampled thaw ponds.

Table 3.S1 Identified zooplankton taxa and their average abundance (ind L^{-1}) through the water column of the sampled thaw ponds.

Taxa	KWK1	KWK2	KWK6	KWK12	SAS2A	SAS2B	SAS2C	SAS2D
Rotifers	**1 341.60**	**2 432.00**	**1 473.00**	**2 300.48**	**1 582.40**	**418.27**	**3 404.53**	**1 286.33**
Anuraeopsis fissa	5.60	135.47	28.00	71.73	6.93	0.13	3.47	5.50
Ascomorpha spp.	1.20	95.20	4.00	92.16	12.00	2.67	22.93	10.83
Asplanchna spp.	0.53	1.07	0.67	0.32	0.53	0.00	0.00	0.00
Cephalodella gibba	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
Euchlanis alata	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.50
Gastropus hyptopus	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Gastropus stylifer	0.00	0.80	0.00	0.00	1.33	0.00	0.00	0.00
Kellicottia longispina	1.47	2.93	0.83	1.33	1.33	0.00	0.00	0.83
Keratella cochlearis	213.60	260.53	870.67	1 422.03	146.40	14.97	46.67	47.00
Keratella earlinae	0.00	0.00	0.67	0.00	78.93	0.00	0.00	0.00
Keratella hiemalis	0.53	2.13	0.17	0.00	1.87	0.57	8.27	6.00
Keratella serrulata	0.00	0.00	0.00	2.13	0.00	0.00	0.00	0.33
Keratella sp.	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Keratella taurocephala	0.00	0.00	0.00	12.85	0.00	0.00	0.00	0.00
Keratella tecta	480.80	1 325.07	34.50	380.11	0.00	30.70	441.87	15.67
Keratella testudo	10.13	9.60	0.17	0.00	62.80	10.40	58.93	168.67
Keratella valga	12.27	21.33	0.00	14.03	0.00	50.33	21.60	8.33
Lecane mira	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Lecane sp.	0.53	0.00	0.00	0.00	3.60	0.27	1.60	0.83
Lecane tenuiesta	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00
Lecane tudicola	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Lepadella sp.	0.00	1.07	0.00	0.00	0.27	0.00	4.27	0.00
Monostyla copeis	0.00	5.07	0.00	0.00	0.27	0.27	2.13	2.00
Monostyla cornuta	0.00	0.00	0.00	0.00	1.73	0.00	0.00	0.00
Monostyla lunaris	0.00	0.00	0.00	0.00	2.80	0.00	0.00	0.00
Notholca foliacea	0.00	1.87	0.67	9.87	0.00	0.00	5.07	0.00
Notholca labis	0.00	1.33	0.00	0.00	0.00	0.27	17.07	0.00
Notholca sp.	0.27	0.00	0.17	0.00	0.00	0.00	0.00	0.00
Ploesma sp.	1.07	0.00	0.00	0.00	1.07	0.00	0.00	0.33
Polyarthra spp.	604.80	490.13	518.67	93.33	1 241.87	302.17	2 721.33	1 008.83
Pompholyx sulcata	2.67	16.00	13.17	18.77	1.33	0.53	4.80	2.17
Synchaeta spp.	0.80	0.27	0.67	0.00	1.33	1.60	0.00	1.50
Trichocerca spp.	4.40	61.33	0.00	181.81	15.20	3.40	44.53	5.50
Trichotria tetractis	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00
Copepods	**26.13**	**37.07**	**2.50**	**41.44**	**41.33**	**21.93**	**134.67**	**26.17**
Cyclopoida nauplii	23.87	34.93	2.50	41.44	40.40	21.93	130.40	23.83
Cyclopoida copepodites	2.27	2.13	0.00	0.00	0.93	0.00	4.27	2.33
Cladocerans	**0.27**	**1.60**	**0.00**	**0.00**	**1.07**	**1.43**	**8.53**	**2.50**
Bosmina sp.	0.00	0.00	0.00	0.00	0.00	0.00	6.93	0.67
Daphnia pulex	0.27	0.53	0.00	0.00	0.80	1.43	1.60	1.33
Holopedium gibberum	0.00	1.07	0.00	0.00	0.27	0.00	0.00	0.50
Total zooplankton	**1 368.00**	**2 470.67**	**1 475.50**	**2 341.92**	**1 624.80**	**441.63**	**3 547.73**	**1 315.00**

Table 3.S2 Relative contribution of variables for all distance-based linear models (DistLM) using a parametric marginal test and a multi-model inference approach.

Variable abbreviation	Variable name	SS (trace)	Pseudo-*F*	*P*	Proportion of variation explained	Summed AICc weights
Total zooplankton						
O_2	Dissolved oxygen	1414.2	4.40	0.05	0.29	0.65
CB	*Chaoborus* biomass	938.1	2.57	0.13	0.19	0.36
BB	Bacteria biomass	170.1	0.39	0.60	0.03	0.24
PB	Phytoplankton biomass	80.9	0.18	0.75	0.02	0.23
EFA	Essential fatty acids (EFA)	4.9	0.01	0.99	0.00	0.19
Rotifera						
O_2	Dissolved oxygen	1489.6	3.65	0.07	0.25	0.62
PB	Phytoplankton biomass	583.8	1.19	0.30	0.10	0.27
CB	*Chaoborus* biomass	329.2	0.64	0.46	0.06	0.22
BB	Bacteria biomass	67.0	0.12	0.84	0.01	0.19
EFA	Essential fatty acids (EFA)	56.7	0.11	0.86	0.01	0.19
Copepoda						
O_2	Dissolved oxygen	1945.9	2.09	0.15	0.16	0.43
CB	*Chaoborus* biomass	1866.2	1.99	0.16	0.15	0.44
BB	Bacteria biomass	213.3	0.20	0.82	0.02	0.21
EFA	Essential fatty acids (EFA)	171.3	0.16	0.84	0.01	0.27
PB	Phytoplankton biomass	71.4	0.06	0.94	0.01	0.24

GENERAL CONCLUSIONS

4.1 Main contributions

Climate change and the associated thawing of permafrost are expected to modify northern landscapes and lead to the drastic alteration of the hydrological regimes, biogeochemical processes, and biological production in high-latitude lakes (Vonk et al. 2015; Wrona et al. 2016). In this context, this thesis aimed to investigate the influence of degrading ice-rich permafrost on the carbon pools and food webs in circumpolar northern freshwaters and fill gaps in knowledge related to the carbon cycle in subarctic and arctic thaw ponds. To our knowledge, this is the first time permafrost thaw influences have been reported in this extent to waterbodies in the circumpolar North. Furthermore, whereas previous studies have focused on microbial activity (e.g., Deshpande et al. 2016) or greenhouse gas emissions (e.g., Matveev et al. 2016) from thermokarst systems, the analysis of the aquatic thermokarst ecosystem as a whole—from dissolved organic matter to the higher levels of the food web—has never been undertaken until now.

More specifically, the following main contributions emerge from the three different research chapters (Fig. 4.1):

I. Permafrost thaw induces rapid changes in northern high-latitude freshwaters, releasing stocks of organic carbon that end up in aquatic systems. This is expressed by higher DOC concentrations and DOM that displays a strong terrestrial imprint in thermokarst ponds in comparison with waterbodies that are not affected by degrading permafrost. This increased terrestrial influence on arctic and subarctic ponds has decreased water column transparency and increased nutrient concentrations. These effects have promoted algal species in the plankton at the expense of benthic algae and have altered biogeochemical processes and the balance between autochthonous and allochthonous production.

II. Despite the marked influence of eroding and degrading permafrost on the chemical and optical properties of the water column and on algae at the base of the trophic chain, the effects of this permafrost thaw on the higher levels of the food web are less pronounced, with relatively low terrestrial organic matter contributing to the biomass of the filter-feeding zooplankton in subarctic thermokarst lakes. Phytoplankton remain the key resource fuelling the food web in thaw ponds despite the overwhelming dominance of terrigenous organic matter. This pattern emphasizes the ability of primary consumers to select for a higher quality diet.

III. In regard to the zooplankton community growing in thermokarst waterbodies, permafrost thaw appears to initially favor rotifer populations; these pelagic micrograzers have a high abundance and biomass in northern Quebec thaw ponds. Furthermore, given the strong thermal structure and hypoxia observed in the hypolimnion of thermokarst freshwaters, the zooplankton populations display a vertical stratification within the water column. This stratification is driven mostly by O_2 needs, but it also reflects a compromise between the avoidance of top-down predation and an accessibility to a high-quality algal food source.

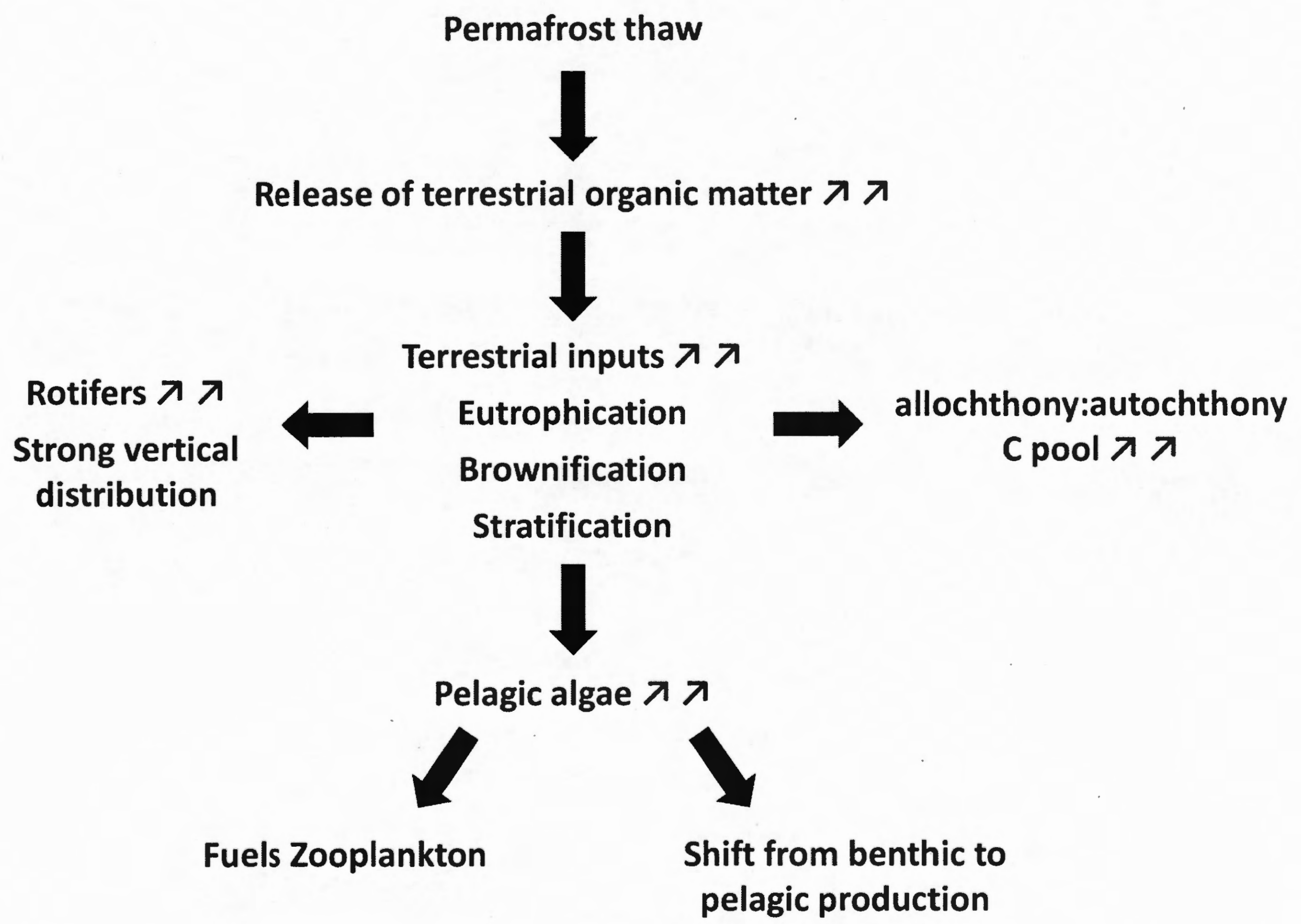

Figure 4.1 Schematic representation of the main conclusions of the thesis.

4.2 Implications of the results and potential avenues for future research

The contributions of this thesis greatly improve our understanding of the effects of permafrost thaw on northern aquatic ecosystems, including the alteration of water quality and the implications for aquatic organisms. Our results provide new knowledge regarding aquatic biogeochemical processes across subarctic and arctic landscapes and offer new observations and tools for future monitoring of these freshwater ecosystems and forecasting the alteration of the circumpolar North in the context of climate change and global warming.

Collectively, our results demonstrate the importance and extent to which thawing permafrost and terrestrial inputs are altering circumpolar freshwaters. These changes may be considered as an example of browning, which is being observed throughout the world with increasing concern (Graneli 2012; Williamson et al. 2015). Because of the erodible and DOM-rich nature of the permafrost landscape, northern browning is an extreme version of this global phenomenon. Coupled with future warming, this heightened browning of northern freshwaters is expected to enhance the thermal stability of waterbodies, increase the risk of anoxia within the water column, and likely shift circumpolar waterbodies toward net heterotrophic conditions and a greater production of greenhouse gases. These consequences once again highlight the importance of including subarctic and arctic lakes and ponds in the assessment of regional and global carbon cycling.

Moreover, given the higher turbidity and the increased amount of nutrients, permafrost thaw is expected to favor phytoplankton growing in the water column at the expense of benthic algae. This shift from benthic to pelagic production is suggested by the higher Chla concentrations in the surface waters of thaw ponds compared with waterbodies not (yet) affected by degrading permafrost. This shift is also evidenced by the extremely low light penetration and the enormous biomass of pelagic algae in the water column of thermokarst freshwaters relative to non-thaw systems. However, the actual measurement of benthic Chla and benthic algal biomass is necessary to properly confirm this shift. In addition, given the notable presence of mixotrophic species observed in thaw ponds, measuring primary production in both the pelagic and benthic zones is required to investigate whether this likely shift of algal biomass from the benthos to the water column has consequences for actual production.

The continually increasing number of thermokarst ponds suggests that global warming will induce the release of more terrigenous material and continue to alter the main metabolic pathways that govern carbon cycling in the North. Previous studies have already emphasized the role of bacteria in the recycling of this old carbon released from thawing permafrost (e.g., Roiha et al. 2015); however, zooplankton and higher trophic organisms were overlooked, although their role in the carbon cycle could be quite important (Yurista 1999). Our results, however, highlight the moderate assimilation of terrestrial carbon by filter-feeding zooplankton within thaw ponds, suggesting a limited role of the pelagic primary consumers in the recycling of this newly released carbon from degrading permafrost. While our research focused on the assimilation of old terrestrial carbon by zooplankton, we do not know about the potential role of zooplankton in the respiration of this carbon. Further research is needed to investigate more fully the role of zooplankton in the carbon cycle of thermokarst systems and to determine if *Daphnia* forage selectively on phytoplankton, or whether the high-quality FA are retained preferentially in zooplankton tissue.

Most studies of thermokarst systems are carried out during single-visit lake surveys. These field samplings are also performed mostly in summer due to the difficult access to these remote sites during the ice-covered period. As such, the seasonally-dynamic nature of terrestrial DOM, primary producers, and zooplankton could be poorly captured, causing potentially erroneous interpretations of the carbon cycle inside these complex ecosystems. Furthermore, the Arctic is currently undergoing fast-paced changes and, as a consequence, uncertainty is quite high regarding future responses of circumpolar freshwaters to global warming and degrading permafrost. More sampling campaigns conducted at different times of the year should be carried out to ensure an accurate picture of what is happening within these rapidly changing arctic lakes and ponds.

Many limnologists consider each lake to be a unique ecosystem. This statement is especially true when it comes to thaw ponds. This thesis emphasizes the unique nature of thermokarst lakes that display some characteristics that have rarely, if ever, been reported for other types of freshwater waterbodies. These atypical aquatic systems challenge conventional perspectives about lake features and their potential role within the global carbon cycle.

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