ÉCOLOGIE DES CYANOBACTÉRIES PLANCTONIQUES DANS LES LACS DE THERMOKARST SUBARCTIQUES

Thèse

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Résumé

Les lacs de thermokarst (lacs et étangs peu profonds causés par le dégel du pergélisol) ont été identifiés comme des écosystèmes d'eau douce d'une importance capitale étant donné leur grande abondance dans le Nord circumpolaire et leur production intense des gaz à effet de serre. L'objectif de cette étude de doctorat était de caractériser le plancton autotrophe de ce type du lac, en mettant l'accent sur deux groupes écologiques de cyanobactéries (les espèces responsables des floraisons et les espèces picoplanctoniques), et d'évaluer leur sensibilité potentielle aux changements climatiques. Des lacs de thermokarst ont été échantillonnés dans une multitude de paysages dans le Nord du Québec où les effets des changements climatiques sont présentement observés. La structure des communautés de phytoplancton ainsi que l'influence des variables environnementales sur celles-ci ont été évaluées par plusieurs méthodes dont le profilage vertical de lacs, l'analyse de pigments photosynthétiques par chromatographie liquide à haute performance, l'analyse de picoplancton par cytométrie en flux, l'analyse moléculaire de la structure des communautés de protistes et l'analyse des échantillons d'eau du lac par microscopie inversée. Aussi, un effet direct (réchauffement) ainsi qu'un effet indirect (enrichissement en phosphore) des changements climatiques sur les lacs de thermokarst ont été évalués par une expérience d'incubation. Enfin, l'effet de la température sur la relation trophique herbivores-cyanobactéries a été évalué à l'aide d'un système en laboratoire. Pour ce faire, des clones tempérés et subarctiques de l'espèce de zooplancton clé *Daphnia pulex* ainsi qu'une souche de picocyanobactéries de haute latitude ont été utilisés.

Les résultats indiquent que les lacs de thermokarst, ainsi qu'un ensemble de lacs de référence, contenaient des pigments photosynthétiques diversifiés provenant du plancton autotrophe. Certains pigments indicateurs pour les cyanobactéries et les bactéries photosynthétiques sulfureuses vertes étaient présents. Les indicateurs de l'état trophique d'un lac (les concentrations de chlorophylle *a* et de phosphore total) ont révélé que les lacs de thermokarst étaient plus eutrophes que les lacs oligotrophes de référence. Les communautés phytoplanctoniques des deux groupes de lacs se composaient de faibles concentrations de cyanobactéries formant des floraisons et de picocyanobactéries,

mais dans des proportions très variables de leur biovolume phototrophe total.

Les résultats des expériences suggèrent que le réchauffement climatique pourrait, à la fois directement et indirectement, stimuler la croissance et la dominance des cyanobactéries ainsi que de détériorer la qualité du phytoplancton dont le zooplancton se nourrit. Les chrysophytes ont également été stimulés par des températures plus chaudes. Le taux de croissance et la performance des daphnies subarctiques ont diminué avec des températures plus élevées et de la nourriture de moins bonne qualité (les seuils d'alimentation ont augmentés), mais dans une moindre mesure que pour le clone tempéré.

Les lacs de thermokarst sont un groupe d'écosystèmes d'eau douce de haute latitude qui sont abondants dans les paysages de fonte du pergélisol et ils se distinguent par quelques caractéristiques limnologiques. Les résultats de cette recherche doctorale démontrent qu'ils contiennent du phytoplancton diversifié en termes de groupes de pigments, de classes de taille et de taxons, et ce malgré la forte atténuation de la lumière par la matière organique dissoute colorée et les particules en suspension, ainsi que par leur caractère fortement hétérotrophe. Les cyanobactéries pourraient devenir plus prédominantes dans les lacs de thermokarst avec les changements climatiques. De plus, l'effet combiné du réchauffement et de l'augmentation des charges en phosphore causeraient des floraisons de cyanobactéries plus fréquentes, ce qui influencerait la diversité du phytoplancton et l'efficacité du réseau alimentaire.

Abstract

Given their great abundance throughout the circumpolar North, and their intense production of greenhouse gases, thermokarst lakes (shallow lakes and ponds caused by thawing permafrost) have been identified as a globally important class of freshwater ecosystems. The objective of this doctoral study was to characterize the autotrophic plankton of this lake type, with emphasis on two ecological groups of cyanobacteria (bloom-formers and picoplankton) and their responsiveness to climate change. Thermokarst lakes were sampled across a range of landscapes in northern Quebec. Phytoplankton community structure and relationships with environmental variables were assessed with a combination of methods including limnological profiling, pigment analysis by high performance liquid chromatography, picoplankton analysis by flow cytometry, molecular assays of protist community structure, and analysis of lake water samples by inverse microscopy. Additionally, an incubation experiment with thermokarst lake water was performed to evaluate the potential direct (warming) and indirect (phosphorus enrichment) effects of climate change. Finally, a laboratory system was designed and applied to test the effects of temperature on herbivore-cyanobacteria feeding relationships using subarctic and temperate clones of the keystone species *Daphnia pulex*, and a high latitude strain of picocyanobacteria.

The results showed that thermokarst lakes as well as a set of reference rock-basin lakes contained diverse pigments originating from autotrophic plankton, including some pigments specific for cyanobacteria and green photosynthetic sulfur bacteria. Indicators of trophic status (chlorophyll *a* and total phosphorus concentrations) showed that the thermokarst lakes were more enriched than the oligotrophic reference lakes. The phytoplankton communities of both groups contained low concentrations of bloom-forming cyanobacteria and picocyanobacteria, but in highly variable proportions of their total phototrophic biovolume.

The experimental results indicated that climate warming may both directly and indirectly stimulate cyanobacterial growth and dominance, and may cause a decrease in phytoplankton food quality for zooplankton. Chrysophytes were also stimulated by

warmer temperatures. The growth rate and performance of the subarctic *Daphnia* clone was negatively affected by higher temperatures and lower food quality (increased feeding thresholds), but to a lesser extent than the temperate clone.

Overall, thermokarst lakes are a class of high latitude freshwater ecosystems that occur in high abundance across thawing permafrost landscapes and that have a number of distinctive limnological properties. Despite the strong attenuation of light by their coloured dissolved organic matter and suspended particles, and their strongly heterotrophic character, the results of this research show that they contain diverse phytoplankton in terms of pigment groups, size classes and taxa. Cyanobacteria may become more prevalent in these waters as a consequence of ongoing climate change. Cyanobacterial blooms are likely to follow the combined effects of warming and increased phosphorus loading, and would in turn affect phytoplankton diversity and the efficiency of food web processes.

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Avant-propos

Cette thèse rassemble les travaux découlant de ma recherche doctorale sous la direction du professeur Warwick F. Vincent. Elle est composée de cinq chapitres, dont trois sont présentés sous forme d'article scientifique. Je suis l'auteur principal de tous ces articles et le responsable de la planification et la réalisation des travaux sur le terrain, la plupart des analyses en laboratoire, l'interprétation de données et la rédaction des articles. L'organisation de cette thèse est la suivante:

Chapitre 1 - Introduction générale

Chapitre 2 - Phototrophic pigment diversity and picophytoplankton abundance in permafrost thaw lakes. **Anna Przytulska**, Jérôme Comte, Sophie Crevecoeur, Connie Lovejoy, Isabelle Laurion et Warwick F. Vincent, est soumis à un numéro spécial "Freshwater ecosystems in changing permafrost landscapes" de Biogeosciences.

Chapitre 3 - Preconditions for cyanobacterial bloom development in northern lakes: Direct and indirect effects of climate change. **Anna Przytulska** et Warwick F. Vincent, sera soumis à Freshwater Biology.

Chapitre 4 - Climate effects on high latitude *Daphnia* via food quality and thresholds. **Anna Przytulska**, Maciej Bartosiewicz, Milla Rautio, France Dufresne et Warwick F. Vincent, est publié dans PLoS ONE, 10: e0126231.

Chapitre 5 - Conclusion générale

Les résultats de ma recherche doctorale ont également donné lieu à des communications scientifiques que j'ai présentées devant des pairs lors de congrès nationaux et internationaux:

Przytulska-Bartosiewicz, A. and Vincent W.F. (2012) The role of planktonic cyanobacteria in the food webs of subarctic freshwater ecosystems. The International Polar Year Conference "From Knowlege to Action", Montreal, Canada.

Przytulska-Bartosiewicz, A. (2012) Northern high latitude lakes, cyanobacterial blooms and the world water crisis. 2012 ASLO Aquatic Sciences Meeting. Lake Biwa, Shiga, Japan.

Przytulska-Bartosiewicz, A. and Vincent W.F. (2012) The effects of warming and nutrient enrichment on bloom-forming cyanobacteria in subarctic lakes. ArcticNet 8th Annual Science Meeting, Vancouver, Canada.

Przytulska-Bartosiewicz, A., Laurion, I. and Vincent, W.F. (2014) Permafrost aquatic ecosystems in the fast changing North: effects of nutrients and temperature on phytoplankton community structure. THAW 2014 Thermokarst Aquatic ecosystems Workshop, Université Laval, Québec City, Canada.

Przytulska-Bartosiewicz, A., Bartosiewicz, M., Rautio, M., Dufresne, F. and Vincent, W.F. (2014) Arctic zooplankton responses to climate change: effects of temperature and cyanobacteria on polyploid *Daphnia pulex*. Genomes to Biomes, Montreal, Canada.

Chapitre 1 Introduction générale

1.1 Introduction

Les régions de pergélisol, qui sont caractérisées par une partie du sol demeurant gelée pendant au moins deux années consécutives, occupent 22% de la superficie des terres de l'hémisphère nord (Zhang et al., 1999). Le pergélisol est beaucoup plus rare dans l'hémisphère sud, où il se retrouve dans les régions de montagne, dans les îles subantarctiques, et dans le continent Antarctique (Schuur et al., 2008). Il est un important réservoir mondial de carbone organique et une source de gaz à effet de serre (CO_2 et CH_4) dans l'atmosphère (Tarnocai et al., 2009). Ces émissions peuvent être intensifiées par la dégradation du pergélisol, à cause de la thermo-érosion, et des processus thermokarstiques (le dégel et l'érosion du pergélisol riche en glace) résultant du réchauffement climatique global. De récentes études, menées à des sites de hautes latitudes, ont clairement démontré que les plans d'eau résultant de l'action thermokarstique (les lacs de thermokarst) agissent comme des conduits efficaces de transfert d'ancien carbone retenu dans le sol vers l'atmosphère dans un contexte de réchauffement climatique (Walter et al., 2006; Laurion et al., 2010; Neghandi et al., 2013).

Les changements climatiques augmentent la fréquence des perturbations, telles que la formation de thermokarst, le drainage des lacs de thermokarst et des glissements de terrain dûs au dégel rapide du pergélisol, ce qui contribue à accroître le taux de dégradation du pergélisol (Grosse et al., 2011). De plus, l'augmentation de la superficie de ces lacs entraîne des émissions de GES plus élevées, ce qui pourrait, par ricochet, stimuler le réchauffement (Walter et al., 2006). La prévision des taux de recyclage du carbone et des nutriments dans les eaux de thermokarst, ainsi que leur sensibilité biogéochimique et leur réponse au réchauffement, nécessitent la compréhension de leurs caractéristiques biologiques, telles que leur diversité, leur fonctionnement écologique et la relation entre les photoautotrophes et les brouteurs planctoniques.

Les lacs situés sur le pergélisol sont généralement de petite taille (10 à 50 m de diamètre; Hobbie, 1973) et peu profonds (<5 m), avec un ensemble unique de

caractéristiques limnologiques (Breton et al., 2009;. Laurion et al., 2010). Ces lacs sont souvent gelés jusqu'au fond ou près du fond pendant l'hiver et ils sont libres de glace pendant seulement trois mois ou moins au cours de l'année entière, comme ils se retrouvent en haute latitude. L'oxygène peut être réduit dans leurs eaux de fond pendant l'été alors que toute la colonne d'eau, sous la glace pendant l'hiver, peut être anoxique (Deshpande et al., 2015). Les lacs de thermokarst sont souvent troubles à cause de solides en suspension et de la matière organique dissoute colorée (CDOM) et ils peuvent présenter une diversité remarquable de coloration (Watanabe et al., 2011; Fig. 1.1). Aussi, les lacs situés dans une région de pergélisol reçoivent la plupart des eaux de ruissellement au début de l'été étant donné que le neige fond rapidement en juin (Hobbie, 1973) et que les précipitations sont faibles en Arctique. La communauté planctonique pourrait donc être fortement rythmée par le moment et la durée de l'impulsion de nutriments apportée par le dégel au printemps (Sheath, 1986), ainsi que par l'étendue du couvert de glace qui contrôle la disponibilité de la lumière pour la photosynthèse.

L'approvisionnement allochtone de nutriments pour les lacs de thermokarst dépend des conditions météorologiques locales, de la stabilité du sol et de la végétation (Prowse et al., 2006). Or, ces trois variables sont sujettes à être affectées par le réchauffement récent des régions arctiques (Wrona et al., 2006). L'augmentation des températures et des précipitations en haute latitude pourraient entraîner une plus grande charge d'éléments nutritifs dans les lacs de thermokarst, une stratification plus stable de la colonne d'eau ainsi qu'une diminution plus prononcée de l'oxygène dans l'hypolimnion (Vonk et al., 2015a). Il a été démontré que l'augmentation de la disponibilité des sels nutritifs dans les lacs tempérés favorise une plus grande production du phytoplancton. Toutefois, leur diversité était réduite et on notait une prévalence accrue de cyanobactéries responsables pour la formation de floraisons (Flanagan et al, 2003; Paerl, 2008; Vincent 2009). Bien que le phytoplancton des lacs de hautes latitudes est limité davantage par la faible lumière et les températures froides que par la disponibilité des éléments nutritifs, il existe aussi des exemples de limitation en nutriments (par exemple, Levine et Whalen, 2001; Bonilla et al., 2005;. Brutemark et al, 2006). Le réchauffement et l'eutrophisation des lacs subarctiques sont susceptibles d'aller de pair. Pourtant, peu d'attention a été accordée aux effets combinés de ces deux facteurs sur le

phytoplancton de hautes latitudes. L'augmentation des nutriments combinée avec la hausse des températures pourraient causer la prolifération de cyanobactéries, comme c'est observé pour des lacs de latitudes tempérées (Kosten et al., 2012). Cette perturbation de la communauté de phytoplancton vers une plus grande contribution des cyanobactéries pourrait engendrer des effets en cascade pour les brouteurs planctoniques.

L'objectif principal de cette étude était d'apporter une meilleure compréhension de l'écologie du plancton des lacs de thermokarst (les lacs et les étangs résultant du dégel du pergélisol). L'accent a été mis sur deux groupes de cyanobactéries du phytoplancton : les taxons qui forment les floraisons nocives et les cellules de petite taille, les picocyanobactéries. Ce choix est justifié par leurs sensibilités potentielles au réchauffement climatique et par leurs implications pour les processus du réseau alimentaire. Les objectifs spécifiques de cette thèse doctorale étaient les suivants: i) caractériser la communauté de phytoplancton des lacs de thermokarst situés dans la zone de pergélisol discontinu du nord du Québec (Canada), en utilisant une combinaison de la cytométrie en flux, ainsi que des analyses des pigments planctoniques, des analyses moléculaires et des énumérations par microscopie; ii) évaluer les effets de l'enrichissement en nutriments et le réchauffement sur la biodiversité du phytoplancton et l'abondance des cyanobactéries dans les lacs de thermokarst; et iii) déterminer comment les effets directs (réchauffement) et indirects (les cyanobactéries comme source de nourriture) des changements climatiques affectent le zooplancton. Dans les sections de l'introduction qui suivent, l'état actuel des connaissances sur les facteurs qui contrôlent la diversité et l'abondance du plancton photoautotrophe dans les lacs de hautes latitudes et les effets directs et indirects potentiels des changements climatiques sur la composition de la communauté de plancton seront exposés. Par la suite, les sites de l'étude, l'organisation de la thèse et les objectifs de chaque chapitre seront présentés.

1.2 La diversité et les facteurs contrôlant le plancton dans les lacs subarctiques

La présence de plancton dans les eaux de hautes latitudes fut signalée pour la première fois au cours des expéditions polaires du 19ème siècle, lorsqu'il fut rapporté que des eaux apparemment sans vie, caractérisées par une couverture de glace permanente, contenaient une «multitude d'êtres vivants à étudier» (Murray, 1910). L'abondance du plancton dans les régions polaires est devenue de plus en plus évidente pendant le 20ème siècle. Les premières recherches limnologiques des lacs situés dans les vallées sèches de McMurdo en Antarctique, effectuées par Goldman et al. (1967), ont signalé que des souches de *Synechococcus* sp. et *Synechocystis* sp. étaient non seulement présentes dans ces lacs, mais qu'elles étaient responsables de maxima de chlorophylle dans les eaux profondes. Des études ultérieures dans différents milieux aquatiques de hautes latitudes ont permis d'établir qu'il y a une abondance de vie, y compris de picocyanobactéries (Vincent, 2000b), sous le couvert de glace et dans les lacs profonds de l'Arctique et de l'Antarctique.

1.2.1 Pigments planctoniques

La composition des pigments de phytoplancton a été utilisée dans les évaluations de la structure de la communauté phytoplanctonique ainsi que dans les études de réponses aux gradients environnementaux dans les mers et les océans (Ecrire et Enden, 2000). Cette approche a également été utilisée dans les études des lacs tropicaux (Descy et al., 2005) et tempérés (Descy et al., 2000), ainsi que dans les régions antarctiques (Volkman et al, 1988;. Lizotte et Priscu, 1992; Hodgson et al., 2004) et arctiques (Quesada et al, 1999; Bonilla et al., 2005;. Bonilla et al, 2009). Les pigments peuvent être classés selon leur fonction comme photoprotecteur ou photosynthétique, bien que certains groupes ont des fonctions mixtes. Les études de Bonilla et al. (2005; 2009), réalisées sur des lacs arctiques peu profonds, ont révélé des différences de pigments entre les communautés benthiques et planctoniques. En effet, les communautés benthiques contenaient des concentrations élevées de phycobiliprotéines qui captent la lumière, des composés protecteurs comme les caroténoïdes, et des pigments qui filtrent le rayonnement UV, tels que scytonémine. En revanche, le phytoplancton en suspension contenait des ratios élevés des pigments Chl *c2* et fucoxanthine par rapport à Chl *a*, ce qui indique des populations abondantes de chrysophytes. Bonilla et al. (2005) ont

également obtenu des réponses contrastées entre les pigments benthiques et les pigments planctoniques lors d'une expérience d'enrichissement avec des sels nutritifs. Les résultats suggéraient que, contrairement au phytoplancton, les phototrophes benthiques des tapis microbiens sont dans un milieu où les éléments nutritifs sont en disponibilité suffisante (il n'y a pas eu de réponse suite à l'addition des éléments nutritifs).

De plus, les études de pigments phytoplanctoniques dans les lacs arctiques ont permis de démontrer l'omniprésence et l'importance des cyanobactéries dans ces eaux. Par exemple, Quesada et al. (1999) a avancé que les assemblages de cyanobactéries, notamment des taxons benthiques, sont les photoautotrophes dominants dans les écosystèmes d'eau douce en Arctique. Une étude réalisée au lac Ward Hunt (Bonilla et al., 2005) a également révélé des ratios élevés de zéaxanthine par rapport à Chl a, ce qui indique la présence de cyanobactéries (conclusion confirmée par la microscopie). De même, l'étude d'un lac méromictique du haut Arctique a indiqué que la perte du couvert de glace était accompagnée par un mélange accru de la colonne d'eau (qui accroît l'apport d'éléments nutritifs), ce qui engendra une grande augmentation de zéaxanthine en profondeur (Veillette al., 2011). Ce résultat fut confirmé par une augmentation concomitante des concentrations de cellules de picocyanobactéries. Les évaluations de la structure des pigments planctoniques fournissent des informations complémentaires à une approche basée sur la microscopie. L'utilisation de ces deux méthodes est pertinente pour évaluer les liens entre les changements climatiques et les cyanobactéries planctoniques.

1.2.2 Analyses moléculaires de la diversité du plancton dans les lacs de thermokarst

Depuis l'avènement du séquençage à haut débit de l'ADN, l'application d'outils moléculaires est une approche privilégiée pour l'analyse des communautés microbiennes des lacs et des océans (Bowler et al., 2009). Ces méthodes sont présentement utilisées dans les études portant sur la diversité des lacs de thermokarst. Toutefois, seuls les procaryotes ont été examinés jusqu'à maintenant dans cet environnement. De récentes études par de Rossi et al. (2013), Negandhi et al. (2014) et Crevecoeur et al. (2015) ont révélé que les lacs formés par le dégel du pergélisol contiennent des communautés bactériennes abondantes et diversifiées. Les concentrations bactériennes mesurées par Rossi et al. (2013), allant jusqu'à 4×10^6

cellules par mL, sont plusieurs fois supérieures à celles rapportées pour les lacs dystrophes (Allgaier et Grossart, 2006) et humiques (Graham et al., 2004). Ces résultats suggèrent que les cyanobactéries peuvent être déjà présentes dans les eaux de thermokarst, et même être une composante importante du phytoplancton dans certains cas. Ces analyses ont également souligné l'importance éventuelle des bactéries photosynthétiques sulfureuses vertes dans le métalimnion et l'hypolimnion de certains lacs de thermokarst qui sont stratifiés. De plus, Negandhi et al. (2013) ont observé des communautés procaryotes abondantes et diversifiées dans la colonne d'eau et les sédiments de lacs polygonaux de hautes latitudes, incluant les bactéries méthanogènes. Par ailleurs, Crevecoeur et al. (2015) ont rapporté que les méthanotrophes représentaient jusqu'à 27% des séquences bactériennes dans les lacs de thermokarst subarctiques, et que des espèces de phototrophes oxygéniques (cyanobactéries) et anoxygéniques (Chlorobi) étaient également présentes. Cependant, les outils moléculaires demeurent jusqu'à maintenant moins utilisés pour évaluer la diversité eucaryote dans les lacs de hautes latitudes. Un exemple d'une telle étude est celle de Charvet et al. (2014) qui ont démontré, par des analyses moléculaires, que les chrysophytes et les dinoflagellés (incluant des taxons mixotrophes) sont particulièrement abondants dans les lacs couverts de glace du haut Arctique (Charvet et al., 2014).

1.2.3 Picophytoplancton

Les picocyanobactéries dominent les communautés photoautotrophes dans les océans et les mers des régions tropicales et tempérées. Cependant, elles sont mystérieusement absentes des eaux océaniques froides des hautes latitudes. Les premières études du phytoplancton dans les lacs de hautes latitudes (Kalff et Welch, 1974; Kalff et al., 1975) étaient orientées sur les échantillons de nanoplancton et de microplancton et n'ont pas considéré l'importance potentielle des picocyanobactéries. Or, l'avènement de la microscopie à fluorescence au cours des dernières décennies a révélé une abondance inconnue de picocyanobactéries dans le phytoplancton des lacs de hautes latitudes (Vincent et al., 2000a). Par exemple, dans les lacs subarctiques du nord du Québec, les picocyanobactéries, comme *Synechococcus* spp., peuvent représenter de 20 à 80% de la production primaire totale (Bergeron et Vincent, 1997). Les picocyanobactéries sont également abondantes ($1{,}65 \times 10^5$ cellules par mL; Veillette et al., 2011) et représentent un groupe génétiquement diversifié (Van Hove et al., 2008) dans les

lacs de l'Île d'Ellesmere. De plus, des concentrations élevées de picocyanobactéries, atteignant 10^7 cellules par mL, ont été signalées dans Ace Lake en Antarctique (Rankin et al., 1997).

Les picoeucaryotes surpassent les picocyanobactéries dans le froid de l'océan Arctique (Lovejoy et al 2007; Balzano et al, 2012). Par contre, peu d'études ont évalué l'abondance de ces eucaryotes microbiens dans les eaux douces arctiques et subarctiques. Rae et Vincent (1998) ont rapporté que les picoeucaryotes contribuent jusqu'à environ 1% du picophytoplancton total au lac Kayouk, un lac glaciaire subarctique dans le nord du Québec (Canada). Vallières et al. (2008) ont constaté que la contribution des picoeucaryotes au picoplancton total augmentait avec la salinité dans un transect rivière-estuaire-mer arctique. Également, dans une étude reliée à celle de Vallières et al. (2008), Waleron et al. (2007) ont démontré que la contribution des picocyanobacteriéries au biovolume de picophytoplancton était de 20% dans le fleuve Mackenzie, de 10% dans l'estuaire et de 5% sur le plateau continental, suggérant que les cellules de picocyanobactéries retrouvées dans l'océan provenaient des eaux intérieures.

1.2.4 Phytoplancton

Les lacs de hautes latitudes peuvent contenir des communautés phytoplanctoniques diversifiées (Tableau 1.1), et ce malgré un climat hostile. Le phytoplancton joue un rôle crucial dans les cycles biogéochimiques des nutriments et du carbone dans ces écosystèmes (Polis et Strong, 1996). Cependant, la vitesse et l'efficacité du transfert de carbone et d'énergie du phytoplancton à des niveaux trophiques supérieurs dépendent de la composition de la communauté (Müller-Navarre et al., 2000). Par exemple, le carbone des cyanobactéries unicellulaires est probablement transféré inefficacement dans le réseau alimentaire en raison de la carence en stérol de ces organismes (von Elert et al., 2003), tandis que le carbone des cyanobactéries filamenteuses possède peu d'utilité trophique en raison de contraintes mécaniques pour l'ingestion (par exemple, Bednarska et Dawidowicz, 2007).

Les premières mentions de cyanobactéries dans les habitats aquatiques de hautes latitudes sont contemporaines et ont eu lieu lors des premières expéditions en Arctique

(Leslie, 1879) et en Antarctique (Murray, 1910). Bien que les cyanobactéries soient généralement considérées comme des organismes préférant les milieux chauds plutôt que froids (Robarts et Zohary, 1987; 1992), les études de Vincent et al. (1993), Vincent et James (1996) et Vézina et Vincent (1997) ont établi qu'elles peuvent être les producteurs primaires dominants dans les eaux froides en Arctique et en Antarctique. Également, les cyanobactéries sont présentes et représentent une composante abondante du phytoplancton des lacs subarctiques (Dupont, 2009; Rautio et al, 2011). Enfin, les cyanobactéries qui forment des tapis microbiens benthiques peuvent atteindre des biomasses élevées et dominer la productivité primaire dans de nombreux lacs de hautes latitudes (Vincent, 2000b; Singh et Elster, 2007; Komárek et Elster, 2008; Komárek et al., 2012).

1.2.5 Zooplancton

Bien qu'ils possèdent une faible biomasse de phytoplancton, les petits lacs subarctiques accueillent souvent une grande abondance de zooplancton (McLaren, 1958). Toutefois, ces plans d'eau sont parmi les milieux aquatiques les plus hostiles de la planète en raison de leur courte saison de croissance, des niveaux élevés de rayonnement ultraviolet dans la colonne d'eau et du gel jusqu'au fond de l'eau chaque hiver. C'est probablement pourquoi les communautés de zooplancton des lacs subarctiques sont moins diversifiées que celles d'habitats similaires des régions tempérées et tropicales. Les poissons sont absents des lacs de hautes latitudes peu profonds qui gèlent complètement ou presque. La prédation par les planctivores est donc limitée, ce qui pourrait expliquer la faible diversité du zooplancton. Dans ces lacs, la communauté de zooplancton est souvent dominée par quelques espèces qui possèdent des cycles de vie distincts et présentent des adaptations à la forte saisonnalité de l'environnement (Gliwicz et al., 2001). Par exemple, le zooplancton de lacs où les poissons sont absents sur les montagnes Tatra (Pologne) est dominé par deux souches de *Daphnia pulex*. De même, le zooplancton de certains lacs subarctiques est dominé par *Daphnia* sp., *Holopedium* sp. ou, une ou deux espèces de copépodes (Swadling et al., 2001).

Dans les lacs arctiques, le zooplancton herbivore (par exemple rotifères) peut être soumis à la prédation par les copépodes, les cladocères prédateurs, les crevettes (mysidacés) et les insectes (Rautio, 2001). La prédation par les invertébrés joue un rôle important dans le

maintien de la diversité du zooplancton des écosystèmes arctiques et alpins lorsque les poissons sont absents (Sprules, 1972; Hébert et Loaring, 1980). Autrement, la diversité du zooplancton des lacs de hautes latitudes peut être maintenue par des mécanismes intrinsèques reliés à des différences dans l'efficacité à exploiter la nourriture dans des concentrations restrictives (Gliwicz, 1990) et des différences dans la capacité de croître en utilisant de la nourriture de différentes qualités (Bukovinszky et al., 2012).

1.3 Facteurs contrôlant le phytoplancton dans les lacs subarctiques

1.3.1 Effets des éléments nutritifs

L'eutrophisation (l'enrichissement en éléments nutritifs) causée par les activités anthropiques affecte les lacs partout sur la planète (Moss et al., 2012). De nombreuses données indiquent que l'augmentation des concentrations de phosphore et d'azote dans les lacs entraîne la croissance rapide du phytoplancton. Dans ces conditions, le phytoplancton est souvent dominé par les cyanobactéries nocives (Paerl et Huisman, 2008). L'eutrophisation anthropique est plus fréquente dans les régions tempérées et tropicales, mais elle est également possible dans certains lacs de hautes latitudes (Antoniades et al., 2011). Or, il existe une différence notable dans la réponse écologique à l'eutrophisation des communautés phytoplanctoniques entre les régions tempérées et polaires (Smol et Douglas, 2007b). En effet, tandis que dans les lacs tempérés et tropicaux, l'enrichissement en nutriments conduit souvent à la dominance des cyanobactéries, les changements pour les communautés phytoplanctoniques des lacs polaires semblent plus variables. Par exemple, Goldman (1960) a démontré que *Ankistrodesmus* sp. était fortement stimulé par l'addition de nitrate et de sulfate dans les lacs de l'Alaska, alors que Kalff et al. (1975) ont révélé que l'eutrophisation du lac Meretta n'a pas abouti à une augmentation de cyanobactéries planctoniques. Toutefois, il est important de mentionner que ces études ont précédé la découverte des picocyanobactéries. En revanche, Moore (1978) a trouvé une corrélation positive entre l'abondance de cyanobactéries dans les lacs de l'Arctique et les concentrations de phosphore au début de la saison de croissance. Finalement, McCoy (1983, l'Alaska) a identifié un changement d'une communauté de phytoplancton diversifiée à la dominance par *Anabaena*,

une cyanobactérie filamenteuse capable de fixer l'azote atmosphérique, suite à l'enrichissement d'un lac en phosphore.

Les concentrations de nutriments peuvent également être un facteur important dans le contrôle de l'abondance du picophytoplancton. Par exemple, Stockner et Shortreed (1988) ont démontré que dans un lac oligotrophe, le lac Kennedy, la cyanobactérie *Anabaena* dominait la communauté de phytoplancton lorsque les ratios de TN: TP variaient de 10: 1 à 15: 1, tandis que le picophytoplancton dominait une fois que ce ratio était de 35: 1. Aussi, l'étude récente de Mackey et al. (2013) a indiqué une forte réponse du picoplancton du lac Tahoe, un lac ultra-oligotrophe, suite à l'addition d'aérosols contenant une grande concentration d'ammonium et de phosphore. Par contre, Drakare et al. (2003) ont observé que la biomasse et la production du picophytoplancton étaient négativement liées à la fois au N et au P sous toutes les formes. Ces auteurs ont plutôt suggéré que la concentration de DOC était plus fiable pour prédire l'abondance du picophytoplancton. Brutemark et al. (2006), quant à eux, ont démontré que les ajouts de phosphore et d'azote causaient une diminution de la diversité du phytoplancton et l'augmentation frappante de picocyanobactéries dans les étangs arctiques peu profonds.

1.3.2 Effets de la température

Le réchauffement climatique peut affecter directement ou indirectement la biomasse et la diversité du phytoplancton dans les lacs. Un effet direct de l'augmentation de la température est la stimulation de la productivité du phytoplancton au printemps et à l'automne (Tadonleke, 2010). Par contre, l'augmentation de la température pendant l'été peut stimuler ou freiner la croissance phytoplanctonique, selon l'optimum thermique des espèces dominantes. Les cyanobactéries semblent être plus résistantes que d'autres groupes du phytoplancton lors de changements graduels ou rapides de la température ambiante (Robarts et Zohary, 1987; Tang et Vincent, 1999). Elles préfèreraient même des conditions plus chaudes (Vincent 2009a b; Paerl et Huisman, 2009). Par conséquent, le réchauffement climatique pourrait entraîner la dominance des cyanobactéries dans les lacs de hautes latitudes. Par ailleurs, l'augmentation de la température pourrait engendrer une diminution de la taille moyenne du zooplancton (Meerhoff et al, 2007;. Manca et DeMott, 2009;. Senerpont

Domis et al, 2013) ainsi qu'une atténuation de sa capacité à contrôler la prolifération d'algues (Gliwicz, 1990). De plus, on a récemment suggéré que le réchauffement stimulaient les processus hétérotrophes chez les organismes mixotrophes (Wilken et al., 2013). Ces changements trophiques ainsi que la compétition entre les espèces impliquent que le réchauffement pourrait avoir des impacts indirects majeurs pour les communautés phytoplanctoniques de hautes latitudes.

Le phytoplancton des lacs de hautes latitudes pourrait être plus sensible au réchauffement que celui des lacs des régions tempérées ou tropicales, compte tenu qu'il est adapté au froid (Douglas et Smol, 1999). Par exemple, Smol et al. (2005) ont rapporté des changements prononcés et rapides dans les communautés de diatomées; les espèces de diatomées planctoniques, appartenant principalement au genre *Cyclotella*, dominent désormais, au détriment des genres benthiques, tels que *Fragilaria* et *Achnanthes*. Alors que *Cyclotella* préfère les conditions de stratification thermique (Sorvari et al., 2002), d'autres diatomées sont favorisées dans les lacs arctiques froids et isothermes qui sont recouverts de glace pendant la majeure partie de l'année (Lotter et Bigler, 2000). Les colonnes d'eau bien mélangées sont probablement favorables aux diatomées, alors qu'une forte stratification estivale ou sous un couvert de glace serait moins propice à leur croissance et à leur succès écologique. Comme les cyanobactéries colonisant les lacs de hautes latitudes sont psychrotrophes plutôt que psychrophiles (Tang et al., 1997), elles pourraient donc être plus fortement stimulées par des températures plus chaudes que les autres groupes du phytoplancton. Certaines cyanobactéries sont également favorisées par des colonnes d'eau stratifiées, comme les espèces responsables des floraisons ont la capacité de créer des vésicules de gaz afin de contrôler leur position dans la colonne d'eau, et que les infimes picocyanobactéries ont un lent taux de sédimentation.

Le réchauffement climatique aurait des implications pour le picophytoplancton de lacs oligotrophes (Callieri et Stockner, 2000) et de lacs boréaux mésotrophes (Jasser et Arvola, 2003). Ces derniers auteurs ont également suggéré que la stabilité de la colonne d'eau déterminerait l'importance relative des contrôles ascendants ou descendants sur l'abondance du picophytoplancton. La température pourrait également influencer la contribution relative

de picocyanobactéries et de picoeucaryotes dans la communauté de picophytoplancton. Par exemple, Ochs et Rhew (1997) ont constaté une diminution de picoeucaryotes suivie par une augmentation de picocyanobactéries avec une augmentation de la température dans réservoir du sud-est des États-Unis. De manière similaire, Hepperle et Krienitz (2001) ont révélé que des températures plus chaudes favorisaient les picocyanobactéries par rapport aux picoeucaryotes dans des lacs allemands. Des études concernant l'eutrophisation du lac Balaton (Mózes et al., 2006;. Vörös et al, 2009) montrent également que les picoeucaryotes sont capables de croître à des températures bien en-dessous de l'optimum connu pour les picocyanobactéries.

1.3.3 Effets interactifs des nutriments et de la température

De nombreuses études suggèrent que la nature et l'amplitude de la réponse du phytoplancton à l'eutrophisation sont modérées par le climat (Carvalho et Kirika, 2003). Par exemple, Elliot et al. (2006) ont constaté que, sans changement de la température, l'augmentation de la charge de phosphore par 50% pourrait accroître la production annuelle nette de phytoplancton de 10%. Or, lorsque cet enrichissement en phosphore était accompagné d'un réchauffement de 4°C, la productivité diminuait de 0,5% et la composition des espèces changeait. Aussi, bien que *Anabaena* prospère dans des conditions chaudes et riches en nutriments, *Asterionella* semble être favorisé par des conditions plus froides et des niveaux réduits d'éléments nutritifs. Également, dans l'étude de Huber et al. (2008), le moment de l'apparition et de l'effondrement des floraisons de diatomées était déterminé par la limitation en silice ou par le broutage du zooplancton, selon le niveau de nutriments et de la température. De plus, la diversité du phytoplancton semble diminuer dans des conditions plus chaudes et eutrophes, particulièrement dans les systèmes bien stratifiés (Elliot et al., 2006). En outre, l'étude récente menée par De Senerpont Domis et al. (2014) a démontré que le réchauffement et l'eutrophisation pourrait conduire non seulement à une nette augmentation de la biomasse du phytoplancton, mais aussi à l'augmentation des stœchiométries carbone-nutriments, avec des conséquences pour l'ensemble du réseau alimentaire aquatique.

Il y a étonnamment peu d'études sur les effets interactifs de l'enrichissement en nutriments et de la température sur le phytoplancton des lacs de hautes latitudes. Bergstrom

et al. (2013) ont démontré qu'en l'absence de réchauffement, l'enrichissement en N causait seulement un changement mineur dans la production totale de phytoplancton dans les lacs subarctiques. En accord avec Bergstrom et al. (2013), Saros et al. (2013) ont révélé que l'enrichissement en nutriments devenait important dans le contrôle de la population de diatomées des lacs de l'Arctique lorsqu'il était accompagné avec un changement de température. Cependant, aucune de ces études n'a exploré les effets interactifs potentiels du réchauffement et de l'enrichissement en nutriments pour la diversité du phytoplancton, et les effets sur les cyanobactéries qui produisent des floraisons ont été omis.

1.4 Facteurs contrôlant le zooplancton dans les lacs subarctiques

1.4.1 Qualité de la nourriture

Une étude pionnière réalisée par Schindler (1971), qui comprenait des expériences avec trois espèces de zooplancton, a révélé que les taux d'assimilation de la nourriture ainsi que les rendements variaient avec la qualité de la nourriture du zooplancton. Le phytoplancton dont se nourrit le zooplancton peut être classifié comme de bonne ou de mauvaise qualité selon son contenu en acides gras (surtout les acides gras polyinsaturés) ou le rapport stoechiométrique de carbone au phosphore (Gulati et DeMott, 1997). La nourriture de haute qualité permet au zooplancton d'atteindre la taille de reproduction plus rapidement que la nourriture de moindre qualité (Sundbum et Vrede, 1997), de produire davantage de nouveau-nés de haute qualité, et de survivre plus longtemps (Lurling et van Donk, 1997). Dans les lacs arctiques et subarctiques, la qualité de la nourriture varie grandement entre les ressources pélagiques et benthiques (Rautio et Vincent, 2007). Bien que la nourriture benthique contient une plus faible concentration d'acides gras que la nourriture planctonique (Mariash et al., 2011), elle améliore l'apport de nourriture disponible lorsque le phytoplancton se fait rare (Mariash et al., 2014). Dans des conditions plus chaudes, la composition du phytoplancton des lacs de l'Arctique pourrait être modifiée avec une contribution plus élevée de cyanobactéries. Toutefois, en général, les cyanobactéries constituent des aliments de mauvaise qualité pour le zooplancton comme elles sont dépourvues d'acides gras essentiels et elles contiennent relativement moins de P que d'autres aliments (DeMott et Muller-Navarre, 1997). Mis à part ces carences biochimiques, les cyanobactéries filamenteuses et coloniales

sont considérées comme une nourriture de mauvaise qualité pour le zooplancton comme leurs caractéristiques morphologiques interfèrent avec l'ingestion (DeMott et al., 2001).

1.4.2 Température

Le zooplancton vit à l'échelle microscopique, à de faibles valeurs de Reynolds. La température, par son influence sur les forces de viscosité, peut donc affecter ses mouvements et son ingestion alimentaire (Vogel, 1981; Loiterton et al., 2004; Bednarska et Dawidowicz, 2007). Aussi, la température influence le temps de passage à travers l'intestin (Geller, 1975) ainsi que la cinétique enzymatique, ce qui affecte la vitesse à laquelle la nourriture est digérée. De plus, des températures plus élevées conduisent à une maturation plus rapide du zooplancton et à une taille adulte réduite, en raison d'une plus grande perte d'énergie métabolique (McKee et Ebert, 1996). Également, la température affecte le ratio entre le taux de respiration et le taux de production, ce qui mène à une hausse du seuil d'alimentation dans des conditions plus chaudes (Achenbach et Lampert, 1997). Enfin, le réchauffement affecte la morphologie du zooplancton en diminuant le ratio entre la largeur et la longueur. Cette altération entraîne une production de petits nouveau-nés et une réduction de la taille corporelle moyenne au sein de la population en seulement quelques générations (De Senerpont Domis et al., 2013).

Une des caractéristiques les plus frappantes du zooplancton des hautes latitudes est la polyploïdie (*Daphnia*: Beaton et Hebert, 1988; Ward et al, 1993; ostracodes: Havel et Hebert, 1989; *Bosmina*: Little et al., 1997). Les effets du réchauffement climatique sur le zooplancton pourrait dépendre du niveau de ploïdie, étant donné que la taille du génome influence la réponse écologique du zooplancton (Colbourne et al., 2011). Par exemple, Dufresne et Hebert (1998) ont mis en évidence que les daphnies arctiques polyploïdes sont mieux adaptées aux températures froides que leurs homologues diploïdes. En accord avec cette conclusion, l'étude de Yurista (1999) a démontré que l'énergie disponible pour les daphnies arctiques atteint un maximum de portée à environ 13°C. Par conséquent, si les températures moyennes des lacs de hautes latitudes augmentaient au-delà cette valeur, les clones tempérés pourraient devenir plus compétitifs et capables de coloniser les lacs de hautes latitudes. Le réchauffement a également réduit la capacité de maintenir des taux métaboliques favorables

pour un copépode marin de l'Arctique en raison du découplage entre la diminution des taux d'ingestion et l'augmentation du taux de respiration à des températures plus élevées (Alcaraz et al., 2013). Les taux de respiration plus élevés dans des conditions plus chaudes ont été accompagné par une augmentation de l'excrétion d'azote et de phosphore par le zooplancton, par un facteur de 2.5 (Alcaraz et al., 2013). Cette implication de l'augmentation de la température sur le recyclage des nutriments chez le zooplancton pourrait également s'appliquer aux habitats d'eau douce de l'Arctique.

1.4.3 Effets interactifs

L'interaction potentielle entre la qualité de la nourriture et la température pour la croissance et la distribution du zooplancton a d'abord été suggérée par Cole et al. (2002). Une étude ultérieure par Masclaux et al. (2009) a démontré que les effets négatifs d'une nourriture de mauvaise qualité diminuent lorsque la température augmente. Cependant, comme le zooplancton de hautes latitudes est adapté à des températures froides, une augmentation de la température ne compenserait pas nécessairement les effets négatifs dus à une nourriture de moindre qualité. Par exemple, le réchauffement a entraîné une diminution des acides gras contenus dans le zooplancton des lacs de hautes latitudes en Sibérie (Gladyshev et al., 2011). Enfin, l'effet combiné de la température et de la qualité de la nourriture sur la performance du zooplancton de hautes latitudes pourrait être plus important que leurs effets considérés séparément.

1.5 Les sites d'étude

Cette étude a été réalisée avec 14 lacs sans poissons situés au Nunavik dans le Québec subarctique (Fig. 1.2), près du village de Whapmagoostui-Kuujjuarapik (latitude 55 ° 16 'N, longitude 77 ° 44' W), et incluait: 6 lacs glaciaires peu profonds à proximité du village - SRB (55 ° 16 'N, 77 ° 44' W), 4 lacs de thermokarst situés près de la rivière Kwakwatanikapistikw - KWK (55 ° 19 'N, 77 ° 30' W) et 4 lacs de thermokarst situés près de la rivière Sasapimakwananisikw - SAS (55 ° 13 'N, 77 ° 42 'W). De plus, l'étude comprenait des lacs de thermokarst situés à la proximité du village d'Umiujaq - 2 lacs BGR (56 ° 36 'N, 76 ° 12' W) et 2 lacs NAS (56 ° 55 'N, 76 ° 22' W). Voici les caractéristiques de chacun des sites d'échantillonnage:

- SRB – Les lacs glaciaires peu profonds situés près de la côte de la Baie d'Hudson. Ils se trouvent sur un substratum de basalte et le couvert végétal est dominé soit par des graminées/herbes, des lichens/bruyères ou par un couvert arbustif.
- KWK – Les lacs de thermokarst situés près de la rivière Kwakwatanikapistikw. Ces étangs thermokarstiques sont formés dans des dépressions de un à trois mètres de profondeur, suite à la fonte du pergélisol dans des monticules de minéraux (Laurion et al., 2010). Ils se retrouvent sur le pergélisol sporadique, entourés principalement par un couvert arbustif avec des bosquets d'arbres.
- SAS – Les lacs de thermokarst situés près de la rivière Sasapimakwananisikw. Ces lacs se trouvent à la frontière du pergélisol sporadique et ils sont situés dans des tourbières, avec une végétation caractérisée par de la mousse, des herbes et des arbustes.
- BGR – Les lacs de thermokarst situés sur le pergélisol discontinu près de la rivière Sheldrake. Ils reposent sur des argiles minérales et ils sont principalement entourés d'un couvert arbustif.
- NAS – Les lacs de thermokarst situés sur le pergélisol discontinu près de la rivière Nastapoka. Ces lacs sont également, principalement entourés d'un couvert arbustif.

1.6 Organisation de la thèse

L'objectif principal de ma recherche doctorale était d'améliorer notre compréhension de l'écologie du phytoplancton dans les écosystèmes lacustres thermokarstiques. Une attention particulière fut accordée aux cyanobactéries étant donné qu'elles sont très répandues dans les habitats aquatiques de hautes latitudes et que les connaissances concernant leur écologie dans les lacs de thermokarst sont limitées. Cette thèse est divisée en cinq chapitres, et les chapitres 2, 3 et 4 sont rédigés sous la forme d'articles scientifiques. L'étude présentée dans le chapitre 2 vise à identifier les principaux groupes d'organismes phototrophes dans les lacs de thermokarst, en analysant les concentrations et l'abondance des pigments phototrophes ainsi que les types et les abondances de cellules composant le picophytoplancton. Le chapitre 3 décrit la composition taxonomique et la biomasse des communautés phytoplanctoniques des lacs de thermokarst. Il présente aussi les résultats d'une expérience ayant comme objectif d'évaluer les implications des effets directs (réchauffement) et indirects (enrichissement du P) des changements climatiques sur la structure de la communauté phytoplanctonique, y compris sur la croissance et la dominance des cyanobactéries responsables des floraisons nocives. Le chapitre 4 présente une évaluation expérimentale de la valeur nutritionnelle des picocyanobactéries et les effets directs des changements climatiques (réchauffement) ainsi que des valeurs de seuils d'alimentation pour une souche de *Daphnia pulex* isolée d'un lac de thermokarst subarctique. Enfin, le chapitre 5 conclut cette thèse, résume les principales conclusions et identifie les perspectives de recherche pour des études futures portant sur l'écologie du plancton des lacs de thermokarst subarctiques qui sont de plus en plus soumis aux effets des changement climatiques.

Table 1.1 Composition taxonomique des communautés de phytoplancton dans les lacs subarctiques selon la littérature.

Classe	Nombre d'espèces	Genres dominants
Chlorophytes	89	*Ankistrodesmus, Chlamydomonas, Chlorella, Closterium, Cosmarium, Gloeococcus, Oocystis*
Chrysophytes	42	*Chromulina, Dinobryon, Kephyrion, Mallomonas, Ochromonas, Pseudokephyrion, Uroglena*
Bacillariophytes	35	*Asterionella, Cymbella, Eunotia, Fragilaria, Aulacoseira, Navicula, Synedra*
Cyanobacteria	32	*Anabaena, Aphanocapsa, Gloeocapsa, Lyngbya, Merismopedia, Nostoc, Oscillatoria*
Euglenophytes	18	*Euglena, Phacus, Trachelomonas*
Dinophytes	16	*Glenodinium, Gymnodinium, Peridinium*
Cryptophytes	14	*Cryptomonas, Rhodomonas*
Xanthophytes	3	*Characiopsis, Pseudostaurastrum, Vaucheria*
Prasinophytes	1	*Pyramimonas, Monomastix*
Prymnesiophytes	1	*Chrysochromulina*
Total	251	

Sources: Lowe, 1923; Kalff, 1967; Yamagishi, 1967, 1969, 1970; Sheath and Hellebust, 1978; Alexander et al., 1980 and Sheath et al., 1982; cette compilation est modifiée de Sheath (1986).

Figure 1.1. Lacs de thermokarst situés dans la vallée de la rivière Sheldrake (sites BGR), du Québec subarctique, Canada. Cette image illustre la diversité de couleurs de l'eau de ces lacs. Ces variations de couleur sont déterminées par les concentrations de sédiments en suspension et de la matière organique dissoute colorée (Watanabe et al., 2011).

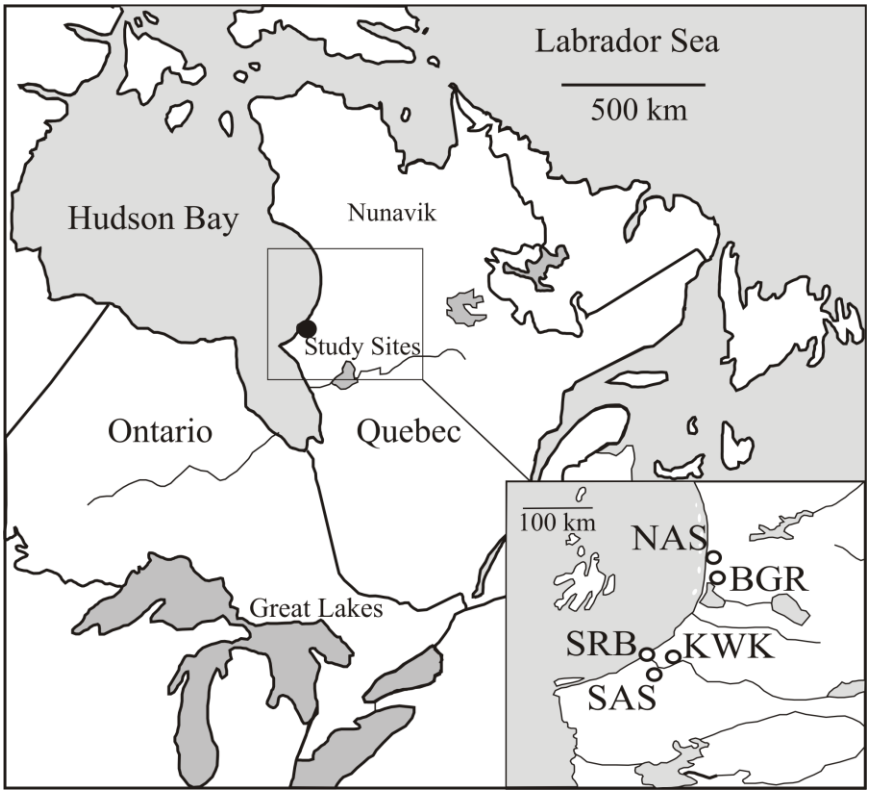

Figure 1.2. La zone d'étude située dans le Québec subarctique (Nunavik).

Chapitre 2 Phototrophic pigments and picophytoplankton in permafrost thaw lakes

2.1 Résumé

Les lacs de dégel du pergélisol (lacs de thermokarst) sont abondamment distribués dans le paysage nordique. Ils sont reconnus comme des sites biogéochimiques actifs qui émettent de grandes quantités de carbone dans l'atmosphère, sous la forme de CH_4 et de CO_2. Toutefois, l'abondance et la composition des communautés photosynthétiques, qui consomment le CO_2, ont été peu explorées pour ce type d'écosystème. Douze lacs de thermokarst, situés le long d'un gradient de dégradation du pergélisol dans le nord du Québec (Canada), ont été échantillonnés afin d'identifier les principaux groupes d'organismes phototrophes ainsi que leurs variables de contrôle. Des échantillons additionnels ont été prélevés dans cinq lacs de référence (qui ne sont pas influencés par le pergélisol) de manière à déterminer si les communautés photosynthétiques et les caractéristiques limnologiques de ces lacs sont différentes de celles des lacs de thermokarst. La structure de la communauté phytoplanctonique a été déterminée par l'analyse de pigments photosynthétiques et photoprotecteurs par chromatographie liquide à haute performance et les concentrations d'autotrophes picoplanctoniques ont été évaluées par la cytométrie en flux. Un des lacs de couleur noire situé dans un paysage de palses (monticules de pergélisol) à dégradation rapide a été sélectionné pour du séquençage de haut débit de ARNr 18S dans le but de faciliter l'interprétation des données de pigments et de cytométrie.

Les résultats ont révélé qu'en plus d'être plus fortement stratifiés, les caractéristiques limnologiques des lacs de thermokarst diffèrent de celles des lacs de référence de manière significative. Néanmoins, des groupes de phytoplancton diversifiés, avec une dominance d'assemblages de pigments pour des taxons contenant de la fucoxanthine, ainsi que des chlorophytes, des cryptophytes et des cyanobactéries, étaient présents dans les deux groupes de lacs. Les concentrations de chlorophylle *a* (Chl *a*) étaient corrélées avec celles du phosphore total (PT). Ces deux indicateurs de l'état trophique d'un lac étaient significativement plus élevés dans les lacs de thermokarst (moyennes globales de 3,3 µg Chl *a* L^{-1} et 34,0 µg TP L^{-1}) que dans les lacs de référence (2,0 µg Chl *a* L-1 et 8,2 µg TP L^{-1}).

Une régression multiple de Chl *a* avec les autres pigments d'algues a indiqué que sa concentration était largement attribuée à la lutéine, la fucoxanthine et la péridinine (R^2 = 0,78). Également, les eaux du fond de deux des lacs de thermokarst contenaient des concentrations élevées de bactériochlorophylle *d*, suggérant la présence de bactéries photosynthétiques sulfureuses vertes.

Les analyses moléculaires ont indiqué une contribution relativement mineure des diatomées, tandis que les chrysophytes, les dinoflagellés et les chlorophytes étaient bien représentés. Ces analyses ont également révélé que la fraction eucaryote hétérotrophe était dominée par de nombreux taxons de ciliés et que des héliozoaires, des Rhizaraires, des chytrides et des flagellés étaient présents. Le picoplancton autotrophe était caractérisé par des biovolume allant jusqu'à 3.1×10^5 μm^3 mL^{-1} pour les picocyanobactéries et de 1.9×10^6 μm^3 mL^{-1} pour les picoeucaryotes. La concentration cellulaire des deux groupes de picophytoplancton était positivement corrélée avec l'abondance totale du phytoplancton (mesurée par la Chl *a*). La concentration cellulaire de picocyanobactéries était inversement corrélée avec la concentration du carbone organique dissous, alors que la concentration cellulaire de picoeucaryotes était corrélée avec la conductivité. Ces résultats suggèrent que les lacs de thermokarst subarctiques sont des habitats importants pour des communautés phototrophes diversifiées et ce, malgré leur caractère hétérotrophe.

2.2 Abstract

Permafrost thaw lakes (thermokarst lakes) are widely distributed across the northern landscape, and are known to be biogeochemically active sites that emit large amounts of carbon to the atmosphere as CH_4 and CO_2. However, the abundance and composition of the photosynthetic communities that consume CO_2 have been little explored in this ecosystem type. In order to identify the major groups of phototrophic organisms and their controlling variables, we sampled 12 permafrost thaw lakes along a permafrost degradation gradient in northern Québec, Canada. Additional samples were taken from 5 rock-basin reference lakes in the region to determine if the thaw waters differed in limnological properties and phototrophs. Phytoplankton community structure was determined by high performance liquid chromatography analysis of their photoprotective and photosynthetic pigments, and autotrophic picoplankton concentrations were assessed by flow cytometry. One of the black colored lakes located in a landscape of rapidly degrading palsas (permafrost mounds) was selected for high-throughput 18S rRNA sequencing to help interpret the pigment and cytometry data. The results showed that the limnological properties of the thaw lakes differed significantly from the reference lakes, and were more highly stratified. However, both waterbody types contained similarly diverse phytoplankton groups, with dominance of the pigment assemblages by fucoxanthin-containing taxa, as well as chlorophytes, cryptophytes and cyanobacteria. Chlorophyll *a* concentrations (Chl *a*) were correlated with total phosphorus (TP), and both were significantly higher in the thaw lakes (overall means of 3.3 µg Chl *a* L^{-1} and 34 µg TP L^{-1}) relative to the reference lakes (2.0 µg Chl *a* L^{-1} and 8.2 µg TP L^{-1}). Stepwise multiple regression of Chl *a* against the other algal pigments showed that it was largely a function of lutein, fucoxanthin and peridinin ($R^2 = 0.78$). The bottom waters of two of the thaw lakes also contained high concentrations of bacteriochlorophyll *d*, showing the presence of green photosynthetic sulphur bacteria. The molecular analyses indicated a relatively minor contribution of diatoms, while chrysophytes, dinoflagellates and chlorophytes were well represented; the heterotrophic eukaryote fraction was dominated by numerous ciliate taxa, and also included Heliozoa, Rhizaria, chytrids and flagellates. Autotrophic picoplankton occurred in biovolume concentrations up to 3.1×10^5 μm^3 mL^{-1} (picocyanobacteria) and 1.9×10^6 μm^3 mL^{-1} (picoeukaryotes). Both groups of picophytoplankton were positively correlated with total phytoplankton abundance, as

measured by Chl *a*; picocyanobacteria were inversely correlated with dissolved organic carbon, while picoeukaryotes were correlated with conductivity. Despite their net heterotrophic character, subarctic thaw lakes are rich habitats for diverse phototrophic communities.

2.3 Introduction

Degradation of ice-rich permafrost leads to the formation of thaw lakes, which are among the most abundant aquatic habitats in high latitude regions (Pienitz et al., 2008; Jones et al., 2012). These environments have attracted increasing scientific interest because of their biogeochemical reactivity. However, although there is rapidly increasing knowledge about their role in greenhouse gas (GHG) emissions (Laurion et al., 2010; Walter et al., 2006), little is known about their photosynthetic communities. Phototrophic organisms consume CO_2 and thereby reduce the net emission to the atmosphere; however, few studies have examined phytoplankton or other phototrophs in these abundant waters. Early studies in the U.S. Tundra Biome Program at Barrow, Alaska, recorded 105 species of algae in tundra lakes and ponds, with dominance of cryptophytes and chrysophytes (Alexander et al., 1980). More recent studies have focused on thaw lake diatoms as paleolimnological indicators, but the dominants in these records are often benthic taxa such as *Pinnularia* and *Fragilaria* (Bouchard et al., 2013). A lake survey in the western Hudson Bay lowlands, including in permafrost catchments, showed that the phytoplankton had diverse communities, primarily composed of cyanobacteria, chrysophytes, chlorophytes, cryptophytes, dinoflagellates and diatoms (Paterson et al., 2014).

Picophytoplankton (PP), consisting of picocyanobacteria and picoeukaryotes (nominally defined as cells 1 to 3 μm in diameter), contribute a major fraction of the total phototrophic biomass across a wide range of aquatic ecosystems (Richardson and Jackson, 2007), including northern lakes and rivers (Waleron et al., 2007; Vallières et al., 2008). In subarctic (Bergeron and Vincent, 1997) and high arctic (van Hove et al., 2008) lakes, picocyanobacteria may dominate the phytoplankton community in terms of biomass as well as cell abundance. For example, in large oligotrophic Clear Water Lake (Lac à l'Eau Claire, Nunavik, Canada), small cell phytoplankton (cell fraction that passed through a 2 μm filter) accounted for 75% of the total phytoplankton Chl *a* (Bergeron and Vincent, 1997). However, the suitability of permafrost thaw lakes as a habitat for picophytoplankton has not been explored.

Our overall aim in the present study was to evaluate by pigment analysis the major groups of phytoplankton in subarctic thaw lakes, and to relate this abundance and community structure to environmental variables. A secondary objective was to determine the abundance and distribution of picocyanobacteria and picoeukaryotes. As a further guide to the composition of the eukaryotic plankton, and in support of the pigment and picoplankton observations, we also applied high throughput 18S rRNA sequencing to surface and bottom waters from one selected lake that was strongly influenced by permafrost degradation. Our study included a wide range of small lakes across the gradient of permafrost degradation in Subarctic Quebec, Canada, from sporadic permafrost landscapes in the south (less than 10% of the area containing permafrost) to discontinuous permafrost in the north (10-90% permafrost). We also took comparative samples from a set of reference, shallow rock-basin lakes that are unaffected by thermokarst processes. Given their limnological variability, as indicated by the variety of water colors among thaw lakes, we hypothesized that there would be large variations in total phytoplankton pigment concentration, pigment diversity and picophytoplankton biovolume. Degrading permafrost soils release dissolved organic carbon (DOC) and fine inorganic particles into the thaw lakes, and these constituents attenuate light down the water column and determine the variability in color (Watanabe et al., 2011). DOC also influences the near surface thermal and stratification regime (Caplanne and Laurion, 2008), and temperature is known to exert a direct effect on phytoplankton community structure, particularly favouring cyanobacterial dominance (Paerl and Huisman, 2008). We therefore hypothesised that DOC and temperature would be the primary drivers of variations in phytoplankton pigmentation and picophytoplankton biovolume.

2.4 Materials and Methods

2.4.1 Study Sites

Twelve thaw lakes (small perennial waterbodies created by thermokarst erosion of the permafrost) were sampled in subarctic Québec during the period of warm open-water conditions, in late summer (August) 2011 and 2012 (Table 2.6). The lakes were distributed along a north-south permafrost degradation gradient and across four geographically distinct locations: the Sasapimakwananisikw River valley (SAS) and the Kwakwatanikapistikw River

valley (KWK) near Whapmagoostui-Kuujjuarapik; and the Sheldrake River valley (BGR) and the Nastapoka River valley (NAS) near Umiujaq. The KWK and SAS valleys occur within the sporadic permafrost landscape, while the BGR and NAS valleys are located in the discontinuous permafrost landscape (Fig. 2.1). Each valley is characterised by distinct vegetation cover and soil structure. Lakes located within the KWK valley are situated on impermeable clay-silt beds where the drainage basin is covered with dense shrub vegetation (Breton et al., 2009), whereas lakes in the SAS valley are located in peatlands in which permafrost mounds (palsas) are thawing and degrading rapidly (Bhiry et al., 2011). The lakes located in the northern valleys (BGR, NAS) are situated on marine clay-silt beds and are surrounded by forest and shrub tundra. In addition to permafrost thaw lakes, a set of five shallow rock-basin lakes (SRB) located on basalt bedrock was sampled in the vicinity of Whapmagoostui-Kuujjuarapik. These provided a set of reference lakes that are located at the same latitude and climatic setting, but without the direct influence of degrading permafrost that is experienced by the thaw lakes. The dates of sampling are given in Table 2.6.

2.4.2 Physicochemical analyses

Profiles of temperature, dissolved oxygen, conductivity, and pH of the 17 lakes were recorded with a 600R multiparametric probe (Yellow Springs Instrument Co.). Additionally temperature and conductivity were recorded with RBR XR620 conductivity-temperature-depth profiler (Richard Brancker Research Ltd). Near surface water samples (0.2 m depth) were collected into dark polyethylene bottles, previously washed with 10% hydrochloric acid and rinsed in MQ water. The samples were stored in coolers and transported to laboratory within 4 h of collection. The total nitrogen (TN) and total phosphorus (TP) measurements were performed on unfiltered water samples collected in 125ml bottles, acidified with sulfuric acid (0.2% final concentration), and stored at 4°C until persulfate digestion. TN concentrations were then measured with a Lachat flow injection analyzer and TP concentrations were measured using a Genesys 10UV spectrophotometer (Thermo Spectronic) and standard techniques (Stainton et al., 1977). Total suspended solids (TSS) were collected onto pre-combusted and pre-weighed glass fiber filters (Advantec MFS) that were dried for 2 h at 60°C and weighed on a Sartorius high precision balance. Dissolved organic carbon (DOC), colored dissolved organic matter (CDOM), soluble reactive

phosphorus (SRP) and nitrate (NO_3^-) measurements were performed on water filtered through 0.2 μm cellulose acetate filters (Advantec MFS). Samples for DOC analyses were stored in 45 mL dark glass bottles that had been previously burned at 450°C for 4 h and rinsed with MQ water to remove any traces of organic substances. The DOC analysis was with a Shimadzu TOC-5000A carbon analyzer calibrated with potassium biphthalate. CDOM was determined by spectrophotometric absorbance of the filtrates at 320 nm, blanked against filtered MQ water and converted to absorption values. SRP and NO_3^- were measured in the filtrates using standard colorimetric methods (Stainton et al., 1977), and major ions were measured using Dionex ICS 2000 ion chromatograph.

2.4.3 Pigment analysis

Surface and near-bottom water samples (50-500 mL) from each lake were filtered onto 25-mm diameter GF/F glass-fibre filters, and immediately frozen and stored at -80°C until pigment extraction in methanol. Pigments were analyzed by high performance liquid chromatography (HPLC) following the protocols and standards described in Bonilla et al. (2005). For some of the statistical analyses, photoprotective pigments (canthaxanthin, diadinoxanthin, echininone, lutein, violaxanthin and zeaxanthin) were separated from light harvesting, photosynthetic pigments (alloxanthin, Chl *b*, fucoxanthin and peridinin) as in Bonilla et al. (2005).

2.4.4 Picophytoplankton enumeration

Unfiltered water samples were transferred to 5mL Cryovials, fixed with glutaraldehyde (10% final concentration) and stored at -80°C until analysis for picophytoplankton abundance. The cells were enumerated using a Becton Dickinson flow cytometer (BD FacsCalibur), equipped with an argon laser. Analyses were done at the lowest flow rate (12 μL min^{-1}), using a solution of 1-μm diameter, yellow-green microspheres (Polysciences, Inc) as an internal standard. Bead concentrations in the calibration solution were controlled using TrueCountAbsolute counting tubes (BD biosciences). Picocyanobacteria and picoeukaryotes were distinguished based on their chlorophyll and phycoerythrin fluorescence. Detection of the two groups was performed by the comparison of flow cytograms where cells were discriminated based on their side scatter signals (SSC) and both red (FL3) and orange

fluorescence (FL2) as well as FL3 versus FL2. Given the low oxygen conditions observed in the bottom layers of the thaw lakes, samples were also analysed for green sulfur bacteria (FL3 vs SCC). The cytograms were analyzed using the Cell Quest Pro software, with manual gating to discriminate the different populations. For the picophytoplankton biovolume estimates, the diameters of 20 cells of each group in a sample from thaw lake KWK12 were measured under epifluorescence microscopy at 1000x magnification, and were then converted to spherical biovolumes. The measured cell diameters (±SD) were 1.0 ± 0.2 μm for picocyanobacteria and 2.0 ± 0.5 μm for picoeukaryotes, giving biovolumes per cell of 0.52 and 4.19 μm^3, respectively.

2.4.5 RNA sampling and analysis

Water samples from the surface and bottom of the black palsa lake SAS2A were first prefiltered through a 20 μm mesh to remove larger organisms and then filtered sequentially through a 3 μm pore size, 47 mm diameter polycarbonate filter (DHI) and a 0.2 μm Sterivex unit (Millipore) with a peristaltic pump. From 100 to 300 mL of water were filtered and the filtration was stopped after 2 hours to minimize RNA degradation. The 3 μm filter for larger cells (L fraction) and the 0.2 μm filter for the smaller fraction (S fraction) were both preserved in RNAlater (Life Technologies) and then stored at -80°C until extraction.

Samples were extracted with the AllPrep DNA/RNA Mini Kit (Qiagen). This protocol was modified by the addition of cross-linked polyvinylpyrrolidone (PVP, Alfa Aesar) (UV light sterilized) to a final concentration of 10% before loading the samples onto the lysate homogenization column. For all samples, the extracted RNA was converted to cDNA immediately with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems-Ambion) and stored at -80°C until analysis. The V4 region of the eukaryotic 18S rRNA that had been converted to cDNA was amplified using the 454 primers as described in Comeau et al. (2011). PCR was carried out in a total volume of 50 μL, the mixture contained HF buffer 1X (NEB), 0.25 μM of each primer, 200 μM of each dNTPs (Life Technology), 0.4 mg mL^{-1} BSA (NEB), 1 U of Phusion High-Fidelity DNA polymerase (NEB) and 1 μL of template cDNA. Two more reactions with 5X and 10X diluted template were also carried out for each sample, to minimize potential primer bias. Thermal cycling began with an initial

denaturation at 98°C for 30 s, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 270 s. The three dilution reactions were pooled and purified with a magnetic bead kit Agencourt AMPure XP (Beckman Coulter) and then quantified spectrophotometerically with the Nanodrop 1000 (Thermo Fisher Scientific). The amplicons were sequenced on 1/8 plates of the Roche 454 GS-FLX using the "PLUS" chemistry at the IBIS/Laval University, plate-forme d'analyses Génomiques (Québec City, QC). The raw 454 sequences have been deposited in the NCBI database under the bioproject name PRJNA286764.

Sequences were analysed using the UPARSE pipeline (Edgar, 2013). For quality filtering, the sequences were truncated at 245 bp to keep 50% of the reads at the 0.5 expected error rate. Singletons as well as chimeras were then removed and operational taxonomic units (OTUs) were determined at the ≥ 98% similarity level. These OTUs were classified using the mothur classifier (Schloss et al., 2009) with a 0.8 confidence threshold based on the SILVA reference database (Pruesse et al., 2007) modified to include sequences from our in-house, curated northern 18S rRNA gene sequence database. In order to compare samples, the OTU tables were each subsampled 100 times at 2200 reads, which corresponded to the lowest number of reads per sample minus 10%; this subsampling used the command multiple_rarefaction_even_depth.py in Qiime (Caporaso et al., 2010). The most abundant and unclassified OTUs were subsequently submitted to a BLASTn search to the nr database in NCBI GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the nearest match.

2.4.6 Statistical analysis

The normal distribution of environmental variables was tested using the Kolmogorov-Smirnov test, and right-skewed variables were normalized by natural logarithm transformation. Given the order of magnitude differences in picophytoplankton abundances and pigment concentrations among samples, the HPLC and flow cytometry data were also normalized by logarithmic transformation. Correlations within and among the phytoplankton, pigment and environmental variables were tested by Pearson correlation analysis, with correction for multi-testing using the false discovery rate procedure as in Benjamini and Hochberg (1995). Statistical relationships among variables were also investigated by

principal component analysis (PCA), stepwise multiple linear regression, cluster analysis and permutation MANOVA (PERMANOVA). Secondary cross-correlated variables were removed prior to these analyses, which were performed using Past 3.04 or Primer 6.

2.5 Results

2.5.1 Environmental heterogeneity

The thaw lakes spanned a wide range of environmental conditions, including water color and CDOM, with the latter strongly correlated with DOC ($R = 0.67$, $p < 0.0001$). The highest DOC concentrations (up to 17 mg L^{-1}) and CDOM (up to 117 m^{-1}) were recorded in the SAS lakes (Table 2.1). These waters were black in color and also had the lowest pH values (6.0 – 6.6). The highest total nutrient concentrations (up to 125 µg TP L^{-1} and 4 mg TN L^{-1}) were recorded in lakes located within the KWK and NAS valleys, and the values were lowest in the shallow rock-basin waters (minima of 1.6 µg TP L^{-1} and 0.1 mg TN L^{-1}). Nitrogen to phosphorus ratios varied greatly among the 17 lakes, from 4 to 131 (g g^{-1}), and total suspended solids were similarly variable, from 1 to 320 mg L^{-1} (Table 2.1). The NAS valley waters contained especially high concentrations of suspended clay particles, producing an opaque milky appearance. Despite their shallowness and small size, the thaw lakes were highly stratified in terms of temperature and oxygen (Fig. 2.2), with anoxic bottom waters in the SAS and KWK lakes. Some had pronounced thermal gradients, with temperature differences up to 10°C between the surface and bottom waters. In contrast, the reference lakes were more mixed (Fig. 2.2).

Principal component analysis (PCA) of environmental variables for surface water samples yielded three components with eigenvalues larger than 1, and these collectively explained 66% of the total variance (Fig. 2.3). The first component explained 31% of the observed variance and was positively correlated with Chl *a*, TSS, TN and TP. The second component was positively correlated with DOC, TN, conductivity and NO_3, and negatively with temperature (Table 2.7).

Cluster analysis of a set of 8 non-autocorrelated environmental variables (TSS, TN, TP, DOC, temperature, pH, conductivity, NO_3) showed some grouping of the lakes, specifically of the SAS waters (Fig. 2.7) and PERMANOVA analysis of these data yielded an F value of 4.60 ($p < 0.0001$). The subsequent pairwise analysis of sites showed that the SRB reference waters differed significantly from KWK, SAS and BGR ($p = 0.0006$, 0.0186 and 0.0468, respectively), however the three sets of thaw lakes did not differ significantly from each other ($p = 0.10$ to 0.18).

2.5.2 Planktonic pigments

Phytoplankton abundance, as measured by Chl *a* concentrations, varied greatly among the waterbodies (Table 1), from 0.4 (SRB1) to 6.8 (KWK6) μg L^{-1} in 2011 and from 0.2 (SRB1) to 9.1 (KWK1) μg L^{-1} in 2012. There was also a small but significant difference in Chl *a* concentrations between years, with means of 3.7 and 2.6 μg L^{-1}, respectively (paired t-test, $t = 2.5$, $p = 0.02$). On average, Chl *a* was significantly higher in the thaw lakes than the reference rock-basin waters: the overall means were 3.3 and 2.0 μg Chl *a* L^{-1}, respectively.

The pigment analyses of the phytoplankton sampled in 2011 (Fig. 2.4, Table 2.2) showed that there were diverse communities including fucoxanthin-containing groups (potentially diatoms, chrysophytes and certain dinoflagellates), chlorophytes (Chl *b*, lutein and violaxanthin), cryptophytes (alloxanthin), dinoflagellates (peridinin) and cyanobacteria (zeaxanthin, canthaxanthin, echinenone). The pigments Chl c_1, c_2, c_3 and crocoxanthin were also present, but generally at trace concentrations, and only in certain lakes. The KWK lakes had high concentrations of zeaxanthin (up to 1.3 μg L^{-1}), accompanied by high concentrations of fucoxanthin and green algal pigments (lutein and violaxanthin). In the SAS lakes, a dominance of dinoflagellates and cryptophytes was indicated by high concentrations of peridinin and alloxanthin. Echinenone was present in KWK and SRB lakes and high concentrations of violaxanthin were also recorded in BGR lakes.

The bottom waters of the thaw lakes also contained diverse planktonic pigments, including high levels of alloxanthin (KWK and BGR) and fucoxanthin (KWK, BGR and SAS). The bottom waters of the shallow rock-basin waters contained high fucoxanthin

concentrations. Diadinoxanthin was abundant in the bottom waters at several sites, particularly those in the KWK valley. High levels of bacteriochlorophyll *d* indicating abundant populations of green photosynthetic sulfur bacteria were recorded in the deeper waters of KWK (Table 2.3, Fig. 2.5).

Similarly diverse pigment assemblages were observed in 2012 (Fig. 2.4, Table 2.2). The abundance of cyanobacterial populations in KWK, BGR and NAS lakes was indicated by high concentrations of zeaxanthin (e.g., NASH) and echinenone (SRB3). Green algal pigments were abundant in the KWK lakes as well as in some shallow rock-basin waters, and high diadinoxanthin concentrations appeared in KWK, SAS and BGR lakes. Fucoxanthin-groups were abundant in SRB and SAS as well as in NASH and BGR2. The turbid thaw lakes within the NAS valley had high concentrations of β,β-carotene. Relatively high levels of ancillary photosynthetic pigments were present in BGR and NAS lakes as well as in clear waters of shallow rock-basin lakes (Table 2.2). Photoprotective pigments were relatively more abundant in KWK lakes (notably KWK6 and KWK23) as well as in the SRB waters (violaxanthin), and less abundant in the DOC-rich SAS lakes (Table 2.2). The bottom waters contained high levels of diadinoxanthin and alloxanthin in KWK lakes, fucoxanthin in BGR2 and Chl *b* in SRB. Bacteriochlorophyll *d* was again present in the bottom and mid-water column of the KWK lakes that had anoxic bottom waters.

For the overall data set, Chl *a* concentrations were significantly correlated with TP ($R = 0.48$; $p = 0.05$), and with TSS ($R = 0.53$; $P = 0.03$), which were themselves strongly correlated ($R = 0.76$; $p < 0.0001$). A forward stepwise linear regression showed that Chl *a* was best described by a combination of the accessory pigments lutein ($p < 0.001$), fucoxanthin ($p = 0.003$) and peridinin ($p = 0.05$): ln Chl *a* = 1.57 ln Lut + 0.58 ln Fuco +0.48 ln Per +0.48 ($R^2 = 0.78$; $p < 0.001$). The regression analyses between principal components and accessory pigments showed a significant relationship for lutein and the first and second principal components: ($R^2 = 0.48$, $p = 0.001$), and zeaxanthin was significantly related to the third component ($R^2 = 0.26$, $p = 0.01$).

Several of the accessory pigments were highly correlated among themselves. These included the chlorophyte pigments violaxanthin and lutein ($R = 0.82$; $p < 0.0001$), and both pigments with Chl *b* ($R = 0.66, 0.90$; $p = 0.0004, < 0.0001$). The cyanobacterial pigments echinenone and canthaxanthin were significantly correlated ($R = 0.70$; $p = 0.0001$), but not with zeaxanthin ($p > 0.1$). Diadinoxathin and violaxanthin were also strongly correlated ($R = 0.72$, $p < 0.001$). The summations within the two categories of pigments, photoprotective and photosynthetic, were also positively correlated ($R = 0.73$; $p < 0.001$).

PERMANOVA analysis was conducted on a data subset of 5 non-autocorrelated accessory pigments: fucoxanthin, peridinin, lutein, echinenone and zeaxanthin. These showed no significant differences among valleys ($F = 1.34$, $p = 0.172$). Similarly, cluster analysis showed no grouping of pigment characteristics according to site, although there was some evidence of grouping according to sample year (Fig. 2.7). The mapping of this cluster matrix on the environmental cluster matrix via the Primer function Relate showed no significant relationship ($R = -0.23$, $p = 0.10$).

Consistent with the multivariate analyses, the accessory pigments were uncorrelated with individual environmental variables (all corrected p values were > 0.05), with the exception of lutein. This chlorophyte pigment was significantly correlated with TP ($R = 0.49$; $p = 0.05$), but this may simply reflect the strong correlation between lutein and Chl a ($R = 0.79$; $p < 0.0001$), which itself correlated with TP (see above).

2.5.3 Picophytoplankton biovolume

Picophytoplankton concentrations varied greatly among the lakes (Fig. 6). In 2011, those located on marine clays (KWK) contained the highest biovolume of total picophytoplankton. The picocyanobacterial abundances ranged from 1.8×10^3 (SAS1B) to 5.9×10^5 (KWK23) cells mL^{-1}, which in biovolume terms was equivalent to 9.5×10^2 (SAS1B) to 3.1×10^5 (KWK23) $\mu m^3\ mL^{-1}$. The BGR lakes also contained abundant picophytoplankton, but with lower contributions from picoeukaryotes. The thaw lakes located on peatlands (SAS) contained up to 10^5 $\mu m^3\ mL^{-1}$ of picophytoplankton biovolume with prevalence of picoeukaryotes over picocyanobacteria. The smaller rock-basin lakes (SRB1, SRB2, SRB4)

contained up to 10^4 μm^3 mL^{-1} picophytoplankton biovolume and two larger rock-basin lakes (SRB3, SRB5) contained up to 10^5 μm^3 mL^{-1} picophytoplankton biovolume, with high contributions from picocyanobacteria. In 2011, the picophytoplankton populations reached 10^6 μm^3 mL^{-1} biovolume in KWK lakes, with the highest cell concentrations of picocyanobacteria, reaching 5.9×10^5 cells mL^{-1} (equivalent to 3.1×10^5 μm^3 mL^{-1}) in KWK23. The highest cell concentrations of picoeukaryotes were also recorded in KWK23, with values up to 2.8×10^5 cells mL^{-1} (1.2×10^6 μm^3 mL^{-1}) in 2011. The shallow rock-basin (SRB) and peatland lakes (SAS) were apparently less favourable, with picoeukaryote biovolume concentrations often below 10^3 μm^3 mL^{-1}. The lower biovolume concentrations of picoeukaryotes were not always accompanied by similar decrease in picocyanobacteria, for example in SRB4 in 2012.

Total picophytoplankton biovolume increased with Chl a concentration ($R = 0.52$; $p = 0.03$), but this relationship was only significant for the eukaryotic component ($R = 0.53$; $p = 0.02$). Picocyanobacteria correlated negatively with DOC ($R = -0.47$; $p = 0.05$), while picoeukaryotes correlated negatively with conductivity ($R = -0.48$; $p = 0.05$). Picocyanobacteria were highly correlated with zeaxanthin ($R = 0.72$; $p = 0.0002$), and there was also a significant, albeit less strong, correlation between picoeukaryotes and zeaxanthin ($R = 0.54$; $p = 0.02$). Stepwise multiple linear regression analysis showed that picophytoplankton (picoeukaryotes, PEuk; picocyanobacteria, PCyan) biovolumes were statistically related to limnological variables according to the relationships: PEuk = 14.9 + 2.9 × Chl a - 1.7 × TN ($R^2 = 0.56$, $p = 0.001$), and PCyan = -2.9 + 4.3 Temp + 1.1 × Chl a - 1.1 × TSS + 1.5 × TP - 1.2 × DOC ($R^2 = 0.67$, $p = 0.001$).

2.5.4 Molecular analyses

The 18S rRNA data set from the palsa thaw lake (SAS2A) contained large numbers of rotifer sequences (400 to 1350 reads per sample, all with closest matches to the genus *Ascomorpha*) and these were removed prior to further analysis. This left a total of 3857 and 3128 reads for the surface L (> 3.0 μm) and S (< 3.0 μm) fractions, and 3522 and 2457 reads for the bottom L and S fractions; 84 to 93% of these eukaryotic sequences could be assigned (≥ 98% identity) to phylum in the modified SILVA database. The largest fraction of total reads was

attributable to ciliates (up to 33% in the surface waters and 74% in the bottom waters; Table 4), including the genus *Stokesia*, especially in the surface waters, and the genera *Cryptocaryon*, *Halteria*, *Peniculida* and *Cyclidium*, especially in the bottom waters (Table 2.5). Among the groups nominally considered as phytoplankton were dinoflagellates, chrysophytes and chlorophytes, with lesser proportions of reads associated with katablepharids, bacillariophytes (diatoms) and cryptophytes (Table 2.4). Cluster analysis of the data set (after subsampling to ensure equal reads per sample) showed that community structure greatly differed with depth (Bray-Curtis dissimilarity index of 0.795 for the large fraction and 0.820 for the small fraction), and to a much lesser extent between large and small fractions (Bray-Curtis dissimilarity index of 0.423 for the surface samples and 0.312 for the bottom samples). Chlorophytes, dinoflagellates, katablepharids and diatoms were more represented in the large, surface water fraction.

2.6 Discussion

Each of the subarctic thaw lakes contained pigments from several phytoplankton phyla, revealing that these abundant waters provide habitats for diverse phototrophic groups. Apart from β,β-carotene found in all algal groups, the most abundant accessory pigment was fucoxanthin, indicating the possible presence of diatoms, chrysophytes and certain dinoflagellates. Peridinin and alloxanthin were also present in many of the samples, indicating the presence of dinoflagellates and cryptophytes, respectively (Jeffrey et al., 2011). Diatoms would be less favoured in these stratified waters given their fast sinking rates in still waters, while flagellated taxa including chrysophytes, dinoflagellates and cryptophytes would be able to maintain their position in the euphotic zone. Mixotrophic chrysophytes and dinoflagellates have been observed in many high latitude lakes (Charvet et al., 2012; and references therein), and may be additionally favored by the high biomass concentrations of heterotrophic bacteria that occur in some of these waters (Breton et al., 2009; Roiha et al., 2015). Green algae were also well represented at most sites, indicating that despite the strong light attenuation by CDOM and TSS in these waters (Watanabe et al., 2011), there is adequate light availability for obligate phototrophs; some of these taxa, however, may also be capable of osmotrophic uptake of dissolved organic compounds.

The concentrations of photoprotective pigments were conspicuously high in the NAS lakes. This was unexpected given that these turbid waters contained elevated concentrations of suspended solids, which indicate a low light availability for photosynthesis, and a lack of need for protection against bright light. It is possible, however, that in this lake, cells suspended in the mixed layer are adapted to intermittent exposure to bright light rather than the average water column irradiance. Such conditions have been observed in a turbid estuarine environment, where the phytoplankton were photosynthetically adapted to high near-surface irradiances rather than the overall shade or dark conditions experienced on average by the cells as they were circulated by turbulent mixing through the water column (Vincent et al., 1994).

The pigment analyses also indicated the abundant presence of cyanobacteria. Echinenone and canthaxanthin are well known photoprotective pigments in cyanobacteria, with the latter especially prevalent in Nostocales, which may suggest the presence of nitrogen-fixing taxa. These results are consistent with bacterial 16S rRNA analyses, which showed the presence of cyanobacterial taxa in some of these lakes that had strong affinities (> 99% sequence similarity) to the Nostocalean taxon *Dolichospermum curvum* (Crevecoeur et al., 2015). Zeaxanthin can occur in high cellular concentrations in cyanobacteria, but it also is found in eukaryotic algal groups. This pigment is a component of photoprotective xanthophyll cycles, and may co-occur with other components of these cycles. For example, studies on the diatom *Phaedactylum tricornutum* have shown the co-occurrence of the diadinoxanthin cycle and the violaxanthin cycle (Lohr and Wilhelm 1999). Consistent with this co-occurrence, we found a strong correlation between diadinoxathin and violaxanthin in the studied lakes ($R = 0.72$, $p < 0.001$). We also observed high concentrations of zeaxanthin, which is often associated with cyanobacteria but also chlorophytes (Jeffrey et al., 2011). Given the molecular analyses results of thaw lake bacterial communities (Crevecoeur et al., 2015) and our flow cytometry data, zeaxanthin was likely to at least in part be associated with the abundant picocyanobacteria in the order Synechococcales. The strong correlation between picocyanobacteria and zeaxanthin further supports this relationship.

HPLC analysis has been used with success in a variety of aquatic ecosystems to not only identify major algal groups, but also to quantify their proportional representation using the software program CHEMTAX (Mackay et al., 1996). However, given the large known variation in pigment ratios in algal cells, this method requires extensive calibration on each class of waters. For example, in shallow a eutrophic lake, CHEMTAX gave a reliable estimation of cyanobacterial and chlorophyte biomass, but not chrysophytes and dinoflagellates (Tamm et al., 2015). The latter were two of the dominant groups in the permafrost thaw lakes, and further work will be required before the CHEMTAX approach can be applied to these waters.

The presence of bacteriochlorophyll *d* in high concentrations in KWK lakes containing anoxic bottom waters indicate that these environments are favourable habitats for photosynthetic sulfur bacteria. These results are consistent with molecular analyses of the bacterial assemblages. 16S rRNA gene clone library analysis of KWK lakes detected the presence of green sulfur bacteria (Rossi et al., 2013), and high throughput 16S rRNA sequencing revealed that the green sulfur bacterium *Pelodictyon* (*Chlorobi*) was one of the most abundant sequences in KWK waters (Crevecoeur et al., 2015). The high concentrations of bacteriochlorophyll *d* suggest that these populations could play an important role in overall primary production of certain thaw lakes, although restricted to deeper water, anoxic conditions.

Picophytoplankton occurred in all of the sampled lakes, but with large differences among waters. In general, the concentrations of both picocyanobacteria and picoeukaryotes increased with increasing total phytoplankton biomass, as measured by Chl *a* concentrations. However, the two groups differed in their correlative relationships with other limnological variables. In partial support of our initial hypothesis that DOC would be a controlling variable, picocyanobacteria, but not eukaryotes, were negatively correlated with DOC. An inverse relationship with DOC was also found for picophytoplankton in Swedish lakes (Drakare et al., 2003). Similarly in Lake Valkea-Kotinen, in the boreal zone of Finland, variations in autotrophic picoplankton were most closely correlated with water column stability, which in turn was strongly regulated by DOC concentration (Pelromaa and Ojala,

2012). High DOC waters are often characterized by low pH, which may be a constraint on certain cyanobacteria, however acid-tolerant picocyanobacteria are known (Jasser et al., 2013). Even in the low pH SAS waters picocyanobacteria were always present, although in low concentrations (e.g., the minimum of 1.8×10^3 mL^{-1} in SAS2B in 2011). Other factors such as zooplankton grazing may also have played a role in controlling picocyanobacteria (Rautio and Vincent, 2006), although this seems less likely for picocyanobacteria given that they are a nutritionally deficient food source for zooplankton in thaw lakes (Przytulska et al., 2015a).

Picocyanobacteria did not show the expected relationship with temperature in the correlation analyses, although temperature was one of the variables retained in the multiple linear regression analysis. Temperature has often been identified as a key variable for cyanobacterial growth and dominance in lakes elsewhere. For example, in reservoirs in the southeastern USA, there was a strong, positive correlation between picocyanobacterial cell concentrations and temperature, while picoeukaryotes showed an inverse correlation, and dominance of the picophytoplankton community shifted from picoeukaryotes in winter to picocyanobacteria in summer (Ochs and Rhew, 1997). Similarly, increasing temperature favoured picocyanobacteria over picoeukaryotes in German lakes (Hepperle and Krienitz, 2001). In experiments with subarctic lake and river water at 10 and 20°C, the concentration of Chl *a* in the picoplankton fraction increased substantially at the warmer temperature (Rae and Vincent, 1998). The temperature range in the present study may have been too restricted to observe such effects.

The molecular data provided further insight into the planktonic diversity of a thaw lake ecosystem. When rotifers were excluded from the analyses, the ciliates were dominant in the RNA sequences of SAS2A. This likely reflected their large cell sizes with a concomitantly large number of ribosomes; for example, *Stokesia vernalis*, the most abundant sequence identified in the surface waters (Table 5), can be >100 µm in length. Ciliates are also known to be fragile cells that are easily broken up during manipulation like pre-filtration through a 20 µm mesh, what could account for their highly abundant sequences in the S as well as L fractions. Chrysophytes and chlorophytes were well represented in the RNA

sequences, particularly in the surface water L fraction, consistent with their abundance as indicated by the pigment data. Dinoflagellates constituted the dominant fraction of the phytoplankton sequences, yet were not detected as peridinin in SAS2A, although this pigment was present in two other SAS lakes. This may indicate the presence of large dinoflagellate cells, for example rigid *Ceratium* cells that can extend up to 100 μm in length, but may also be due to the presence of dinoflagellates that lack the accessory pigment peridinin. Diatoms can also include large cell types, but their representation in the sequences was small, suggesting that most of the fucoxanthin that we measured was associated with chrysophytes such as *Uroglena* (Table 5) rather than diatoms. It is of interest that diatoms from the genus *Urosolenia* were the closest match following a BLAST search. This diatom is known to be lightly silicified and may be less susceptible to sedimentation in these well stratified waters. The cryptophyte pigment alloxanthin was in high concentration in SAS2A, as in the other thaw lakes, yet cryptophyte sequences accounted for < 1.5% of the total RNA reads. This might reflect the small cell-size and accordingly low rRNA content of certain cryptophyte taxa, for example *Chroomonas*.

The molecular data also provided insight into the nature of the picoeukaryotic communities in the SAS lakes. The taxonomic identities (Table 5) indicated the presence of several chlorophyte genera that are known to produce small cells, notably *Choricystis*, *Lemmermannia Monoraphidium* and *Chlorella*. For example, in subalpine Lake Tahoe (USA), *Choricystis coccoides* produces cells that are only 0.5 μm^3 in volume, too small to be grazed by calanoid copepods in that lake (Vincent, 1982). Among the chrysophytes, *Spumella* and related genera are known to produce small cells. For all of these analyses, the many unidentified eukaryotic reads add an extra element of uncertainty to the interpretation, but collectively these data underscore the planktonic diversity of the thaw lake ecosystem.

Permafrost thaw lakes receive large quantities of allochthonous organic carbon from their surrounding catchments and this is reflected in their high DOC and CDOM concentrations, as observed in the present study. These waters have high respiratory oxygen demands and are net heterotrophic, resulting in prolonged hypoxia or anoxia in the bottom waters during summer, and anoxia throughout the water column once ice covers the lake in

winter (Deshpande et al., 2015). The abundant cilate and nanoflagellate sequences in our molecular analyses also point to high productivity by bacterial heterotrophs, their likely prey in these waters. However, despite these multiple signs of intense heterotrophy, the pigment, cytometry and molecular results in the present study show that these ecosystems are also the habitats for abundant phototrophs from diverse taxonomic groups.

2.7 Conclusions

The wide range of thaw lakes sampled in the present study significantly differed from the reference rock basin lakes in their limnological properties. On average, they contained higher phytoplankton (Chl *a*) and TP concentrations than the reference lakes, but had a comparable diversity of pigments, dominated by chlorophyte, chrysophyte and dinoflagellate pigments. Cyanobacteria and cryptophytes were also well represented, but the thaw waters appeared to be less favorable for diatoms, at least during the highly stratified late-summer period. Picophytoplankton occurred in all of the thaw lakes, in some of the waters at biovolume values up to 10^6 μm^3 mL^{-1}, and the molecular analysis of samples from one of the lakes types indicated that small cell chlorophytes may be among the dominants in the picoeukaryotic fraction. Despite the heterotrophic nature of these organic-rich ecosystems, with respiration likely exceeding photosynthesis throughout the year, permafrost thaw lakes contain abundant, diverse phototrophs that potentially support higher trophic levels, and that will lessen the net CO_2 release from these waters to the atmosphere.

2.8 Acknowledgments

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Table 2.1. Limnological characteristics in studied subarctic lakes, including surface values for temperature (T), pH, dissolved organic carbon concentration (DOC), colored dissolved organic matter (CDOM), total suspended solids (TSS), soluble reactive phosphorus (SRP), total phosphorus (TP), total nitrogen (TN), nitrate (NO_3) and Chlorophyll *a* (Chl *a*). Mean values from 2011 and 2012 (+/- range in brackets; nd = no data from 2011).

Sites	T(°C)	pH	DOC (mg L^{-1})	CDOM (m^{-1})	TSS (mg L^{-1})	SRP (µg L^{-1})	TP (µg L^{-1})	TN (mg L^{-1})	NO_3 (mg N L^{-1})	Chl *a* (µg L^{-1})
Thaw lakes on marine clays										
BGR1	15.1 (0.7)	7.5 (0.2)	3.9 (0.4)	6.1 (1.7)	2.9 (0.5)	1.8 (0.6)	17.3 (3.5)	0.1 (0.0)	0.05 (0.0)	1.8 (1.0)
BGR2	14.5 (0.4)	7.0 (0.3)	9.0 (0.3)	39.1 (5.7)	19.2 (6.1)	2.4 (1.0)	45.7 (0.2)	0.3 (0.0)	0.05 (0.0)	3.4 (1.4)
NASA	15.6 (nd)	7.0 (nd)	3.0 (nd)	12.3 (nd)	319.3 (nd)	2.9 (nd)	124.5 (nd)	3.7 (nd)	0.25 (nd)	4.1 (nd)
NASH	18.3 (nd)	7.6 (nd)	4.1 (nd)	22.6 (nd)	18.2 (nd)	6.2 (nd)	28.5 (nd)	0.4 (nd)	0.04 (nd)	1.7 (nd)
Thaw lakes on mineral clays										
KWK1	17.1 (4.4)	6.1 (0.8)	9.2 (2.8)	39.0 (16.4)	14.1 (12.0)	2.2 (1.5)	36.8 (26.7)	0.3 (0.1)	0.05 (0.0)	8.0 (1.2)
KWK6	14.9 (0.8)	6.8 (0.4)	5.2 (0.0)	10.7 (0.7)	9.3 (1.1)	1.0 (0.4)	30.9 (3.0)	0.2 (0.0)	0.06 (0.0)	4.4 (1.6)
KWK12	16.8 (0.8)	8.0 (1.1)	8.6 (0.7)	39.6 (3.7)	13.8 (2.6)	1.6 (0.0)	27.7 (2.1)	0.2 (0.0)	0.08 (0.0)	2.5 (0.1)
KWK23	14.9 (0.2)	6.7 (0.2)	7.2 (0.6)	35.8 (1.9)	10.2 (1.9)	4.9 (0.6)	47.7 (5.6)	0.2 (0.0)	0.04 (0.0)	3.5 (1.9)
Thaw lakes on peatlands										
SAS1A	14.3 (0.2)	6.6 (0.3)	10.7 (0.8)	68.7 (2.7)	7.6 (2.6)	1.6 (0.3)	14.3 (0.9)	0.5 (0.1)	0.17 (0.0)	3.6 (1.2)
SAS1B	13.6 (0.1)	6.3 (0.3)	15.9 (0.4)	109.5 (3.9)	21.8 (5.4)	1.9 (0.4)	12.7 (2.2)	0.6 (0.1)	0.07 (0.0)	4.3 (0.1)
SAS2A	19.9 (nd)	6.2 (nd)	14.9 (nd)	98.4 (nd)	2.6 (nd)	3.1 (nd)	9.6 (nd)	0.7 (nd)	0.04 (nd)	1.2 (nd)
SAS2B	16.0 (nd)	6.0 (nd)	17.1 (nd)	116.9 (nd)	5.2 (nd)	1.3 (nd)	10.3 (nd)	0.5 (nd)	0.11 (nd)	0.9 (nd)
Shallow rocky basins										
SRB1	15.8 (2.2)	7.6 (0.6)	9.9 (0.0)	46.0 (6.5)	2.6 (1.2)	2.1 (0.6)	7.9 (2.8)	0.2 (0.1)	0.06 (0.0)	0.3 (0.1)
SRB2	13.8 (0.5)	7.6 (1.1)	13.2 (2.7)	68.8 (21.5)	1.3 (0.3)	1.4 (0.5)	11.2 (2.6)	0.2 (0.1)	0.14 (0.1)	1.4 (0.4)
SRB3	15.6 (0.2)	6.6 (0.2)	7.8 (1.4)	34.5 (9.5)	5.4 (2.0)	1.1 (0.1)	13.4 (2.8)	0.3 (0.1)	0.05 (0.0)	5.2 (0.6)
SRB4	15.0 (0.3)	7.9 (0.5)	10.4 (1.6)	20.0 (0.5)	5.0 (1.9)	0.8 (0.1)	5.7 (2.6)	0.8 (0.4)	0.44 (0.4)	2.4 (1.0)
SRB5	18.7 (1.8)	7.1 (0.9)	3.7 (0.1)	9.4 (2.7)	0.7 (0.1)	0.5 (0.2)	2.9 (1.3)	0.1 (0.0)	0.32 (0.2)	0.8 (0.0)

Table 2.2. Phytoplankton planktonic pigments as ratios to chlorophyll *a* (µg µg $^{-1}$) in subarctic water bodies sampled in 2012. Key: Allo, alloxanthin; Chl *b*, chlorophyll *b*; Fuco, fucoxathin; Perid, peridinin; Cantha, canthaxanthin; Diadino, diadinoxanthin; Echin, echinenone; Lut, lutein; Viola, violaxanthin; Zea, zeaxanthin.

Sites	Photosynthetic				Photoprotective					
	Allo	Chl *b*	Fuco	Perid	Cantha	Diadino	Echin	Lut	Viola	Zea
Thaw lakes on marine clays										
BGR1	0.195	0.120	0.140	0.026	0.000	0.081	0.031	0.088	0.097	0.056
BGR2	0.015	0.072	0.202	0.109	0.021	0.141	0.024	0.106	0.133	0.193
NASA	0.287	0.089	0.016	0.000	0.000	0.000	0.000	0.104	0.045	0.000
NASH	0.052	0.107	0.280	0.000	0.060	0.155	0.066	0.068	0.196	0.553
Thaw lakes on mineral clays										
KWK1	0.040	0.167	0.051	0.032	0.009	0.245	0.012	0.090	0.056	0.023
KWK6	0.051	0.251	0.103	0.017	0.019	0.084	0.000	0.276	0.169	0.116
KWK12	0.067	0.075	0.154	0.039	0.011	0.086	0.020	0.079	0.087	0.026
KWK23	0.099	0.133	0.204	0.023	0.016	0.160	0.009	0.228	0.168	0.243
Thaw lakes on peatlands										
SAS1A	0.118	0.049	0.299	0.029	0.020	0.071	0.000	0.038	0.092	0.022
SAS1B	0.145	0.059	0.236	0.055	0.006	0.061	0.000	0.040	0.061	0.011
SAS2A	0.166	0.042	0.112	0.000	0.000	0.023	0.000	0.023	0.000	0.000
SAS2B	0.370	0.160	0.357	0.000	0.022	0.031	0.027	0.135	0.111	0.000
Shallow rock-basin lakes										
SRB1	0.049	0.167	0.160	0.051	0.000	0.073	0.022	0.124	0.199	0.071
SRB2	0.109	0.196	0.233	0.025	0.020	0.045	0.031	0.128	0.108	0.034
SRB3	0.127	0.070	0.291	0.036	0.013	0.059	0.027	0.069	0.122	0.066
SRB4	0.037	0.129	0.258	0.022	0.006	0.065	0.031	0.133	0.143	0.040
SRB5	0.047	0.055	0.349	0.003	0.007	0.063	0.022	0.046	0.151	0.062

Table 2.3. The relative concentration of bacteriochlorophyll *d* (BChl *d*, µg L^{-1}) based on the maximum peak area at 430 nm. The lakes have been arranged from lowest to highest concentrations.

Site	Date	Depth (m)	BChl *d* (µg L^{-1})
KWK6	04/08/2012	3.1	1.2
KWK6	21/08/2011	3.1	1.3
KWK23	04/08/2012	2.0	1.9
KWK12	03/08/2012	2.0	8.2
KWK12	19/08/2011	2.5	24.6
KWK1	19/08/2011	2.0	28.9
KWK23	04/08/2012	3.3	36.2
KWK23	21/08/2011	3.3	44.3
KWK1	03/08/2012	2.0	44.7
KWK12	03/08/2012	2.5	47.3

Table 2.4. RNA sequence analysis of eukaryotes in samples from permafrost thaw lake SAS2A, sampled in 2012. Each value is the % number of reads of the total for each sample (total number of reads minus rotifer sequences). The large fraction was retained on a 3 μm filter and the small fraction was on a 0.2 μm filter after filtration through the 3 μm pre-filter.

Taxonomic group	Percentage of reads			
	Surface		Bottom	
	Large	Small	Large	Small
Ciliophora	22.94	43.55	62.95	83.90
Dinophyta	17.11	4.02	8.71	1.90
Chrysophyta*	14.47	14.81	9.07	3.60
Chlorophyta**	9.97	2.03	2.11	0.40
Cercozoa	6.17	12.37	2.50	1.22
Cryptophyta	3.10	1.71	2.39	0.65
Katablepharidophyta	2.71	1.00	0.11	0.20
Bacillariophyta	2.47	0.44	0.96	0.45
Fungi***	1.47	0.61	0.32	0.04
Centroheliozoa	1.44	0.80	0.11	0.12
Choanoflagellida	1.32	3.12	0.23	0.20
Raphidophyceae	0.51	0.33	0.00	0.04
Pavlovales	0.44	0.19	0.18	0.04
Prymnesiales	0.15	0.03	0.06	0.00
Perkinsea	0.00	0.00	0.03	0.12
Unknown affinities	15.75	15.00	10.28	7.11

*includes Chrysophyceae, Synurophyceae and Bicosoecida
**includes Chlorophyceae and Trebouxiophyceae
***includes Chytridiomycota, Oomycota and Ascomycota

Table 2.5. Closest identity (ID) of eukaryotic RNA sequences from permafrost thaw lake SAS2A to GenBank sequences (following a BLASTn search), at the lowest taxonomic level identified.

GenBank Taxonomy	Accession number	Isolation source	% ID	Percentage of reads	
				Surface	Bottom
Ciliophora					
Stokesia vernalis	HM030738	Freshwater	99	8.65	0.04
Cryptocaryon sp.	JF317699	Drinking water	99	0.89	5.61
Peniculida sp.	GQ330632	Peat bog water	98	0.83	1.85
Halteria sp.	GU067995	Lake Esch-sur-Sure	99	0.49	6.38
Cyclidium marinum	JQ956553	Marine coast	99	0.00	34.42
Chrysophyta					
Uroglena sp.	EU024983	FU44-26	99	6.03	0.03
Paraphysomonas sp.	JQ967316	Freshwater	99	0.67	3.78
Dinobryon divergens	KJ579346	WO33_4	99	0.40	0.00
Spumella-like flagellate	AY651098	Lake Mondsee	99	0.03	0.76
Rhizaria					
Cercozoa	AB771834	Lake Kusaki	99	3.83	0.28
Cryptophyta					
Cryptomonas tetrapyrenoidosa	KF907407	Deokam032610	99	1.71	0.87
Cryptomonas pyrenoidifera	KF907397	CNUCRY 166	99	0.33	0.00
Cryptomonas curvata	KF907377	CNUCRY 90	99	0.06	0.28
Dinophyta					
Dinophyceae sp.	GQ423577	Lake Baikal	99	1.31	0.58
Peridinium wierzejskii	KF446619	Baikal region	99	1.03	2.07
Gyrodiniellum shiwhaense	FR720082	Shiwha Bay	98	0.49	0.01
Bacillariophyta					
Urosolenia eriensis	HQ912577	Y98-8	98	1.22	0.58
Urosolenia eriensis	HQ912577	Y98-8	99	0.22	0.10
Chlorophyta					
Lemmermannia punctata	JQ356704	SAG 25.81	99	1.07	0.08
Chlorella sp.	Y12816	OvS/Ger1	99	1.00	0.00
Choricystis sp.	AY195972	AS-29	99	0.89	0.11
Koliella longiseta	HE610126	SAG 470-1	99	0.72	0.04
Monoraphidium sp.	KP017571	LB59	99	0.39	0.00
Raphidophyta					
Gonyostomum semen	KP200894	Freshwater	100	0.23	0.02
Fungi					
Saprolegnia sp.	FJ794911	Lake (parasite)	99	0.11	0.00
Penicillium brevicompactum	KP981369	ATCC 16024	99	0.00	0.10
Prymnesiales					
Chrysochromulina parva	EU024987	FU44-40	100	0.09	0.03

Table 2.6. Location (longitude and latitude), maximum depth (Z) of the subarctic lakes and sampling dates. All sampling was ca. 20 cm below the surface and ca. 20 cm above the maximum depth. The shallow rock basin lakes have been referred elsewhere as follows: WP1 (SRB1), WP2 (SRB2), Olsha (SRB3), 4 KM (SRB4), Iqalusiuvik (SRB5).

Sites	Latitude	Longitude	Z (m)	Sampling dates	
				2011	2012
Thaw lakes on marine clays					
BGR1	56°36.650'N	76°12.900'W	3.5	20/08	09/08
BGR2	56°36.632'N	76°12.937'W	1.0	20/08	09/08
NASA	56°55.434'N	76°22.708'W	3.2		07/08
NASH	56°55.452'N	76°22.636'W	3.6		07/08
Thaw lakes on mineral clays					
KWK1	55°19.890'N	77°30.241'W	2.1	19/08	03/08
KWK6	55°19.937'N	77°30.117'W	3.2	21/08	04/08
KWK12	55°19.808'N	77°30.239'W	2.6	19/08	03/08
KWK23	55°19.947'N	77°30.131'W	3.4	21/08	04/08
Thaw lakes on peatlands					
SAS1A	55°13.128'N	77°42.477'W	1.9	23/08	05/08
SAS1B	55°13.143'N	77°42.475'W	1.7	23/08	05/08
SAS2A	55°13.591'N	77°41.815'W	2.6		13/08
SAS2B	55°13.600'N	77°41.806'W	2.0		13/08
Shallow rock-basin lakes					
SRB1	55°16.982'N	77°44.187'W	0.4	24/08	11/08
SRB2	55°16.970'N	77°44.122'W	0.8	24/08	11/08
SRB3	55°16.958'N	77°44.387'W	1.6	24/08	14/08
SRB4	55°19.907'N	77°41.959'W	0.7	16/08	08/08
SRB5	55°22.262'N	77°37.072'W	1.8	12/08	08/08

Table 2.7. Principal Component Analysis (PCA) for the environmental variables in the subarctic lakes.

Variables	PC 1	PC 2	PC 3
DOC	0.126	**0.285**	**-0.673**
TSS	**0.520**	0.014	**0.282**
TP	**0.484**	-0.220	0.189
TN	**0.392**	**0.479**	0.116
Temp	-0.246	**-0.253**	**0.367**
Cond	-0.157	**0.406**	**0.424**
pH	-0.257	-0.009	0.275
$N\text{-}NO_3$	-0.047	**0.620**	0.143
Chl a	**0.416**	-0.220	0.084

Values in bold are different from 0
with a significance level of p = 0.05

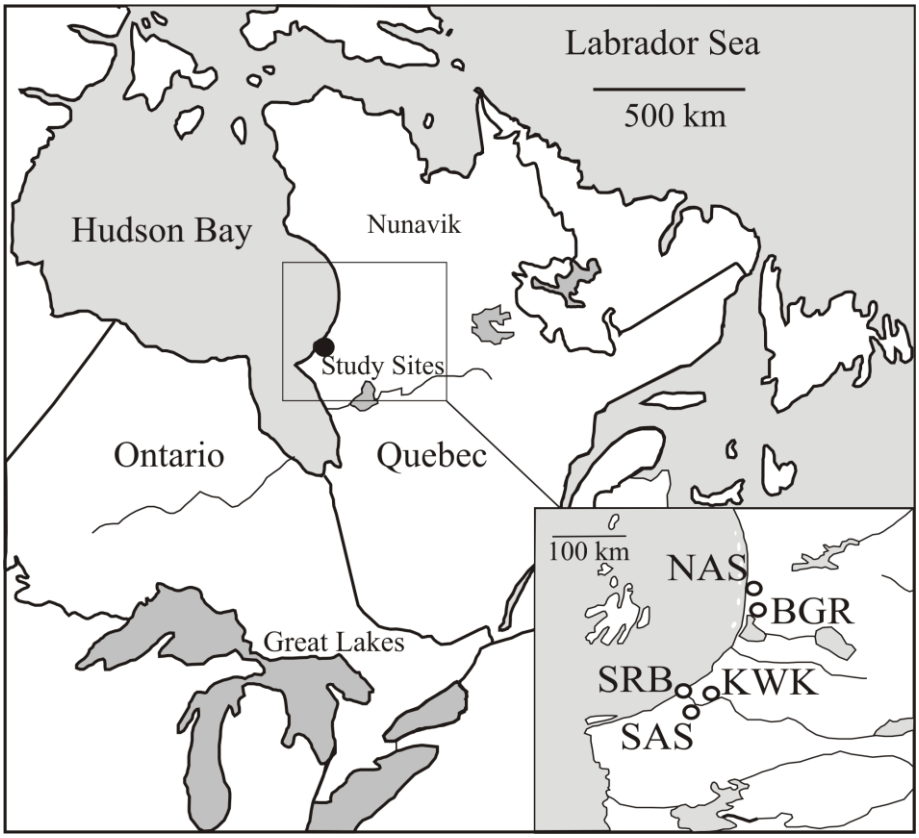

Figure 2.1. The location of the study area in Subarctic Quebec.

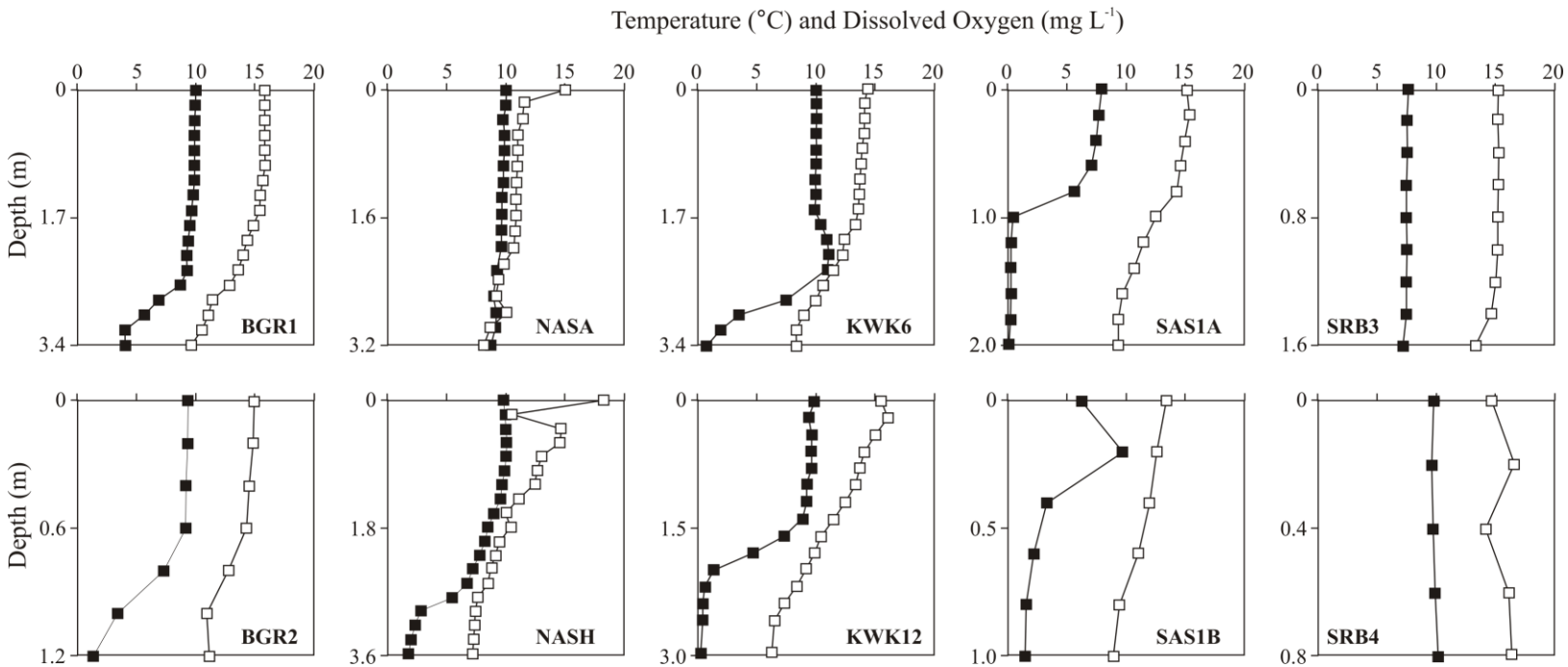

Figure 2.2. Temperature (white squares) and oxygen (black squares) stratification in permafrost thaw lakes (BGR, KWK, NAS, SAS) and shallow rock-basin lakes (SRB) during the summer 2012.

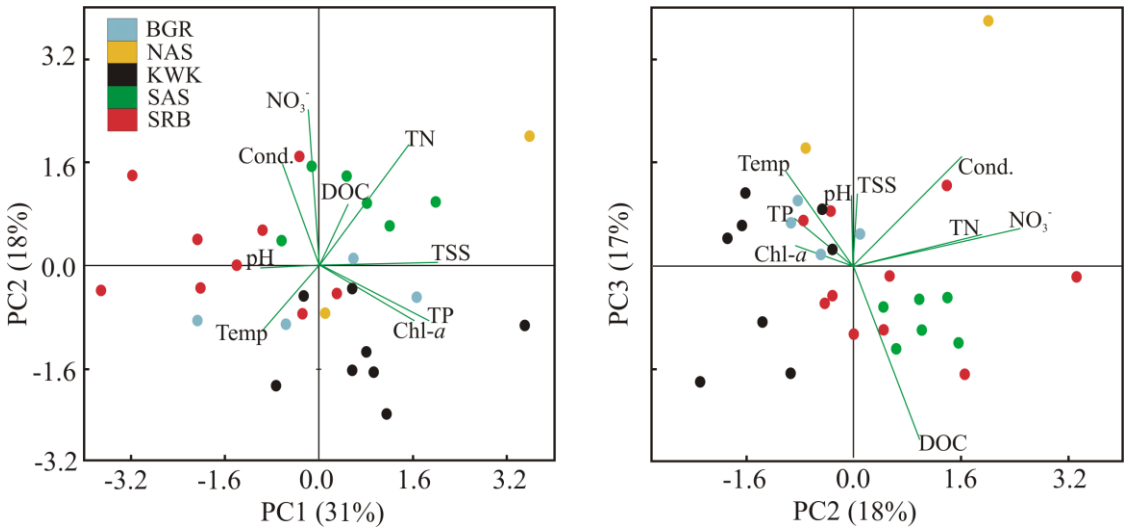

Figure 2.3. Principal component analysis of limnological variables in the thaw and SRB lakes. The first and second factors (left hand panel) account for 49% of the variability and the second and third factors (right hand panel) account for 35% of the variability.

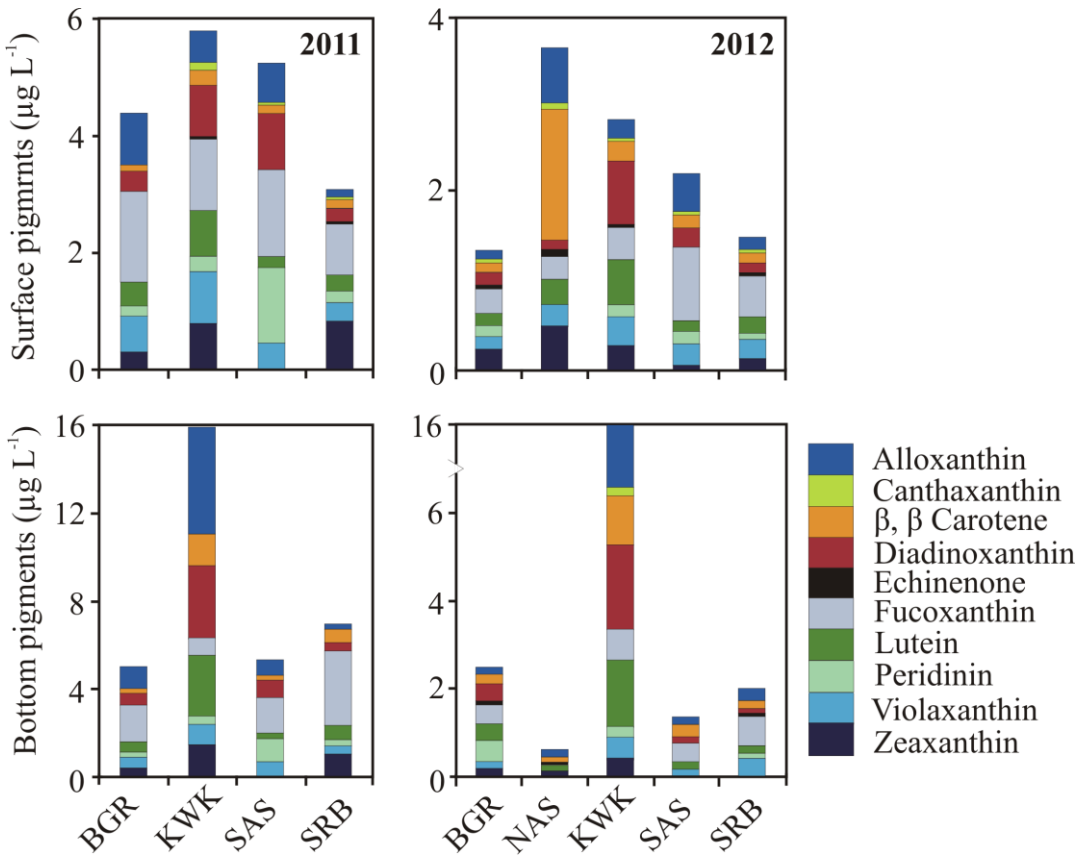

Figure 2.4. Average phytoplankton pigment compositions in the permafrost thaw and SRB lakes in surface and bottom waters.

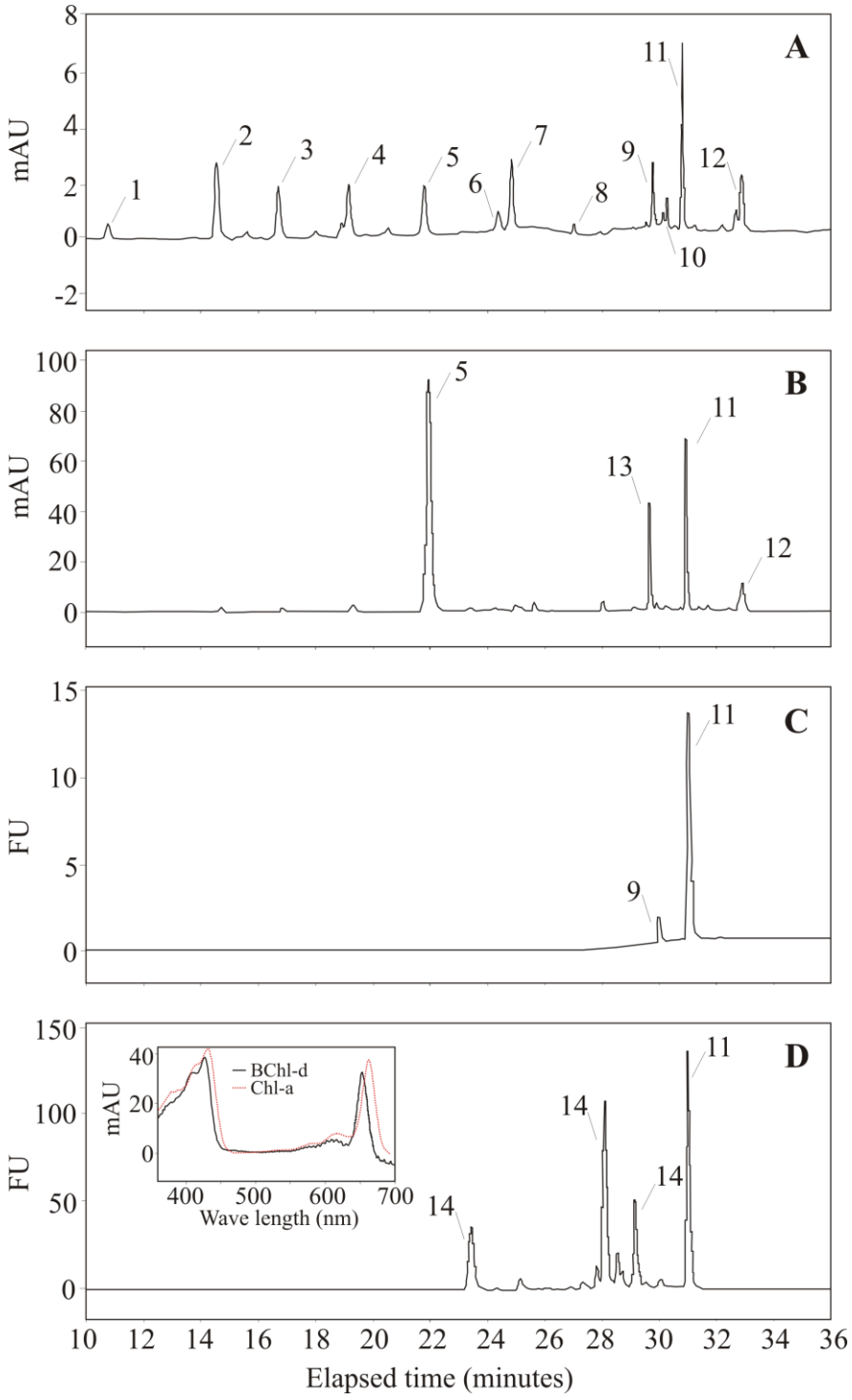

Figure 2.5. High-performance liquid chromatograms of the KWK12 lake, sampled 3 August 2012: absorbance for the surface (A) or bottom (B) water layers and fluorescence for the surface (C) or bottom (D) water layers. Pigments from left to right: 1. Perid, 2. Fuco, 3. Viola, 4. Diadino, 5. Allo, 6. Zea, 7. Lut, 8. Cantha, 9. Chl *b*, 10. Echin, 11. Chl *a*, 12. β,β-Carotene, 13. Croco and 14. BChl *d*. Insert in panel D: Bacteriochlorophyll Chl *d* (BCHl *d*, black line) and Chl *a* (red line) absorption spectra (mAU = measured absorption units).

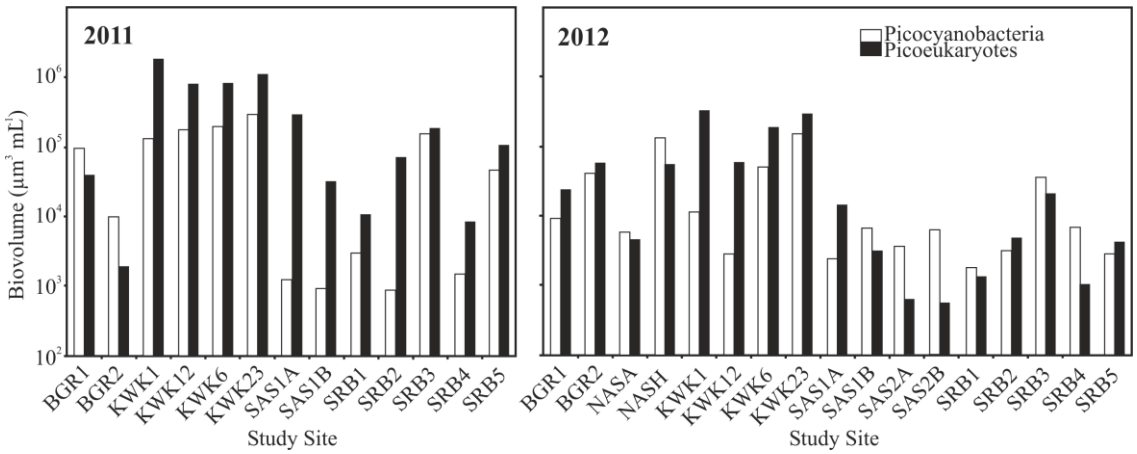

Figure 2.6. Picophytoplankton biovolume in the surface water of shallow rock-basin (SRB) and permafrost thaw lakes located on marine clays (KWK, BGR, NAS) and peatlands (SAS).

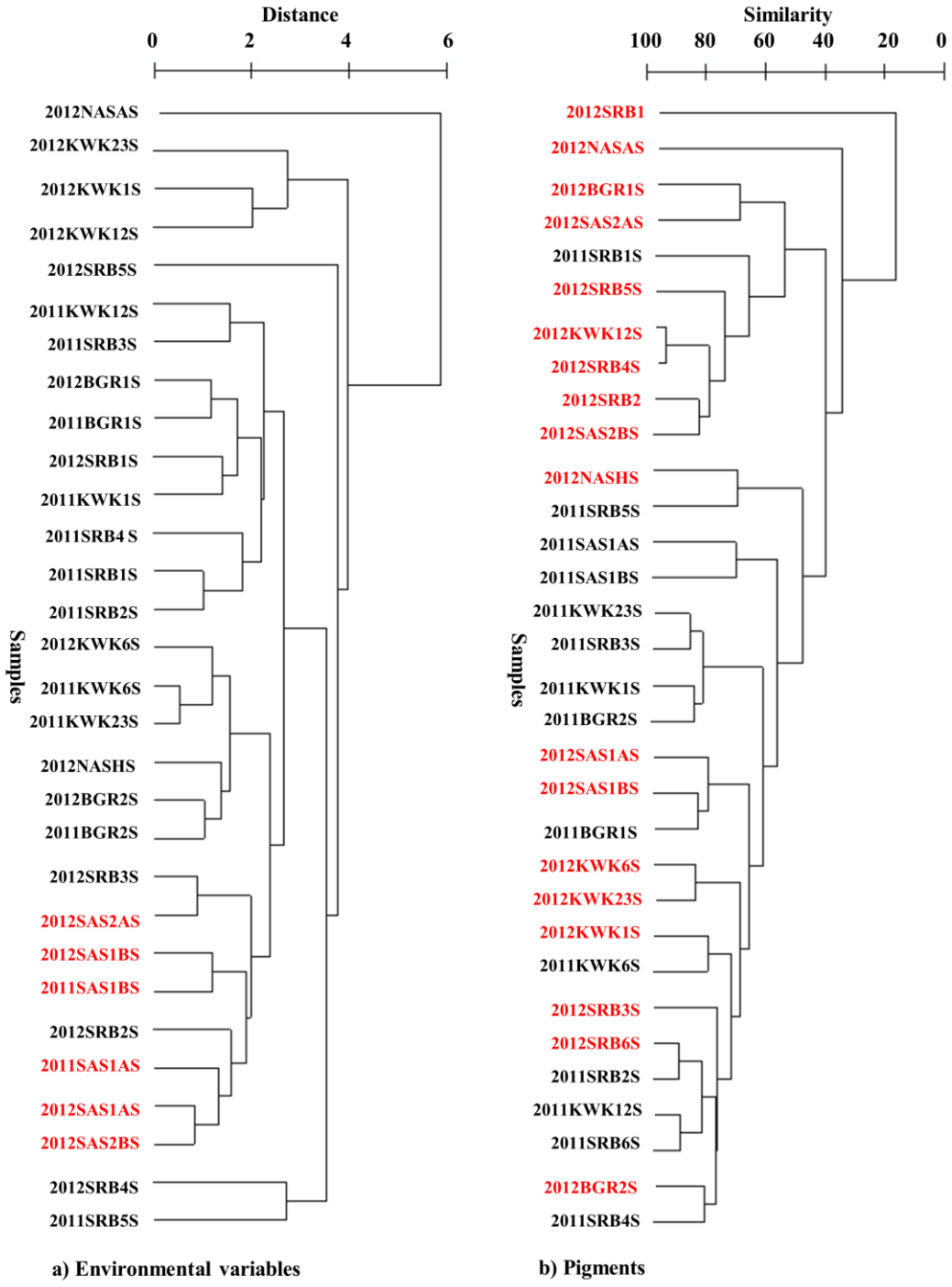

Figure 2.7. Cluster analyses of the environmental (left) and pigment (right) data. Distance is Euclidean distance.

Chapitre 3 Preconditions for cyanobacterial bloom development in northern lakes: Direct and indirect effects of climate change

3.1 Résumé

Les floraisons de cyanobactéries sont une préoccupation grandissante dans de nombreux endroits à travers le monde. Or, on en signale que très rarement dans des habitats aquatiques de hautes latitudes. Les lacs sont une composante majeure du paysage nordique. Les effets directs (par exemple, la hausse des températures) et indirects (par exemple, l'augmentation de la charge de nutriments provenant de la fonte du pergélisol) des changements climatiques sont susceptibles d'entraîner de grands changements pour les caractéristiques limnologiques de ces lacs dans le futur.

L'objectif de cette étude était d'examiner les impacts potentiels de ces changements environnementaux pour la structure de la communauté phytoplanctonique ainsi que le développement des floraisons de cyanobactéries. Spécifiquement, la question de quelles conditions préalables sont nécessaires pour stimuler la croissance et la dominance des espèces de cyanobactéries responsables de floraisons dans les eaux douces nordiques a été posée. Pour ce faire, le phytoplancton de 17 lacs de thermokarst et de lacs glaciaires (dont le fond est en roche) du Québec subarctique (Canada) échantillonnés pendant l'été a été analysé afin de déterminer sa composition taxonomique et la contribution de la biomasse des cyanobactéries. Aussi, une expérience de conception factorielle a été réalisée pour évaluer si un effet direct (le réchauffement) et un effet indirect (l'enrichissement en phosphore) des changements climatiques peuvent fournir les conditions préalables à la prolifération des cyanobactéries.

Tous les lacs contenaient des communautés phytoplanctoniques diversifiées qui étaient souvent dominées par des chrysophytes, des dinoflagellés et des chlorophytes. Les cyanobactéries étaient présentes dans tous les plans d'eau mais leur contribution était grandement variable, allant de 0,1 à 47% du biovolume total de la communauté. Le biovolume des cyanobactéries corrélait positivement avec la température de l'eau et le carbone organique dissous, tandis qu'il corrélait négativement avec les concentrations de phosphore soluble réactif, de fer et de manganèse.

L'enrichissement avec du phosphore a fait quadruplé la concentration en chlorophylle *a* (Chl *a*) et augmenté les concentrations des pigments cyanobactériens zéaxanthine et échinénone. Aussi, les énumérations de phytoplancton ont révélé une diminution majeure de la diversité (baisse de l'indice de Shannon Wiener de 1,69 à 0,16) accompagnée d'une augmentation des cyanobactéries, notamment l'espèce hétérocystée *Dolichospermum planctonica*. Des eaux plus chaudes ont initialement fait proliférer les cyanobactéries (de 0,25 à 0,55 x 10^6 μm^3 mL^{-1}, au jour 6), et une floraison de chrysophytes s'est développée par la suite (de 0,31 à 1,35 x 10^6 μm^3 mL^{-1}, au jour 12). Par conséquent, les effets combinés du réchauffement et de l'enrichissement au phosphore ont mené à une biodiversité du phytoplancton réduite, avec une communauté composée de cyanobactéries et de chrysophytes. Par ailleurs, la communauté picophytoplanctonique a grandement répondu lors de cette expérience ; les picocyanobactéries ont été fortement stimulées par l'enrichissement avec le phosphore et les picoeucaryotes ont augmenté avec le réchauffement.

Les niveaux d'inoculum de cyanobactéries actuels et leur sensibilité à la température et, surtout, au phosphore fournissent les pré-conditions nécessaires pour le développement de floraisons de cyanobactéries. Les changements climatiques en cours engendreront fort probablement la prévalence accrue des cyanobactéries, la détérioration de la qualité de l'eau ainsi que la diminution de la diversité du phytoplancton dans ces lacs du grand Nord.

3.2 Abstract

Cyanobacterial blooms are an increasing problem at many locations throughout the world but are rarely reported in aquatic habitats at high latitudes. Lakes are a major feature of northern landscapes, and these waters are likely to experience largescale changes in their limnological properties in the future as a result of the direct (increased temperatures) and indirect (increased nutrient loading from thawing permafrost) effects of climate warming.

In the present study, we examined the potential impact of these environmental changes on phytoplankton community structure and bloom development. Specifically, we addressed the question of what preconditions would be necessary to stimulate the growth and dominance of bloom-forming cyanobacteria in northern freshwaters. We analyzed the summer phytoplankton of 17 shallow lakes on eroded permafrost (thaw lakes) and glacier scoured rock (rock basin lakes) in subarctic Québec, Canada, to determine their summer taxonomic composition and the biomass contribution of cyanobacteria. We also conducted a factorial design experiment to evaluate if direct (warming) and indirect (P-enrichment) effects of climate change may additionally set the preconditions for cyanobacterial blooms.

All lakes contained diverse phytoplankton communities, often dominated by chrysophytes, dinoflagellates and chlorophytes. Cyanobacteria were present in all water bodies, but their contribution was highly variable, ranging from 0.1 to 47% of the total community biovolume. The biovolume of cyanobacteria correlated positively with DOC and surface water temperatures, and negatively with SRP, Fe and Mn.

P-enrichment resulted in a four-fold increase in Chlorophyll *a* (Chl *a*) and an increase in the cyanobacterial pigment zeaxanthin and echinenone. The phytoplankton enumerations showed that there was a decrease in diversity (drop in the Shannon Wiener index applied to genera, from 1.69 to 0.16) accompanied by increased cyanobacteria, notably the heterocystous species *Dolichospermum planctonica.* Increased temperature led to an initial increase of cyanobacteria (from 0.25 to 0.55 x 10^6 μm^3 mL^{-1} at day 6) followed by the development of a chrysophyte bloom (from 0.31 to 1.35 x 10^6 μm^3 mL^{-1}, at day 12). Combined warming and P-enrichment led to reduced phytoplankton

biodiversity, with a community composed of cyanobacteria and chrysophytes. There was also a pronounced response by the picophytoplankton community; picocyanobacteria were strongly stimulated by P-enrichment and picoeukaryotes increased in response to warming.

The current inoculum levels of cyanobacteria and their responsiveness to temperature and especially phosphorus provide the necessary pre-conditions for development of cyanobacterial blooms. Ongoing climate change will likely result in an increased prevalence of cyanobacteria, and decreased water quality and phytoplankton diversity in these northern lakes.

3.3 Introduction

The effects of climate warming are most pronounced at high northern latitudes and are resulting in perturbation of weather regimes and landscape processes. These changes include higher frequencies of severe rain and snow storm events, warmer lake temperatures, delays in freeze-up and advances in ice break-up, earlier snowmelt including periods of winter thaw, increased evapotranspiration following changes in plant communities, a deepening of the permafrost active layer and increases in thermokarst erosion (Prowse et al., 2006; Wrona et al., 2006; Natali et al., 2014). Accompanying these changes are increased fluxes of dissolved organic carbon (DOC) and inorganic nutrients to arctic freshwaters from their more productive and eroding catchments (Vonk et al., 2015b; and references therein). However, little is known about how these direct effects of warming combined with increased solute fluxes may affect biological processes in northern lakes (Medeiros et al., 2014).

Warming of aquatic habitats is expected to increase rates of enzymatically controlled light-saturated photosynthesis (Talling, 1957) and may directly lead to higher primary productivity in the surface waters (Falkowski and Raven, 2007). However, several studies show that such effects of increasing temperature may be offset by nutrient limitation of the phytoplankton (Behrenfeld et al., 2006; Boyce et al., 2010). The relationship between temperature and nutrient requirements can result in a negative response of marine phytoplankton to climate change via effects on N to P stoichiometry of algal cells (Toseland et al., 2013). While the relationship between climate and plankton has been intensively investigated at tropical (Sotero-Santos et al., 2008; Liu et al., 2011; Zhu et al., 2014) and temperate latitudes (Davis et al., 2009; Gkelis et al., 2014), there is little information about the combined effects of warming and nutrient supply on the phytoplankton in high latitude aquatic habitats (e.g., Lundholm et al., 2010).

The response of cold-adapted arctic plankton communities to environmental change may be moderated by the effects of community structure. According to the diversity–stability hypothesis, ecosystem stability and resistance to environmental stress is correlated with the taxonomic, functional, and genetic diversity of the community (McCann, 2000). More diverse communities often have higher productivities (Abrams, 1995). Ambient temperature, through its effects on physiological rates and on functional

group specialization, can influence planktonic diversity (Beaugranda et al., 2010; Liu et al., 2011; Thienpont et al., 2015). For example, increasing temperature has been associated with positive effects on phytoplankton diversity in some marine (Beaugrand et al., 2010; Tittensor et al., 2010) and freshwater ecosystems (Smol et al., 2005). Conversely, studies by Elliott et al. (2006) and Schabhüttl et al. (2013) showed that increasing temperature is followed by a decrease in phytoplankton diversity with prevalence of cyanobacteria and exclusion of cold-adapted algae. Either of these effects may be operating in high latitudes lakes exposed to a warmer climate regime.

Cyanobacteria thrive under warm conditions in lakes at tropical (Sotero-Santos et al., 2008; Wilhelm et al., 2011; Zhu et al., 2014) and temperate latitudes (Paerl and Huisman, 2008; Báez et al., 2014). In lower latitudes, the warming of lakes is often directly (via increased nutrients release from the sediments, Wilhelm and Adrian, 2008) or indirectly (via changes in the efficiency on nutrients retention in the food web, Huber et al., 2008) related to eutrophication and is accompanied by occurrence of noxious cyanobacteria blooms (O'Neil et al., 2012, Gkelis et al., 2014). Development of bloom forming cyanobacteria can be accompanied by a decline in phytoplankton biodiversity (Figueiredo et al., 2006; Krienitz et al., 2013). As climate warms and nutrient enrichment follows, high latitude lakes might therefore have a higher abundance of cyanobacteria and decreased phytoplankton diversity.

Psychrophilic phytoplankton, taxa with temperature optima at or below 15°C and a maximum growth temperature below 20°C, are known to occur in cold marine (Lovejoy et al., 2007) and freshwater (Priscu and Goldman, 1984) aquatic habitats. Warming of Arctic and Subarctic freshwaters may restrict the seasonal window for optimal growth and development of planktonic psychrophiles. As a result of warming, diverse diatom communities including some large species may be replaced by more uniform communities consisting mainly of smaller species (Winder et al., 2009). Benthic cyanobacteria inhabiting high latitude lakes are psychrotrophs rather that psychrophiles (Tang et al., 1997), and planktonic cyanobacteria in these waters may be similarly responsive to warmer temperatures.

The aim of the present study was to evaluate the set of preconditions required for cyanobacterial bloom development in northern lakes, specifically shallow subarctic

waters including the thermokarst lakes and ponds that are abundantly distributed across permafrost landscapes. First we addressed the question of whether such waters contain an inoculum of colonial taxa that could seed the development of cyanobacterial blooms in the future. Secondly, by way of a factorial incubation experiment with northern lake water samples, we evaluated the hypothesis that the combined effects of warming and nutrient enrichment would stimulate the growth of bloom-forming cyanobacteria. Specifically we aimed to test whether: i) primary production and phytoplankton diversity in high latitude lakes is regulated by ambient temperature and nutrient availability; ii) increased temperature results in decreased diversity of high latitude phytoplankton communities; iii) increased temperature coupled with increased concentrations of limiting nutrient (P) results in a decreased phytoplankton diversity and dominance of cyanobacteria.

3.4 Materials and methods

3.4.1 Study sites

Field sampling was conducted in August 2011 and 2012 on 18 water bodies in five locations near the village of Whapmagoostui-Kuujjuarapik (N 55°19', W 77°30'; Fig. 3.1). These included six shallow rock basin lakes located in the vicinity of the village (SRB; N 55°16', W 77°44'), four permafrost thaw lakes based upon mineral clays (KWK; N 55°19', W 77°30'), two highly turbid permafrost thaw lakes (NAS; N 56°55', W 76°22', in 2012 only) and four thaw lakes in peatlands (SAS; N 55°13', W 77°42'). The study also included two thaw lakes, on mineral clays, located near the village of Umiujaq (BGR; N 56°36', W 76°12').

3.4.2 Physicochemistry

Profiling of temperature, dissolved oxygen, conductivity, redox potential and pH was done with a multiparametric probe (Model 600R, YSI Inc.), with additional profiling of temperature and conductivity with a RBR XR620 probe (Richard Brancker Research Ltd.) The surface and bottom water samples were filtered through 0.2 μm membrane filters to measure colored (chromophoric) dissolved organic matter (CDOM) by absorbance scans, from 250 to 800 nm with a Varian Cary 100 spectrophotometer. The filtrates were also analyzed for dissolved organic carbon (DOC) with a Shimadzu TOC-

5000A carbon analyzer calibrated with potassium biphthalate, soluble reactive phosphorus (SRP) after acidification with 15% H_2SO_4 and then using a standard colorimetric method; and major ions using a Dionex ICS 2000 ion chromatograph. The unfiltered water samples were used to assess the total nitrogen (TN) and total phosphorus (TP) concentrations after digestion with persulfate (Stainton et al., 1977). Total suspended solids (TSS) were collected onto pre-combusted and pre-weighed 25 mm glass fiber filters (Advantec MFS) that were oven dried for 2 h at 60°C and then reweighed.

3.4.3 Phytoplankton community structure

Water samples were collected from just beneath the surface (0.2 m depth) and were transported to the laboratory in 1 L dark polyethylene bottles, then fixed with Lugol's iodine (5% final concentration). Samples were then transferred to sedimentation columns (Utermöhl, 1958) and the cyanobacteria and other phytoplankton were enumerated to genus level with an inverted microscope (Zeiss Axiovert 200). For each phytoplankton species at least 20 individual organisms were measured and biovolume was calculated following Hillebrand et al. (1999). Phytoplankton biodiversity indices were calculated for each sampling site in 2011 and 2012 following Begon et al. (2006).

3.4.4 Effects of warming and P-enrichment

In August 2012, 320 L of water from a permafrost thaw lake located within the KWK valley (KWK12, Fig. 3.1) was collected, transported by helicopter to the field station, filtered through 50 µm Nitex to remove large zooplankton and distributed among sixteen 20 L polythene Cubitainers. A factorial design was used to test the effects of warming and nutrient enrichment. The temperature treatment was effected by placing 8 of the Cubitainers in a greenhouse to provide a 4-5°C temperature increase compared to ambient conditions, with the 8 remaining Cubitainers incubated outside of the greenhouse. For the nutrient enrichment, 4 Cubitainers at each of the two temperatures were amended daily with inorganic phosphorus (K_2HPO_4); each addition was to a final concentration of 2 µg P L^{-1}. Temperature in the Cubitainers was monitored in separate containers using the RBR CTD in logging mode. The Cubitainers were incubated for 12 d and subsampled (250ml) every third day, after shaking to ensure homogenous conditions, for chemical and phytoplankton analyses. In addition, water samples were filtered onto 25-mm diameter GF/F glass-fibre filters and immediately frozen at -80°C for planktonic pigment analyses. The pigments were extracted in methanol and analyzed by high performance liquid

chromatography (HPLC) following protocols given in Bonilla et al. (2005).

During the experiments, picoautotrophic microbes were sampled into 3.5 mL Cryovials, fixed with glutaraldehyde (final concentration of 10%), and immediately frozen at -60°C. The samples were later thawed and analyzed in a Becton Dickinson flow cytometer equipped with an argon laser, at the lowest flow rate (12 µl min^{-1}), using 1-µm, yellow-green microspheres (Polysciences, Inc) as an internal standard. The bead concentration was controlled using TrueCountAbsolute counting tubes (BD biosciences). Picocyanobacteria and picoeukaryotes were distinguished based on their chlorophyll *a* and phycobiliprotein fluorescence. Detection of the two groups was performed by the comparison of flow cytograms where cells were discriminated based on their side scatter signals (SSC) and both red (FL3) and orange fluorescence (FL2) as well as FL3 versus FL2.

3.4.5 Statistical analyses

The data were normalized by logarithm transformation, and the biovolume of each phytoplankton group was related to environmental variables by Pearson correlation analysis. Pearson correlations were also used to analyze autocorrelations between environmental variables. Mutual relationships among variables were then investigated by principal component analysis. Stepwise multiple linear regression analysis was used to correlate phytoplankton biomass (for each group separately) with the principal components (Camdevyren et al., 2005). The effects of the experimental factors (phosphorus enrichment and increased temperature; P and T) on phytoplankton abundance and biodiversity were tested using 2-way factorial ANOVA followed by post hoc Tukey test. All analyses were performed using PAST statistical software.

3.5 Results

3.5.1 Environmental heterogeneity

The subarctic lakes showed variable limnological conditions, with their trophic status ranging from oligotrophic to eutrophic based on TP values (1.6 to 64.5 µg L^{-1}) and oligotrophic to mesotrophic based on Chl *a* concentrations (0.3 to 9 µg L^{-1}). The extent of water column stratification was also variable, with temperature differences from 1 to

10°C between the surface and bottom waters. Further limnological details are given in Przytulska et al. (2015b) and Deshpande et al. (2015).

3.5.2 Phytoplankton community structure

Each lake contained diverse phytoplankton communities in both 2011 and 2012 (Fig. 3.2). In 2011, lakes located in the KWK valley contained abundant populations of chrysophytes and euglenophytes, including a large contribution from cyanobacteria in one of these waterbodies (KWK6). Lakes in BGR and SAS valleys contained high numbers of chrysophytes and dinoflagellates with prevalence of the latter group in SAS1B. Cyanobacteria were most abundant in the shallow rock-basin lakes (SRB). In 2012, lakes located in KWK valleys contained abundant populations of euglenophytes and chlorophytes. Cyanobacteria were present and abundant in lakes KWK1 and KWK6. Chrysophytes were abundant in BGR and SAS lakes along with diatoms and zygnematophytes in the SAS lakes. During this second summer only SRB3 and SRB5 contained large numbers of cyanobacteria, notably the genus *Dolichospermum*.

For the overall study, more than 60 genera were identified, within a total of 8 phyla. The Shannon diversity index ranged from 0.67 (KWK6) to 2.39 (SRB1), and Simpson's index ranged from 1.48 to 8.91. Stepwise multiple linear regression analysis indicated that the variability in Shannon biodiversity index was related to a combination of the following limnological variables: Diversity = 17.14 - 0.532 SRP - 3.11 Temp + 0.673 Fe, ($R^2 = 0.31$, $p = 0.01$).

3.5.3 Environmental effects on phytoplankton community structure

Pearson's correlation analysis showed that cyanobacterial biomass in subarctic lakes was positively correlated with surface temperature and Cl^- concentrations (Table 3.1). There was a negative correlation between cyanobacteria and SRP, DOC, Fe and Mn. Chlorophyte biovolume was negatively correlated with Fe and Ca; and for the zygnematophytes there was negative correlation with temperature and a positive correlation with pH and Ca. There were no significant correlations between other phytoplankton groups and environmental conditions.

Principal component analysis (PCA) of the environmental variables resulted in four components with eigenvalues larger than 1, which explained 80% of total variance

(Table 3.2). The first component explained about 37% of the observed variance. Several variables were strongly correlated with this first component, namely DOC, SRP, Fe and Temp (Fig. 3.3; Table 3.4). The second component explained about 17% of observed variance. The variables correlated strongly with this component were DOC, Cl^-, NO_3^- and Mn concentrations. The third component explained only 14% of the variance and was correlated with DOC, conductivity and NO_3^-. The fourth component explained only 11% of the variance and was related mainly to pH. Cyanobacteria biomass in subarctic lakes was statistically related to PC1 and PC3 ($R^2 = 0.29$, $p = 0.01$) as: ln (Cyanobacteria) = 4.552 + (0.236 × PC1) - (0.318 × PC3). Chlorophyte biovolume was also related to PC3 ($R^2 = 0.21$, $p = 0.01$) as: ln (Chlorophytes) = 4.827 - (0.439 × PC3). Zygnematophytes were related to PC3 and PC4 ($R^2 = 0.44$, $p = 0.01$) as: ln (Zygnemophytes) = 4.617 + (0.316 × PC3) + (0.335 × PC4).

3.5.4 Effects of warming and P-enrichment on the phytoplankton community

The water temperature in the containers located in the greenhouse averaged 23.7 ± 3.2°C and under outside ambient conditions averaged 18.4 ± 2.7°C, giving an average difference of 5.3 ± 0.9°C. This warming was associated with 57.7 % more phytoplankton biomass at day 12 relative to the control (+T versus C; $p = 0.001$, ANOVA with Tukey HSD). The P addition resulted in a much higher increase of phytoplankton by 470 % in comparison to the control (+P versus C, $p = 0.01$, ANOVA with Tukey HSD). There was also a significant response of phytoplankton community structure to increased temperature and enrichment. Warming resulted in a slight decrease of the biodiversity (Table 3.3) with an accompanying increase of cyanobacteria after day 6 and chrysophytes after day 12 (Fig. 3.4). Warming combined with P-addition resulted in a strong decrease of the biodiversity and a significant ($p = 0.01$) increase of the chrysophytes after day 6, and the emergence of a cyanobacterial bloom by day 12. A sharp decrease of phytoplankton biodiversity was also observed in the treatment exposed to higher P-addition only. This was accompanied by a fourfold increase of the planktonic biomass, but with a community consisting largely of cyanobacteria and chrysophytes and an algal bloom apparent by day 12.

Pigment analysis confirmed the striking effect of increased temperature and nutrient enrichment on the phytoplankton community structure (Fig. 3.5). Warming resulted in an increase of planktonic pigments associated with cyanobacteria (zeaxanthin),

and chrysophytes/dinoflagellates (fucoxanthin). Combined warming and P-enrichment resulted in an increase of cyanobacterial pigments as well as those of green algae (violaxanthin and chlorophyll *b*). Changes in the planktonic pigment structure indicated that the community was most stimulated by the phosphorus addition alone with, in comparison to control, almost six-fold higher concentrations of the cyanobacterial pigment echinenone as well as two-fold higher concentrations of diadinoxanthin and chlorophyll *b*.

3.5.5 Effects of warming and P-enrichment on the picophytoplankton

The two-way factorial ANOVA analysis followed by Tukey post-hoc test revealed a significant positive effect of temperature ($p < 0.001$) on picoeukaryotes, particularly in combination with phosphorus enrichment (Fig. 3.4). Phosphorus addition alone did not result in a significant difference in the abundance of picoeukaryotes when compared to the control treatment after day 12. The abundance of picocyanobacteria was highest in the cultures subjected to enrichment, with and without warming. The warming itself did not result in a significant difference in their abundance ($p > 0.05$) when compared to the control treatment at the end of the experiment.

3.6 Discussion

The field survey revealed that the 18 studied subarctic lakes contain diverse phytoplankton communities including species from all main taxonomic groups. This finding is consistent with the diverse pigment assemblages previously reported for these lakes (Przytulska et al. 2015a). Cyanobacteria were present in all of the studied lakes and sometimes contributed up to half of the total phytoplankton biovolume. The first precondition for cyanobacterial bloom development was met: the existence of an inoculum of bloom-forming taxa, specifically *Dolichospermum* (formerly *Anabaena*; Komárek and Zapomělová 2007). In contrast to other taxonomic groups, cyanobacterial biomass was negatively related to SRP concentrations and positively related to surface water temperature. Cyanobacterial biovolume was also correlated with Fe and Mn concentrations, suggesting that these metals may play a role in the cyanobacterial ecology of subarctic lakes. Fe is known to be a factor limiting nitrogen-fixing cyanobacteria in some lake ecosystems (e.g., Wurtsbaugh et al., 1985).

The factorial experiment revealed striking effects of warming and P enrichment on the phytoplankton, including the picoplanktonic component. The most pronounced changes in phytoplankton were the increase in chrysophyte biomass by day 6 and the increase in cyanobacterial biomass by day 12 of the incubations. An interactive effect was observed in +P+T treatment, where after initial bloom of the chrysophytes (day 6), a cyanobacterial bloom was recorded at day 12. Cyanobacteria bloom development also occurred in the +P treatment, with biovolumes reaching 5×10^6 μm^3 mL^{-1}. Contrary to expectation, warming itself did not result in an increased dominance of cyanobacteria. In this case, the initial increase of cyanobacteria was followed by a much faster increase of chrysophytes after day 6.

Warming and P enrichment also affected the picophytoplankton. Picocyanobacteria achieved high abundances in the P-replete conditions, and an increase in picoeukaryotes was observed when warming accompanied the P enrichment. Temperature has often been identified as a key variable controlling picophytoplankton growth and abundance. For example, Ochs and Rhew (1997) reported a strong correlation between picocyanobacterial abundance and temperature in reservoirs of the southeastern USA. Conversely, these authors also found that picoeukaryotes were negatively correlated to temperature, with dominance shifting from picoeukaryotes in winter to picocyanobacteria in summer. Increasing temperature favoured picocyanobacteria over picoeukaryotes in German lakes (Hepperle and Krienitz, 2001) and in experiments with subarctic river and lake waters (Rae and Vincent, 1998). Phosphorus availability has been recognized as a second important variable controlling picophytoplankton dynamics (Burns and Schallenberg, 1998; Tzaras et al., 1999). Recently Mackey et al. (2013) found that an increase in N to P ratios was accompanied by a strong positive increase in picophytoplankton abundance in ultra-oligotrophic Lake Tahoe (USA). The positive response of picoeukaryotes to warming indicates stimulation of these microbes by direct effects of climate changes. In contrast, the strong positive effect of P enrichment itself showsthe potential for indirect stimulation of picocyanobacteria by climate change in northern lakes.

The results from this ‘bottom up’ experiment must be interpreted with caution. Sampling was from a single lake during the open water period, and T0 conditions would

differ at other sampling times. The enclosure of communities, irrespective of 'bottle size', is known to affect plankton communities, and the sharp decrease in Euglenophytes in the control provides evidence of this effect. The removal of large grazers by pre-filtration, to focus on the bottom up controls and reduced within-treatment variability, would have released the community from a pressure that might otherwise influence community structure. Finally, although the phosphorus treatments were randomly distributed and independent, the experimental temperature units were clustered by necessity (inside and outside the greenhouse) and were not independent, leading to an inevitable weakness of design (Hurlbert, 1984). Nonetheless, the results indicated a potentially strong response by the phytoplankton community to increased P supply and warming.

In the phytoplankton survey of these subarctic waters, cyanobacterial biovolume was positively correlated with surface water temperatures, and in the culture experiment with thaw lake water, cyanobacteria responded positively to increased temperature during the first 6 d of incubation. There is a general agreement in literature that increased epilimnetic temperatures favour cyanobacteria in aquatic ecosystems of tropical and temperate latitudes (Mooij et al., 2005; Pearl and Huisman, 2008; Jeppesen et al., 2007). This effect may, however, result from direct stimulation of cyanobacteria by warmer conditions for growth, or indirectly by enhanced stratification of the water column, which favours buoyant taxa that can regulate their vertical position (Vincent, 2009; Carey et al., 2012).

Bloom-forming cyanobacteria are known to be more responsive to temperature than other phytoplankton groups (Reynolds, 1989; 2006). For example, growth of *Microcystis areuginosa* responded positively to increasing temperature above 28°C, with a Q_{10} value (acceleration over a 10°C step) up to 9.5. However, our experimental results showed that while warmer temperatures stimulated filamentous cyanobacteria, notably in the genus *Dolichospermum* (formerly *Anabaena*; see: Komárek and Zapomelova, 2007), the longer incubation time in warmer conditions (day 6-12) resulted in dominance of planktonic chrysophytes. This result was unexpected, but is consistent with certain field observations. For example, a warming trend in seven Canadian lakes between 1981 and 2003 was accompanied by increasing chrysophyte biovolume, with a greater abundance of mixotrophic taxa (Paterson et al., 2008). Recently Wilken et al. (2012) found that planktonic mixotrophs may be favoured over other phytoplankton by increasing

temperature as it enhances the rates of heterotrophic feeding. In part this may be related to increased growth rates of the prey species for mixotrophs, specifically picocyanobacteria and heterotrophic bacteria. The observed increased abundance of *Ochromonas* sp. in the lake water exposed to warming could also have exerted a top-down control on the picocyanobacteria, given the known ability of mixotrophic chrysophytes to graze on small celled planktonic cyanobacteria (van Donk et al., 2009).

In contrast to the response to the effects of increased temperature, phosphorus addition resulted in an initial increase of chrysophytes, followed by the shift towards cyanobacterial dominace by day 12. Nutrient enrichment is a well-known factor leading to cyanobacterial blooms in temperate lakes (Paerl et al., 2001; Schindler, 2001; Calandrino and Paerl, 2011), with increasing proportional representation of cyanobacteria above 20 μg TP L^{-1} (Watson et al., 1997). The warming of permafrost landscapes may result in an increased loading of phosphorus and other nutrients to thaw lakes (Vonk et al., 2015a). Some earlier studies in high latitudes indicated strong responses of the phytoplankton to whole lake fertilizations with great increases in biovolume of chrysophytes and some cyanobacterial species (Holmgren, 1984; Welch et al., 1989). Filamentous cyanobacteria appear to be more strongly stimulated by P enrichment in high latitude lakes than unicellular or colonial taxa (Schindler et al., 1974), particularly nitrogen fixing forms. The shift toward *Dolichospermum* in the experiment may in part have been the result of a shift to nitrogen deficiency, and the competitive advantage of this N_2-fixing cyanobacterium. Indeed, we have observed higher frequency of heterocysts in *Dolichospermum* from P-enriched mesocosms, which suggest higher rates of atmospheric N_2 fixation (Horne and Goldman 1972, Vintila and El-Shehawy, 2007) when nitrate availability became limited.

At a global level, the combined effects of climate warming and eutrophication are predicted to enhance the frequency and magnitude of cyanobacterial blooms (Johnk et al., 2008; Vincent 2009; Huber et al., 2012; O'Neil et al., 2012). The synergistic nature of this effect has been questioned, however, with evidence that nutrient enrichment may play the greater role (Rigosi et al., 2014). In the present study, the combined effect of P addition and warming resulted in an increase of phytoplankton and cyanobacterial dominance by day 12, however the combination stimulated cyanobacteria to a lesser extent that P addition alone (Fig. 5). Palaeolimnological analysis of fossil pigments in a

high Arctic lake indicated that cultural eutrophication and climate warming resulted in an increased abundance of chromophyte algae and cyanobacteria (Antoniades et al., 2011), consistent with our experimental results.

The dominance of cyanobacteria in the combined +T&P treatment was accompanied by an almost complete disappearance of dinoflagellates and a striking decrease in phytoplankton biodiversity. In contrast, warming did not result in decreased biodiversity, and the Shannon-Wiener index slightly increased in the course of incubations at the warmer temperature as compared to the control treatment. There is an ongoing debate in the literature about the effects of warming and eutrophication on phytoplankton diversity. Pomati et al. (2011; 2015) concluded that indeed eutrophication but not warming will result in a decreased phytoplankton diversity, while other authors (e.g., Paerl and Huisman, 2008; Schabhüttl et al., 2012) have suggested that warming may mimic eutrophication effects and result in decreased diversity. These disparate results might be reconciled if diversity follows a hump-shaped pattern, similar to the dependence of species richness on productivity (Mittelbach et al., 2001). In meso- to eutrophic systems exposed to relatively low temperature, as in the high latitude lakes studied here, short term effects of warming may result in increased productivity and phytoplankton diversity. Such stimulating effects may, however, be offset by indirect results of climate change leading to higher nutrient availability in these systems. In the latter case, as indicated by our incubations, development of abundant bloom forming cyanobacteria will result in a sharp decrease in plankton biodiversity and deterioration of water quality.

3.7 Acknowledgments

The authors thank Isabelle Laurion for help in planning the 2011 field campaign, Sophie Crevecoeur, Alex Matveev, Jérome Comte, Paschale N. Bégin and Bethany Deshpande for their help in the field, and Marie-Josée Martineau for help in planktonic pigments analyses. Comments from Maciej Bartosiewicz helped improve earlier drafts of this manuscript.

Table 3.1. Pearson's correlation between phytoplankton biomass and environmental variables in subarctic lakes. Phytoplankton phyla: Cyanobacteria (Cyano), Chlorophytes (Chloro), Chrysophytes (Chryso), Zygnematophytes (Zygn).

Factor	Cyano	Chloro	Chryso	Zygn
DOC	**-0.460**	-0.264	-0.296	0.009
SRP	**-0.408**	-0.240	0.041	0.056
Temp	**0.445**	0.232	0.069	**-0.749**
NO_3^-	0.005	-0.300	-0.037	0.151
pH	0.219	0.240	0.263	**0.372**
Cl	**0.454**	0.08	0.103	0.074
Fe	**-0.373**	**-0.369**	-0.190	0.054
Mn	**-0.382**	-0.280	-0.078	0.013
Ca	-0.204	**-0.374**	0.107	**0.388**

Table 3.2. Results of the principal component analysis for the subarctic lakes.

Factor	Eigenvalues.	var. %
PC1	3.85986	42.887
PC2	1.38978	15.442
PC3	1.04809	11.645

Table 3.3. Biodiversity indices for experimental treatments after day 12.

	Control	+T	+P	+T&P
Total number of genera	13	10	12	6
Simpson's index	4.25	3.97	2.45	1.06
Shannon-Wiener index	1.69	1.79	1.30	0.16

Table 3.4. Pearson's correlation analysis (loadings) between principal components (PC) and environmental variables (dissolved organic carbon – DOC, chlorides – Cl, nitrates – NO_3, soluble reactive phosphorus – SRP, iron – Fe, manganese – Mn, surface water temperature – Temp, pH and conductivity – Cond) in subarctic lakes.

	PC 1	PC 2	PC 3	PC 4
DOC	**-0.39**	**0.35**	**0.34**	-0.16
Cl	**0.29**	**0.53**	-0.01	0.02
NO_3	0.20	**0.38**	**0.55**	0.00
SRP	**-0.46**	-0.18	-0.04	0.16
Fe	**-0.48**	-0.02	0.06	0.03
Mn	0.25	**-0.56**	**0.32**	0.19
Temp	**0.30**	0.02	**-0.52**	**-0.40**
pH	0.28	0.05	0.01	**0.72**
Cond	0.22	**-0.32**	**0.45**	**-0.48**

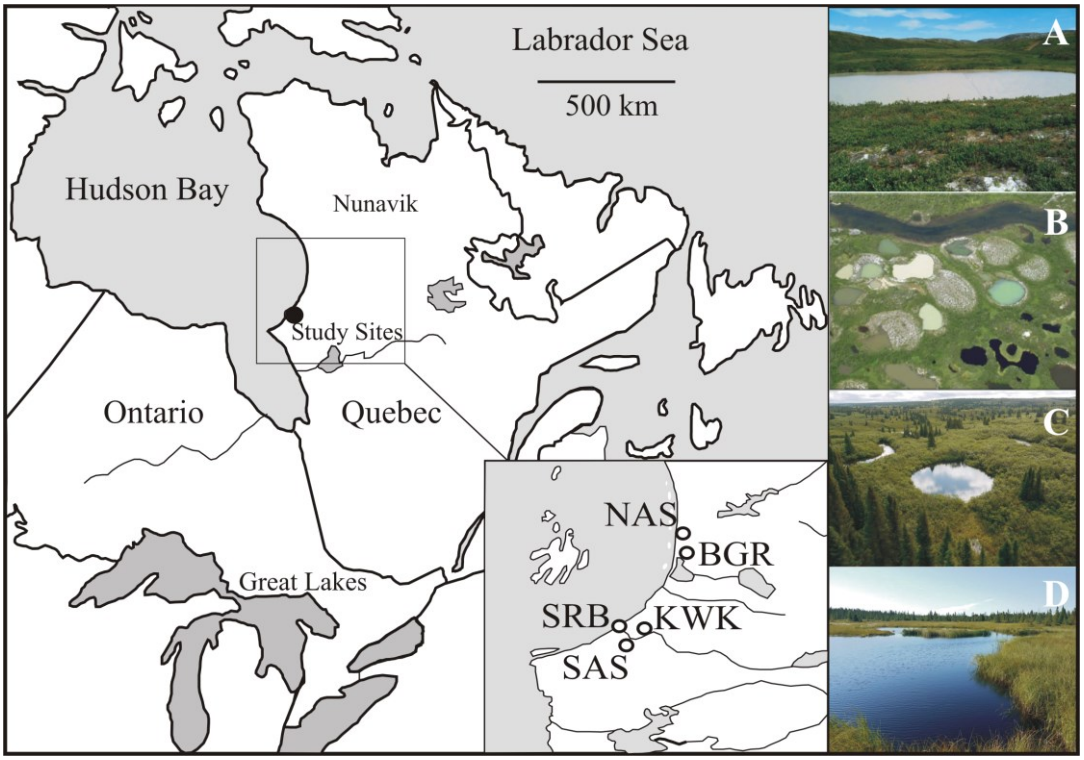

Figure 3.1. Location of the study area in subarctic Quebec, Canada. The photographs illustrate the different permafrost landscapes: A – NAS valley, B – BGR valley, C – KWK valley and D – SAS valley.

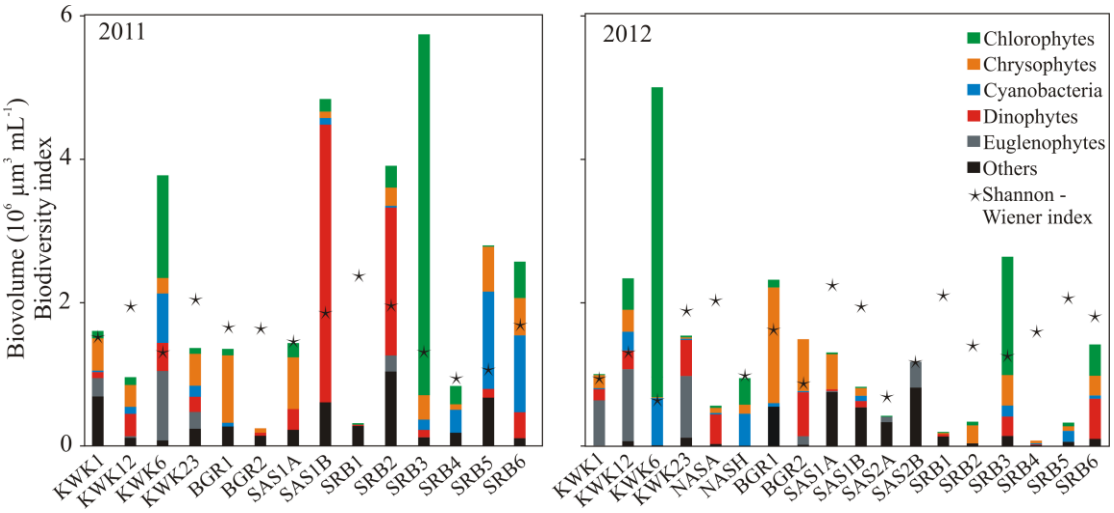

Figure 3.2. Phytoplankton biovolume and diversity in subarctic lakes.

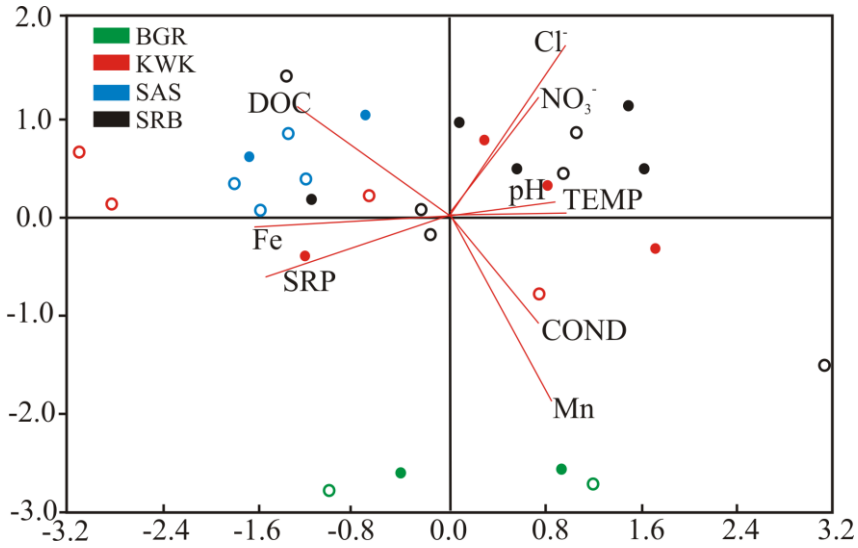

Figure 3.3. Biplot of PC1 and PC2 for principal component analysis of the environmental variables in subarctic lakes. Closed circles – 2011 data, open circles – 2012 data.

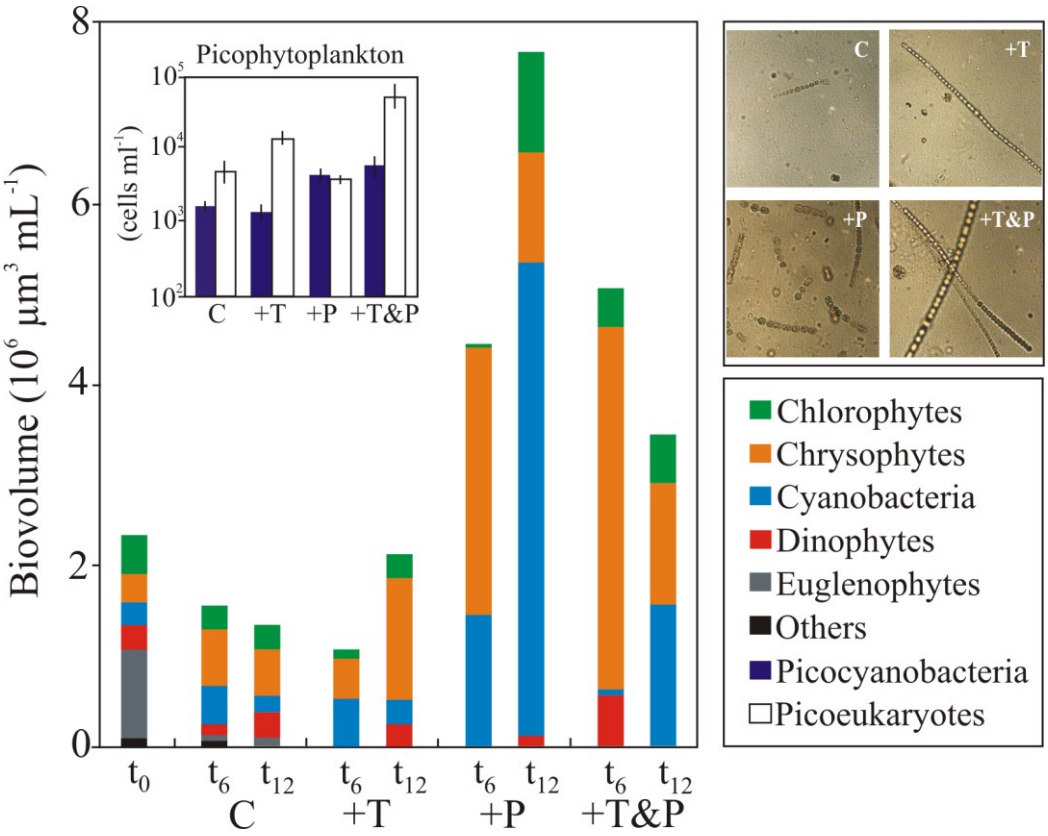

Figure 3.4. The effects of warming (+T) and nutrient enrichment (+P) and both (+T&P) on phytoplankton community structure (picocyanobacteria and picoeukaryotes given as an insert, at day 12 only) in the permafrost thaw lake water cultures. The photomicrographs (at x400 magnification) show the effect of experimental factors on *Dolichospermum* sp. abundance after 12 days of incubation.

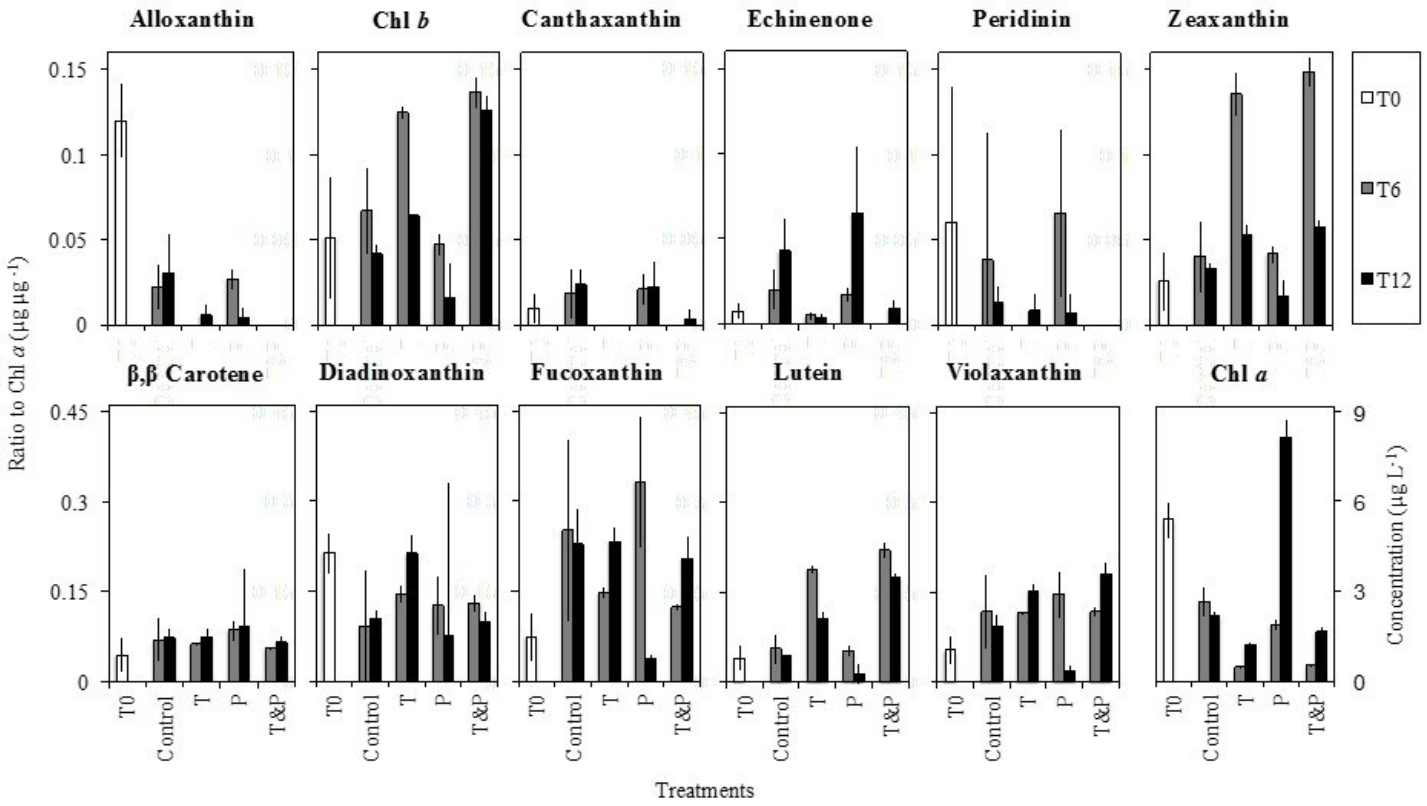

Figure 3.5. The effect of warming (+T) and nutrient enrichment (+P) on phytoplankton pigment structure (ratios to Chl *a*) in the permafrost thaw lake water cultures. The secondary axis is provided for Chl *a* only. T0 is the white bar, T6 is in grey and T12 in black.

Chapitre 4 Climate effects on high latitude *Daphnia* via food quality and thresholds

4.1 Résumé

L'importance et la rapidité du réchauffement climatique dans les hautes latitudes nordiques pourraient engendrer de nombreux effets directs et indirects pour les réseaux alimentaires aquatiques. Un des effets prédits est un changement dans la communauté de phytoplancton avec une abondance accrue de cyanobactéries. Étant donné que les cyanobactéries sont reconnues pour être une source de nourriture pauvre en nutriments, l'hypothèse qu'un tel changement réduirait l'efficacité de l'alimentation et la croissance du zooplancton nordique a été posée. Afin de vérifier cette hypothèse, un clone de *Daphnia pulex* vivant dans un lac de thermokarst du Québec subarctique a d'abord été isolé. On a confirmé que ce clone était triploïde, mais sinon, génétiquement semblable à un clone diploïde de référence (clone de la même espèce provenant d'un étang d'eau douce du sud du Québec). Par la suite, un système à écoulement contrôlé a été utilisé pour étudier l'effet direct de la température et l'effet indirect des picocyanobactéries subarctiques (*Synechococcus*) comme source de nourriture pour les seuils de concentrations pour l'alimentation et le taux de croissance du clone subarctique. De plus, l'effet direct de la température sur l'alimentation en picoplancton eucaryote (*Nannochloropsis*) des deux clones de *Daphnia* a été comparé.

Les résultats ont révélé que le clone subarctique avait un seuil alimentaire significativement plus faible pour la croissance que le clone tempéré, et ce à 18°C et à 26°C. Ce résultat implique que le clone subarctique est adapté pour de faibles concentrations de nourriture, même dans des conditions plus chaudes. La faible qualité des cyanobactéries comme source de nourriture a été confirmée. En effet, les picoeucaryotes contenaient des acides gras polyinsaturés, en contraste avec les cyanobactéries qui n'en renfermaient pas. Les seuils alimentaires pour la croissance des daphnies subarctiques étaient de 3,7 (18°C) à 4,2 (26°C) fois plus élevés lorsque le zooplancton était nourris de *Synechococcus* par rapport à *Nannochloropsis*. Également, il y avait un effet négatif significatif de l'augmentation de la température et du changement d'alimentation (utiliser des cyanobactéries comme source de nourriture) sur le contenu et la composition des acides gras du zooplancton. Enfin, les

résultats ont démontré que les effets de la température et de la qualité de la nourriture pour les daphnies agissent en synergie ; l'effet combiné de la température et de la qualité de la nourriture sur la performance des daphnies subarctiques était plus important que leurs effets additionnés séparément. Cette étude suggère les effets indirects, potentiellement puissants, du réchauffement climatique pour les réseaux alimentaires aquatiques.

4.2 Abstract

Climate change is proceeding rapidly at high northern latitudes and may have a variety of direct and indirect effects on aquatic food webs. One predicted effect is the potential shift in phytoplankton community structure towards increased cyanobacterial abundance. Given that cyanobacteria are known to be a nutritionally poor food source, we hypothesized that such a shift would reduce the efficiency of feeding and growth of northern zooplankton. To test this hypothesis, we first isolated a clone of *Daphnia pulex* from a permafrost thaw pond in subarctic Québec, and confirmed that it was triploid but otherwise genetically similar to a diploid, reference clone of the same species isolated from a freshwater pond in southern Québec. We used a controlled flow-through system to investigate the direct effect of temperature and indirect effect of subarctic picocyanobacteria (*Synechococcus*) on threshold food concentrations and growth rate of the high latitude clone. We also compared the direct effect of temperature on both *Daphnia* clones feeding on eukaryotic picoplankton (*Nannochloropsis*). The high latitude clone had a significantly lower food threshold for growth than the temperate clone at both 18 and 26°C, implying adaptation to lower food availability even under warmer conditions. Polyunsaturated fatty acids were present in the picoeukaryote but not the cyanobacterium, confirming the large difference in food quality. The food threshold for growth of the high latitude *Daphnia* was 3.7 (18°C) to 4.2 (26°C) times higher when fed *Synechococcus* versus *Nannochloropsis*, and there was also a significant negative effect of increased temperature and cyanobacterial food on zooplankton fatty acid content and composition. The combined effect of temperature and food quality on the performance of the high latitude *Daphnia* was greater than their effects added separately, further indicating the potentially strong indirect effects of climate warming on aquatic food web processes.

4.3 Introduction

The Arctic is currently warming at much faster rates than the global average and many physical effects including reduced seasonal ice cover over lakes and seas, a deepening of the permafrost active layer, and changes in snowfall and hydrology, have become apparent in northern environments (Wrona et al., 2006). High latitude lakes have been identified as systems that are particularly vulnerable to warming because of the wide-ranging influence of low temperatures and persistent ice cover on their ecosystem structure and function (Vincent et al., 2013). The effects of loss of ice cover on aquatic food webs and productivity have been investigated via field observations, simulated changes in underwater irradiance, and by paleolimnological inferences (Comeau et al., 2011; Charvet et al., 2014; Smol and Douglas, 2007a). Similarly, temperature effects have been examined by observation, experimental manipulations and modeling (Kaplan et al., 2003; Quinlan et al., 2005; Kirchman et al., 2009). Collectively, these studies imply that climate change has the potential to directly affect aquatic communities and processes through changes in light and temperature conditions, but may also exert indirect effects via changes in species composition and trophic relationships. However, despite increasing interest in climate impacts on high latitude ecosystems, the combined influences of such direct and indirect effects on trophic processes, and specifically phytoplankton–zooplankton interactions, remain poorly understood.

Picocyanobacteria are a ubiquitous component of the phytoplankton in high latitude freshwaters (Vincent et al., 2000a), and may be increasingly favored by climate warming. At temperate latitudes, cyanobacterial growth is known to respond strongly to warmer temperatures and increased nutrient supply (Paerl and Huisman, 2009), and high latitude cyanobacteria may be similarly responsive. For example, loss of ice from a High Arctic lake resulted in increased mixing and nutrient entrainment from lower depths, and these conditions were accompanied by a 5-fold increase in picocyanobacterial concentrations (Veillette et al., 2008). In experimental analyses of temperature and UVR effects on microbial communities in Arctic lakes, warmer temperatures resulted in a more rapid net growth (chlorophyll *a*) of smaller (< 2 μm) than larger phytoplankton, with noticeable dominance of picocyanobacteria (Rae and Vincent, 1998). More recent studies have

indicated that warming may not only stimulate some cyanobacterial species in cold benthic environments, but may also lead to lower cyanobacterial diversity and increased toxin production (Kleinteich et al., 2012).

An increased prevalence of cyanobacteria and the associated shift towards a less diverse phytoplankton community could potentially affect food web processes in northern waters given that many of these prokaryotic taxa are known to be a poor food source for zooplankton grazers. For filamentous and colonial cyanobacteria this is in part because their morphological traits interfere with ingestion (Gliwicz and Lampert, 1990), but single celled picocyanobacteria may also be a less favorable food supply than other phytoplankton. A diet consisting of cyanobacterial cells was shown to lead to a decreased growth rates and higher threshold food concentrations in temperate zooplankton (Gliwicz and Lampert, 1990), likely due to decreased food quality. The latter includes low phosphorus to carbon ratios (DeMott, 1998) and the absence of polyunsaturated fatty acids (DeMott and Muller-Navarra, 1997).

Zooplankton in high latitude lakes and ponds are exposed to a variety of environmental stresses ranging from extreme UVR and low water levels to prolonged periods of low temperatures and food shortages (Rautio et al., 2011). An interesting feature of northern crustaceans is the trend towards polyploidy at high latitudes (Little et al., 1997; Dufresne and Hebert, 1994), which may be accompanied by increased cell and body size (Kozłowski et al., 2003). The larger body sizes in zooplankton, and particularly in cladocerans, may result in competitive superiority due to lower threshold food concentrations (Gliwicz, 1990). Cladocerans are often the dominant constituents of the zooplankton communities and keystone herbivores in high latitude ponds (Rautio et al., 2011), and polyploidy is well known in northern taxa in this group (Rautio et al., 2011), including in the genus *Daphnia* (Dufresne and Hebert, 1994).

Our aim in the present study was to evaluate the direct effect of temperature on zooplankton feeding, and indirect effects that may operate in high latitude freshwaters through a shift of the phytoplankton community to increased dominance by picocyanobacteria. We hypothesized that high latitude cladocerans will respond negatively to

picocyanobacteria as a food source. Given evidence that high latitude *Daphnia* populations are adapted to colder temperatures (Dufresne and Hebert, 1994; Yurista, 1999), we also hypothesized that subarctic *Daphnia pulex* will be negatively affected by warming to a greater extent than its southern counterparts. To address these hypotheses, we compared the effects of temperature on food thresholds and growth rates of a high latitude versus temperate clone of *Daphnia pulex*, and the effects of picoplankton food type (picoeukaryote versus picocyanobacterium) on the high latitude clone, including its fatty acid content and composition.

4.4 Material and Methods

We isolated a high latitude clone of *Daphnia pulex* (*Daphnia*-HL) from a permafrost thaw lake (SAS2C; 55°13'N, 77°42'W) in the Sasapimakwananisikw River Valley (SAS Valley) located near the village of Kuujjuarapik-Whapmagoostui at the edge of Hudson Bay, subarctic Quebec, Canada (Fig. 4.1). No collection permits were required, but consultation about this work was undertaken with the Whapmagoostui First Nation and the Kuujjuarapik Inuit community via Centre d'études nordiques (CEN). The *Daphnia*-HL clone was maintained in 0.45 μm filtered temperate lake water (Lake Saint Charles, Quebec, Canada), supplemented with selenium (7 μg SeO_2 L^{-1}), at room temperature (21°C) over 12 months prior to the start of the experiments. There was unlikely to be any genetic change in the *Daphnia* population within this time frame since no sexual reproduction occurred and asexually produced ephippia were removed. The temperate clone of *Daphnia pulex* (*Daphnia*-T) was isolated from a shallow temperate pond in the vicinity of Metis, southern Quebec, Canada (48°37'N, 68°06'W) and was maintained in the same laboratory conditions as the high latitude clone. This "common garden" approach allowed direct comparisons between the two genotypes with the same starting conditions. The two pond sites are 980 km and 6 degrees of latitude apart, and this translates into a large climatic difference. For the high latitude clone, the duration of the growing (open water) season would likely be 4 months (June to September), with 12-13°C as an average surface water temperature at the beginning of the season rising to 16-17°C by mid-summer (Laurion et al., 2010), and extremes to around 20°C (Crevecoeur et al., 2015). For the temperate clone, the average

growing season would likely be 6 months (May to October) with 12-13°C as an average surface water temperature at the beginning and by the end of the season and 22-24°C in the middle of the summer (Vergilino et al., 2009).

4.4.1 Genetic analyses

In order to assign the two *Daphnia* clones to their respective lineages in the *Daphnia pulex* complex, we sequenced the NADH dehydrogenase subunit 5 (ND5) mitochondrial gene. DNA extractions were performed using 40 µL of Quick extract (Epicentre Biotechnologies) solution. Individual *Daphnia* were incubated for 2 hours and a half at 65 °C followed by 15 minutes at 95°C. The ND5 gene was amplified using the primers DpuND5b (5′-GGGGTGTATCTATTAATTCG-3′) as reverse primer and DpuND5a (5′-ATAAAACTCCAATCAACCTTG-3′) as in (Vergilino et al., 2009). DNA sequencing was done by the Genome Quebec laboratory at McGill University. DNA sequences were aligned using MUSCLE (Edgar, 2004) and a maximum-likelihood phylogeny was produced using phyML 3.0 (Guindon et al., 2010) to identify the position of both clones in the ND5 phylogeny and ascertain their mitochondrial origin. Sequences from a number of clones belonging to the major lineages of the *D. pulex* complex (available in GenBank) were added to the tree.

The genome size of both clones was assessed using flow cytometry with *Artemia franciscana* as a standard. Two *Daphnia* were ground using a Kontes Dounce tissue grinder for 20 strokes with an 'A' pestle in 1 mL of modified Galbraith buffer (Bennett et al., 2003). After filtering twice through a 40-µm mesh, 20uL of cell suspension of *Artemia franciscana* were added to each sample. Cell suspensions were stained with propidium iodide (50 ppm; Invitrogen) at 4 °C in the dark for 10 h. The nuclear DNA content of each clone was assessed using an Epics Altra flow cytometer (Beckman-Coulter) with an argon laser emitting 14 mW of light at 488 nm; DNA-PI fluorescence emission was measured at 600–640 nm. Instrument alignment and stability were monitored by adding 5 µL of a solution of red-fluorescing beads (Linear Flow Carmine; Molecular Probes). Nuclear DNA content was calculated from the mean fluorescence intensity (FL, arbitrary units) on the gated data of the first peak as: Total genome size = (sample FL/ *Artemia* FL) x 2.61pg where 2.61 pg corresponds to the diploid

genome size of *Artemia franciscana*.

4.4.2 Growth experiments

Juveniles of the high latitude *Daphnia pulex* (*Daphnia*-HL) clone were exposed to seven abundances of either picoeukaryotic (*Nannochloropsis limnetica*, strain 18.99, SAG culture collection; Eustigmatophyceae) or picocyanobacterial (subarctic *Synechococcus* sp., strain ULCCC211, Université Laval; Chroococcales) food, at either 18°C or 26°C. The selection of food levels was based on two preliminary experiments indicating that animals did not survive at elevated temperature in food concentrations below 0.01 mg C L^{-1} and that they produced eggs before the end of the experiment in food concentrations higher than 0.2 mg C L^{-1}. Following the experiments using *Daphnia*-HL, two additional experiments (*Nannochloropsis* only, at 18 and 26°C) were conducted in the same experimental system using the temperate clone of *Daphnia pulex* (*Daphnia*-T). All of the experiments were performed in a set of flow-through vessels (Stich and Lampert, 1984) installed in a water-bath with the temperature controlled to within ±0.5°C. The phytoplankton cell abundance was maintained at the required food carbon levels with a multichannel peristaltic pump providing fresh medium from a magnetic stirred, covered, black PVC reservoir, at a rate of 2.2 L d^{-1} per chamber. The medium was prepared daily during each of the experiments in a two-step lake water filtration procedure (through 0.45 and 0.2 µm Advantec membrane filters) to remove all bacteria and detritus that could be a possible additional food source for *Daphnia* (Maszczyk and Bartosiewicz, 2012).

The neonates used in each experiment were derived from fourth brood *Daphnia* females cultured at 21°C in three 80-L tanks and fed daily *ad libitum* with equal mixture of picoeukaryotic cells and picocyanobacterial cells. Prior to each experiment, the neonates (0 to 8 hours old) were separated from females and placed for 2 days in 2.5 L glass beakers within a water-bath maintaining temperatures similar to those used during subsequent experiment, containing fresh experimental medium, but supplemented only with phytoplankton food that was later used in the experiment (1 mg C L^{-1} of either phytoplankton species).

The picophytoplankton were cultured in chemostats containing Z8 medium (Kótai, 1972) and harvested daily for the zooplankton medium. The organic carbon content of these cell suspensions used in each experiment was calculated using a calibration curve relating organic carbon to absorbance at 800 nm.

Each of the experiments was started with 700, 2-d old (±8h) juveniles (25 animals per flow-through chamber) that were distributed at random into 28 flow-through vessels (4 replicates for each of the 7 food concentrations) and kept under experimental conditions for 4 days. At the same time 100, 2-d old, neonates were measured under a dissecting microscope (Zeiss) and freeze-dried for analysis of fatty acids (FA), separated by fraction: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), as in (Mariash, 2014). Additional animals were dried at 60°C for 12 h (10×10 individuals in a pre-weighed silver cups) for mass estimation using a Sartorius high precision balance. After the 4th day, 10 random *Daphnia* from each flow-through vessel were harvested and dried in 60°C (12h) for mass estimation and the remaining 15 animals from each chamber were freeze dried in a 4 mL cryovial for fatty acid analysis. The growth rates were calculated from body dry mass as $g = (\ln M_t - \ln M_0)/t$ where M_t is body mass of 6-d old individuals, and M_0 represents the body mass of 2-d old individuals.

Threshold food concentrations (TFC) were calculated as the *x*-axis intercepts for linear regressions of growth rate versus natural log (*ln*) phytoplankton abundance (Gliwicz, 1990). The TFC and slopes of the regressions among treatments were tested for differences using 95% confidence intervals (CI): significant differences were identified by non-overlapping CIs. The TFC confidence interval for each treatment was estimated with the slope and y-axis intercept coefficients from the regression equations. A three-way ANOVA (food concentration, temperature and food type) followed by Tukey HSD was used to test for significant effects of experimental factors on the growth rate in *Daphnia*-HL in the first series of experiments and three-way ANOVA (food concentration, temperature and clone) was used to test for significant effects of temperature and differences in growth rate between the *Daphnia*-HL and *Daphnia-T* clones in the second series of experiments. The fatty acid content and composition (FA, total fatty acids; SAFA, MUFA and PUFA contents) were

compared using two-way ANOVA (temperature, food type) followed by Tukey HSD, with measurements of animals from the 5 intermediate food abundances used as replicates. All statistical analyses were performed using JMP Software from SAS.

4.5 Results

4.5.1 Phytoplankton and zooplankton characteristics

The strain of *Nannochloropsis* used in this study contained almost twice the quantity of fatty acids (FA) per unit biomass as the subarctic *Synechococcus* (43.7 and 24.0 µg FA mg^{-1} dry $mass^{-1}$, respectively), including PUFAs that were completely absent from the picocyanobacteria (Fig. 4.2). The *Daphnia* neonates kept at 26°C for 2 d after birth were slightly heavier than those kept at 18°C, but the difference was significant only for animals at 26°C fed *ad libitum* with *Synechococcus* (mean=11.7 µg, n=10, p=0.001). There was also a small but significant difference in body length between the two clones: 0.71 (±0.033) mm for *Daphnia*-HL versus 0.63 (±0.019) mm for *Daphnia*-T (t=3.73, p=0.03, df=18).

Both *Daphnia* clones clustered in the *Daphnia pulex* lineage on the maximum-likelihood tree (Fig. 4.5) and showed a >99% similarity in their mitochondrial ND5 gene sequences. The high latitude clone had a genome size of 0.51 pg, whereas the temperate clone had a genome size of 0.34 pg. The ratio of these values (1.5) indicated that the high latitude *Daphnia* is a triploid clone while the temperate *Daphnia* clone is diploid.

4.5.2 Growth rate and threshold food concentration responses

There was a significant effect of temperature and food type on the growth rates in *Daphnia*-HL (three-way ANOVA with Tukey HSD, Table 4.1, Fig. 4.3) in the first series of experiments and a significant effect of experimental clone and temperature, as well as their interactions, on growth rates in the second series of experiments (three-way ANOVA with Tukey HSD, Fig. 4.3). There was also a significant effect of temperature and clone on TFC in *Daphnia* (Fig. 4.3), and a significant effect of temperature and food type on TFC in *Daphnia*-HL (Fig. 4.3). The food threshold for growth in the *Daphnia*-HL and –T was higher at 26°C than at 18°C. The TFC was also significantly higher with *Synechococcus* than with

Nannochloropsis food at both temperatures (Fig. 4.3). When fed *Nannochloropsis*, the high latitude *Daphnia* was able to maintain positive growth at food abundances lower than the temperate clone at both temperatures (Fig. 4.3).

The slope of the growth rate versus food abundance (*ln*) regression line was greater at 18 than 26 °C, but this difference was significant only in *Daphnia*-T (based on CI). The slope of the regression was also significantly greater when *Daphnia*-HL were fed with *Synechococcus* than with *Nannochloropsis*. The slope for *Daphnia*-T was higher than for *Daphnia*-HL at both temperatures (Fig. 4.3). The growth rates for both clones at the highest food concentration used in this study (0.2 mg C L^{-1}) were significantly lower at 26 than 18°C, and lower for *Daphnia*-HL fed *Synechococcus* than *Nannochloropsis* (Fig. 4.3). *Daphnia*-T fed *Nannochloropsis* (0.2 mg C L^{-1}) grew faster than *Daphnia*-HL fed the same food, but only at 26°C (Fig 4.3).

4.5.3 Fatty acid responses

The elevated temperature resulted in a significant decrease of body fatty acids (FA) in *Daphnia*-HL feeding on either *Nannochloropsis* or *Synechococcus* (Table 4.2, Fig. 4.4). The animals feeding on *Nannochloropsis* accumulated more FA than those feeding on *Synechococcus* at each temperature (Fig. 4.4). There was also a significant effect of the interaction of food and temperature on total body FA content (Table 4.2).

The warmer temperature resulted in a significant differences in FA composition in *Daphnia*-HL (Fig. 4.4). The MUFA content was higher in animals grown at 18 than 26°C, but this difference was significant only in the *Nannochloropsis* food treatment (p=0.002, df=20, MS=27, two-way ANOVA with Tukey HSD, Fig. 4.4). The *Synechococcus* food resulted in a significantly lower MUFA content in *Daphnia* HL body than *Nannochloropsis* food at each respective temperature (p=0.0001 at 18°C and p=0.0002 at 26°C, df=20, MS=27). Although the PUFA content in *Daphnia* HL body was lower in *Synechococcus* food and at elevated temperatures (Fig. 4.4), only the combined effect of increased temperature and the lesser quality of cyanobacterial food resulted in a significantly lower body PUFA

content in 6-d old individuals (FQ × T, p=0.007, df=20, MS=25, two-way ANOVA with Tukey HSD).

4.6 Discussion

The high latitude *Daphnia* isolated in the present study (*Daphnia*-HL) was identified to be a triploid representative of the *D. pulex* species complex. This is consistent with previous observations that a large number of subarctic clones of this species are triploid while temperate clones, including the *Daphnia*-T used in our experiments, are diploid (Vergilino et al., 2009). Our results showed that the *Daphnia*-HL feeding curve was strikingly different from that for *Daphnia*-T, with a much lower threshold food concentration (TFC) for growth. *Daphnia*-HL was negatively affected by subarctic picocyanobacteria as a food source, and this effect greatly worsened at the increased temperature. This implies that high latitude aquatic ecosystems may be affected to a greater extent by indirect trophic effects than by the direct physiological impacts of climate warming. It should be noted, however, that these results are for a single subarctic clone of *Daphnia pulex*, and whether they are general features of high latitude *Daphnia* will require more detailed studies on a wide range of species and clones.

4.6.1 Clonal origin effects

The growth rate – food curves for both *Daphnia* clones closely fitted the expected relationship, but with major differences in the curve parameters, especially the TFC values. *Daphnia*-T at 18°C had a TFC of 17.5 µg C L^{-1}, which is similar to that of other temperate *Daphnia pulex* clones fed nutrient replete phytoplankton (e.g., 20 µg C L^{-1}, using larger phytoplankton cells; Gliwicz, 1990). In contrast, *Daphnia*-HL maintained positive growth rates above a threshold of 8.1 µg C L^{-1}. This TFC value was half that of the temperate clone and well below most values reported in the literature. This pronounced difference implies that the high latitude daphnids may be well adapted to low food concentrations, which is a feature of oligotrophic northern lake and pond environments (Rautio et al., 2011). The slope of the growth-food curves for the two clones also differed significantly, with the slope for *Daphnia*-HL 43% below that for *Daphnia*-T. This muted response to increases in food

concentration by the high latitude clone might also be a reflection of the persistently low phytoplankton biomass concentrations in northern waters.

4.6.2 Temperature effects

The distinct growth-food characteristics of *Daphnia*-HL implied that this clone is well attuned to cold subarctic environment, hence we hypothesized that it would be more negatively affected by warming than the temperate counterparts. The 8ºC increase in temperature indeed resulted in a significant increase in TFC, but there was no significant change in slope. The temperate clone showed both a significant increase in TFC and a significant decline in slope. An increase in TFC values as a function of temperature has been previously reported in zooplankton and suggested as an explanation for the dominance of small cladocerans in tropical climates (Moore and Folt, 1993). However, in an experimental comparison of several cladocerans, although TFC values were found to increase with increasing temperature, there was no significant effect of body size (Achenbach and Lampert, 1997). Our results lend further support to the view that high latitude zooplankton have a number of distinctive features, including better competitive ability at low food concentrations (Dzialowski and O'Brien, 2004).

4.6.3 Polyploidy effects

Many hypotheses have been proposed to account for the shift in crustacean zooplankton towards polyploidy at high latitudes. Proximal hypotheses invoke higher rates of unreduced gamete production at lower temperatures (Mable, 2004) or hybridization between refugial species following Pleistocene glaciations (Dufresne and Hebert, 1997). Functional hypotheses include greater flexibility of polyploid organisms under a suite of environmental conditions (Stebbins, 1984) and better competitive abilities of polyploid organisms against inbred sexual populations in marginal habitats (Haag and Ebert, 2004). The results obtained here provide some support for the functional hypotheses, in that *Daphnia*-HL was competitive at low food concentrations. However, the growth and feeding performance of *Daphnia pulex* is known to be variable among clones (Jose and Dufresne, 2010), and additional experiments are required to assess the interclonal variability in temperature and food effects on northern and southern communities.

4.6.4 Food type effects

There was a clear difference in food quality between the two picophytoplankton food types, specifically in total fatty acid content and in presence or absence of PUFAs. Consistent with this difference in food quality, *Daphnia*-HL showed a strong negative response to the picocyanobacterial food supply, with a pronounced increase in its TFC. The nutritional status of the animals was also impaired by their feeding on picocyanobacteria, as shown by the decreased total fatty acid content in all lipid classes. The inadequacy of cyanobacterial food for zooplankton is well known (von Elert et al., 2003; Wilson et al., 2005), with effects that include lower fecundity, survival and growth, and a decline in the size structure of the population (Ghadouani et al., 2006). Our results showed that cyanobacteria not only strongly affected the feeding threshold, but also had a significant effect on the slope of the growth rate - food abundance curves. This combined change in both TFC and slope may imply compensatory feeding (Iwabuchi and Urabe, 2012), and such a response by *Daphnia*-HL encountering picocyanobacteria could have other indirect effects, for example increased ingestion and bioaccumulation of cyanotoxins and toxic metals in a warmer climate (Chételat and Amyot, 2009; Dinh et al., 2013).

4.6.5 Interactions between temperature and food type

The possibility of interactions between temperature and food quality on zooplankton feeding efficiency was first suggested by Cole et al. (2002) and has been increasingly recognized as a potentially important response to environmental change (Masclaux et al., 2009; De Senerpont Domis et al., 2013). In the present study, the temperature increase did not result in a significant change in slope of the growth-food curve for *Daphnia*-HL, but the cumulative effect of warming and picocyanobacterial food resulted in a significant change of slope and in a greater than 6-fold increase of the TFC. This result implies a synergistic rather than additive effect of these two factors on high latitude zooplankton competitiveness, and indicates a potentially greater importance of indirect relative to direct effects of climate warming. The indirect effects of temperature change also included the decrease in polyunsaturated fatty acid content, which in turn may have led to lower survival, fecundity and growth rates. Schlechtriem et al. (2006) showed that daphnids cultured at elevated temperatures and fed cyanobacteria food suffered from decreased fatty acid content and

composition, which shifted to that reflecting their food (Brett et al., 2006). Our study support these findings in *Daphnia*-HL and also points to preferential retention or biosynthesis of unsaturated fatty acids in this zooplankton strain (Taipale et al., 2011). However, temperature changes that stimulate cyanobacterial development in high latitude lakes and ponds (Rae and Vincent, 1998) may also alter the composition and dynamics of fatty acids in picocyanobacterial food (Wada et al., 1990; Beaugrand et al., 2014). The indirect effects of global warming on high latitude zooplankton communities may thus be stronger than indicated by our results, and climate-zooplankton models of the direct effects of temperature and food quantity (e.g., Kosten et al., 2012) could be usefully modified to include a food quality component.

4.6.6. Implications for subarctic aquatic ecosystems in a warming climate

Global climate change is likely to strongly alter the community structure of high latitude aquatic ecosystems (Wrona et al., 2006). Higher air temperatures and accelerated permafrost thawing will likely result in greater nutrient and organic carbon loading to high latitude lakes and ponds. Cyanobacteria are already a common element of the phytoplankton in many northern lakes and rivers (Vincent et al., 2000a; Veillette et al., 2008; Rae and Vincent, 1998), and this combination of warming and enrichment may stimulate their development and dominance in a manner similar to that observed in temperate climates (Kosten et al., 2012). Although the present study was limited to one clone of high latitude *Daphnia* and one strain of high latitude cyanobacteria, the pronounced nature of the responses implies that these effects deserve close attention.

Daphnia has been used for many decades as a model organism to study the effects of environmental stressors on zooplankton in various aquatic ecosystems (Lampert, 2006), and it plays a key trophic role in the food webs of arctic lakes and ponds (Rautio et al., 2011). It has been suggested that *Daphnia* populations will further expand in high latitude lakes as a result of climate-induced increases in lake productivity (Chételat and Amyot, 2009). However this effect may be offset by a phytoplankton shift towards cyanobacteria, and a synergistic negative effect of lesser food quality and warming on *Daphnia* feeding and growth. Such effects would impair the transfer of carbon to high trophic levels, and may

reduce the impact of grazing on cyanobacteria, further contributing to their growth and dominance. As shown here, these indirect impacts of temperature change on phytoplankton–zooplankton interactions may greatly exceed the direct positive effects of temperature on zooplankton growth and physiology.

4.7 Acknowledgments

We thank Isabelle Laurion for her advice and provision of laboratory facilities, Heather Mariash for assistance in lipid analysis, Claude Belzile for genome size analyses, Mathieu Cusson for statistical guidance, Marie-Josée Martineau for assistance with the cyanobacterial culture, Roxanne Maranger for kindly providing equipment support, Piotr Maszczyk for discussions that helped formulate this study, and the Associate Editor and two anonymous reviewers for their insightful comments and suggestions.

Table 4.1. Effects on *Daphnia* growth rates. The first three-way ANOVA tested for the effects of temperature (T), food type (F), food concentration (FC) and their interactions on growth rate of *Daphnia*-HL, and the second tested the effects of temperature (T), clone (Cn), food concentration (FC) and their interactions on growth rate of the two *D. pulex* clones, *Daphnia*-HL and *Daphnia*-T.

Source	df	MS	F	p
***Daphnia*-HL**				
T	1	0.0371	440	**<0.0001**
F	1	0.4673	5547	**<0.0001**
T × F	1	0.0002	3	0.0931
FC	5	0.1216	1444	**<0.0001**
T × FC	5	0.0013	16	**<0.0001**
F × FC	5	0.0072	85	**<0.0001**
T× F × FC	5	0.0001	1	0.2754
Error	72	0.0001		
***Daphnia*-HL&T**				
T	1	0.0215	163	**<0.0001**
FC	5	0.1436	1088	**<0.0001**
T × FC	5	0.0019	15	**<0.0001**
Cn	1	0.0379	287	**<0.0001**
T × Cn	1	0.0009	7	**0.01**
FC × Cn	5	0.0128	97	**<0.0001**
T × FC × Cn	5	0.0003	2	**0.044**
Error	72	0.0001		

Table 4.2. Effects on *Daphnia* fatty acid content. The two-way ANOVA tested the effects of temperature (T), food type (F) and their interaction on total body fatty acids in *Daphnia*-HL.

Source	df	MS	F	P
T	1	2690.7	24	**<0.001**
F	1	6114.3	55	**<0.0001**
F × T	1	913.7	8	**0.011**

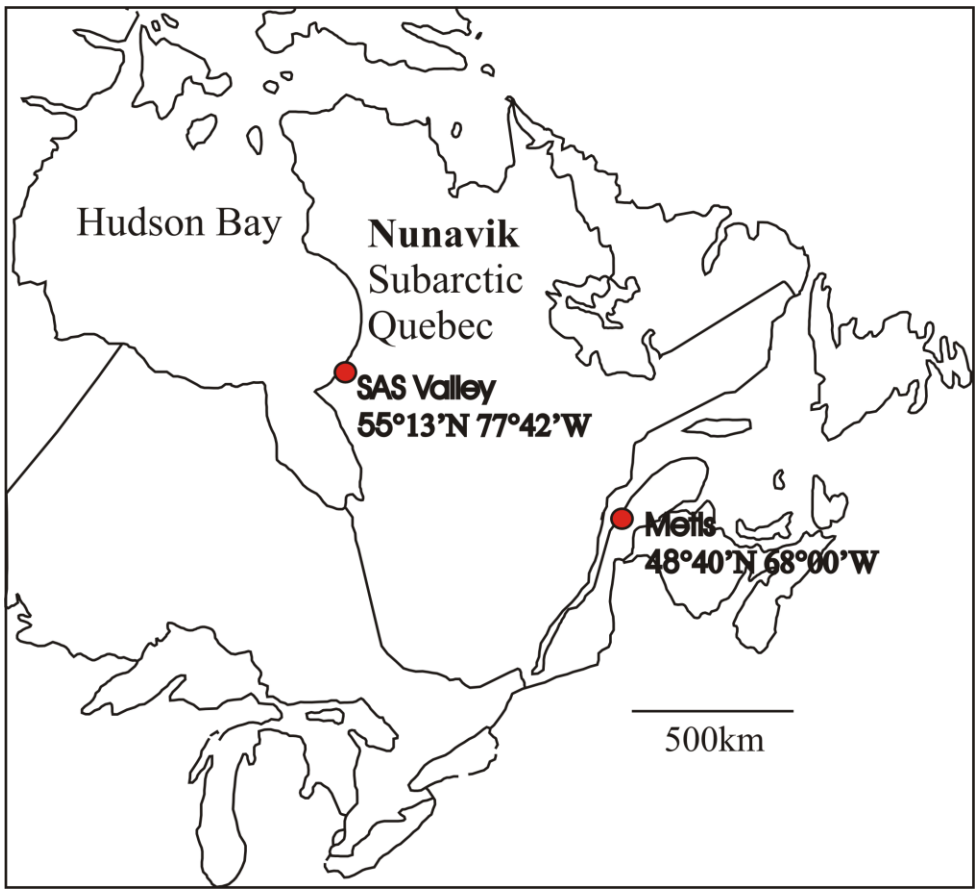

Figure 4.1. Location of the collection site for the zooplankton used in the growth experiments. The high latitude clone of *D. pulex* (*Daphnia*-HL) originated from a thermokarst pond in the SAS Valley (subarctic Quebec) and the temperate clone from Metis in southern Québec (*Daphnia*-T).

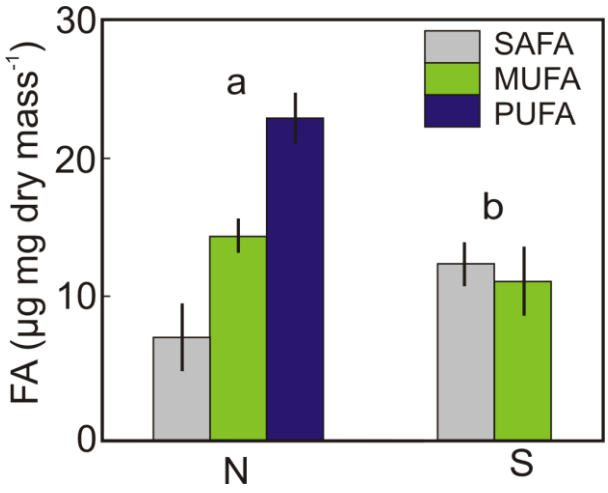

Figure 4.2. The FA content and composition of two picophytoplankton food types used in the growth experiments. The concentrations of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (±SE, n=5) in *Nannochloropsis* (N) and *Synechococcus* (S). The different letters indicate a significant difference in total fatty acid content at $p<0.001$.

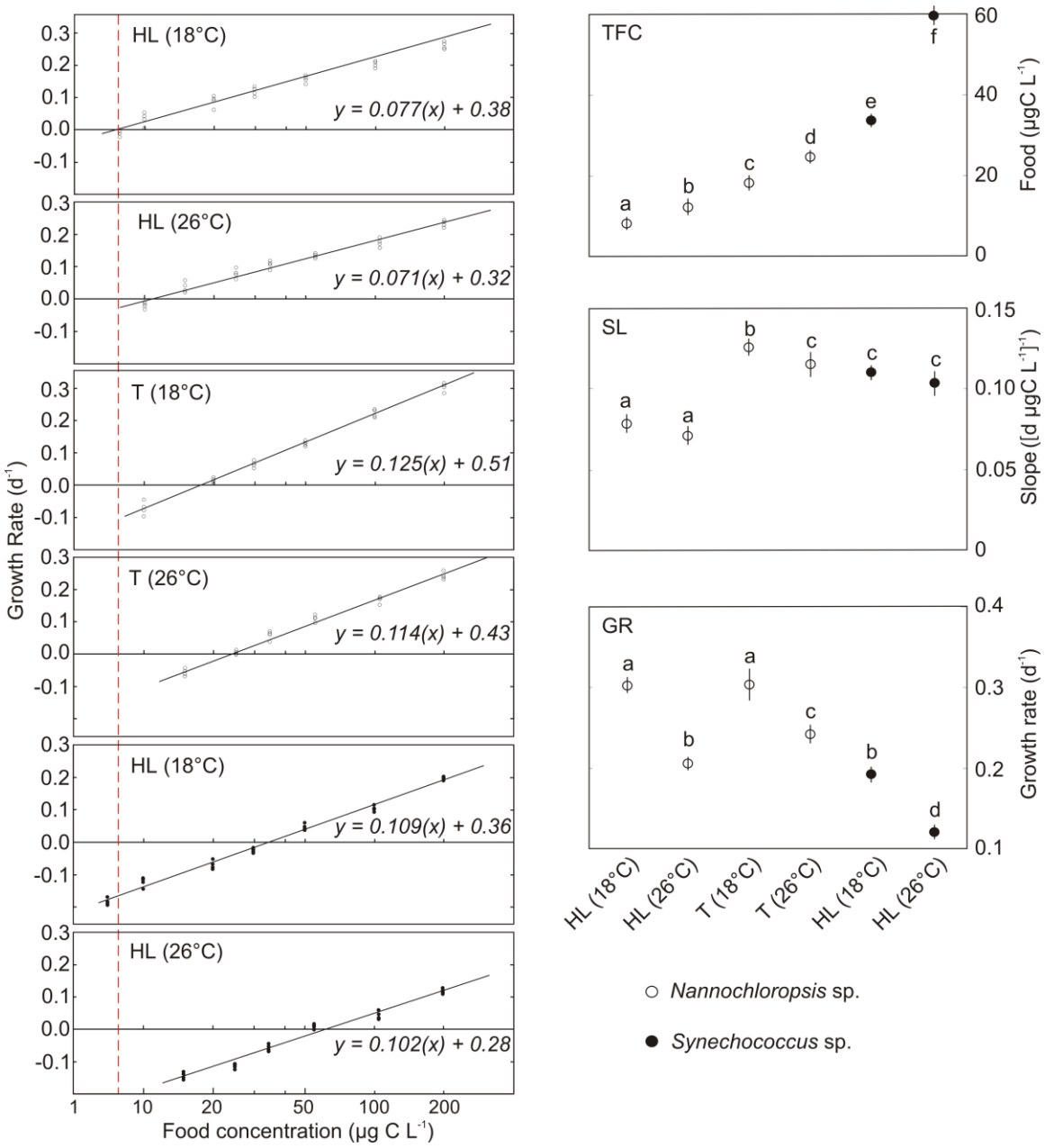

Figure 4.3. Growth versus food relationships. The left hand graphs shows growth rates against *ln* food concentration in each experiment, with the fitted regression equations giving growth rate as a function of *ln* food concentration in mg C L^{-1}; each point in the graphs is the mean of 4 replicates at each food concentration. The right hand graphs show threshold food concentrations (TFC, mean ± 95% CI), slopes of the regression lines of growth rate on food concentration (SL, mean ± 95% CI) and growth rates at 0.2 mg C L^{-1} (GR, mean ± 95% CI) in high latitude (HL) and temperate *Daphnia* (T). The animals were fed *Nannochloropsis* or *Synechococcus* (HL clone only) at 18°C or 26°C. The different letters indicate statistically different values ($p<0.05$, ANOVA with Tukey HSD or confidence intervals comparisons).

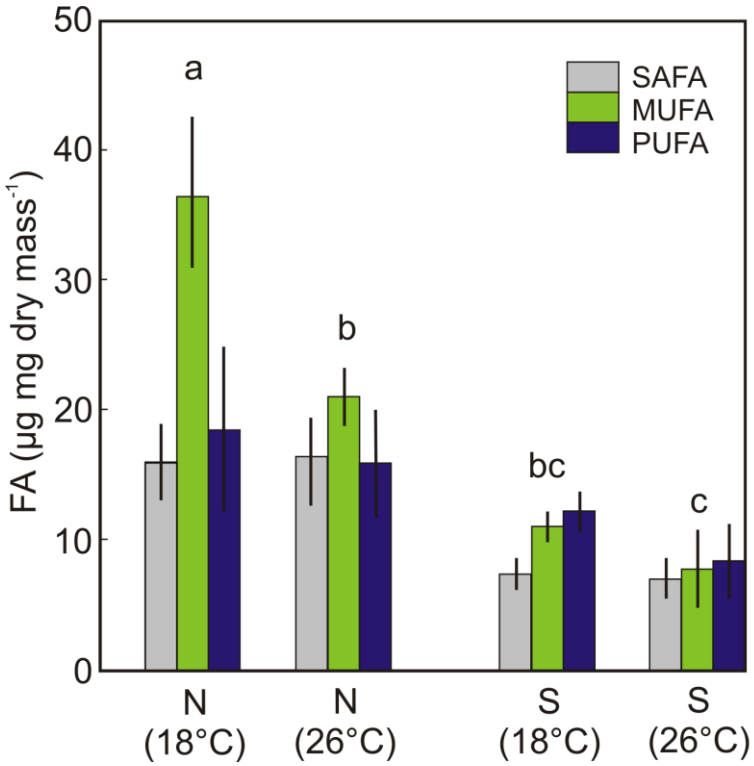

Figure 4.4. The content and composition of FA in *Daphnia*-HL fed either *Nannochloropsis* (N) or *Synechococcus* (S) at 18°C or 26°C. Each values is a mean of 5 replicates ±SE. Different letters indicate significant differences in total fatty acid contents at $p<0.01$ (two-way ANOVA with Tukey HSD).

Figure 4.5. Maximum likelihood of phylogenetic relationships among members of *D. pulex* complex using partial sequences of the ND5. The tree is rooted through the European *D. pulex* group. Maximum likelihood bootstrap values for PhyML are indicated for major groups. Arrows show the position of the two *Daphnia* clones used in this study.

Chapitre 5 Conclusion générale

Les lacs sont une caractéristique importante du paysage nordique et les eaux de thermokarst sont parmi les types d'écosystèmes d'eau douce les plus abondants de la Terre (Pienitz et al, 2008; Grosse et al, 2013). L'environnement arctique est désormais soumis à un rapide réchauffement climatique qui entraînera divers impacts potentiels sur les lacs du Nord (Vincent et al., 2013). Cette thèse, qui a mis l'accent sur les cyanobactéries, a généré de nouvelles connaissances sur les caractéristiques du plancton des lacs de thermokarst. Des observations sur le terrain combinées à des expériences ont permis de révéler la présence d'espèces responsables de floraisons et de cyanobactéries picoplanctoniques, de démontrer la sensibilité de ce plancton aux changements de l'environnement, et les effets négatifs des picocyanobactéries pour l'alimentation du zooplancton. L'ensemble de ces résultats originaux implique que les effets indirects du réchauffement climatique, tels que la mobilisation des éléments nutritifs à partir de la fonte du pergélisol et la diminution de la qualité de la nourriture pour le zooplancton, pourraient avoir des implications importantes pour le fonctionnement des écosystèmes de thermokarst. Ces effets indirects exacerberaient l'effet direct du réchauffement climatique qui est d'augmenter la température de l'eau de ces lacs.

Les analyses de phytoplancton ont révélé que les lacs de thermokarst contiennent des taxons abondants et diversifiés : 62 genres sur un total de 8 embranchements. La combinaison des résultats des analyses de pigments photosynthétiques, de la cytométrie en flux et des analyses moléculaires et microscopiques démontre que les écosystèmes des lacs de thermokarst représentent des « points chauds » de la biodiversité planctonique dans le paysage du pergélisol. La plupart des lacs étudiés contenaient des cyanobactéries dont certains genres potentiellement nocifs tels que *Dolichospermum* (anciennement *Anabaena*). Toutefois, leurs concentrations variaient considérablement entre différents endroits du pergélisol. L'éventail de lacs de thermokarst étudiés, situé dans des paysages de pergélisol sporadique à discontinu, contenait des picocyanobactéries en abondance. Par conséquent, ces lacs devraient désormais être considérés comme un habitat important pour ces photoautotrophes de petite taille. Enfin, un élément fascinant des résultats de cette thèse est que certains lacs contenaient des concentrations élevées de bactériochlorophylle *d*, ce qui

indique que les bactéries photosynthétiques sulfureuses vertes sont abondantes et pourraient jouer un rôle important dans la production primaire de ces écosystèmes.

L'incubation expérimentale décrite au chapitre 3 a révélé que les communautés de phytoplancton des lacs de thermokarst sont sensibles à un effet direct (réchauffement) et à un effet indirect (enrichissement en éléments nutritifs) des changements climatiques. En effet, des changements dans la température et la disponibilité des nutriments ont conduit à une prolifération de cyanobactéries dans la deuxième phase d'incubation, à la suite du développement de chrysophytes dans la première phase. Ces nouvelles conditions ont également induit une diminution de la diversité du phytoplancton. De plus, tel qu'expliqué dans le chapitre 4, un effet direct (réchauffement) ainsi qu'un effet indirect (diminution de la qualité de la nourriture) des changements climatiques affectent négativement la croissance et la performance du zooplancton des lacs de thermokarst. Toutefois, le zooplancton polyploïde de ces hautes latitudes est probablement mieux outillé pour faire face aux effets des changements de température brusques que leurs homologues diploïdes des latitudes tempérées.

Les conditions limnologiques des lacs de thermokarst étudiées dans cette étude révélaient une hétérogénéité frappante et variaient en fonction des caractéristiques du paysage et de la végétation. Aussi, les propriétés limnologiques des lacs de thermokarst étaient différentes de celles des lacs glaciaires de référence. Les concentrations de phytoplancton (Chl *a*) et de phosphore total étaient supérieures dans les lacs de thermokarst que dans les lacs glaciaires. Cependant, la diversité de pigments planctoniques était comparable dans les deux types de lacs et les chlorophytes, les chrysophytes et les dinoflagellés dominaient. La structure des pigments planctoniques a également indiqué la présence abondante de cyanobactéries, avec des concentrations élevées d'échinénone et de canthaxanthine. La présence de ces pigments, bien connus comme pigments photoprotecteurs dans les cyanobactéries et surtout répandu dans l'ordre Nostocales, pourrait suggérer l'abondance de certains taxons qui sont des fixateurs d'azote. Ces résultats concordaient avec les résultats de l'analyse de l'ARN 16S bactérienne (Crevecoeur et al., 2015) qui indiquaient

la présence des taxons de cyanobactéries dans certains de ces lacs avec une ressemblance (> 99% de similarité de séquence) au taxon de Nostocales *Dolichospermum curvum*.

Les concentrations élevées de zéaxanthine dans certains lacs correspondaient à des concentrations élevées de picophytoplanton, et il y avait une corrélation statistiquement significative entre ce pigment et les énumérations cellulaires de picocyanobactéries. Les résultats des analyses moléculaires des communautés procaryotes (Crevecoeur et al., 2015) et les données de la cytométrie en flux (Chapitre 2) impliquent que les concentrations de zéaxanthine étaient, au moins en partie, associées avec les abondantes picocyanobactéries de l'ordre Synechococcales. Le picophytoplancton était présent dans tous les lacs étudiés et il était très abondant dans certains lacs de thermokarst, atteignant des concentrations très élevées (jusqu'à 10^6 cellules ml^{-1}). Ces résultats sont en accord avec des études menées dans d'autres écosystèmes d'eau douce de hautes latitudes, mais ils contrastent avec les très faibles concentrations de picocyanobactéries qui ont été observées dans les eaux froides océaniques (Vincent, 2000). Le picophytoplancton de tous les lacs était composé de picocyanobactéries et de picoeucaryotes. Ces deux groupes différaient dans leurs relations avec des variables limnologiques. En effet, le carbone organique dissous était une variable de contrôle pour les picocyanobactéries, mais pas pour les eucaryotes. En revanche, l'abondance des picoeucaryotes corrélait négativement avec la conductivité, mais pas celle des picocyanobactéries. Le picophytoplancton contribuait une proportion très variable (de 1 à 56%) à la biomasse photoautotrophe totale dans les lacs étudiés.

Les résultats de cette étude procurent une compréhension détaillée de nombreux aspects du phytoplancton dans les écosystèmes de thermokarst. Cependant, les observations ont été limitées dans le temps et l'espace. Les observations sur le terrain ont été réalisées au cours de la période la plus chaude de l'année et avec des conditions d'eau libre. Cette période est susceptible d'être la plus propice pour le phytoplancton et le développement du zooplancton. Il serait très utile de prolonger ces observations aux autres saisons, en particulier au printemps lors du début de la croissance du phytoplancton. L'évaluation des impacts des changements climatiques pour la structure des pigments planctoniques serait approfondie en comprenant comment des changements dans l'environnement l'ont altéré

dans le passé. Cette recherche pourrait être réalisée avec des analyses de structure de pigments dans les enregistrements sédimentaires des lacs de thermokarst. Une telle approche paléolimnologique a été utilisée avec succès dans les études de Meretta Lake, dans le haut Arctique canadien (Antoniades et al., 2007) et des méthodes similaires pourraient être appliquées à des lacs de thermokarst dans le futur.

La compréhension globale des relations entre les variables limnologiques et la structure des pigments planctoniques nécessite l'étude de lacs situés dans des lieux géographiques diversifiés, y compris dans les zones de pergélisol continu, discontinu et sporadique de différentes régions du Nord circumpolaire. Les analyses moléculaires présentées au chapitre 2 ont montré le potentiel prometteur de cette approche. Entre autres, ces méthodes pourraient être utilisées pour déterminer si la diversité des pigments planctoniques est associée à la diversité génétique de la communauté, et si les changements saisonniers du plancton (les protistes phototrophes et hétérotrophes) des lacs subarctiques sont similaires à ceux connus pour les latitudes tempérées et tropicales. Les outils moléculaires, tels que la génomique fonctionnelle, offrent une approche prometteuse pour comprendre les rôles et les relations entre les communautés procaryotes et eucaryotes.

Les résultats de l'analyse microscopique soutiennent les résultats de l'analyse moléculaire et de l'analyse de pigments. Les cyanobactéries étaient bien représentées dans la plupart des lacs étudiés et elles ont contribué jusqu'à 50% du biovolume total du phytoplancton. L'étude de terrain a révélé que les cyanobactéries sont plus abondantes dans les lacs plus chauds et que leur abondance était inversement proportionnelle à la concentration de phosphore dissous. La concentration des métaux traces (Fe et Mn) corrélaient également avec le biovolume de cyanobactéries et ils pourraient jouer un rôle dans l'écologie des cyanobactéries des lacs subarctiques.

L'incubation d'échantillons d'eau d'un lac de thermokarst dans des conditions plus chaudes a stimulé les cyanobactéries filamenteuses, notamment du genre *Dolichospermum,* pendant la première phase de l'expérience (de 0 à 6 jours) et des chrysophytes planctoniques pendant la deuxième phase (jours 6 à 12). Aussi, les effets directs du réchauffement

comprenaient une augmentation des picoeucaryotes, mais pas des picocyanobactéries. L'augmentation de la biomasse de phytoplancton dans des conditions plus chaudes a été accompagnée par une diminution de la concentration de Chl *a*. Ce résultat pourrait indiquer que le phytoplancton contenait relativement moins de Chl *a* par cellule dans des conditions plus chaudes. Alternativement, ce résultat suggère que des chrysophytes mixotrophes, qui dominaient la communauté à la fin de l'expérience, ont graduellement transféré à un métabolisme hétérotrophe. Cette interprétation est basée sur la théorie générale en écologie qui stipule que le métabolisme hétérotrophe répond plus fortement à une augmentation de la température que le métabolisme autotrophe. Par conséquent, les effets directs potentiels des changements climatiques pour les lacs de thermokarst ne se limitent pas qu'à des communautés plus abondantes et plus uniformes ; des altérations de l'efficacité de la photosynthèse, de l'alimentation et des fonctions de certains taxons de phytoplancton pourraient se produire.

L'enrichissement en phosphore des échantillons de lacs de thermokarst a entraîné la prolifération de cyanobactéries à la fin de l'expérience. Ce résultat indique que le réchauffement pourrait ne pas être nécessaire à l'apparition de floraisons nuisibles, dans des conditions où les éléments nutritifs sont disponibles en quantité suffisante. Cependant, lors de nos expériences, il n'a pas été possible d'examiner les effets d'une plus forte stratification thermique sur le développement de floraisons dans les lacs de thermokarst. Une perspective de recherche primordiale serait d'examiner quels sont les effets des changements dans la structure physique des lacs subarctiques pour les cycles des éléments nutritifs ainsi que pour le phytoplancton. Aussi, à cause de la plus grande productivité de leurs bassins versants, les lacs de hautes latitudes contiendront des concentrations plus élevées de matière organique dissoute. Ce changement pourrait entraîner des modifications brusques dans la lumière sous-marine, dans les budgets de chaleur et dans la stratification. Ces effets seraient probablement accompagnés par des changements dans les communautés autotrophes, vers des taxons avec des taux de sédimentation plus lents (par exemple, les petites diatomées et les picocyanobactéries) ou des taxons qui peuvent ajuster leur position dans la colonne d'eau supérieure (par exemple, les phytoflagellés et les cyanobactéries contenant des vésicules de

gaz) et vers des phototrophes qui se développent dans des conditions anoxiques dans la colonne d'eau inférieure (par exemple, les bactéries photosynthétiques sulfureuses vertes).

Une diminution brutale de la biodiversité du phytoplancton, tel qu'indiqué par l'indice de Shannon-Wiener, était l'effet le plus frappant du réchauffement qui accompagnait l'enrichissement en phosphore. La littérature indique que la diminution de la richesse spécifique (le nombre d'espèces présentes dans un milieu donné) et de la biodiversité peuvent entraver le bon fonctionnement des écosystèmes, soit directement (par exemple, en diminuant la productivité), ou indirectement en affectant leur capacité de s'adapter à de nouvelles conditions environnementales. La stabilité de la production de phytoplancton, qui peut être définie par la résistance et la résilience face aux ajouts d'éléments nutritifs, à la sécheresse et au broutage, a été corrélée à l'indice de la diversité Shannon-Wiener. Cette relation entre la diversité et la stabilité a été observée dans de nombreux réseaux trophiques, et la variation de la diversité des espèces à tout niveau trophique peut entraîner des effets en cascade au sein de l'ensemble de l'écosystème. La déstabilisation de réseaux alimentaires à cause des activités humaines ou des changements environnementaux, dans les latitudes tropicales et tempérées, ont entraîné des changements catastrophiques pour la biogéochimie régionale.

Les lacs de thermokarst sont des voies de transfert importants pour le transport d'ancien carbone emmagasiné dans le sol vers l'atmosphère, sous la forme de CH_4 et CO_2. Toutefois, malgré l'hétérotrophie nette causée par la respiration du carbone allochtone (Deshpande et al., 2015), les résultats de cette thèse démontrent que les lacs de thermokarst contiennent une communauté de phytoplancton abondante et diversifiée qui libère de l'oxygène (potentiellement disponible pour l'oxydation du CH_4), et qui consomme du CO_2 (qui peut ensuite être séquestré par sédimentation dans le fond du lac). Des changements dans les communautés de phytoplancton, y compris une réduction de la biodiversité et l'apparition saisonnière de floraisons nuisibles, pourraient affecter cet équilibre entre des gaz à effet de serre et leurs émissions nettes dans l'atmosphère.

Les effets directs et indirects des changements climatiques pour les écosystèmes d'eau douce de hautes latitudes comprennent également des conséquences pour la croissance et la performance des herbivores aquatiques. Les effets directs du réchauffement pour le clone de *Daphnia pulex* triploïde provenant d'un lac du Québec subarctique incluaient une diminution du taux de croissance et des seuils alimentaires plus élevés. Le seuil alimentaire, qui se définit comme la concentration à laquelle l'assimilation égale la respiration, serait l'un des déterminants les plus importants des capacités compétitives du zooplancton. Ainsi, la capacité des daphnies triploïdes (du Québec subarctique) de maintenir un taux de croissance positif malgré des concentrations alimentaires bien en-dessous de celles observées pour les daphnies diploïdes (sud du Québec), et ce aux deux températures étudiées (à 18 °C et à 26 °C), implique que ce génotype est susceptible de persister et de dominer le zooplancton même dans un climat plus chaud. Or, la plus importante contribution des cyanobactéries, qui sont reconnues pour être une source de nourriture pauvre en nutriments, pourrait favoriser d'autres espèces de zooplancton ou d'autres clones qui sont mieux adaptés à exploiter de tels aliments. En plus, cette conclusion est appuyée par les résultats expérimentaux de cette thèse qui ont suggéré que le réchauffement et la qualité de la nourriture agissaient en synergie sur la croissance de zooplancton.

D'autres études sont nécessaires afin d'étendre cette compréhension à d'autres espèces de zooplancton de hautes latitudes, y compris des taxons littoraux (par exemple, *Simocephalus*) et pélagiques (par exemple, *Holopedium*) de différentes tailles, ainsi que le microzooplancton (par exemple, les ciliés). Il serait important d'examiner quel rôle la polyploïdie joue dans les réponses du zooplancton de hautes latitudes face aux facteurs de stress environnementaux. Pour ce faire, une approche appropriée serait de comparer expérimentalement les niveaux d'expression des gènes des clones polyploïdes avec des clones diploïdes de référence, sous différentes conditions environnementales.

Enfin, les résultats de cette thèse ont permis d'accroître nos connaissances sur les communautés planctoniques des lacs de thermokarst et leurs réponses possibles aux changements climatiques. Les effets directs et indirects des changements climatiques ont été identifiés et ils impliquent des altérations aux communautés de phytoplancton et de

zooplancton. Une relation entre les changements dans la qualité du phytoplancton comme source de nourriture (augmentation de l'abondance des cyanobactéries) et de la compétitivité du zooplancton (seuil d'alimentation accru) a été clairement démontrée. De plus, de nombreux lacs de hautes latitudes pourraient recevoir davantage de nutriments d'une manière naturelle ou anthropique. Ce changement est susceptible d'affecter le fonctionnement des écosystèmes aquatiques. La préservation et la gestion des lacs de thermokarst devraient avoir comme objectif d'atténuer les impacts indirects des changements climatiques et de l'eutrophisation en cours. Les plans d'action et les stratégies à mettre en place afin de conserver la diversité du plancton ainsi que les fonctions écologiques devraient être développées avant que les conséquences de l'eutrophisation et du réchauffement climatique deviennent trop importantes. Les tentatives de restauration post-factum de lacs situés à des latitudes tropicales et tempérées se sont avérées coûteuses et souvent inadéquates pour inverser des changements déjà amorcés.

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- http://cfb.unh.edu/cfbkey/html/index.html (An image-based key to the zooplankton of the Northeast (USA));
- Freshwater Algae of North America: Ecology and Classification. Edited by Wehr, J.D. and Sheath, R.G. 918 pages, Academic Press;
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