



Diversité et dynamique des communautés de protistes dans le haut Arctique canadien

Thèse

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Résumé

Le paysage arctique est un mélange d'étendues désertiques, fouettées par les vents, et d'une grande diversité d'écosystèmes aquatiques. Confronté à des augmentations de température nettement supérieures à la moyenne globale qui modifient les propriétés de ces paysages, l'Arctique est un site d'étude stratégique pour suivre les impacts des changements climatiques sur les communautés microbiennes endogènes. Caractéristique importante des écosystèmes arctiques, la cryosphère commence déjà à atteindre des seuils de non-retour le long de la côte nord de l'île d'Ellesmere. Dans les lacs, la combinaison naturelle des basses températures, de la variabilité de l'irradiance et de la faible teneur en nutriments inorganiques limite la production primaire et la croissance de nombreux organismes. La richesse des microorganismes dans ces systèmes est le résultat d'une diversité de stratégies adaptatives et nutritives. Ainsi, les changements observés dans les couverts de glace auront des impacts sur l'activité biologique de ces lacs. Contribuant à celle-ci, les protistes, eucaryotes unicellulaires microscopiques, exploitent une large gamme des ressources de carbone et d'énergie, incluant la phototrophie, la préation et la combinaison des deux, la mixotrophie. Cette thèse cherche à déterminer la contribution des mixotrophes à la structure des communautés de protistes dans les lacs arctiques, et à développer la connaissance sur leurs réponses potentielles aux conditions environnementales changeantes. Le lac Char, le lac A et le lac Ward Hunt ont été sélectionnés pour leurs propriétés limnologiques distinctes et pour investiguer la biodiversité de leurs communautés de protistes en Aout 2008. La microscopie, les pigments et les banques de clones du gène de l'ARN de la petite sous-unité ribosomale 18S indiquent la dominance des chrysophytes, des protistes principalement mixotrophes, dans les trois lacs. Pour le lac A, l'été 2008 était marqué par la perte d'un couvert de glace permanent, créant des conditions d'eau libre inhabituelles. Le séquençage à haut-débit de la région V4 du gène 18S ARNr révèle le contraste entre les communautés homogènes dans la colonne d'eau marquée d'une stratification méromictique sous le couvert de glace en mai 2008 et les variabilités spatiales établies en août 2008 dans la colonne d'eau libre de glace ainsi qu'en Juillet 2009 sous un couvert de glace normal. Ces résultats illustrent l'importance qu'ont les facteurs environnementaux, tel que l'irradiance, sur les communautés de protistes. Pour examiner plus attentivement le rôle de la lumière et investiguer l'impact de la disponibilité des proies, nous avons entrepris une expérience de lumière/dilution au lac Ward Hunt. Le pyroséquençage de la région V4 de l'ARN ribosomal ainsi que son gène révèlent des différences taxonomiques entre les deux traitements d'irradiance, suggérant une divergence du type de mixotrophie entre une dominance de dinoflagellés essentiellement brouteurs à faible irradiance et de chrysophytes bactéritivores sous une forte lumière. Cette thèse révèle la diversité des

protistes et leur variation saisonnière au sein des lacs arctiques, et offre un aperçu de l’importance des conditions environnementales sur la stratégie mixotrophe adoptée par les communautés de protistes.

Abstract

The Arctic region is a blend of stark windswept landscapes interwoven with a wide diversity of freshwater ecosystems. Presently confronted by temperature increases well above global average, causing changes in landscape and aquatic properties, the Arctic is a strategic area to study the impact of climate change on endogenous microbial communities. Ice is a crucial characteristic of Arctic ecosystems and has already begun to cross thresholds along the northern coastline of Ellesmere Island. In lakes, the effects of cold temperatures, variable irradiance and low inorganic nutrients combine to restrict primary production and growth of most organisms. The established richness of microorganisms present in these systems is due to the high diversity of their adaptive and nutritive strategies. Hence, the observed shifts in ice cover regimes of lakes will have impacts on their biological activity. Of these microbial components, the protists, unicellular eukaryotes, exploit a wide range of carbon and energy resources from phototrophy to predation and the combination of both, mixotrophy. The subject of this research was to determine the contribution of mixotrophs to protist community structure in Arctic lakes, and to develop knowledge of their potential response to the changing environmental conditions. Char Lake, Lake A and Ward Hunt Lake, three limnologically different lakes, were chosen to investigate the biodiversity of protists in August 2008. Microscopy, pigments and 18S gene clone libraries revealed a dominance of each lake by chrysophytes, prominent mixotrophic protists. At Lake A, the summer of 2008 was marked by a loss of ice-cover, creating atypical open-water conditions. High-throughput sequencing of the V4 region of the 18S ribosomal RNA gene revealed the contrast between the homogenous community structure within the ice-covered water column of May 2008, despite the sharp physico-chemical meromictic stratification within the lake, and the established spatial variability of the protist communities under the ice-free conditions of August 2008 and ice-covered conditions of July 2009. These results illustrate the importance of varying environmental factors, such as underwater irradiance, in shaping protist communities. To further examine the role of light and to investigate the impact of low prey resources, we conducted a light/dilution experiment at Ward Hunt Lake. Pyrosequencing of the V4 region of the 18S ribosomal RNA, along with the gene, showed taxonomic differences under the two light conditions, suggesting a divergence in the dominant type of mixotrophy, with dominance of primarily microflagellate grazers, the dinoflagellates, under low irradiance, and of bacterivorous chrysophytes in the high light treatment. This thesis research underscored the diversity of mixotrophs and their seasonal variations in Arctic lakes, and provided insights into the importance of environmental conditions on the mixotrophic strategy adopted by protist communities.

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Liste des abréviations et des sigles

- 18S : Petite sous-unité du ribosome des eucaryotes
- ADN ou DNA : Acides désoxyribonucléiques
- ADNc ou cDNA : Acides désoxyribonucléiques complémentaires
- AMOVA : Analysis of molecular variance
- ARN ou RNA : Acides ribonucléiques
- ARNr ou rRNA : Acides ribonucléiques ribosomaux
- BLAST : Basic local alignment search tool
- pb ou bp : paires de bases
- C : Carbone
- CCA : Canonical correspondence analysis
- CCD : Charge-coupled device camera
- CCMP : Provassoli-Guillard National Center for Culture of Marine Phytoplankton (National Center for Marine Algae and Microbiota)
- Chl α : Chlorophylle α
- CH : Char Lake
- CLUMEQ : Consortium de recherche pour le calcul scientifique de haute performance
- C:N : Carbon to nitrogen ratio
- CTD : Conductivity-Temperature-Depth
- DAPI : 4'6-diamidino-2-phenylindole
- DGGE : Denaturing gradient gel electrophoresis
- dNTP : Désoxyribonucléotide triphosphate
- EDTA : Acide éthylènediaminetetraacétique
- FNU : Fluorescence-Utermöhl-Nomarski
- FW : Filtered water
- GF/F : Glass microfiber filter
- HPLC : High performance liquid chromatography
- IBIS : Institut de Biologie Intégrative et des Systèmes
- LA : Lake A
- LW : Lake water
- MDA : Multiple displacement amplification
- MID : Multiplex identifier
- ML : Maximum-likelihood

NCBI : National Center for Biotechnology Information

NJ : Neighbour-joining

NOx : Nitrate et nitrite

NRI : Net-relatedness index

NTI : Nearest taxon index

N:P : Nitrogen to phosphorus ratio

OTU : Operational taxonomic unit

PAR : Photosynthetically active radiation

PAST : PAleontological STatistics software

PC : Polycarbonate

PCA : Principal component analysis

PCR : Polymerase chain reaction

PTFE: Polytetrafluoro thylene

SRP : Soluble reactive phosphorus

TN : Total nitrogen

Tris : Tris (hydroxymethyl) aminomethane

TP : Total phosphorus

V4 : région hypervariable 4 de l'ARN ribosomal

WH : Ward Hunt Lake

*À ma mère, d'une biologiste moléculaire à l'autre
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Chapitre 1 : Introduction générale

Chapitre 2 : *Chrysophytes and other protists in High Arctic lakes: molecular gene surveys, pigment signatures and microscopy.* Sophie Charvet, Warwick F Vincent et Connie Lovejoy.

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Chapitre 4 : *Effects of light and prey availability on High Arctic protist communities measured by high-throughput DNA and RNA sequencing.* Sophie Charvet, Warwick F Vincent et Connie Lovejoy.

Soumis à *FEMS : Microbiology Ecology*

Chapitre 5 : Conclusion générale

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Charvet S, Vincent W F, Lovejoy C. 2012. *Protist diversity and community dynamics in lakes of the Canadian High Arctic*. Invited Guest Student Seminar, Woods Hole Oceanographic Institution, Woods Hole, MA, USA.

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Charvet S, Vincent W F, Lovejoy C. 2011. *Mixotrophic protists in High Arctic Lakes*. Phycological Society of America Annual Meeting, Seattle, WA, USA.

Charvet S, Vincent W F, Lovejoy C. 2010. *Algae that behave like animals: Dominance of High Arctic lakes by mixotrophic chrysophytes*. ArcticNet Annual Scientific Meeting, Ottawa, Canada.

Charvet S. 2009. *Between trophic worlds: Impacts of light on mixotrophy in Arctic lakes*. MERGE-International Polar Year conference, Montreal, Canada.

Pendant mon doctorat, j'ai contribué à un autre article lors d'un stage au Woods Hole Oceanographic Institution, à Woods Hole, MA, dans le laboratoire de Virginia P Edgcomb.

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Chapitre 1 Introduction

L'Arctique est une vaste région dont la majeure partie est sillonnée de lacs et de rivières, représentant une grande diversité limnologique propice aux études de ces systèmes complexes. Les microorganismes constituent la principale biomasse dans ces écosystèmes aquatiques. Entre autres, les protistes, eucaryotes unicellulaires d'une grande diversité, accomplissent de multiples fonctions au sein des réseaux trophiques et contribuent aux cycles biogéochimiques. Les changements qui ont présentement lieu en Arctique, dont un réchauffement sévère et une augmentation des précipitations (Anisimov et al., 2007), rendent ces systèmes vulnérables. Plusieurs seuils critiques ont déjà été atteints dans la région (Vincent et al., 2009) modifiant les propriétés physiques et chimiques mais également biologiques de ces systèmes. Ainsi, les lacs arctiques doivent être étudiés avant qu'ils ne soient altérés à jamais (Mueller et al., 2009).

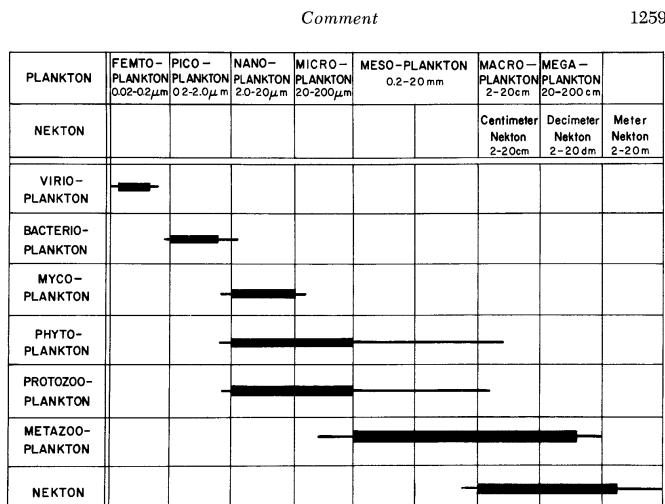
1.1 Diversité des protistes

Au sein du groupe des protistes il existe une vaste diversité de tailles (Sieburth et al., 1978; Table 1.1), de structures cellulaires et de morphologies externes (Miller, 2004), ainsi que de fonctions et d'adaptations à des habitats extrêmes (Mueller et al., 2005; Edgcomb et al., 2011a, 2011b; Amaral-Zettler, 2013). La diversité d'adaptation est telle que, malgré le fort potentiel de dispersion de ces microorganismes (Finlay, 2002), il y a très peu de chevauchement entre les espèces marines et celles d'eau douce (Scheckenbach et al., 2006; Logares et al., 2007). En effet, la transition de l'eau salée à l'eau douce était un phénomène rare au cours de l'évolution des différentes lignées de protistes (Logares et al., 2007). Avec les Cryptophyta et quelques genres de dinoflagellés, les chrysophytes et les ciliés sont des groupes importants dans les milieux lacustres (Slapeta et al., 2005; Medinger et al., 2010). Les Chlorophyta sont également caractéristiques de ces milieux, avec certaines familles de diatomées (Round et al., 1990; Medinger et al., 2010) de Cercozoa, de Centroheliozoa, et d'Haptophyta (Slapeta et al., 2005).

Le développement des technologies de microscopie a permis des avancées importantes dans l'identification, la classification, et la compréhension des fonctions des microorganismes dans l'environnement. Ces méthodes, telles que la sédimentation d'Utermöhl (Utermöhl, 1958; Reid, 1983), ont permis l'identification taxonomique, le comptage ainsi que l'estimation de biomasse de protistes. Les techniques de microscopie à fluorescence se sont raffinées avec l'emploi de l'acridine orange (Daley & Hobbie, 1975) et du 4'6-diamidino-2-phenylindole (DAPI; Porter & Feig, 1980) et sont utilisées pour compter les bactéries dans un échantillon environnemental. La méthode du FNU,

qui est une combinaison des techniques de fluorescence, du contraste interférentiel différentiel de Nomarski et de la sédimentation par Utermöhl, est utilisée pour l'identification, le comptage et l'estimation de la biomasse des espèces au sein des communautés de protistes (Lovejoy et al., 1993; Vallières et al., 2008). La chromatographie en phase liquide (HPLC ; *High performance liquid chromatography*) a également été développée pour des applications biologiques, entre autres l'identification des organismes par leur contenu en pigments (Jeffrey et al., 1997; Zapata et al., 2000; Roy et al., 2011).

Table 1.1 Classes de taille des cellules de protistes (tiré de Sieburth et al., 1978).



Bien que ces techniques classiques aient évolué et se soient perfectionnées, l'identification des protistes reste limitée par les infimes distinctions morphologiques entre les organismes unicellulaires ou par le partage de pigments identiques par différents taxons. Par exemple, les diatomées et les chrysophytes sont tous les deux caractérisés par la fucoxanthine (Roy et al., 2011). Le paradigme selon lequel les organismes sont classifiés par leurs ressemblances morphologiques ou physiologiques ne représente pas fidèlement la véritable diversité de chaque taxon ni les relations évolutives entre eux (Pace et al., 2012). Dans ce sens, les investigations sur la systématique, l'écologie et l'évolution des microorganismes ont été particulièrement favorisées par l'avènement des techniques de biologie moléculaire. Le gène de la petite sous-unité ARN ribosomale 18S fournit des informations essentielles et fiables sur les liens évolutifs entre les organismes (Woese & Fox, 1977; Pace et al., 1986; Woese et al., 1990) et a permis de voir que la

phylogénie des protistes (Figure 1.1) ne reflète pas forcément la classification fonctionnelle (Baldauf et al., 2000; Baldauf, 2003).

Les phototrophes et les hétérotrophes constituent des groupes paraphylétiques (Figure 1.1) dont les membres sont répartis entre plusieurs phylums de l'arbre des eucaryotes (Falkowski & Knoll, 2007). Une appréciation du contexte évolutif de l'acquisition de la photosynthèse chez les eucaryotes permet d'éclaircir la distribution phylogénétique complexe de ces organismes. Selon la théorie de l'endosymbiogenèse, la phototrophie chez les eucaryotes provient de l'endocytose d'une cyanobactérie par un protozoaire, qui s'est transformée en symbiose entre les deux organismes (Margulis, 1993a; Margulis, 1993b). Au cours de l'évolution, l'endosymbionte est devenue une organelle photosynthétique ; le chloroplaste (McFadden, 2002). Cette endosymbiose primaire a donné lieu aux Archaeplastida (Glaucophyta, Chlorophyta, Rhodophyta). Les phototrophes retrouvés parmi les stramenopiles, Cryptophyta, Haptophyta, Euglenophyta, et alvéolés sont issus d'une endosymbiose secondaire, soit la phagocytose d'une micro-algue eucaryote (Rhodophyta) par un hétérotrophe, suivie du maintien du chloroplaste de la proie. De plus, parmi les alvéolés, certains dinoflagellés sont pourvus d'un chloroplaste d'origine cryptophyte ou stramenopile obtenu lors d'une endosymbiose tertiaire (Bhattacharya et al., 2004).

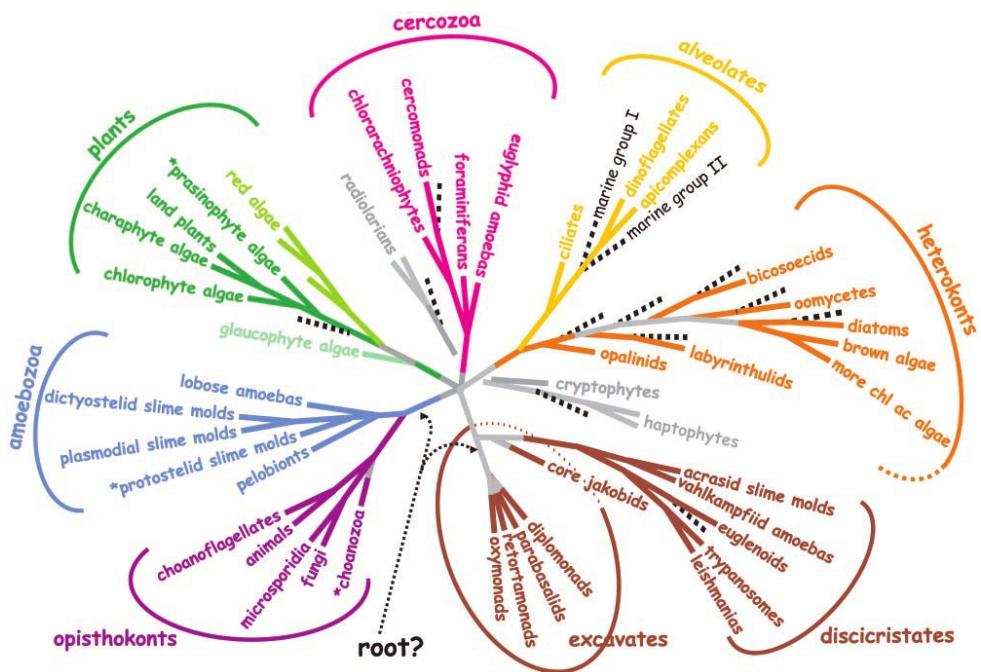


Figure 1.1 Arbre phylogénétique des eucaryotes (tiré de Baldauf, 2003).

La phagotrophie correspond donc à un caractère ancestral alors que la phototrophie stricte est un caractère dérivé (Troost et al., 2005). La mixotrophie (i.e. la capacité d'utiliser la photosynthèse et l'hétérotrophie pour répondre aux besoins énergétiques et en carbone) représente une étape évolutive le long d'un gradient de l'hétérotrophie à la photoautotrophie stricte, beaucoup plus rare (Kooijman et al., 2003). Les phylums aux tendances mixotrophes, distribués au travers de l'arbre phylogénétique, peuvent également contenir des organismes exclusivement phototrophes ou hétérotrophes. Les chrysophytes (Raven, 2009) et dinoflagellés (Stoecker, 1999) sont les groupes les plus représentées parmi les mixotrophes, mais la mixotrophie a également été observée pour des Haptophyta (Skovgaard et al., 2003), des Cryptophyta (Urabe et al., 2000; Marshall & Laybourn-Parry, 2002), des Euglenophyta (Epstein & Shiaris, 1992), des ciliés (Esteban et al., 2010) et certains Chlorophyta (González et al., 1993; Sanders & Gast, 2012). En raison de cette évolution mouvementée des protistes, les groupes fonctionnels ne sont pas toujours monophylétiques, ce qui rend difficile la déduction des informations sur la fonction écologique d'un organisme à partir de données taxonomiques. Plusieurs espèces de différents groupes de protistes ont tout de même été caractérisées et leurs fonctions bien définies (Laliberté & De la Noüe, 1993; Schnepf & Kühn, 2000; Skovgaard et al., 2000; Lafarga-De la Cruz et al., 2006; Rehman et al., 2007; Brutemark & Granéli, 2011). Il est donc possible de relier les organismes à une fonction spéculative par association à un ordre ou un genre particulier. Ainsi, au cours de cette thèse, une combinaison de techniques « classiques » de microbiologie et du gène marqueur de la petite sous-unité ribosomale ARN 18S ont tout d'abord permis de caractériser l'appartenance phylogénétique ou taxonomique des organismes à l'étude et ensuite de déduire leur fonction dans le milieu.

1.2 Groupes fonctionnels et processus microbiens

Par la photosynthèse, l'énergie lumineuse est transformée en énergie chimique utilisée pour incorporer le carbone inorganique à la biomasse. Les phototrophes eucaryotes et procaryotes permettent ainsi l'entrée du carbone dans le réseau trophique. Ces organismes incorporent également les nutriments dissous (i.e. le phosphore et l'azote) dans leurs molécules organiques, telles que les acides nucléiques, acides aminés et phospholipides (Prescott et al., 2002), ce qui permet une entrée de ces éléments essentiels dans la matière organique. Le phytoplancton eucaryote contribue à l'activité photosynthétique des lacs, sans toutefois supporter entièrement le réseau trophique de l'écosystème dans tous les cas (Del Giorgio & Peters, 1993). Dans les régions polaires, le phytoplancton est régulièrement dominé par des nanoflagellés phototrophes représentant la source eucaryote de production primaire (Laybourn-Parry & Marshall, 2003). Les cyanobactéries contribuent majoritairement à la biomasse et à la production primaire des écosystèmes arctiques et

antarctiques, des fois comme picocyanobactéries dans la colonne d'eau et souvent sous forme de tapis microbien (Vincent et al., 2000). Cette association en tapis offre une protection contre les ultraviolets (Quesada et al., 1999; Jungblut et al., 2010) et permet une concentration et un recyclage des nutriments inorganiques, rendant ces communautés phototrophes benthiques indépendantes de la limitation en nutriments que subit le phytoplancton eucaryote dans la colonne d'eau (Bonilla et al., 2005).

L'hétérotrophie correspond à l'utilisation de carbone organique pour subvenir aux besoins en carbone, et en énergie. Parmi les protistes hétérotrophes, on retrouve les herbivores qui effectuent la production secondaire en se nourrissant de la matière organique particulière contenue dans le phytoplancton, des cyanobactéries aux dinoflagellés (Sherr & Sherr, 2002). Ces herbivores sont ensuite les proies de protistes omnivores (Sherr & Sherr, 2002) tels que les ciliés et dinoflagellés, qui capturent et ingèrent des flagellés hétérotrophes (Jurgens et al., 1996). Le processus de prédation implique une succession d'étapes au niveau desquelles il peut y avoir une sélection active ou passive des proies (Montagnes et al., 2008). Suite à la quête, au contact et au traitement de la proie, l'ingestion constitue la principale étape de la prédation par phagocytose, soit l'internalisation de la cellule entière ou par aspiration de son contenu cellulaire (Montagnes et al., 2008).

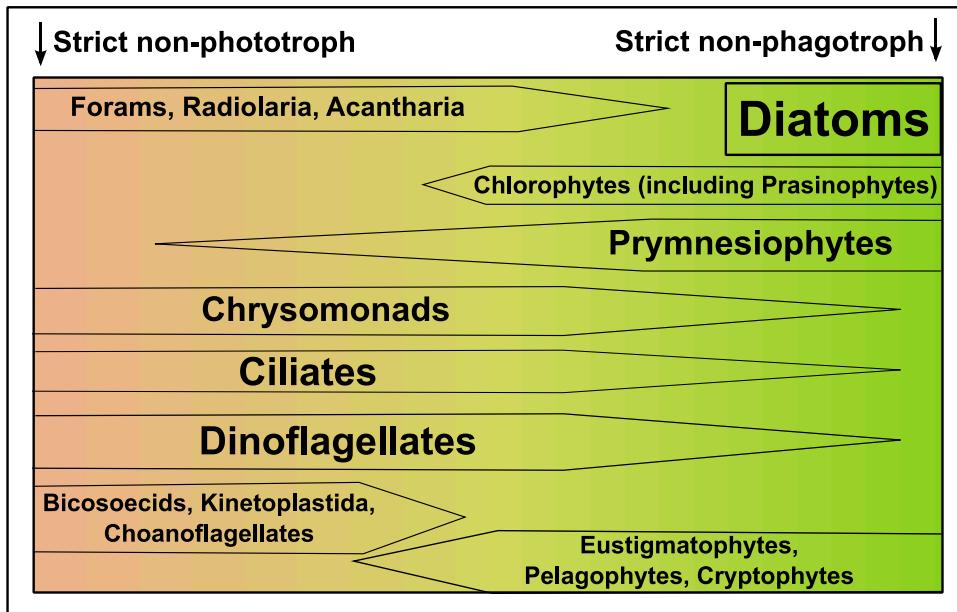


Figure 1.2 Gradient de mixotrophie chez les eucaryotes (tiré de Flynn et al., 2012).

La mixotrophie est une stratégie utilisée par certains protistes qui implique un recours à la photosynthèse et à l'hétérotrophie pour subvenir aux besoins en carbone, en nutriments et en énergie. Cette double stratégie est reconnue comme une réalité non-négligeable des écosystèmes marins et lacustres (Hartmann et al., 2012; Tittel et al., 2003) et est utilisée par une grande majorité des espèces phytoplanctoniques (Stoecker, 1998; Flynn et al., 2012). La mixotrophie peut se manifester soit par une capacité à absorber et à utiliser le carbone organique dissout (osmo-mixotrophie), soit par le recours à la phagotrophie en parallèle à la photosynthèse (Sanders, 2011). L'observation de l'osmo-mixotrophie provient principalement d'études en laboratoire au cours desquelles les concentrations de carbone dissout, notamment sous forme de glucose (Laliberté & De la Noüe, 1993) ou d'acétate (Markager & Sand-Jensen, 1990), étaient beaucoup plus élevées que dans les systèmes naturels (Bennett & Hobbie, 1972), particulièrement les lacs oligotropes arctiques. Dans cette thèse le terme mixotrophie porte exclusivement sur les organismes qui combinent la photosynthèse et la phagocytose (Figure 1.2) et ne considère pas l'osmo-mixotrophie.

Table 1.2 Classification des protistes mixotrophes en fonction de leurs comportements (basé sur Stoecker 1998).

Modèle	Groupe	Mode de nutrition principal	Mode de nutrition supplémentaire	Conditions de mixotrophie	Exemples
I	-	Phototrophie / Phagotrophie	-	Les deux modes de nutrition sont utilisés à proportions égales	-
II	A	Phototrophie	Phagotrophie	Quand les nutriments sont limitants	<i>Dinobryon cylindricum</i> , <i>Ochromonas minima</i>
	B	Phototrophie	Phagotrophie	Pour des substances essentielles à la croissance	<i>Uroglena americana</i> , <i>Cryptomonas</i> spp.
	C	Phototrophie	Phagotrophie	Quand la lumière est limitante, pour le carbone et l'énergie	<i>Chrysochromulina brevifilum</i> , <i>Amphidinium cryophilum</i>
III	A	Phagotrophie	Phototrophie	Quand les proies sont limitantes	<i>Poterioochromonas malhamensis</i>
	B	Phagotrophie	Kleptoplastidie	Pour supplémerter les apports en carbone	<i>Pfiesteria piscicida</i> , <i>Laboea strobilia</i>

Étant un groupe polyphylétique, l'identification des mixotrophes nécessite une classification fonctionnelle (Jones, 1997; Stoecker, 1998) basée sur les différentes stratégies mixotrophes (Table 1.2) et garantissant une détermination de leur fonction écologique plus appropriée que ce que la classification taxonomique ne permettrait. En effet, pour certains groupes de phytoplancton la mixotrophie offre un moyen d'éviter la compétition pour les nutriments dissous, tels que le phosphore (Jones et al., 1993; Kamjunke et al., 2007) et l'azote (Marshall & Laybourn-Parry, 2002), ou pour contourner une carence en oligoéléments, tels que le fer (Maranger et al., 1998), qui limitent la photosynthèse. D'autres phototrophes utilisent cette stratégie afin de contourner le

manque de lumière (Jones, 1997) qui peut être très prolongé dans certains milieux (Roberts & Laybourn-Parry, 1999; Marshall & Laybourn-Parry, 2002). De plus, certains mixotrophes principalement hétérotrophes adoptent la photosynthèse comme alternative à la phagotrophie lorsque les concentrations de proies sont trop faibles (Caron et al., 1990; Jones, 1997; Stoecker, 1998). Une telle variabilité de stratégies mixotrophes implique que la fonction de ces organismes, au sein d'un réseau trophique soit différente d'un milieu à l'autre, dépendamment de son contenu en ressources organiques et inorganiques.

L'utilisation par une même cellule de la photosynthèse et du carbone organique particulaire comme sources de carbone et d'énergie est un avantage compétitif pour les mixotrophes comparativement aux phototrophes et hétérotrophes strictes. Certains mixotrophes, tels que les modèles IIIA et IIC selon Stoecker (1998), peuvent utiliser ces deux ressources de façon substituable (Jones, 1997). Ainsi, quand le nombre de proies devient limitant pour le groupe IIIA, la photosynthèse permet de maintenir la croissance. Inversement, quand la lumière devient limitante les mixotrophes de type IIC complètent leur régime avec des proies. Par conséquent, ces derniers peuvent abaisser la densité de proies au dessous du seuil de prédation de leurs compétiteurs hétérotrophes stricts, en présence de lumière (Rothhaupt, 1996a; Tittel et al., 2003). Les deux autres catégories de mixotrophes (modèles IIA et B) ont des besoins énergétiques essentiels et sont incapables de compenser entièrement l'absence totale de lumière par la prédation (Stoecker, 1998). Néanmoins, le groupe IIA utilise la phagotrophie comme source de nutriments lorsque les nutriments inorganiques dissous sont limitants pour la photosynthèse. Le groupe IIB dépend de la phagotrophie pour un apport en éléments de croissance essentiels. Ainsi, ces groupes sont plus compétitifs que les phototrophes strictes en milieux oligotrophes ou à faible luminosité (Jones, 2000). Puisque certains mixotrophes sont favorisés lors d'une limitation en nutriments ou en éclairement, ils développent des populations dominantes dans les milieux oligotrophes (Rothhaupt, 1997), et dans les lacs humiques (Jansson et al., 1996) ou couverts de glace (Laybourn-Parry & Marshall, 2003).

En termes de compétition, cependant, l'assemblage et le maintien des appareils de photosynthèse et de phagocytose implique un coût énergétique très élevé pour la cellule (Raven, 1997). Par conséquent, les mixotrophes ne sont pas aussi performants dans leur utilisation des ressources énergétiques de base que les spécialistes (Jones, 2000; Tittel et al., 2003). Leur avantage, par rapport aux phototrophes ou hétérotrophes obligatoires, est de pouvoir s'adapter aux conditions restrictives de leur environnement. Les influences du phytoplancton mixotrophe sur les communautés microbiennes sont variées, allant de la production primaire à la facilitation en passant

par la prédation. Dans les milieux particulièrement oligotrophes tels que les lacs arctiques, les mixotrophes contribuent significativement à la production primaire totale (Laybourn-Parry et al., 2005). En effet, les mixotrophes ayant conservé leur activité métabolique sous la glace sont capables de croître rapidement, ce qui crée un pic de production primaire au moment de la fonte du couvert de glace, quand l'éclairage permet de soutenir une forte activité photosynthétique. Il arrive qu'en fin de saison de croissance les mixotrophes profitent d'une nouvelle niche créée par l'épuisement des nutriments lorsque les phototrophes deviennent limités par le manque d'azote ou de phosphore. Ainsi, la présence de mixotrophes est influencée par les facteurs environnementaux et les interactions de compétition et a le potentiel d'affecter le réseau trophique aquatique et la structure des communautés microbiennes.

1.3 Réseaux trophiques

Le transfert de carbone et d'énergie dans les réseaux trophiques (Figure 1.3) commence par le broutage du phytoplancton (eucaryote et procaryote) par les consommateurs primaires, nanoplancton ou microplancton hétérotrophes ou mixotrophes. À leur tour, ces herbivores sont consommés par le microzooplancton ou le mesozooplancton omnivore. De plus, une partie du transfert de carbone se fait par la prédation sur les bactéries hétérotrophes qui représentent également une source de carbone et d'énergie pour les protistes hétérotrophes (Sherr & Sherr, 2002). Le microplancton constitue un lien vers le mesozooplancton et assure une mise à disposition directe de l'énergie et des nutriments pour les niveaux trophiques supérieurs (Sherr & Sherr, 2002). Néanmoins, les rotifères, certains copépodes et des larves de poissons et autres metazoaires filtreurs peuvent également se nourrir de picoplancton ou de nanoplancton (Pedrós-Alió et al., 1995; Hobbie et al., 1999).

Les bactéries hétérotrophes et les archées utilisent la matière organique dissoute issue de l'exsudation par le phytoplancton (Sarmento & Gasol, 2012), la production de pellettes fécales par le zooplancton (Møller et al., 2003), les déchets de la prédation (Strom et al., 1997; Møller et al., 2003) ou de la lyse de cellules mortes suite à une infection virale (Wilhelm & Suttle, 1999). Cette boucle microbienne (Azam et al., 1983) a deux rôles importants au sein du réseau trophique aquatique. Premièrement, les communautés procaryotes contribuent au recyclage et au transfert du carbone et des nutriments à travers le réseau alimentaire aquatique par l'assimilation de ces composés dans leur biomasse. Deuxièmement, ces organismes effectuent la reminéralisation de la matière organique, approvisionnant le phytoplancton en carbone et en nutriments inorganiques (Pomeroy et al., 1998). De fait, le phytoplancton et les procaryotes interagissent dans un lien de compétition-facilitation pour les nutriments inorganiques d'une part, et d'autre part par l'exsudation

de matière organique par les algues ou la reminéralisation par les bactéries. En outre, les virus interviennent à chaque étape de ce réseau trophique et participent au recyclage du carbone et des nutriments. Le court-circuit viral (*viral shunt*) constitue une portion importante du retour du carbone organique particulaire à l'état de matière organique dissoute (Wilhelm & Suttle, 1999).

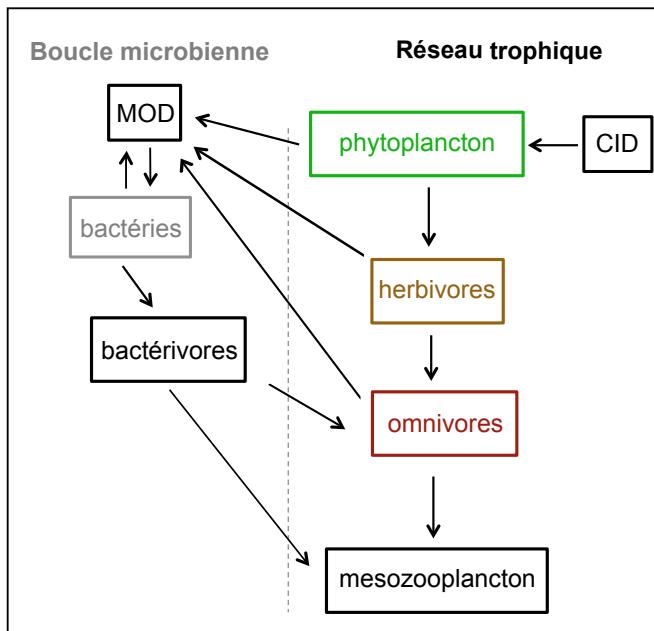


Figure 1.3 Transfert de carbone dans le réseau trophique aquatique. MOD, matière organique dissoute; CID, carbone inorganique dissout.

Aux pôles, où l'apport de matière organique est limité par l'absence de végétation dans les bassins versants, comparativement aux régions tempérées, la majorité de la matière organique disponible pour la chaîne alimentaire est d'origine autochtone (Vincent et al., 2008b) et provient de la production primaire interne du lac effectuée principalement par des picocyanobactéries (Vincent et al., 2000; Van Hove et al., 2008). L'absence totale de lumière en hiver rend la photosynthèse impossible (Vincent et al., 2008b) alors qu'en été la production primaire dans certains lacs reste largement restreinte par la présence de couverts de glace. Les lacs qui perdent leur couvert de glace au cours de l'été ont tout de même une période de croissance relativement courte et la photosynthèse reste limitée par les nutriments (Vincent et al., 2008b). Les systèmes aquatiques sont donc principalement hétérotrophes et dépendants de la faible biomasse autochtone. La boucle microbienne y est particulièrement importante, approvisionnant la production primaire en nutriments essentiels et représentant un substrat pour les protozoaires. Selon une étude circumpolaire, les lacs subarctiques présentent 4 types d'assemblages de communautés phytoplanctoniques au cours de la saison de croissance (Table 1.3), chacun dominé par des

chrysophytes à un moment de l'année (Holmgren, 1984). La dynamique des populations de planctons dans les lacs arctiques est plus restreinte que dans les lacs des régions tempérées et présente des chaînes trophiques plus courtes où, par exemple, les rotifères constituent le niveau trophique le plus élevé (Panzenböck et al., 2000). Certains lacs arborent de faibles populations de ciliés, de rotifères, de nauplii de copépodes et de cladocères, supportées par les communautés microbiennes et les nanoflagellés (Hobbie et al., 1999).

Table 1.3 Types d'assemblages de phytoplancton dans les lacs arctiques, subarctiques et alpins. Les groupes sont listés par ordre de leur dominance (basé sur Holmgren, 1984).

Type d'assemblage	Printemps	Été	Automne
1. Chrysophyceae	Chrysophyceae Dinophyceae Volvocales	Chrysophyceae Dinophyceae Chlorococcales	Chrysophyceae
2. Chrysophyceae-Diatomées	Chrysophyceae Cryptophyceae Dinophyceae	Diatomées	Diatomées Cryptophyceae Cyanobactéries
3. Chrysophyceae-Cryptophyceae	Chrysophyceae Volvocales	Cryptophyceae Diatomées	
4. Chrysophyceae-Dinophyceae	Dinophyceae	Chrysophyceae Diatomées Chlorococcales	Dinophyceae

Le phytoplancton qui domine ces lacs polaires a des capacités mixotrophes lui permettant d'avoir recours au broutage lorsque la fixation photosynthétique du carbone est restreinte par la lumière ou que les nutriments sont limitants (Roberts & Laybourn-Parry, 1999; Marshall & Laybourn-Parry, 2002; Laybourn-Parry et al., 2005; Christoffersen et al., 2008). Par exemple, le lac Fryxell dans les vallées sèches de l'Antarctique possède une population dominante de cryptophytes mixotrophes. Ceux-ci utilisent la bactéritorophie en été sous le couvert de glace permanent afin de compléter la photosynthèse limitée par la faible luminosité pénétrant sous le couvert de glace. Lors de l'hiver obscur des régions polaires, la population de cryptophytes peut se maintenir et pourra reprendre une photosynthèse complémentée par la phagotrophie en été (Roberts & Laybourn-Parry, 1999; Marshall & Laybourn-Parry, 2002; Laybourn-Parry, 2002). De plus, les mixotrophes ont la capacité de survivre dans le noir pendant six mois (Jones et al., 2009), une durée nettement plus longue que celle de l'obscurité hivernale dans le haut Arctique canadien. Ainsi, malgré un bilan à dominance hétérotrophe les lacs arctiques recèlent un potentiel de photosynthèse grâce à leurs communautés de protistes dominées par des mixotrophes.

Ces mixotrophes ont donc un impact considérable sur les communautés de protistes et de bactéries dans les lacs arctiques. Par une prédation sur les bactéries qui peut surpasser la bactéritivorie par les niveaux trophiques supérieurs (Bird & Kalf, 1986), les mixotrophes nanoflagellés ont le potentiel de modifier la morphologie et la composition de la communauté microbienne (Hahn & Höfle, 1999). La prédation par les mixotrophes peut également affecter les niveaux trophiques supérieurs, notamment en entrant en compétition avec leurs propres prédateurs pour des proies qu'ils ont en commun (prédation intra-guilde ; Ptacnik et al., 2004). Inversement, certaines études ont démontré le phénomène de facilitation des mixotrophes sur leurs compétiteurs autotrophes (Rothhaupt, 1996a; Rothhaupt, 1996b). Enfin, le mode de nutrition des mixotrophes modifie leur qualité nutritive en tant que proie pour le mesozooplancton (Weithoff & Wacker, 2007; Wacker & Weithoff, 2009) et la présence de mixotrophes cause une augmentation du ratio C :N du seston, permettant un transfert plus efficace du carbone à travers la chaîne trophique (Ptacnik et al., 2004).

1.4 Question principale et hypothèses

Le fil directeur de cette thèse est la détermination de la contribution relative des mixotrophes aux communautés de protistes et la compréhension de l'effet des conditions environnementales changeantes dans les lacs Arctiques sur ces communautés. Une première hypothèse porte sur la dominance des communautés de protistes par les organismes mixotrophes (chapitre 1). Selon la deuxième hypothèse, les mixotrophes subiraient des modifications en fonction de la profondeur et du temps suivant les conditions physico-chimiques (chapitre 2). Ce qui mène à une troisième hypothèse selon laquelle les modifications des conditions environnementales par le réchauffement climatique auront un effet sur les communautés de protistes dans ces systèmes. Les communautés de mixotrophes seraient affectées plus particulièrement par la disponibilité de la lumière et des proies (chapitre 3).

1.5 Sites d'étude

Les lacs, mares et cours d'eau constituent la caractéristique principale des paysages arctiques et antarctiques et englobent une vaste diversité limnologique (Vincent et al., 2008b). Les pôles sont caractérisés par de forts gradients d'éclairement et des lacs répartis le long d'un gradient latitudinal du subarctique au haut Arctique reçoivent différentes doses d'irradiation solaire pendant l'année. L'extrême saisonnalité de l'éclairement du haut Arctique est combinée à des couverts de glace permanents qui atténuent la transmission de la lumière dans la colonne d'eau, et ce même en été. Les propriétés de ce couvert de glace jouent également un rôle dans l'établissement du régime lumineux. Outre son épaisseur, la glace possède plusieurs caractéristiques optiques, telles que

l'orientation des cristaux, la porosité et le contenu en bulles d'air ou en sédiments (Mullen & Warren, 1988). Ce couvert de glace limite également l'exposition au vent et par conséquent le mélange de la colonne d'eau. En particulier, les lacs méromictiques ou épi-plateforme de la côte nord de l'Île d'Ellesmere (Mueller et al., 2009) et des Vestfold Hills de l'Antarctique (Lauro et al., 2011) possèdent deux couches d'eau qui ne se mélangent jamais à cause du gradient de salinité et du couvert de glace permanent qui augmente la stabilité de leur stratification. Inversement, les lacs ayant un couvert de glace saisonnier, tel que les lacs monomictiques froids de l'Île de Cornwallis (Rigler, 1974), sont plus propices au mélange durant l'été.

La majorité de l'Arctique est considéré comme un désert gelé. Par exemple, l'archipel Arctique canadien ne reçoit pas plus que 200 mm/an de précipitations (Thomas et al., 2012). Combiné aux faibles températures, cette déshydratation influence la distribution de la végétation terrestre. Le manque de végétation affecte le contenu en matière organique des sols des bassins versants et par conséquent celui du ruissèlement vers les lacs. Avec les faibles températures et le gel fréquent qui réduisent l'activité microbienne des sols, la dégradation de la matière organique végétale est fortement limitée, ce qui restreint sa reminéralisation en nutriments inorganiques. Ainsi, les lacs arctiques sont faiblement alimentés en matière organique d'origine externe (allochtone) et sont généralement oligotrophes, voir même ultraoligotrophes (Panzenböck et al., 2000; Laybourn-Parry & Marshall, 2003; Bonilla et al., 2005). Étant sensibles au moindre changement dans leur bassin versant, la simple présence d'animaux aux alentours des lacs, tel qu'une colonie d'oies des neiges, peut en modifier le statut trophique (Côté et al., 2010). Les activités anthropiques ont également eu des impacts sur la région. Par exemple, le lac Meretta à Resolute Bay est eutrophisé depuis les années 1950 par des déversements d'égouts provenant d'une base du Département des Transports (Douglas & Smol, 2000).

En raison de la faible productivité et du peu d'apports allochtones dans les lacs arctiques et antarctiques, les taux de sédimentation y sont très faibles (Pienitz et al., 2004; Doran et al., 1994). Ainsi la résolution temporelle des sédiments lacustres est faible, car une couche de 5 mm peut contenir les sédiments déposés au cours de 50 ans (Antoniades et al., 2007), et une coupe si fine constitue un défi analytique (Pienitz et al., 2004). Néanmoins, les études paléolimnologiques offrent une vision incomparable du passé de ces lacs, montrant majoritairement les impacts récents des changements environnementaux dans la région (Michelutti et al., 2003; Antoniades et al., 2007). En outre, les conséquences physiques (Vincent et al., 2008a) ou biologiques (Vincent et al., 2009; Veillette et al., 2011) des changements climatiques contemporains peuvent être étudiées en temps

réel puisque ces lacs sont des sentinelles des changements à venir (Williamson et al., 2009). Ainsi, l'Arctique est un site d'étude approprié pour l'exploration d'une large gamme de propriétés limnologiques et de leurs conséquences sur la biologie des lacs. Ces habitats aquatiques, aux réseaux trophiques élémentaires, constituent des écosystèmes modèles pour étudier les communautés de protistes et investiguer leurs fonctions.

Table 1.4 Descriptifs des lacs étudiés. Z représente la profondeur.

Site	Latitude	Longitude	Surface (km ²)	Z moyen. (m)	Z max. (m)
Lac Char*	74.42°N	94.50°W	0.53	10.2	28
Lac Ward Hunt [§]	83.05° N	74.10° W	0.37	2.0	8
Lac A*	83.00°N	75.30°W	5.00	-	120

* informations de Van Hove et al. (2006).

§ informations de Bonilla et al. (2005) et Vincent et al (2011).

Les trois lacs choisis pour les investigations rapportées ci-dessous sont situés dans l'archipel Arctique canadien au-delà du Cercle Polaire (latitude 66°33' N) et illustrent la grande diversité limnologique de l'Arctique (Table 1.4). Le lac Char est situé dans la baie de Resolute (74°42' N ; 94°50' W) sur l'île de Cornwallis, une région considérée comme un désert polaire. Avec une surface de 0.53 km² et une profondeur maximale de 28 m, le lac Char est un lac monomictique caractérisé par un phénomène de mélange en été suite à la perte de son couvert de glace annuel, des températures froides et une colonne d'eau bien aérée (Rigler, 1974). Particulièrement oligotrophe et légèrement basique (Michelutti et al., 2003), le lac Char devient encore plus oligotrophe pendant l'été, à cause du ruissellement de l'eau de fonte qui dilue les ions Ca⁺⁺, Mg⁺⁺ et K⁺ présents sous la glace (Schindler et al., 1974). Le lac Char, qui est la source d'eau potable du village de Resolute, fût intensivement étudié de 1968 à 1972 dans le cadre du Programme Biologique International (Rigler, 1972). Quelques études ont été effectuées sur la production primaire (Markager et al., 1999) et les diatomées (Michelutti et al., 2003), mais on constate un manque de données sur les populations de procaryotes et de protistes. De plus, la biodiversité du lac est affectée par les récents changements climatiques (Michelutti et al., 2003).

Les deux autres lacs de l'étude sont situés dans le parc national Quttinirpaaq (« le sommet du monde » en Inuktituk), sur la côte Nord de l'île d'Ellesmere. Le lac A (83°00' N ; 75°30' W), avec une profondeur maximale de 128 m, est un lac méromictique. Sous un couvert de glace permanent, le lac A présente une stratification stable depuis plus de quarante ans avec un fort gradient physico-chimique le long de la colonne d'eau (Vincent et al., 2008a). Les apports d'eau douce au lac A proviennent de la fonte du couvert de neige dans le large bassin de drainage, renforçant le

phénomène méromictique puisque la zone saline en profondeur reste stagnante alors que la couche supérieure d'eau douce est constamment renouvelée (Veillette et al., 2011). Une récente augmentation de la fréquence de perte de son couvert de glace cause des changements dans la structure physico-chimique du lac A (Veillette et al., 2011). Le lac Ward Hunt ($83^{\circ}05'N$; $74^{\circ}10'W$), sur l'île de Ward Hunt, est caractérisé par une petite superficie de 0.37 km^2 (Villeneuve et al., 2001) et une profondeur maximale de 8 m (Vincent et al., 2011). Le lac Ward Hunt est ultraoligotrophe (Bonilla et al., 2005) et habituellement recouvert de glace avec des températures de 0 à 5°C , même durant l'été où les températures de l'air n'atteignent pas plus de 10°C en moyenne. La colonne d'eau de ce lac a possiblement été entièrement gelée dans le passé, mais une étude paléolimnologique de ses sédiments démontre une récente augmentation de sa productivité (Antoniades et al., 2007).

1.6 Méthodologies utilisées

Tel que discuté plus haut, la microscopie et le HPLC sont des méthodes qui servent à la quantification des cellules et permettent d'identifier les organismes comptés (chapitre 2). La méthode du FNU pour le comptage microscopique est avantageuse car l'utilisation du marqueur DAPI en complément de la microscopie à contraste interférentiel différentiel (DIC) permet de distinguer les cellules des particules abiotiques et l'autofluorescence de la chlorophylle *a* dissocie les organismes phototrophes des hétérotrophes (Lovejoy et al., 1993). Utilisé séparément, le marquage des cellules au DAPI permet également de faire le comptage des cellules sous épifluorescence en distinguant procaryotes et eucaryotes (chapitre 4). Le HPLC a servi à identifier des groupes majeurs grâce à leurs signatures uniques en pigments photosynthétiques et photoprotecteurs (Roy et al., 2011). Cette technique permet également une estimation de la biomasse phytoplanctonique par quantification de la chlorophylle *a* (Bonilla et al., 2005).

L'outil principal de cette thèse est le séquençage du gène de la petite sous-unité ribosomale ARN 18S (Woese et Fox, 1977; Pace et al., 1986; Woese et al., 1990). Les ribosomes sont des organites présents chez tous les organismes vivants, qui effectuent la traduction des ARN messagers en protéines. Ainsi, ce gène de la petite sous-unité ribosomale est un gène constitutif essentiel à toute forme de vie. Certaines parties de ce gène sont très conservées et se retrouvent dans tous les domaines, alors que d'autres parties du gène sont plus variables, traduisant une distance phylogénétique entre les organismes (Figure 1.4). Par conséquent, les techniques de séquençage ont pris beaucoup d'importance en microbiologie systématique et en écologie microbienne. L'utilisation classique de banques de clones pour isoler les gènes d'intérêt de l'ADN

communautaire, précédemment amplifiés par PCR, suivit du séquençage par la technique de Sanger, permet l'obtention de la séquence complète du gène de l'ARNr 18S (Lovejoy et al., 2007). Cette technique de séquençage basée sur la méthode de Sanger et al (1977) se fait maintenant par une réaction d'amplification du brin d'ADN contenant des nucléotides-terminateurs, suivie d'une migration capillaire des fragments de tailles différentes et de la détection par fluorescence du nucléotide de fin (Smith et al., 1986). Cependant, le passage par les banques de clones est un facteur limitant pour le rendement en terme du nombre de séquences car il s'agit d'une étape laborieuse qui ne fournit pas toujours beaucoup de fragments modèles (*template*) pour l'étape subséquente de séquençage.

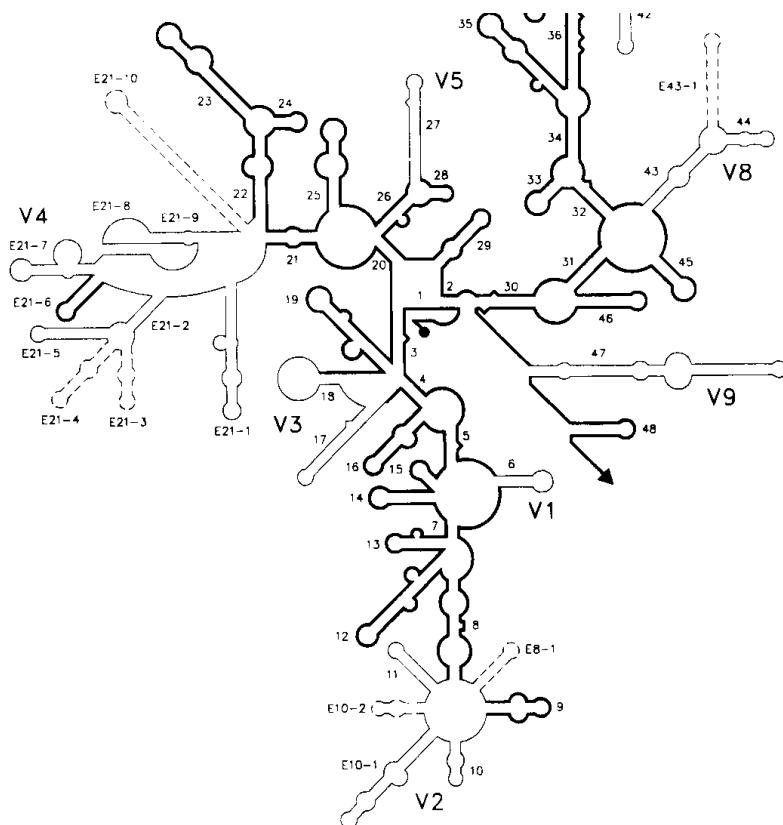


Figure 1.4 Structure secondaire de l'ARN ribosomale de la petite sous-unité du ribosome (tiré de De Rijk et al., 1992).

La plupart des études en écologie microbienne ont maintenant recours aux techniques de séquençage haut-débit. Cette nouvelle génération de technologies de séquençage, telles que le pyroséquençage-454 ou l'Illumina, a révolutionné la microbiologie environnementale en montrant

la profondeur jusqu'alors inexplorée de la diversité microbienne (Sogin et al., 2006; Lazarevic et al., 2009). Le pyroséquençage, nommé pour le pyrophosphate libéré suite à la réaction d'incorporation d'un nucléotide triphosphate dans une chaîne d'ADN (Ronaghi et al., 1996), est une technique de séquençage-par-synthèse, permettant le suivi de l'assemblage des nucléotides au cours de l'élongation par l'ADN-polymérase. La détection du pyrophosphate au moment de sa libération, par émission de fluorescence suite à une cascade de réactions impliquant la sulfurylase et la luciférase (Ronaghi et al., 1998), permet de savoir quel nucléotide a été incorporé à une position précise de la séquence. Brièvement, chaque amplicon modèle (*template*) est fixé sur une bille puis amplifié dans un micro-incubateur créé par émulsion. Ensuite les billes recouvertes de copies du même amplicon sont réparties dans des puits sur une plaque PicoTiterPlateTM. Les réactions de PCR permettant le séquençage ont lieu dans ces puits. La fluorescence émise lors de l'incorporation d'un nucléotide est détectée par une caméra CCD et enregistrée par un ordinateur pour créer un flowgramme à partir duquel la séquence sera reconstituée (Huse et al., 2007).

Table 1.5 Récapitulatif de l'échantillonnage et des analyses effectuées pour chaque chapitre.

Chapitre	Lacs	Date	Échantillons	Analyses	Référence
2	Lac Char	Aout 2008	1 échantillon à 2 m (surface du lac)	- Microscopie (FNU) - Pigments (HPLC) - Banque de clones (ADN)	Charvet et al. (2012) <i>Polar Biology</i>
	Lac A				
	Lac Ward Hunt				
3	Lac A	Mai 2008	2, 5, 10, 12, 20, 29, 32, 60 m	Pyroséquençage du gène de la petite sous-unité ribosomale ARN 18S	Charvet et al. (2012)
		Aout 2008	2, 10, 12, 29 m		
		Juillet 2009	2, 5, 10, 12, 29 m		
4	Lac Ward Hunt	Juillet 2009	Eau de surface (sous la glace) utilisée pour l'expérience de lumière/dilution	- Expérience de dilution - Pyroséquençage sur ADN et ARN - Pigments (HPLC) - Microscopie (DAPI)	Charvet et al. (chapitre 4)

1.7 Développement de la thèse

L'objectif principal de cette thèse est d'évaluer la composition taxonomique des communautés de protistes et sa variabilité au sein de différents lacs et sous différentes conditions environnementales afin de qualifier l'impact potentiel des changements climatiques sur ces écosystèmes. Les chapitres 2, 3 et 4 composent le corps de la thèse et sont présentés sous formes d'articles scientifiques (Table 1.5 et Figure 1.5). Le deuxième chapitre équivaut à une description qualitative de la structure des communautés naturelles de protistes de trois lacs arctiques. Cette étude comporte une comparaison des techniques de microscopie et HPLC avec le séquençage par Sanger du gène de l'ARNr 18S. Le

troisième chapitre est une étude spatio-temporelle des communautés de protistes du lac A par pyroséquençage du gène de l'ARNr 18S avant, pendant et après un évènement inhabituel de fonte totale du couvert de glace. Le quatrième chapitre relate une analyse plus empirique des populations de protistes et de la façon dont elles sont affectées par les conditions d'éclairement et d'abondance des proies. La composition des communautés de protistes au cours de cette expérience est déterminée avec le pyroséquençage du gène codant pour l'ARN de la petite sous-unité ribosomale et de l'ARN ribosomal 18S lui-même. Ayant déterminé la composition des communautés de protistes en période de transition environnementale aux deuxième et troisième chapitres, la dernière étude suggère ensuite une explication du comportement des protistes, probablement à l'origine de leurs réponses aux changements climatiques. Pour conclure cette thèse, un résumé des résultats clés est présenté à la lumière de la question initiale suivie d'une discussion des perspectives de recherches sur les mixotrophes dans les milieux lacustres.

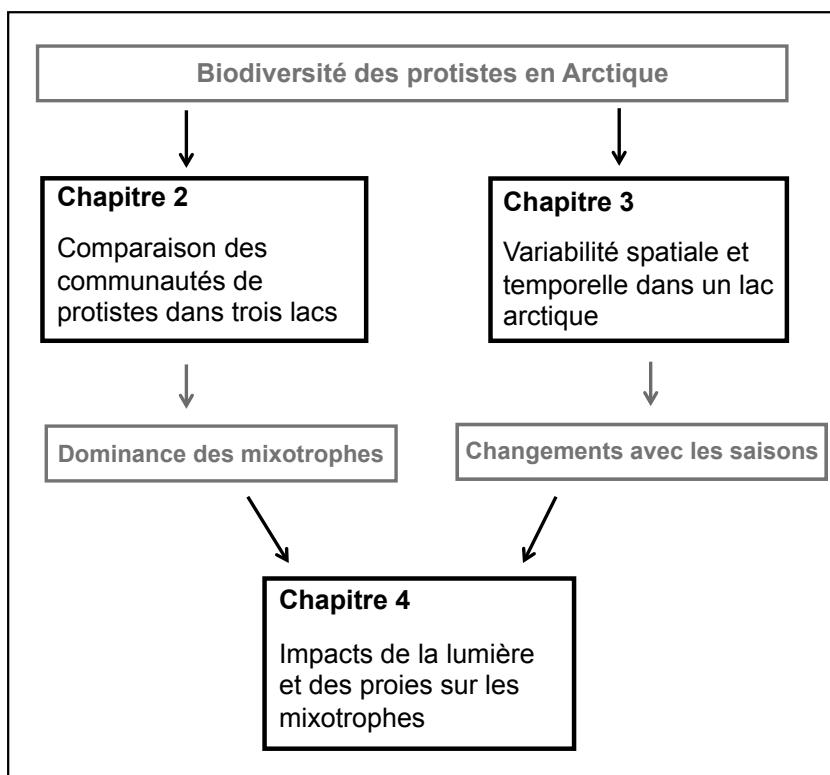


Figure 1.5 Schéma récapitulatif de la structure de la thèse montrant les liens entre les chapitres.

Chapitre 2 - Chrysophytes and other protists in High Arctic lakes: molecular gene surveys, pigment signatures and microscopy

Résumé

Des analyses de microscopie démontrent que les communautés de phytoplancton et autres protistes dans les lacs du haut Arctique continent souvent des chrysophytes. De telles études sont davantage appuyées par des analyses de pigments par chromatographie liquide à haute performance (HPLC), permettant d'identifier les groupes d'algues les plus abondants. Cependant, l'utilisation du gène 18S d'ARNr indique que dans d'autres écosystèmes beaucoup de protistes, particulièrement les petits hétérotrophes, sont sous-représentés ou négligés par la microscopie et le HPLC. Avec une combinaison de microscopie, d'analyses de pigments et d'une étude du gène 18S ARNr nous avons investigué la structure des communautés de protistes de trois lacs situés dans le désert polaire du haut Arctique (le lac Char à 74°42' N, le Lac A à 83°00' N et le lac Ward Hunt à 83°05' N) en fin d'été. Les trois méthodes ont indiqué que les chrysophytes étaient abondamment représentés et constituaient 50-70% de la biomasse totale de protistes et 25-50% des séquences du gène 18S ARNr. Les analyses de HPLC corroborent ces résultats en révélant la présence ubiquitaire de pigments caractéristiques des chrysophytes. Cependant, les banques de clones ont dévoilé une plus grande contribution des hétérotrophes dans les communautés de protistes que ce qui était suggéré par la microscopie. Le flagellé *Telonema* et les ciliés étaient communs aux trois lacs, et une séquence de fongus a été isolée du lac Char. Ces approches ont fourni des informations complémentaires sur la structure des communautés de protistes parmi les trois lacs et soulignent l'importance des chrysophytes, suggérant que ceux-ci sont bien adaptés aux faibles concentrations de nutriments et à la forte saisonnalité qui caractérisent l'environnement du haut Arctique.

Abstract

Microscopic analysis of the phytoplankton and other protist communities in High Arctic lakes has shown that they often contain taxa in the Chrysophyceae. Such studies have been increasingly supported by pigment analysis using high performance liquid chromatography (HPLC) to identify the major algal groups. However, the use of 18S rRNA gene surveys in other systems indicates that many protists, especially small heterotrophs, are underreported or missed by microscopy and HPLC. Here we investigated the late summer protist community structure of three contrasting lakes in High Arctic polar desert catchments (Char Lake at 74°42' N, Lake A at 83°00' N and Ward Hunt Lake at 83°05' N) with a combination of microscopy, pigment analysis and small subunit 18S ribosomal RNA gene surveys. All three methods showed that chrysophytes were well represented, accounting for 50-70% of total protist community biomass and 25-50% of total 18S rRNA gene sequences. HPLC analysis supported these observations by showing the ubiquitous presence of chrysophyte pigments. The clone libraries revealed a greater contribution of heterotrophs to the protist communities than suggested by microscopy. The flagellate *Telonema* and ciliates were common in all three lakes, and one fungal sequence was recovered from Char Lake. The approaches yielded complementary information about the protist community structure in the three lakes, and underscored the importance of chrysophytes, suggesting that they are well adapted to cope with the low nutrient supply and strong seasonality that characterize the High Arctic environment.

2.1 Introduction

The protist taxonomic composition of lakes is highly variable and influenced by local conditions of irradiance and nutrient supply. The majority of high latitude lakes are oligotrophic or even ultra-oligotrophic because slow weathering in cold polar soils results in low catchment inputs of nutrients (Vincent et al., 2008b). Irradiance conditions of these lakes differ from temperate lakes, with continuous darkness throughout the winter months and low irradiance over much of the rest of the year due to ice cover. These particular features of Arctic lakes and their relative isolation provide an opportunity to investigate communities adapted to such low nutrient and energy conditions.

The phytoplankton community composition of Canadian High Arctic waters has been previously examined by microscopy (Kalff et al., 1975; Vallières et al., 2008), and pigment analysis using High Performance Liquid Chromatography (HPLC) has also been applied to infer the major phytoplankton groups present in several High Arctic lakes (Bonilla et al., 2005; Mueller et al., 2005). Both approaches have indicated the common occurrence of chrysophytes in high latitude lakes in general (Panzenböck et al., 2000; Laybourn-Parry & Marshall, 2003; Bonilla et al., 2005; Forsström et al., 2005). Short 18S rRNA gene sequences of chrysophytes from denaturing gradient gel electrophoresis (DGGE) bands have also been reported from oligotrophic Antarctic maritime lakes (Unrein et al., 2005). However, these techniques are thought to underestimate community diversity compared to the more recent environmental gene surveys (Moreira & López-Garcia, 2002). Such molecular techniques can be used to identify smaller organisms than is possible using light microscopy, and with more precision than by pigment signatures alone (Vaulot et al., 2008). Hence, gene surveys possibly allow for a more complete assessment of the microbial community, including heterotrophs, living in these lakes. Sequence information may also enable phylogenetic comparisons of plankton from different studies and over global scales (Jungblut et al., 2010).

Although microscopy and pigment analyses have indicated the potential importance of chrysophytes in polar environments, there has been little recent taxonomic work on the organisms inhabiting Arctic lakes and genetic investigations have been lacking. The aim of the present study was to identify and compare the contribution of different phytoplankton to the protist community structure of three limnologically contrasting High Arctic lakes using 18S rRNA gene surveys, classic microscopic observations and pigment analyses. Given the previous records based on microscopy from Arctic (Welch, 1973; Panzenböck et al., 2000; Bonilla et al., 2005), Subarctic (Forsström et al., 2005) and Antarctic (Butler et al., 2000; Unrein et al., 2005) lakes, a second objective was to examine the detailed phylogeny of the chrysophytes in the different lakes. Heterotrophic protists have been little examined in such waters, although heterotrophic

nanoflagellates have been identified as an important food web component (Vincent et al., 2008b). Hence a third objective was to examine the biodiversity of heterotrophic protists in the lakes using both microscopy and gene surveys.

2.2 Materials and Methods

2.2.1 Study sites

The three lakes were located in Nunavut, in the Canadian Arctic Archipelago. Char Lake (CH) is on Cornwallis Island, near Resolute Bay, at latitude 74°42' N and longitude 94°50' W (Figure 2.1). The limnology of the lake was extensively described during the Char Lake Project, as part of the International Biological Program (IBP), in the early 1970s (Rigler, 1974). It has a drainage basin of 4.35 km², a surface area of 0.53 km², a maximum depth of 28 m and was considered ultra-oligotrophic (Schindler et al., 1974). The lake is normally ice-free for less than 2 months (Rigler, 1974) during which time the water column is entirely mixed, so the water temperature rarely exceeds 4 °C.

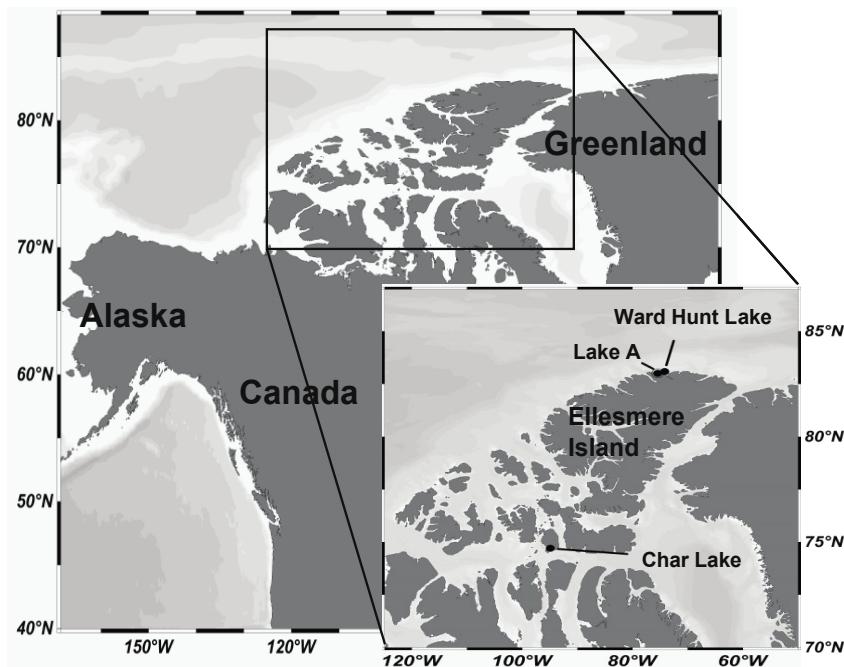


Figure 2.1 Map of sampling sites in Eastern Canadian Arctic.

Lake A (LA) lies 1000 km north of Cornwallis Island, on the northern coast of Ellesmere Island at latitude 83°00' N, and longitude 75°30' W (Figure 2.1). It has a drainage basin of about 36 km² (Jeffries et al., 1984), a surface area of 5 km² and a maximum depth of 120 m (Van Hove et al., 2006). Lake A is a permanently stratified, meromictic lake and was classed as oligotrophic to ultra-

oligotrophic by Van Hove et al. (2006). The upper 10 m is freshwater while the metalimnion is characterized by a conductivity of 4.5 mS cm^{-1} at 12 m and the monimolimnion reaches a maximum of 30.2 mS cm^{-1} in the deeper waters. Wind mixing is further inhibited by the perennial ice-cover. During an exceptional period of warming in August 2008, this ice cover melted entirely (Vincent et al., 2009), but the salinity stratification was maintained (Veillette et al., 2011).

Ward Hunt Lake (WH) is located on Ward Hunt Island (Figure 2.1), and at latitude $83^{\circ}05' \text{ N}$ and longitude $74^{\circ}10' \text{ W}$ is the northernmost lake of North America (Villeneuve et al., 2001). The lake's maximum depth is 8 m, and with a total area of 0.37 km^2 it was the smallest lake of this study. Ward Hunt Lake is ultra-oligotrophic and usually completely covered by thick perennial ice throughout the summer. However, in August 2008, for the first time on record, more than 25% of this ice had melted and detached from the eastern side and the western side was entirely devoid of ice-cover (Vincent et al., 2009).

2.2.2 Sampling and analyses

All three lakes were sampled in August 2008. Physico-chemical profiles of the water columns were taken using a conductivity-temperature-depth (CTD) profiler (XR-420 CTD-RBR profiler; RBR Ltd, Ottawa, Canada). Approximately 12 L of water for chemical and biological analyses were collected from the surface (1-2.5 m) using a closing Kemmerer bottle (Wildlife Supply Company, Yulee, FL, USA) emptied directly into lake-rinsed polypropylene containers, with no pre-filtration; our experience is that pre-filtration presents a contamination risk in these ultra-oligotrophic waters. The samples were kept cool and in the dark and transported back to a field laboratory, within four hours. The water was subsampled for nutrients, microscopy, HPLC pigments determination, and DNA analysis.

2.2.3 Nutrients

Aliquots of 120 mL of sampled water were stored in glass bottles with polypropylene caps. These were later analysed at the Canadian Center for Inland Waters (Burlington, Ontario) after being transported in the dark at *ca.* 4°C . Concentrations of nitrate and nitrite ($\text{NO}_x\text{-N}$), total nitrogen (TN), ammonia (NH_3) and soluble reactive phosphorus (SRP) were determined using standard colorimetric techniques (Gibson et al., 2002). Total phosphorus was determined from a separate 125 mL aliquot by the continuous flow analyser stannous chloride method. The detection limit for $\text{NO}_x\text{-N}$ was $0.005 \text{ mg N L}^{-1}$ and for SRP was 0.001 mg L^{-1} .

2.2.4 HPLC pigments

Samples for HPLC analyses were filtered (0.5-1 L) in our field laboratory under dim ambient light on GF/F 25 mm filters (Fisher Scientific), which were then folded and wrapped in aluminum foil and immediately placed in a Dry Shipper (nominal temperature -180 °C). This was then shipped back for analysis at Université Laval, and the samples stored at -80°C until analysis. Pigments were extracted from frozen filters by sonication (3 times at 17 W, for 20 s) in 2.5 mL of 95% methanol, followed by centrifugation, and filtration with PTFE syringe filters (pore size 0.2 µm) into HPLC vials. Shortly following the extraction, 100 µL of the extracts were injected into a ProStar HPLC (Varian, Palo Alto, CA, USA) equipped with a Symmetry C8 column (3.5 µm pore size, 4.6 X 150 mm, Waters Corporation, Milford, MA, USA) with the solvent and detection protocols as in Bonilla et al. (2005).

2.2.5 Microscopy

Samples for microscopy were collected for analysis by the Fluorescence-Nomarski-Utermöhl (FNU; Lovejoy et al., 1993) technique. Briefly, 90 mL of lake water was fixed with 10 mL of a mix of buffered paraformaldehyde and glutaraldehyde (final concentrations of 0.1 and 1% respectively; Tsuji & Yanagita, 1981) and stored in the dark at 4 °C. Samples were examined within 8 months at Université Laval, where 16 - 60 mL were left for 24 h in a sedimentation chamber (Hasle, 1978). The sedimented samples were then stained with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen Inc.; 5 µg/mL final concentration) and examined using a Zeiss Axiovert 100 inverted microscope at 400X, for larger cells and 1000X magnification for smaller cells, under visible light and ultra-violet excitation, to visualise DAPI stained nuclei. Blue excitation was used to confirm the presence or absence of red fluorescing chloroplasts. Taxonomic identification of phytoplankton was based on Findlay and Kling (1979), Canter-Lund and Lund (1995) and Wehr and Sheath (2003) on specimens viewed at 1000X. Heterotrophic protists were poorly identified using the above references but they were separated by shape and size, and diversity estimates were based on the separate categories.

Taxon-specific biovolumes were estimated from the two visible dimensions measured directly with an ocular micrometer or from images captured using a Qimaging Fast 2000R system (Qimaging, Surrey BC, Canada) and processed using Image-Pro (v. 5.1.1, Acton MA). Geometry was inferred from the literature, for example to differentiate between oblate spheres and ovoids. The biovolumes of complex cell shapes were estimated using the equations proposed by Hillebrand et al. (1999). Cell biovolumes were then transformed to carbon biomass (pg C L^{-1}) based on the equations in Menden-Deuer and Lessard (2000).

2.2.6 DNA collection and clone libraries

To examine the nanoplankton community, approximately 3-4 L of water were filtered onto 47 mm diameter 3.0 μm pore size polycarbonate filters (Millipore) with no initial pre-filtration. The filters were then submerged in buffer (50 mM Tris, 40 mM EDTA, 0.75 M sucrose), in 2-mL cryovials before being stored at -80 °C until further manipulation.

Community DNA was extracted using a salt (NaCl) extraction protocol modified from Aljanabi and Martinez (1997). Briefly, filters and buffer were transferred from cryovials to 15-mL tubes, with lysozyme (1 mg mL⁻¹) for 45 minutes at 37°C (Diez et al., 2001). Proteinase K (0.2 mg mL⁻¹) and SDS 1% were then added and cells were incubated for one hour at 55°C (Diez et al., 2001). Concentrated NaCl (6 M) was then added to the tubes (final concentration of 2.3 M), which were vortexed for 1 min and centrifuged for 10 min at 7000 g. The supernatant was transferred into a new 15-mL tube and 5 mL of cold 70% ethanol was added into each sample, mixed and left overnight at -20°C. For each sample, 1.8 mL of the total volume was transferred into a 2-mL microcentrifuge tube and centrifuged at 14000 rpm for 10 min at 4°C and the supernatant discarded. This was repeated for the entire volume of the sample. The DNA was then washed with 200 μL of 70% ethanol and pellets dried and finally resuspended in 100 μL of 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA). Genomic DNA was stored at -20°C until subsequent polymerase chain reaction (PCR) and cloning.

Clone libraries were constructed targeting the small subunit 18S ribosomal RNA gene. Community genomic DNA was amplified with the eukaryote specific primers NSF4/18 (5'-CTGGTTGATYCTGCCAGT-3') and NSR 1787/18 (5'-CYGCAGGTTCACCTACRG-3') (Hendriks et al., 1991) using the iCycler™ Thermal Cycler (Bio-Rad Laboratories, Inc., CA, USA). One PCR reaction consisted of 1X Feldan PCR Buffer (Feldan Bio Laboratories, Inc., Québec, Canada), 200 μM of dNTPs, 0.3 μM of each primer, 1 μL of Bovine Serum Albumin, 1.25 units of Feldan Taq polymerase and 1-4 ng of genomic DNA. The PCR product was purified using a QIAquick PCR Purification Kit (QIAGEN Sciences, Maryland, USA) and a polyadenosine tail was added on each end (24 μL of PCR product, 5 μL of 10X Feldan PCR buffer, 1 μL of 10 mM dATP and 0.2 μL Feldan Taq polymerase, for 10 minutes at 72 °C). The ligation of the product into the StrataClone vector, pSC-A-amp/kan, and transformation of *Escherichia coli* competent cells with the recombinant vectors were done according to the StrataClone PCR Cloning Kit instruction manual (Stratagen, La Jolla, CA, USA). Cells were plated on Luria-Bertani (LB) and selected for recombinant transformants that were then picked and grown as per Diez et al. (2001). The cloned PCR inserts were verified and screened as in Potvin and Lovejoy (2009), then sequenced at the

Centre Hospitalier de l'Université Laval (CHUL, QC, Canada) using the forward (NSF4/18) and reverse (NSR1787/18) primers, that had been used to make the clone libraries, with an ABI 3730xl system (Applied Biosystems, Foster City, CA, USA).

The same protocols as above were used to obtain the 18S rRNA gene sequence from *Kephyrion* strain CCMP 3057, as a reference “cultured” sequence for the present study. This strain was isolated by J Boenigk in 2006 from a freshwater lake in Austria. We obtained the strain from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). It was cultured in DY-V for 5 weeks, then cells were centrifuged and DNA extracted, cloned and sequenced using the same 18S rRNA primers as above.

Forward and reverse sequence segments were compiled, edited and trimmed using ChromasPro (Technelysium Pty Australia, version 1.5). The approximately 1700-nt sequences were then aligned with ClustalW Multiple Alignment tool and visually checked using the BioEdit Sequence Alignment Editor (Hall, 1999). Operational taxonomic units (OTUs) were generated using Mothur (Schloss et al., 2009). Sequences were deposited in GenBank under accession numbers JF730750-JF730878. Reference alignments were constructed using additional sequences selected from the closest match to our sequences based on a BLAST search (Altschul et al., 1990) of GenBank. If the closest match was an uncultured clone, the closest isolated strain was also included. Phylogenetic trees were created using ClustalX (Thompson et al., 1997) and NJPlot (Perrière & Gouy, 1996). OTUs that Blasted close to organisms reported from both marine and freshwater origins were further investigated with an alignment and guide tree from Multiple Sequence Comparison by Log-Expectation (MUSCLE; European Molecular Biology Laboratory).

2.2.7 Diversity analyses and comparison of samples

UniFrac analyses were conducted with Mothur to compare the phylogenetic diversity among the protist communities (Lozupone & Knight, 2005). The Bray-Curtis index was applied to abundance data from both microscopic cell counts and OTUs from the clone libraries, using the PAST software package (Hammer et al., 2001). The same data matrix was used to estimate Shannon diversity index (Pielou, 1966) and Simpson's dominance index (Simpson, 1949). For the microscopy data, indices were based on the proportion of individuals for each species to the total number of cells counted. For the 18S rRNA gene sequence data, indices were based on proportion of sequences for each distinct OTU to the total number of sequences analysed.

2.3 Results

2.3.1 Physical and chemical properties

The physico-chemical results indicated different surface water properties among the three lakes (Table 2.1). Char Lake was warmest whereas Ward Hunt Lake was still under its perennial ice-cover and had the coldest waters. Conductivity was highest in Lake A, while Char Lake and Ward Hunt Lake both had similar values around 0.15 mS cm^{-1} . Nutrient values were low in all three lakes, with total nitrogen (TN) concentrations $< 0.1 \text{ mg L}^{-1}$ and total phosphorus (TP) $< 0.004 \text{ mg L}^{-1}$. Char Lake had lower concentrations of TN compared to the other two lakes. Conversely, phosphorus concentrations were lowest in Lake A compared to Char Lake and Ward Hunt Lake. The Char Lake N: P ratio of 15:1 was close to the Redfield ratio, whereas Lake A (32:1) and Ward Hunt Lake (24:1) N: P ratios were much higher, implying a more severe phosphorus limitation in these latter two lakes.

Table 2.1 Physico-chemical characteristics of the surface waters of the three lakes at the time of sampling for protist analysis, August 2008. Temperature in $^{\circ}\text{C}$, conductivity in mS cm^{-1} Nutrient concentrations in $\mu\text{g L}^{-1}$ (TN: total nitrogen; $\text{NO}_x\text{-N}$: nitrogen in nitrate and nitrite; NH_3 : ammonia; TP: total phosphorus; SRP: soluble reactive phosphorus)

Site	Temperature	Conductivity	TN	$\text{NO}_x\text{-N}$	$\text{NH}_3\text{-N}$	TP	SRP
Char Lake	6.6	0.15	56	6	12	3.8	1.3
Lake A	3.4	0.43	80	13	5	2.5	1.1
Ward Hunt Lake	0.9	0.15	87	16	<5	3.8	0.9

2.3.2 General protist biomass and diversity

Chlorophyll (*chl*) *a* concentrations were extremely low ($< 1 \mu\text{g L}^{-1}$) as were biomass levels estimated from cell counts (Table 2.2). This, in combination with the low nutrient concentrations, would classify the lakes as ultra-oligotrophic. The accessory pigment data (Table 2.2) indicated extremely low concentrations of chlorophyll *b*, a pigment characteristic of green algae. Chlorophylls *c2* and *c3* concentrations were also low. Chl *c1*, characteristic of chrysophytes, diatoms and prymnesiophytes, had the highest concentration among the accessory chlorophylls. The dominant carotenoid was fucoxanthin, a pigment found in chrysophytes, diatoms and dinoflagellates. Char Lake also contained violaxanthin (11%) and diadinoxanthin (8%) as well as smaller concentrations of alloxanthin, zeaxanthin, lutein and β -carotene. Aside from the fucoxanthin, Ward Hunt Lake only contained violaxanthin, diadinoxanthin and zeaxanthin. The pigment diversity was lowest in Lake A, with zeaxanthin, a signature pigment of cyanobacteria (but also green algae), being the most

prominent carotenoid. We looked for the dinoflagellate marker peridinin in the chromatograms but did not detect it in any of the lakes.

Table 2.2 Total cell count (10^5 cells L^{-1}), biomass concentrations ($\mu\text{g C L}^{-1}$) as determined by microscopy, and chlorophyll and carotenoid concentrations ($\mu\text{g L}^{-1}$) as determined by High Performance Liquid Chromatography (ND: not detectable).

Variable	Char Lake	Lake A	Ward Hunt Lake
Total cell count	8.00	3.15	7.67
Biomass	2.2	0.5	1.2
Chl <i>a</i>	0.682	0.283	0.542
Chl <i>b</i>	0.009	0.003	0.012
Chl <i>c1</i>	0.047	0.010	0.035
Chl <i>c2</i>	0.014	0.000	0.027
Chl <i>c3</i>	0.003	0.002	0.002
β -carotene	0.020	0.020	ND
lutein	0.016	ND	ND
zeaxanthin	0.030	0.199	0.015
alloxanthin	0.027	ND	ND
diadinoxanthin	0.060	ND	0.127
violaxanthin	0.096	ND	0.058
fucoxanthin	0.486	0.192	0.335

Microscopy and gene surveys revealed different aspects of the protist diversity (Figure 2.2). The microscopic analyses indicated that chrysophytes represented 50-70% of the total planktonic biomass, with dinoflagellates representing <1-3% in the three lakes (Figure 2.2a). The Char Lake community included other identifiable photosynthetic groups including species of Bacillariophyceae, Dictyochophyceae, Chlorophyceae and Cryptophyceae. These classes were also detected in Ward Hunt Lake, albeit in much smaller proportions. In contrast, Lake A was less diverse with microscopic data indicating, aside from the chrysophytes, the presence of Cryptophyceae (5%) and Chlorophyceae (2%). Dictyochophyceae (*Pseudopedinella* sp.) were observed in Char Lake (3%) and Ward Hunt Lake (2%) but not Lake A. Unidentified chloroplast-containing flagellates accounted for *ca.* 20% of the Char Lake and Ward Hunt Lake protist biomass, but only 5% of Lake A biomass. Heterotrophic protists including ciliates (<1%) accounted for the remaining small proportion of biomass in the three lakes, with unidentified colourless eukaryotic single cells relatively more important in Lake A and Ward Hunt Lake compared to Char Lake. The diversity indices (Table 2.3) were consistent with these observations, indicating that Char Lake and Ward Hunt Lake had the most diverse communities. Shannon's Index for Lake A was much lower while the dominance index was two times higher than for Char Lake (Table 2.3).

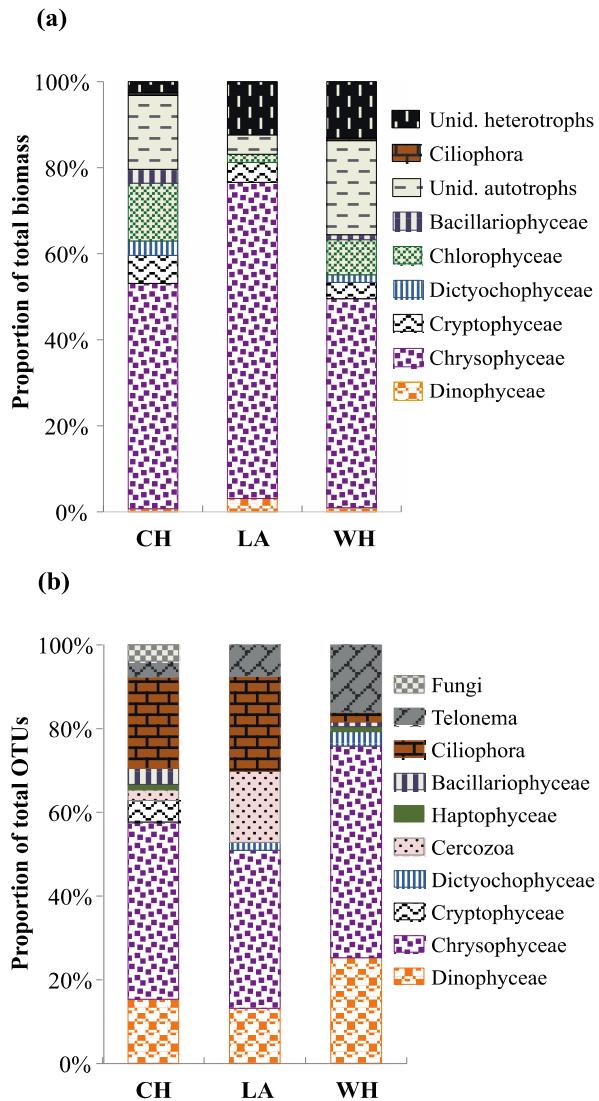


Figure 2.2 Protist community composition of surface waters of Char Lake (CH), Lake A (LA), and Ward Hunt Lake (WH) in August 2008, identified by (a) microscopic counts and (b) 18S rRNA gene sequencing.

Chrysophyte sequences were also predominant in the molecular surveys, representing 25-50% of the sequences. The diversity indices differed from those estimated with the microscopy (Figure 2.2b, Table 2.3). The sequences of protists other than chrysophytes indicate that different organisms contributed to this diversity (Table 2.4). Dictyochophyceae were detected in clone libraries from Lake A and Ward Hunt Lake (Figure 2.2b, Table 2.4). Cercozoa sequences were recovered from Lake A, rare in Char Lake and not found in Ward Hunt Lake. In contrast Bacillariophyceae sequences were recovered from Char Lake and Ward Hunt Lake, but not from Lake A. Dinoflagellates accounted for 13-25% of the clones at the three sites, and ciliates represented over

20% of sequences from Char Lake and Lake A but were much less common in Ward Hunt Lake (Figure 2.2b).

Most dinoflagellate operational taxonomic units (OTUs, defined at 98% similarity) had closest matches to either phototrophic (*Gymnodinium aureolum*; GenBank accession AY999082) or heterotrophic (*Pfiesteria*-like dinoflagellate; AM050344) organisms, both usually reported from marine environments (Table 2.4). One Char Lake clone grouped with *Woloszynskia pascheri*, the only freshwater dinoflagellate that was related to any of our polar lake clones (Annexe 1). Sequences closely matching the bipolar ice-associated *Polarella glacialis* were recovered from Lake A and Ward Hunt Lake (Annexe 1). Most ciliates from Char Lake grouped within the Oligotrichia with closest matches to uncultured strombolids, whereas Lake A sequences were clearly dominated by *Halteria grandinella* (Stichotrichia), which was also present in Ward Hunt Lake (Table 2.4, Annexe 2).

Table 2.3 Richness, diversity and dominance indices obtained from microscopy and molecular approaches

	Char Lake	Lake A	Ward Hunt Lake
<i>Microscopy</i>			
Number of taxa	58	14	37
Shannon Diversity Index	2.61	1.61	2.37
Simpson Dominance Index	0.15	0.34	0.12
<i>Molecular</i>			
Number of OTUs	33	16	19
Number of clones	78	53	88
Shannon Diversity Index	3.15	2.35	2.17
Simpson Dominance Index	0.06	0.60	0.21

The recently proposed phagotrophic eukaryote phylum Telonemia (Shalchian-Tabrizi et al. 2006) was represented by close sequence matches (95-96% similarity) to one of its two named species, *Telonema antarcticum* Thomsen. These sequences were detected in all three lakes, with a considerable proportion in Ward Hunt Lake. Fungi, however, were only detected in Char Lake.

Shannon diversity indices for the molecular analyses indicated that Char Lake was most diverse, while Lake A and Ward Hunt Lake had comparable Shannon indices (Table 2.3). The dominance index was also greater for the latter two lakes compared to Char Lake (Table 2.3). The Bray-Curtis cluster analysis based on microscopic data indicated that protist community compositions of the lakes were all >60% different from each other, with Char Lake and Ward Hunt Lake the most similar (40%) compared to Lake A. This analysis implied that Char Lake and Ward Hunt Lake had

more species in common than either had with Lake A. The molecular data suggested differently, with the cluster analysis grouping Lake A and Ward Hunt Lake although only at 20% similarity. UniFrac analysis indicated that each lake contained a distinct assemblage of species ($p < 0.05$).

2.3.3 Chrysophyte diversity

Results from both microscopy and molecular techniques indicated that chrysophytes largely dominated the protist community in all three lakes (Figure 2.2). The microscopic observations revealed that each lake was characterised by different genera (Figure 2.3a). Ward Hunt Lake was dominated by the morphospecies *Erkenia subaequiciliata* Skuja (Annexe 3), a small 3-5 μm spherical cell with one long and one short flagellum and two distinct chloroplasts, and *Dinobryon sociale* Ehrenberg (Annexe 4), a colonial lorica-forming species with typical heterokont flagella. *D. sociale* also seemed to have formed cysts, which made up to 3.5% of the chrysophyte population in Ward Hunt Lake. The most prevalent Lake A chrysophytes were identified as either *Pseudokephyrion* Pasher, a small cell in a bottle shaped lorica that has two visible flagella, or *Kephrion* Pasher (Annexe 5), which looks similar to *Pseudokephyrion* except with a single visible flagellum. We were not able to consistently see this difference. The classical taxonomy of these two genera is based on lorica shape (Bourrelly, 1968) and there were at least four distinct lorica forms in the Arctic samples. Chrysophyte cysts were common in Lake A, representing *ca.* 20% of the chrysophytes cells. Char Lake was dominated by a *Dinobryon* sp., most closely resembling *D. sociale*. Both *Kephrion/Pseudokephyrion* spp. and *E. subaequiciliata* were also recorded in Char Lake. Chrysophyte cysts that could not be matched to a particular species constituted 5% of the Char Lake chrysophytes while *Dinobryon sociale* cysts recognized by their association with *D. sociale* loricas represented another 5% of chrysophyte cells.

The gene survey identified different chrysophyte taxa, relative to the microscopic analysis (Figure 2.3b). Most sequences fell into two clusters, designated Cluster I and Cluster II (Figure 2.4), which were not assignable to known taxonomic groups. Both clades were strongly supported by bootstrap values of 100% in the neighbour-joining (NJ) tree and 70-99% in the maximum-likelihood (ML) tree. Sequences from all three lakes were found in Cluster I, which had no matches closer than 92% similarity to any cultured organism. The highest BLAST match was to *Cyclonexis annularis*, but Cluster I did not group near the *C. annularis* sequence and even fell outside of the main chrysophyte phylogeny (Figure 2.4). The second unidentified group, Cluster II, was predominantly found in Ward Hunt Lake and Char Lake. These sequences were 99% similar among themselves, and were matched with 96% similarity to *Ochromonas tuberculata* (AF123293). In the NJ and ML

trees, the cluster fell within a larger clade that included *O. tuberculata* and several other Char Lake clones (Figure 2.4).

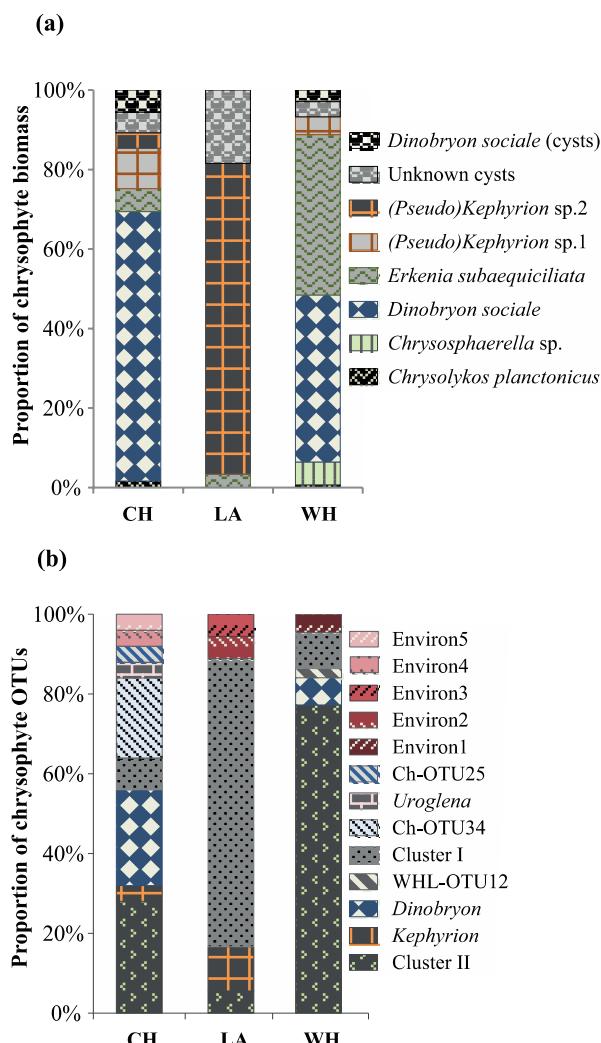


Figure 2.3 Chrysophyte communities of surface waters of Char Lake (CH), Lake A (LA) and Ward Hunt Lake (WH) in August 2008, identified by (a) microscopic counts and (b) 18S rDNA sequencing. Ch OTUs are from Char Lake; WHL OTUs are from Ward Hunt Lake.

Four sequences from different lakes fell into different, previously identified environmental clades (Figure 2.4). Three of these environmental clades contained sequences from oligotrophic Lake George, in the Adirondacks Park (Richards et al., 2005). Other sequences from Ward Hunt and Char Lakes clustered with *Dinobryon* sequences from several different species (Figure 2.4). The NCBI BLAST search indicated 96% similarity with the *Dinobryon cylindricum* sequence (EF165140), yet in the NJ and ML trees all three sequences appeared more closely related to *D.*

bavaricum and *D. divergens* (FN662758 and FN662756) than to *D. cylindricum*. Two of the Lake A sequences and one Char Lake sequence clustered with *Kephryion* CCMP 3057 as part of a sub-cluster with several sequences of undescribed *Ochromonas*-*Spumella*-like flagellates (Figure 2.4).

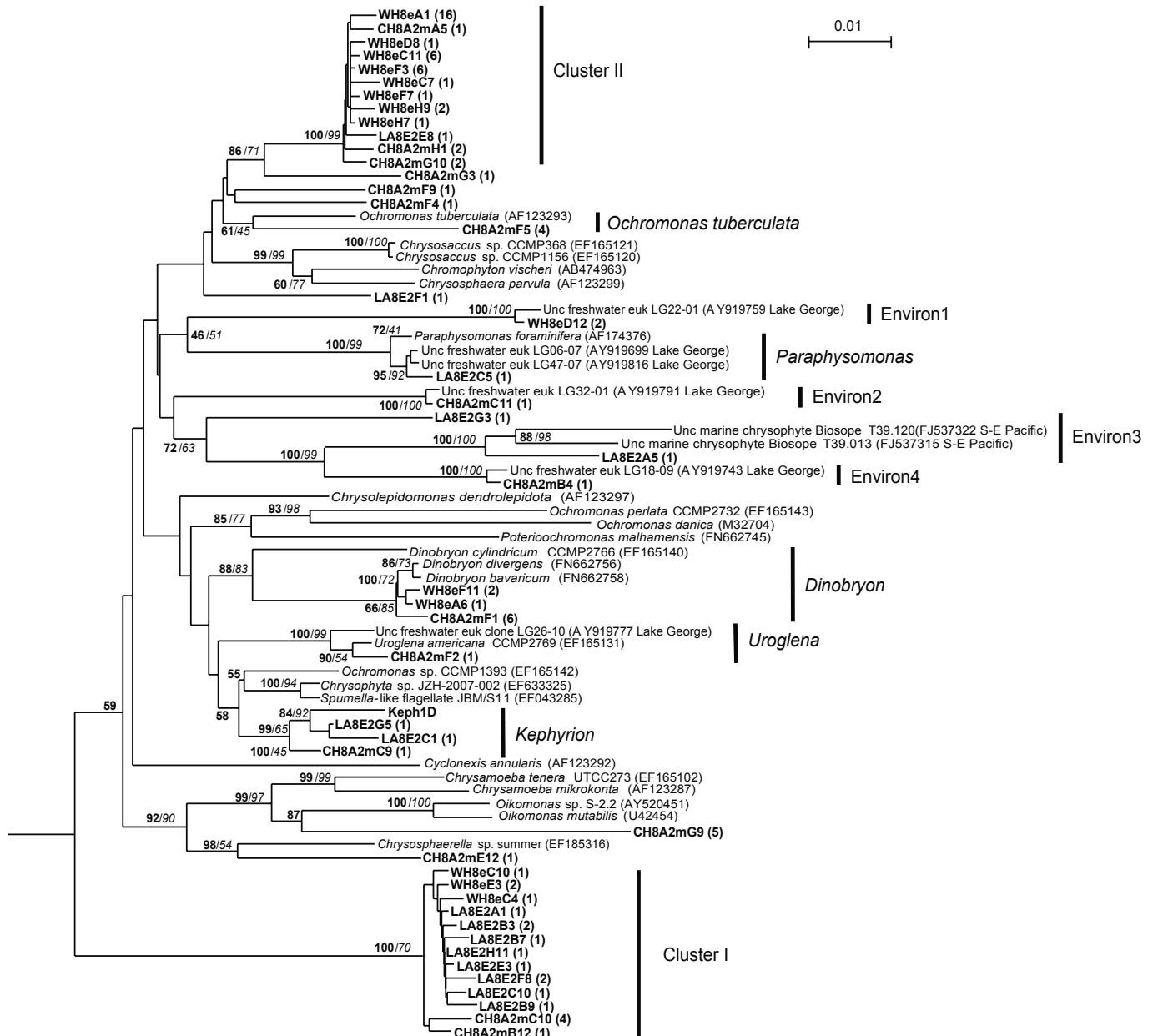


Figure 2.4 Chrysophyte 18S rDNA neighbour-joining phylogenetic tree. The outgroup (not shown) used to root this tree was the dinoflagellate *Paulsenella vonstoschii*. Bootstrap values (>50%) from the neighbour-joining tree are in bold and those from the maximum-likelihood tree are in italics. Sequences from Char Lake (CH), Lake A (LA) and Ward Hunt Lake (WH) are in bold, with the number of clones for each sequence indicated in parentheses. CH8A2mG9 represents OTU Ch-OTU34, and CH8A5mE12 represents OTU Ch-OTU25 referred to in Figure 2.3.

2.4 Discussion

2.4.1 Protist dominance and diversity

Irrespective of approach, chrysophytes dominated the protist communities of the three lakes examined. Chrysophytes are found across diverse aquatic habitats (Sandgren et al., 2009) but species composition varies, as do community associations of chrysophytes with other protists. Chrysophyte stomatocysts (Smol, 1988), and silica-scaled chrysophytes (Wilken et al., 1995), accumulate in the sediments and are used to reconstruct past climate trends, illustrating the importance of these organisms in Arctic regions. Chrysophytes have been previously recorded in many High Arctic and Subarctic lakes such as in the Franz-Joseph Archipelago (Panzenböck et al., 2000), oligotrophic Greenland lakes (Christoffersen et al., 2008) and pingos (Kristiansen et al., 1995), and Canadian Subarctic lakes where chrysophytes and cryptophytes dominate the two annual phytoplankton peaks (Sheath, 1986). Holmgren (1984) suggested the phytoplankton communities in oligotrophic Arctic and Subarctic lakes could be classified as four distinct assemblages: (1) Chrysophyceae, (2) Chrysophyceae-Diatoms, (3) Chrysophyceae-Cryptophyceae, (4) Chrysophyceae-Dinophyceae. The oligotrophic High Arctic lakes of the present study all fall into the Type 1 assemblage category.

All methods utilized showed that chrysophytes were ubiquitous and abundant, suggesting their importance for biological productivity and trophic links in Arctic lakes. The HPLC results showing high levels of fucoxanthin supported the predominance of chrysophytes. This carotenoid is the signature pigment of chrysophytes, but Bacillariophyceae and Dictyochophyceae can also contribute to a fucoxanthin peak, and microscopy and clone libraries detected both classes. However, none of the signature pigments of diatoms (diatoxanthin, diadinoxanthin), Dictyochophyceae (19'-butanoyloxyfucoxanthin), or dinoflagellates (peridinin, peridinol, dinoxanthin) were detected, indicating that as recorded using microscopy, these groups were much less abundant, and that most of the fucoxanthin was from the chrysophytes.

The molecular and microscopic techniques both indicated that there were different chrysophyte genera in each of the three lakes. The two shallower lakes, Char Lake and Ward Hunt Lake, were dominated by *Dinobryon* spp. according to microscopic observations. *Dinobryon* and *Uroglena* are common in summer waters of Finnish Lake Saanajärvi (Forsström et al., 2005), a high latitude lake with some limnological similarities to Char Lake. The gene surveys diverged from the microscopy to some extent, although they also detected *Dinobryon* spp. as being prominent in both Ward Hunt and Char Lakes. Microscopy indicated that Lake A was dominated by *Pseudokephyrion-Kephyrion*

spp. with no *Dinobryon* recorded, despite its geographical proximity to Ward Hunt Lake. Similarly, Laybourn-Parry and Marshall (2003) reported the genus *Dinobryon* in some Spitzbergen lakes while in others *Kephrion* was dominant.

Erkenia subaequiciliata was an important morphological species in all three lakes. The genus *Erkenia* was described by Skuja (1948), but has apparently not been deposited in any culture collection as yet. Furthermore, no record exists for *Erkenia* 18S rDNA, although it is often reported in plankton communities of freshwater lakes worldwide (Gerhart & Likens, 1975; Pollingher, 1981; Jacquet et al., 2005; Kozak, 2005). In general, existing 18S rRNA gene records are far from exhaustive and lack many key morphospecies (Richards et al., 2005). Based on distribution patterns emerging from both the morphological and sequence data we can speculate on the identity of our Cluster II (Figure 2.4), which grouped with *Ochromonas tuberculata*. The distribution of Cluster II between the three lakes was similar to that of *E. subaequiciliata* (Figures 2.2b, 2.3b), which has two visible flagella. *O. tuberculata* itself has a short second flagellum, but in phylogenetic studies it consistently groups with the Chrysosphaerales, which have one single visible flagellum (Andersen et al., 1999). This suggests that despite a similar distribution, it is unlikely that Cluster II corresponds to *Erkenia*. However, the morphologically-based taxonomy of many single-cell chrysophytes hides much genetic diversity, and many current genera are polyphyletic (Andersen et al., 1999; Boenigk et al., 2005; Pfandl et al., 2009). *O. tuberculata*, for example, is phylogenetically dissimilar to any other species of the polyphyletic genus *Ochromonas* (Andersen et al., 1999).

The sequences in Cluster I did not group with any sequences currently in GenBank, whether cultured or uncultured. This Cluster I was more diverse than Cluster II and the Lake A and Char Lake sequences grouped apart. We speculated that Cluster I could represent a loricate organism related to *Kephrion*, as this genus was frequently observed in our microscopic counts, especially in Lake A. We sequenced the 18S rRNA gene from the only well-identified culture listed in CCMP as *Kephrion* (isolated by J. Boenigk from a high altitude Austrian lake). However, this culture (CCMP 3057) grouped far from Cluster I, within a clade of *Spumella*-like chrysophytes. Cluster I branched at the base of the chrysophytes in our phylogenies, implying that it may be ancestral or a sister clade to the Chrysophyceae. It possibly represents a novel taxon, which has previously been overlooked and is potentially restricted to Arctic freshwaters. The unresolved morphological identity of this cluster suggests it may be among the small nondescript flagellates and represented by the “unidentified flagellates” category recovered by microscopy in this study.

Table 2.4 Char Lake, Lake A and Ward Hunt Lake number of clones (#) and corresponding highest BLAST match (accession number in parentheses) and similarity (%) for protist operational taxonomic units (OTU) to environmental and cultured sequences.

Char Lake	Lake	Number of clones	Group	Highest Match (accession number)	Similarity (%)	Origin	Highest cultured match (accession number)	Similarity (%)	OTU Accession number
	3	Dinoflagellates	<i>Pfiesteria</i> -like dinoflagellate (AM050344)	96	Massan Bay, Korea	<i>Paulsenella vonstoschii</i> (A1968729)	96	JF730751	
	1		Uncultured alveolate clone PAA10SP2005 (EU162621)	99	Lake Pavin (France)	<i>Rana sphenocephala</i> pathogen MJY-2007 (EF675616)	89	JF730760	
	1		Uncultured freshwater eukaryote clone LG10-02 (AY919716)	99	Lake George (USA)	<i>Woloszynska pascheri</i> strain CCAC0075 (EF058253)	97	JF730777	
	1		Uncultured freshwater eukaryote clone LG33-11 (AY919799)	96	Lake George (USA)	<i>Gyrodinium helveticum</i> (AB120004)	96	JF730756	
	5		Uncultured freshwater eukaryote clone LG33-11 (AY919799)	99	Lake George (USA)	<i>Gyrodinium helveticum</i> (AB120004)	99	JF730801	
	1	Ciliates	<i>Woloszynska pascheri</i> strain CCAC0075 (EF058253)	97		<i>Asterias</i> sp. FU44-31 (EU024985)	96	JF730800	
	1		Uncultured eukaryotic picoplankton clone P1.24 (AY642698)	98	Lake Pavin (France)	<i>Histiobalantium natans viridis</i> (AB450957)	98	JF730793	
	1		<i>Srobilidium caudatum</i> (AY1435573)	98		<i>Varstrombdium</i> sp. <i>kieulum</i> (DQ811090)	95	JF730763	
	5		Uncultured alveolate clone PAB12A1/2004 (DQ244025)	99	Lake Pavin (France)	<i>Protaspis grandis</i> (DQ303924)	95	JF730790	
	10		Uncultured freshwater eukaryote clone LG17-12 (AY919741)	99	Lake George (USA)	<i>Cavernomonas mira</i> Cav-A (EFJ790718)	88	JF730787	
	3	Bacillariophyceae	<i>Stephanodiscus hanzezii</i> isolate UTCC 267 (CQ844874)	98		<i>Plagioeselminis nanoplectica</i> (FM876311)	95	JF730764	
	1	Cercozoa	Uncultured marine eukaryote clone SA1_F02 (EF526980)	96	Framvaren Fjord (Norway)	<i>Cryptomonas curvata</i> strain CCAC 0080 (AM051189)	99	JF730755	
	1		Uncultured cercozoan clone PCA3SP2005 (EU162633)	93	Lake Pavin (France)	<i>Geminigera acyphilia</i> (DQ452092)	95	JF730776	
	2	Cryptophytes	Uncultured freshwater eukaryote clone LG05-02 (AY919695)	99	Lake George (USA)	<i>Phagocystis namoplectica</i> (FM876311)	99	JF730799	
	1		Uncultured freshwater eukaryote clone LG03-02 (AY919686)	99	Lake George (USA)	<i>Cryptomonas curvata</i> strain CCAC 0080 (AM051189)	99	JF730771	
	1		Uncultured freshwater cryptophyte clone LG07-11 (AY919703)	99	Lake George (USA)	<i>Geminigera acyphilia</i> (DQ452092)	95	JF730775	
	1	Haptophyceae	<i>Chrysophromulina parva</i> strain CCMP 291 (AM491019)	99		<i>Tetomonas antarcticum</i> (AJ564773)	95	JF730780	
	3	Telomonia	Uncultured eukaryote clone BL010625.25 (AJ564770)	98	Marine (Spain)	<i>Chytromyces angularis</i> isolate AFTOL-ID 630 (AF164253)	91	JF730770	
	1	Fungi	<i>Rhizophycis rosea</i> isolate AFTOL-ID 43 (AY655829)	94	High-elevation soils	<i>Chytromyces angularis</i> isolate AFTOL-ID 630 (AF164253)	91	JF730792	
	2		Uncultured Chytridiomycota clone T2P_AeA04 (GQ995409)	91				JF730794	

Table 2.4 suite

Lake A	Ward Hunt Lake	Number	Phylogenetic group	Accession number	Location	Reference
2	1	Dinoflagellates	Uncultured eukaryote clone NPK97 (EU371756) <i>Polaris glacialis</i> strain Abra-E (EF434276)	98 99	Austre Broggerbreen (Svalbard)	<i>Gymnodinium aureolum</i> strain SWA 16 (AY999082) JF730834 JF730821
4	6	Ciliates	Uncultured eukaryote clone SS1_E_01_38 (EU050964) Uncultured alveolate clone PAA2AU2004 (DQ24028)	97 92 94	Kings Bay sediments (Svalbard) Lake Pavin (France)	<i>Parastrobilidinopsis nimina</i> (DQ392786) <i>Strobilidium caudatum</i> (AY143573) JF730817 JF730829 JF730819
5	1	Cercozoa	<i>Halteria grandinella</i> (AF194410) Uncultured eukaryote clone: CYSGM-12 (AB275095)	99	Sagami Bay sediments (Japan)	<i>Protaspidia</i> sp. CC-2009b (FI824125) JF730812 JF730810
4	9	Telomonia	Uncultured eukaryote clone BL010625.25 (AJ564770) <i>Pseudopediastrum elastica</i> (U14387)	98 94	marine (Spain)	<i>Telomera antarcticum</i> (AJ564773) JF730836 JF730835
1	1	Dictyochophyceae	Uncultured alveolate clone PAB10AU2004 (DQ244026) Uncultured eukaryote clone NPK97_180 (EU371756)	97 98	Lake Pavin (France) Austre Broggerbreen (Svalbard)	Uncultured <i>Amoeobophrya</i> clone F (AY820526) <i>Gymnodinium aureolum</i> strain SWA 16 (AY999082) JF730842 JF730873
16	2	Ciliates	<i>Pfesteria</i> -like dinoflagellate (AM050344)	97		JF730848
2	2		<i>Amoeobophrya</i> sp. 'Dinophysis' (AF239260)	93		JF730871
1	1	Bacillariophyceae	<i>Polaris glacialis</i> strain Abra-E (EF434276) <i>Halteria grandinella</i> (AF194410)	99 98	oxic pool, Chevreuse (France)	JF730863 JF730840
1	1		Uncultured choreotrichid ciliate clone CH1_2A_10 (AY821916)	98		JF730875
1	1		<i>Navicula pellucida</i> strain SAG 1050-3 (AJ544657)	99		JF730867
1	1	Heterotrophic Stramenopile Haptophyceae	Eukaryote marine clone ME1-17 (AF363186)	99	Mediterranean	<i>Pirsonia verrucosa</i> isolate P847 (AJ561113) JF730854
1	1	Dictyochophyceae	<i>Chrysosphaera parva</i> strain CCMP 291 (AM491019)	99	Coastal marine (Norway)	JF730872
2	2		Uncultured marine eukaryote clone CD8.07 (DQ647512)	96		<i>Pedinella</i> sp. <i>sequamata</i> (AB081517) JF730839
1	11	Telomonia	<i>Pseudopediastrum elastica</i> (U14387)	94	Marine (Spain)	<i>Telomera antarcticum</i> (AJ564773) JF730861
3	11		Uncultured eukaryote clone BL010625.25 (AJ564770)	98	Marine (Spain)	<i>Telomera antarcticum</i> (AJ564773) JF730877
			(AJ564770)	94	Marine (Spain)	JF730870

The number of environmental sequences in publically available databases continues to grow and although there is scant morphological information to be inferred from the small ribosomal subunit gene biogeographical studies are possible by comparing sequences from different regions. In addition, some ecological information can be gleaned by comparing environmental conditions where specific sequences and clades are found. The grouping of some of our sequences with other environmental sequences (Environ1-4) hints at the distribution and ecology of these organisms. Three of these environmental clades were first recovered from the temperate oligotrophic Lake George, in the Adirondack Park (Richards et al., 2005), suggesting that they are common in low nutrient freshwater environments. The fourth clade, Environ-3, included marine sequences from the South-Eastern Pacific, but these were only 96% similar to our sequence, which could therefore represent a novel Arctic group. However, the number of 18S rRNA gene studies of freshwater lakes is far fewer than those from marine systems, and many of these taxa could well be globally dispersed.

Chrysophyte dominance in Arctic lakes may be due to a number of adaptations to low nutrient availability or to the pronounced seasonal changes in light availability. For example, chrysophytes form cysts, protecting cells during unfavourable conditions (Nicholls, 2009), that in Arctic lakes could include nutrient exhaustion and winter darkness. We found that cysts represented 7-20% of total chrysophytes in the three lakes, with the greatest proportion in Lake A. Yubuki et al. (2008) suggested that formation of spores in planktonic chrysophytes is directly influenced by cell density and that cells encyst once a critical density is reached. Nicholls (2009) also noted that the small size of many chrysophytes compared to diatoms and dinoflagellates affords a relatively high surface to volume ratio, which could favour the uptake of nutrients at low concentrations. The most common chrysophyte found in Ward Hunt Lake, the morphospecies *Erkenia subeaquiciliata*, was 2-5 µm in diameter, and as few picocyanobacteria were present in that lake, it may have been the dominant small phytoplankton cell type. Lake A has high pelagic concentrations of picocyanobacteria (Van Hove et al., 2008) and picocyanobacteria were frequently observed in Char Lake (S. Charvet personal observations). HPLC pigment profiles also indicated the presence of cyanobacteria in these two lakes. These 1-2 µm diameter prokaryotic cells would out compete the chrysophytes for nutrients on the basis of size since chrysophytes in the two lakes were generally larger than 10 µm in diameter, however these abundant picocyanobacteria could provide an N- and P-containing food source for mixotrophic chrysophytes (see below).

Another factor that could favour chrysophytes in oligotrophic waters is their capacity to swim. Several genera (*Dinobryon*, *Synura*, *Uroglena* and *Mallomonas*) can actively maintain their position

at strategic depths. Motility confers a relative advantage over sessile algae in seeking favourable irradiance conditions (Pick & Lean, 1984) or avoiding zooplankton predators (Nicholls, 2009). Furthermore, cells able to migrate to deeper depths where nutrients are more abundant have an advantage within a stratified water column. Another potential adaptation to low nutrients and periodic light limitation is phagotrophic mixotrophy, whereby nutrients and energy can be obtained from bacterial prey, circumventing direct energy dependency on solar radiation and the reliance on dissolved inorganic nutrients (Raven, 2009). A number of studies have documented that phototrophic chrysophytes prey on bacterial or algal cells (Bird & Kalf, 1986; Rothhaupt, 1996a; Katechakis & Stibor, 2006). Hence when nutrients are scarce at the end of the growing season, as in Lake A (Veillette et al., 2011), chrysophyte mixotrophy would be favoured.

While many OTUs recovered from the present study were closest to sequences from freshwater and ice, others grouped closest to sequences previously reported only from marine systems. The transition from marine to freshwater is thought to be rare (Logares et al., 2007) and the putative marine groups recovered from the freshwater lakes may well have been transient. Among the alveolates, all were from nominal marine groups, with the exception of one freshwater dinoflagellate *Woloszynskia pascheri*. Another dinoflagellate, *Polarella glacialis* was the sole known cold ecotype represented, and was recovered from Ward Hunt Lake and Lake A. *P. glacialis* was originally isolated from the sea ice in the Ross Sea, Antarctica and subsequently from Northern Baffin Bay, in the Arctic Ocean (Montresor et al., 1999; Montresor et al., 2003). Using PCR and cloning techniques, it has also been detected in saline Antarctic lakes (Rengefors et al., 2008) and, notably, in Arctic snow on Ward Hunt Island (Harding et al., 2011). This ice-associated dinoflagellate readily encysts and local atmospheric transport from the sea or sea ice to the lakes is likely. Other dinoflagellate sequences, with matches to *Gymnodinium aureolum* (AY99082), and the *Pfeisteria* group, as well as ciliate sequences belonging to the marine *Strombidium*, are nearly always picked up in Arctic marine 18S rRNA gene surveys (Lovejoy et al., 2006; Lovejoy & Potvin, 2011), and their presence in our clone libraries could indicate local transport as well.

Overall, Char Lake was more taxonomically diverse than the other two lakes. This was the southernmost lake we examined, with a longer growing season that lasts from April to the end of August (Schindler et al., 1974). The N:P ratio of inorganic nutrients in Char Lake corresponded to the Redfield ratio, while the ratios of the other lakes indicated strong phosphorus limitation. These observations imply that protist diversity might increase with increasing nutrient levels in our three oligotrophic Arctic systems, however much wider sampling is required to test this relationship. Furthermore, this difference in diversity could be due to the geographic distance between

Cornwallis Island and the northern coast of Ellesmere Island. Char Lake is not influenced by the same winds as Lake A and Ward Hunt Lake, nor is it subjected to the same allochthonous inputs. For example, Char Lake lies closer to anthropogenic activity, near the hamlet of Resolute Bay.

2.4.2 Comparison of methods

There have been few studies where both detailed microscopy and small subunit rRNA gene surveys have been carried out on the same sample. Jungblut et al. (2010) targeted polar cyanobacteria living in microbial mats using a multi-phasic approach. Even within this narrow taxon sampling and the addition of many new sequences from morphologically well-identified polar cultures, there were mismatches between environmental sequences and the morphotypes within the mats. That study did however imply a global distribution of cyanobacterial genotypes throughout the cold biosphere. The low similarity of the environmental sequences to cultured representatives of most of the protists in our Arctic lakes reflects the poor representation of different groups that have been sequenced and may also indicate that as for cyanobacteria, some protists may be characteristic of polar or cold regions and these represent genuine new records.

Pigment analysis, microscopy and the 18S RNA gene survey identified chrysophytes as common, but there were differences in taxonomic detail. Microscopy also highlighted the abundance of cysts, which would not be separated from vegetative cells by their 18S rRNA gene sequences. Other less common taxa such as dictyochophytes were noted by both methods but again the resolution of the species present was poor, with *Pseudopedinella* noted from Char Lake under light microscopy (Annexe 6), while *Pedinella* was the closest match to dictyochophyte sequences from Lake A and Ward Hunt Lake. Whether this species difference is real or due to inadequate reference sequences available cannot be resolved without additional culturing and sequencing studies. Microscopy and HPLC pigment analyses are intrinsically more quantitative than 18S rRNA gene surveys because copy number of 18S rRNA genes among different groups varies widely (Zhu et al., 2005). As has been found in other gene surveys, we recovered higher proportions of dinoflagellates and ciliates compared to microscopic observations. Dinoflagellates and ciliates have many more copies of this gene than most small algae, so even if rare, they are more likely to be detected using PCR-based cloning and sequencing (Potvin & Lovejoy, 2009). Because of this, the 18S rRNA gene detected likely numerically rare species, such as *Polarella*, and the ciliates *Halteria* and *Strobilidium*, which were not recorded in our microscopy survey.

A major insight provided by the 18S rRNA gene libraries, in contrast to microscopy, was in the diversity of heterotrophic protists. Most heterotrophic protists are colourless, and many have little in

the way of distinguishing morphological features (Caron, 1983) and are vulnerable to loss from fixation and preservation processes (Hara et al., 1986). Furthermore, taxonomic studies have previously concentrated on phototrophs and the number of classic phycologists exceeds the number of protozoologists. This has resulted in a limited number of reference texts on heterotrophic protists, which has impeded their identification (Vors et al., 1995; Packroff & Woelfl, 2000). Clone libraries also detected more heterotrophic protists than microscopy from Lake Stechlin (Luo et al., 2011).

The comparison of sequences from different geographic regions and habitats is less ambiguous than comparing microscopically identified morphospecies that depend on expert identification (Jungblut et al., 2010). For example *Telonema* has been previously reported from marine, ice and freshwater environments (Bråte et al., 2010). This genus was present in Char Lake and relatively common in both Lake A and Ward Hunt Lake. Three of the four *Telonema* OTUs recovered from our sites, had best matches to *Telonema antarcticum* (98%), which was first recorded in microscope studies from Antarctica but was also isolated and described from coastal waters of Norway (Klaveness et al., 2005). When aligned in MUSCLE with the sequences used in Bråte et al. (2010), our *Telonema* OTUs all grouped within the sole freshwater clade (Bråte et al., 2010). Telonemia cells were probably among the unidentified heterotrophic nanoflagellates in the microscopy data. The sole fungal sequence, detected from Char Lake, was 94% similar to *Rhizophlyctis rosea*, a fungus that is mostly reported from soil environments (Willoughby, 2001). There exists scant information about fungi in Arctic aquatic environments (Voronin, 1997; Hodson et al., 2008), and as in the case of heterotrophic protists few researchers study these and they have been largely ignored in microscopic surveys of plankton.

Overall the combined approach provided deeper insight into the lake communities than any single analysis. HPLC provides some taxonomic information at the phylum level for photosynthetic taxa and information that can be used to infer adaptation to different irradiance regimes. Microscopy was biased against heterotrophs, whereas the 18S rRNA gene survey was limited by the lack of reference sequences to known organisms. This may be a temporary shortcoming since as sequences are added to reference databases more species names will be matched to environmental sequences. Although the 18S rRNA gene survey approach may be biased against autotrophic communities (Vaulot et al., 2008), accurate assessments in the future will require both microscopy and genetic analysis.

2.4.3 Implications for food webs and climate change

Polar lakes are diverse and harbour a continuum of food webs, from systems that support both zooplankton and fish communities to those where ciliates and rotifers comprise the highest trophic level (Vincent et al., 2008b). Cyanobacteria often dominate polar freshwater productivity in these extreme ecosystems, either as benthic microbial mats in streams, lakes and ponds (Jungblut et al., 2010) or as picocyanobacteria in the phytoplankton communities (Lizotte, 2008; Van Hove et al. 2008). The high zeaxanthin concentrations from Lake A were consistent with previous reports of large picocyanobacterial populations in this lake (Veillette et al., 2011). These picocyanobacteria likely represent the primary food source of phagotrophic protists, belonging to both heterotrophic and mixotrophic groups. The dominance of chrysophytes, accompanied by the significant presence of heterotrophs, in these aquatic ecosystems suggests their importance for the local food webs. Chrysophytes, such as *Dinobryon* spp. and *Ochromonas tuberculata*, feed on bacterial cells (Bird & Kalff, 1986; Rothhaupt, 1996a). In these lakes we also recovered phagotrophic species such as *Telonema antarcticum*, and ciliates that feed on flagellates and small algae. The different phagotrophs may differ in their trophic position within the food web of High Arctic lakes. From the data presented here, we can deduce a simple food web that would be based on the primary production of picocyanobacteria, consumed by the mixotrophic chrysophytes, which in turn may become prey to the larger *Telonema* and ciliates.

Arctic aquatic food webs are likely to be altered by climate change. The increases in temperature and precipitation result in thawing of permafrost, followed by increasing terrestrial vegetation in the surrounding catchments. These changes will likely result in major shifts in allochthonous carbon and nutrient inputs (Vincent et al., 2008b) that may stimulate the production of bacterial prey for microbial grazers. Longer ice-free periods and thermal stratification of the lakes may result in earlier nutrient depletion in the upper waters, creating favourable conditions for mixotrophic chrysophytes. Alternatively, these conditions may cause a shift towards co-dominance with dinoflagellates, as suggested by Holmgren (1984). How such shifts in lake trophic status influence higher food webs, nutrient and carbon cycling will be important questions for future studies.

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Chapitre 3 - Pyrosequencing analysis of the protist communities in a High Arctic meromictic lake: DNA preservation and change

Résumé

Les lacs méromictiques du haut Arctique canadien sont des environnements extrêmes, caractérisés par des températures froides, des apports faibles en nutriments, et des périodes prolongées de faible irradiance et d'obscurité. Ces lacs sont perpétuellement stratifiés, avec une couche d'eau douce oxygénée (mixolimnion) au-dessus d'une couche d'eau salée anoxique (monimolimnion). Les propriétés physiques et chimiques du lac A, le lac méromictique le plus profond de l'Arctique situé sur la côte nord de l'île d'Ellesmere, sont étudiées depuis plus de 15 ans. Cependant, les communautés biologiques de ce lac sont beaucoup moins connues. Par séquençage à haut-débit du gène de l'ARN ribosomal 18S, nous avons investigué les communautés de protistes dans la colonne d'eau du lac A, à trois points temporels : sous la glace de printemps en mai 2008, durant une période de réchauffement et de perte de glace en août 2008, puis sous un couvert de glace estival en juillet 2009. Les séquences de plusieurs groupes de protistes furent obtenues à partir d'échantillons pris à travers la colonne d'eau, aux différents temps d'échantillonnage, même dans la couche profonde anoxique où la croissance est peu probable. De plus, ces séquences contenaient des séquences de diatomées et de taxons marins, qui n'ont encore jamais été observés par microscopie dans le lac A. Cependant, les séquences d'autres taxons tels que les ciliés, les chrysophytes, les Cercozoa et *Telonema* variaient entre les profondeurs, entre les années et entre les différentes conditions de couvert de glace. Ces résultats impliquent une communauté saisonnière active dans les eaux de surface qui sont sensibles à la profondeur et aux changements temporels. L'ADN de ces organismes se superpose à un bruit de fond causé par la détection d'acides nucléiques provenant de multiples sources internes et externes, préservés par les eaux froides et anoxiques de la couche profonde.

Abstract

High Arctic meromictic lakes are extreme environments characterized by cold temperatures, low nutrient inputs from their polar desert catchments and prolonged periods of low irradiance and darkness. These lakes are permanently stratified with an oxygenated freshwater layer (mixolimnion) overlying a saline, anoxic water column (monimolimnion). The physical and chemical properties of the deepest known lake of this type in the circumpolar Arctic, Lake A on the far northern coast of Ellesmere Island, Canada, have been studied over the last 15 years, but much less is known about its biological communities. We applied high-throughput sequencing of the V4 region of the 18S ribosomal RNA gene, to investigate the protist communities down the water column, under the ice at the end of winter in 2008, during an unusual period of warming and ice-out the same year, and again under the ice in mid-summer 2009. Sequences of many protist taxa occurred throughout the water column at all sampling times, including in the deep anoxic layer where growth is highly unlikely. Furthermore, there were sequences for taxonomic groups including diatoms and marine taxa, which have never been observed in Lake A by microscopic analysis. However the sequences of other taxa such as ciliates, chrysophytes, Cercozoa and *Telonema* varied with depth, between years and during the transition to ice-free conditions. These results imply that there are seasonally active taxa in the surface waters of the lake that are sensitive to depth and change with time. DNA from these taxa is superimposed upon background DNA from multiple internal and external sources that is preserved in the deep, cold, largely anoxic water column.

3.1 Introduction

Meromictic lakes with saline deep water overlain by fresh water are known from both the north and south polar regions (Vincent et al., 2008b). Protists living in these perennially stratified environments encounter a range of extreme conditions. Those in the surface waters must tolerate cold temperatures, low nutrients and reduced light due to the prolonged winter darkness and ice cover. The saline, usually anoxic, waters below the freshwater layer create another extreme environment. The pronounced vertical gradients in light, temperature, salinity, nutrients, oxygen and other terminal electron acceptors, provide a range of conditions for life within the same lake that could select for distinct communities down the water column.

Several meromictic lakes occur along the northern coast of Ellesmere Island, Canada, and were formed when seawater was trapped by isostatic uplift following the last de-glaciation and subsequent inflow of meltwater (Jeffries & Krouse, 1985). These lakes owe much of their continued water column stability to year-round ice cover and protection from wind-driven mixing (Vincent et al., 2008a). The deepest meromictic lake in the region, Lake A (83.00°N , 75.30°W), originated about 4000 years ago following the retreat of the Ellesmere Island glaciers (Jeffries & Krouse, 1985). The dense saline waters of the monimolimnion are separated from the surface fresh waters by a stable halocline. These deeper waters derived from the original seawater are mostly anoxic and would be predicted to harbour very different species compared to the freshwater surface layer (mixolimnion) originating from the surface runoff of catchment snowmelt that flows into the moat region and under the ice cover during summer (Veillette et al., 2012).

Previous studies of Lake A revealed that picocyanobacteria were abundant in the mixolimnion (Van Hove et al., 2008; Antoniades et al., 2009) with high concentrations of other bacteria in the deep monimolimnion, including green sulfur bacteria (Antoniades et al., 2009). Using 18S rRNA gene clone libraries and Sanger sequencing, Charvet et al. (2012b) reported that the late summer protist communities in surface waters were dominated by chrysophytes and dinoflagellates, similar to Arctic non-meromictic lakes. Little is known, however, of the taxonomic makeup of protists down the water column (Veillette et al., 2011), with no previously published reports on the communities in the monimolimnion, and much of the protist diversity of this and similarly isolated far northern lakes remains unknown.

In the present study, we used high-throughput amplicon tag pyrosequencing of the V4 region of the 18S rRNA gene to examine protist communities down the water column. Specifically we compared communities from the surface mixed layer (the mixolimnion), the pycnocline and the saline deep

layer (the monimolimnion). Cold, anoxic waters have been found to preserve extracellular DNA (e.g. Danovaro et al., 2005), and dead or dormant cells could accumulate in dense saline waters. These effects may create a background genetic signal that could mask the sequences from living organisms. Our aim was, therefore, to assess the extent of protist variations with depth and time relative to the background stocks of DNA that may be preserved in the deep waters of Lake A. Samples were collected from under the ice in spring (May 2008) and mid-summer (July 2009), and during a period of unusual warming and complete ice-out in late summer (August 2008). Since polar lakes are particularly vulnerable to ongoing climate change (Williamson et al., 2009), our results may indicate the type of community shifts that could follow the more regular loss of summer ice from these High Arctic ecosystems in the future.

3.2 Materials and Methods

3.2.1 Study site, sampling, nutrients and photosynthetically active radiation

Meromictic Lake A ($83^{\circ}00' \text{ N}$, $75^{\circ}30' \text{ W}$) is located along the northern coast of Ellesmere Island, Nunavut, Canada. The surface area is 5 km^2 , with a drainage basin of 36 km^2 and maximum depth of 128 m (Tomkins et al., 2009). Further details about this region are given in Vincent et al. (2011). Sampling was conducted on 30 May 2008, 20 August 2008 and 20 July 2009. In May and July, the lake was covered by 1.5-1.6 m of ice and 5-10 cm of snow; and in August 2008, it was exceptionally entirely free of ice. Physicochemical water column profiles were taken using a conductivity-temperature-depth (CTD) profiler (XR-420 CTD-RBR profiler; RBR Ltd, Ottawa, Canada). The potential density (sigma-theta) of the water was calculated using the *oce* package for the R program, based on the data for pressure, salinity and temperature.

Water was collected at discrete depths with a Kemmerer bottle (Wildlife Supply Company, Yulee, FL, USA) and contents emptied directly into cleaned polypropylene containers after rinsing with sample water. Samples were collected from 2, 5, 10, 12, 20, 29, 32 and 60 meters in May 2008, from 2, 10, 12, and 29 m in August 2008 and from 2, 5, 10, 12 and 29 m in July 2009. The *ca.* 12 L of collected water from each depth was kept cool, in the dark and transported back to a field laboratory within four hours. For DNA analysis, 3-4 L of water from the separate depths was sequentially filtered onto 47 mm diameter $3.0 \mu\text{m}$ pore size polycarbonate filters (Millipore) and $0.2 \mu\text{m}$ Sterivex units (Millipore). Lysis buffer (50 mM Tris, 40 mM EDTA, 0.75 M sucrose) was added to the cryovials containing the filters and to the Sterivex units, which were then stored at -80°C until DNA extraction.

Samples for nutrients were collected in 120 mL glass bottles with polypropylene caps and kept in the dark at *ca.* 4°C until analyses at the Canadian Center for Inland Waters (Burlington, Ontario). Concentrations of nitrate and nitrite (NO_x-N) and soluble reactive phosphorus (SRP) were determined using standard colorimetric techniques (Gibson et al., 2002). The detection limit for NO_x-N was 0.005 mg N L⁻¹ and for SRP was 0.001 mg L⁻¹.

Table 3.1 Estimates of irradiance within the water column, under the ice in May and directly under the surface in August 2008. Ez is in mol photons m⁻² d⁻¹, Notes specify the PAR under specific conditions, and values were calculated from the equations given in the text below.

Depth (m)	Notes	Irradiance (Ez)		
		May 2008	Aug 2008	July 2009
0	Incident PAR	53*	33*	65
2	Under ice	0.38*	-	1.03
	Under the surface	-	14.97	-
5	Water column	0.132		0.359
10	irradiance	0.022	0.90	0.062
12		0.011	0.443	0.0305
20		0.0007	-	-
29		2.8×10 ⁻⁵	1.1×10 ⁻³	7.7×10 ⁻³
32		9.8×10 ⁻⁶	-	-
60		5.1×10 ⁻¹⁰	-	-

Photosynthetically active radiation (PAR) values within the Lake A water column were derived from incident PAR data collected at Lake A in 2008 and 2009. Incident PAR was 53 and 33 mol photon m⁻² d⁻¹ in May and August 2008, respectively (Veillette et al., 2011), and 65 mol photon m⁻² d⁻¹ in July 2009. PAR immediately under the ice in May was estimated as described by Belzile et al. (2001). We estimated PAR levels at the sampled depths of our study in May and August 2008 and July 2009 (Table 3.1) based on the albedo and attenuation coefficient measurements from Belzile et al. (2001). The irradiance under the snow was calculated using the following equation:

$$E_{d(snow)} = E_{inc} \times (1 - \alpha_{(snow)}) \times e^{(-Kd(snow) \times Z)}$$

where E_{inc} is the incident irradiance at the surface of the snow, α is the albedo of the snow, K_{d(snow)} is the attenuation coefficient and Z the depth of the snow cover. The irradiance under the ice was obtained from the following equation:

$$E_{d(ice)} = E_{d(snow)} \times e^{(-Kd(ice) \times Z)}$$

where $K_{d(\text{ice})}$ is the attenuation coefficient of the ice and Z the depth of the ice cover. The irradiance at the different sampling depths of the water column was estimated from the following equation:

$$E_z = E_{d(\text{surface})} \times e^{(-K_d(\text{water}) \times Z)}$$

where E_z is the irradiance at depth Z , $E_{d(\text{surface})}$ is the irradiance at the surface, whether immediately under the ice or at the surface of the open water, and $K_d(\text{water})$ is the diffuse attenuation coefficient of the water column.

3.2.2 Chlorophyll *a* and biomass

Extracted chlorophyll *a* concentrations (Chl *a*) were derived from high performance liquid chromatography (HPLC) as detailed in Veillette et al. (2011) and Bonilla et al. (2005). Protist biomass was estimated from the light microscopy counts in Veillette et al. (2011). Taxon-specific biovolumes were calculated from the two dimensions noted either directly with an ocular micrometer or from images captured using a Qimaging Fast 2000R system (Qimaging, Surrey BC, Canada). Geometric differences between oblate spheres and ovoids, for example, were inferred from the literature. The biovolumes of more complex cell shapes were estimated following Hillebrand et al. (1999). Cell biovolumes were then transformed to carbon biomass (ng C L⁻¹) based on the equations in Menden-Deuer and Lessard (2000).

3.2.3 DNA extractions

Community DNA was extracted using a salt (NaCl) based method modified from Aljanabi and Martinez (1997) with lysozyme and proteinase K steps (Diez et al., 2001) as detailed in Charvet et al. (2012b). The final ethanol-rinsed DNA pellets were dried and resuspended in 100 µL of 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at -80°C.

3.2.4 PCR amplifications and sequencing

Both large (3 µm) and small (0.2 µm) fractions from May and August 2008 were amplified separately then mixed in equal volumes for subsequent sequencing. Only the large fraction was amplified for the July 2009 sample. The V4 region of the 18S rRNA gene was targeted with primers E572F and E1009R as described in Comeau et al. (2011). The V4 region is the longest variable region of the 18S rRNA gene and has relatively high taxonomic resolution (Dunthorn et al., 2012); even species can be distinguished within some groups, such as centric diatoms (Luddington et al., 2012). The forward primers included the Roche A adaptor and multiplex identifiers (MID-1 to -12) and the reverse primer included the Roche B adaptor. Amplicon DNA concentrations were

measured using a Nanodrop ND-1000 spectrometer (Thermo Scientific, Wilmington, DE, USA) and equal quantities of the DNA from the individual samples were mixed and run on one eighth of a plate using the Roche 454 GS-FLX Titanium platform at the Plateforme d'Analyses Génomiques de l'Université Laval, at the Institut de Biologie Intégrative et des Systèmes, Québec, Canada. The raw reads were deposited in the NCBI Sequence Read Archive, they are published under the accession number SRA057195.

3.2.5 Pre-processing, quality control and taxonomy analyses

Raw sequence reads were initially filtered for unidentified nucleotides (Ns), bad primer and short reads (Comeau et al., 2011). Reads were randomly re-sampled to ensure the same number of reads for each MID tag, these were then pooled and aligned in Mothur against the SILVA reference alignment (Schloss et al., 2009; http://www.mothur.org/wiki/Silva_reference_files) using the ksize=9 parameter. Misaligned reads were removed at this point and aligned reads were clustered into Operational Taxonomic Units (OTUs) at the $\geq 98\%$ similarity level using furthest-neighbour clustering (Mothur). OTUs represented by only one sequence, singletons, may be part of the rare biosphere within a sample (Sogin et al., 2006), but may also arise from sequencing errors (Kunin et al., 2010; Huse et al., 2010). Our decision to discard these singleton-reads was therefore conservative, and the true diversity may be underestimated (Sogin et al., 2006). Read and OTU yields are presented in Table 3.2.

The taxonomic assignation was refined by assigning reads, using a 50% bootstrap cut-off, against our user-designed V4 reference sequence database. This reference database (available upon request) is based on the NCBI taxonomy database with added curated Arctic-specific sequences (Comeau et al., 2011) including those from Arctic lakes (Charvet et al., 2012b). Common remaining “unclassified sequences” were further investigated using BLASTn (Altschul et al., 1990) against the GenBank nr database (NCBI).

3.2.6 OTU-based analyses

Communities from the different samples and depths were clustered using a Bray-Curtis analysis based on relative abundance of OTUs and using the Sorenson index based on presence-absence data (Mothur). Similarly, an un-weighted UniFrac analysis (Lozupone & Knight, 2005) was also carried out to take into account the fact that the samples from July only contained the 3 μm fractions of the communities, while the May and August samples had the 0.2 and 3 μm size fractions. An analysis of molecular variance (AMOVA) was also conducted (Mothur) to determine if there were significant differences among the communities of OTUs.

Table 3.2 Total sequences and operational taxonomic unit (OTU) yields for each samples. The number of reads after equalization of samples was 5193; reads were binned into OTUs at 98% similarity. The clean # reads is the number of reads left per sample after filtering badly aligned sequences and discarding singleton OTUs. Clean # OTUs specifies the final number of OTUs obtained from the clean number of reads.

Sample	Depth (m)	Initial # reads	Clean # reads	Clean # OTUs
May	2	9498	4471	2073
	5	8381	4195	2431
	10	8646	4099	2366
	12	8201	4217	2275
	20	9158	4094	2294
	29	8285	3269	1990
	32	10609	3868	2446
August	60	9946	3961	2433
	2	8421	4014	1752
	10	9134	4208	2334
	12	8891	4067	2192
July	29	9270	3612	2309
	2	13368	4451	1062
	5	13716	4548	950
	10	14648	4813	861
	12	16438	4658	1041
	29	7960	4298	908

3.2.7 Statistical analyses

The sequence abundance data were transformed to relative proportions before conducting multivariate analyses. A principal component analysis (PCA) was conducted on the physicochemical data (temperature, salinity, NO_x, SRP, Chl *a* and PAR). A canonical correspondence analysis (CCA) was performed to determine which environmental variables were correlated with changes among protist communities. We selected the most frequently occurring genera (representing $\geq 5\%$ of the sequences belonging to a group) within the major protist groups representing $\geq 10\%$ of the total sequences for at least one sample (ciliates, dinoflagellates, chrysophytes, diatoms, chlorophytes, Cercozoa and Telonemia) to reduce the number of taxa used in the CCA. The PCA and CCA were performed using PAST software (Hammer et al., 2001). A correlation analysis was conducted in PAST on the environmental variables to avoid redundancy in the CCA, and none of the variables were significantly correlated, so all were kept for the ordination analysis. Evaluation of the significance of differences between the community structures, using the data at the genus level, was conducted with Metastats (White et al., 2009; <http://metastats.cbcn.umd.edu>).

3.3 Results

3.3.1 Environmental parameters

The physicochemical profiles of Lake A, in May and August 2008, were previously reported by Veillette et al. (2011) and are summarized along with the July 2009 data (Figure 3.1). Salinity was lower at 12 m, in August 2008 and July 2009 compared to May 2008, indicating erosion of the halocline. The sigma-theta calculations reflected the strong stratification of the water column, with a two order of magnitude increase from 0.25 to 23 kg m^{-3} , over the depth interval of 12 to 29 m. Nutrient concentrations reflected the physical stratification, with much higher concentrations of SRP in the monimolimnion than in the freshwater mixolimnion (Figure 3.1). Chl a concentrations were low, ranging from 0.03 to $0.48 \mu\text{g L}^{-1}$ and overall, greater in the mixolimnion in August and July compared to May (Figure 3.1). In July, the Chl a concentrations were more homogenous throughout the mixolimnion with 0.29 - $0.30 \mu\text{g L}^{-1}$. Protist biomass increased in August compared to May by a factor of 2.5, and followed the same trends as Chl a , except at 12 m (Figure 3.1). At this depth, the biomass decreased while the Chl a concentrations increased to reach its maximum concentration of $0.48 \mu\text{g L}^{-1}$.

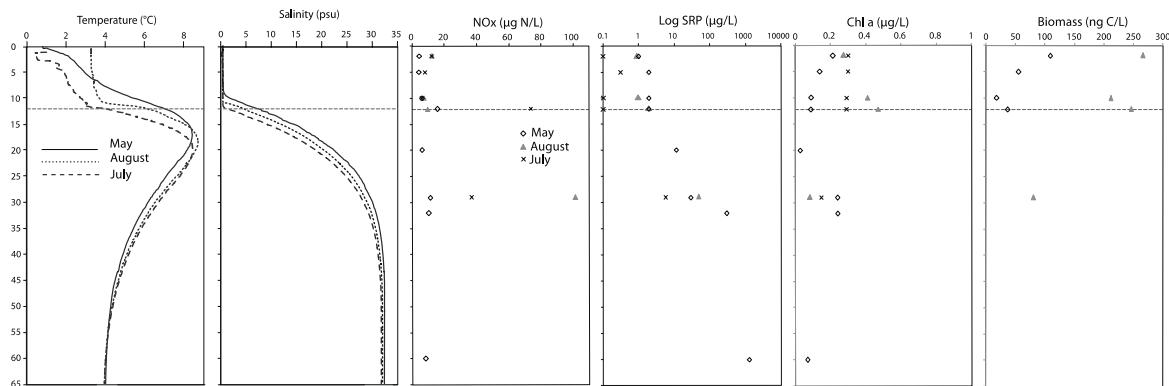


Figure 3.1 Environmental variables of the lake water column, in May 2008, August 2008 and July 2009. NO_x, nitrate and nitrite concentrations; SRP, soluble reactive phosphorus (note the logarithmic scale); Chl a , chlorophyll a . The depth axis is in meters.

3.3.2 Protist communities

The Bray-Curtis clustering indicated a tendency of the communities to group by sampling date (Figure 3.2a) and the dendrogram obtained from the Sorenson index provided a similar clustering of samples (not shown). The May 2008 communities clustered together, except for May 29 m, which grouped with the August 29 m sample. The samples from July grouped apart from May and August

2008 samples. The unweighted UniFrac dendrogram showed a similar separation of the communities by year (Figure 3.2b). However, the July 29 m sample grouped with the 32 m May 2008 sample. At the phylum level (Figure 3.3), no trends down the water column were evident, but given the Bray-Curtis and UniFrac clustering patterns, we investigated differences at finer taxonomic scales. Phyla were selected for detailed analysis on the basis of their particularly high sequence representation (dinoflagellates) and low variability (diatoms), or for their marked vertical and temporal changes (ciliates and chrysophytes).

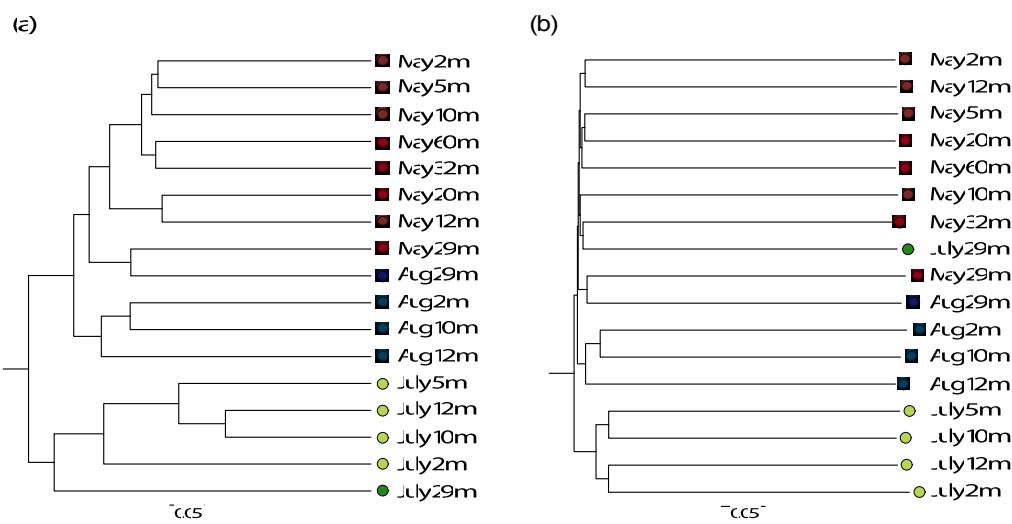


Figure 3.2 Bray-Curtis (a) and un-weighted Unifrac (b) dendograms based on OTUs (98% similarity) from May 2008, August 2008 and July 2009.

The Dinophyceae accounted for the greatest proportion of sequences throughout the water column in May, contributing 30-50% of sequences in the mixolimnion and >50% in the monimolimnion (Figure 3.3). At the genus level, *Scrippsiella* and unclassified Peridiniales sequences were recovered from the mixolimnion but not the monimolimnion (Figure 3.4a). In August, the overall proportion of dinoflagellates was less but the relative proportion of *Scrippsiella* sequences was greater compared to May. Sequences with best matches to *Polarella* were also recovered in the August mixolimnion (Figure 3.4b). In July 2009, the relative dinoflagellate abundance varied, representing >30% at 2, 10 and 29 m, and 7 and 19% at 5 and 12 m, respectively. Genera also varied with depth, with increased proportions of a brackish *Scrippsiella*, the appearance of the freshwater genus *Woloszynskia* in the mixolimnion and the dominance of the ice-associated *Polarella* sequences at 10 m (Figure 3.4c).

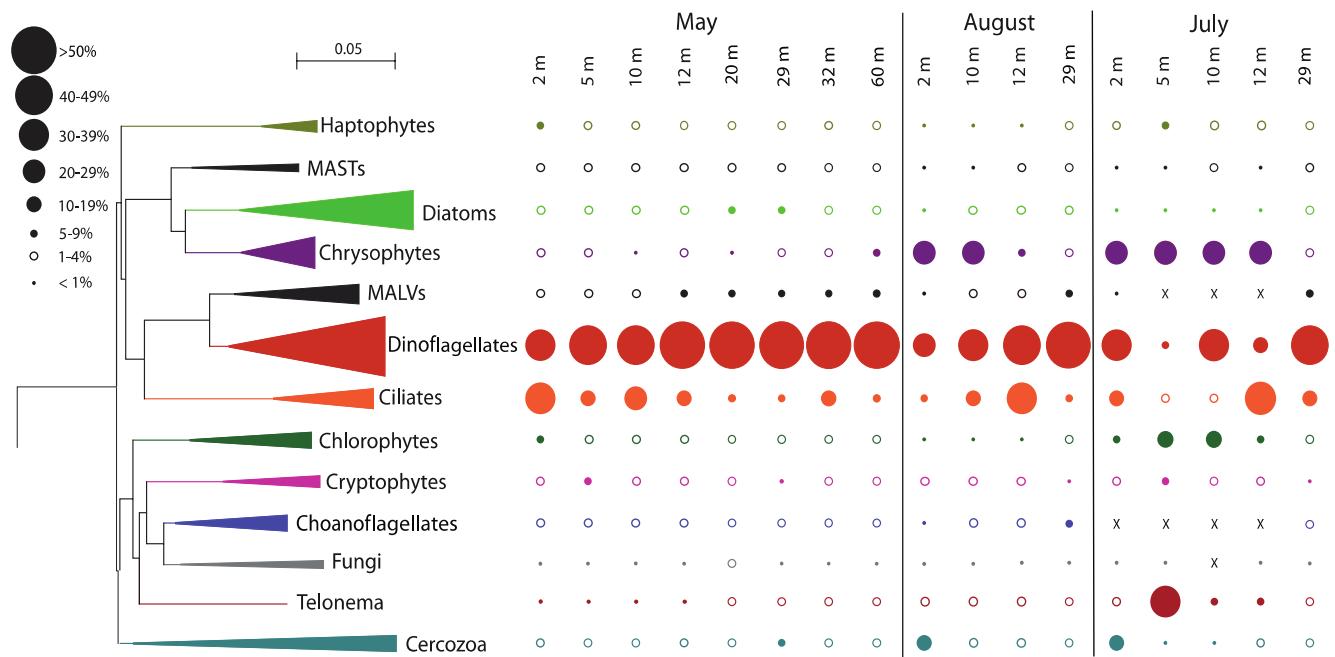


Figure 3.3 Neighbour-joining eukaryote tree indicating the proportion of each phylum from the water column of Lake A at the three dates. The size of the leaves is proportional to the number of genera within the groups from all samples. The sizes of the circles show proportions of sequence groups from each sample (scale in upper left corner).

Diatoms dominated the stramenopile sequences (38-80%) in May, representing 4% of total protist sequences in the mixolimnion and up to 9% in the monimolimnion (Figure 3.3). There was little taxonomic change among depths (Figure 3.4d). Diatom proportions were lower in August, with <2% in the mixolimnion (Figure 3.4e). However, at 29 m diatoms represented 4% of the total sequences and accounted for 50% of the stramenopile sequences. In July, the diatoms represented <0.5% of the total sequences from the mixolimnion and a BLAST analysis against the GenBank nr database showed that most of those sequences were closest to (95-97% similarity) to uncultured freshwater environmental sequences (Table 3.3). At 29 m, the diatom sequences represented close to 2.5% of total sequences (Figure 3.4f).

In May 2008, the ciliate sequences were proportionally more abundant in the mixolimnion, especially at 2 m, and fewer deeper down the water column (Figure 3.3). The most commonly represented genera were *Halteria*, *Parastrombidinopsis* and *Strombidium* and the proportional representation of these taxa varied with depth (Figure 3.5a). In May, the ciliate community at 12 m resembled those of the underlying monimolimnion rather than the mixolimnion. In August, a change in relative representation of ciliates was observed with ciliates accounting for a greater

proportion of sequences at the bottom of mixolimnion (12 m; Figure 3.3) marked by a relative increase of *Strombidium* sequences (Figure 3.5b). In July 2009 *Halteria* was again common at 2 and 5 m, while sequences related to *Strombidium* were mostly at 12 m (Figure 3.5c). At 29 m, in May 2008 ciliates were diverse, whereas in July 2009 novel currently unclassified ciliate sequences had highest representation (Figure 3.5c).

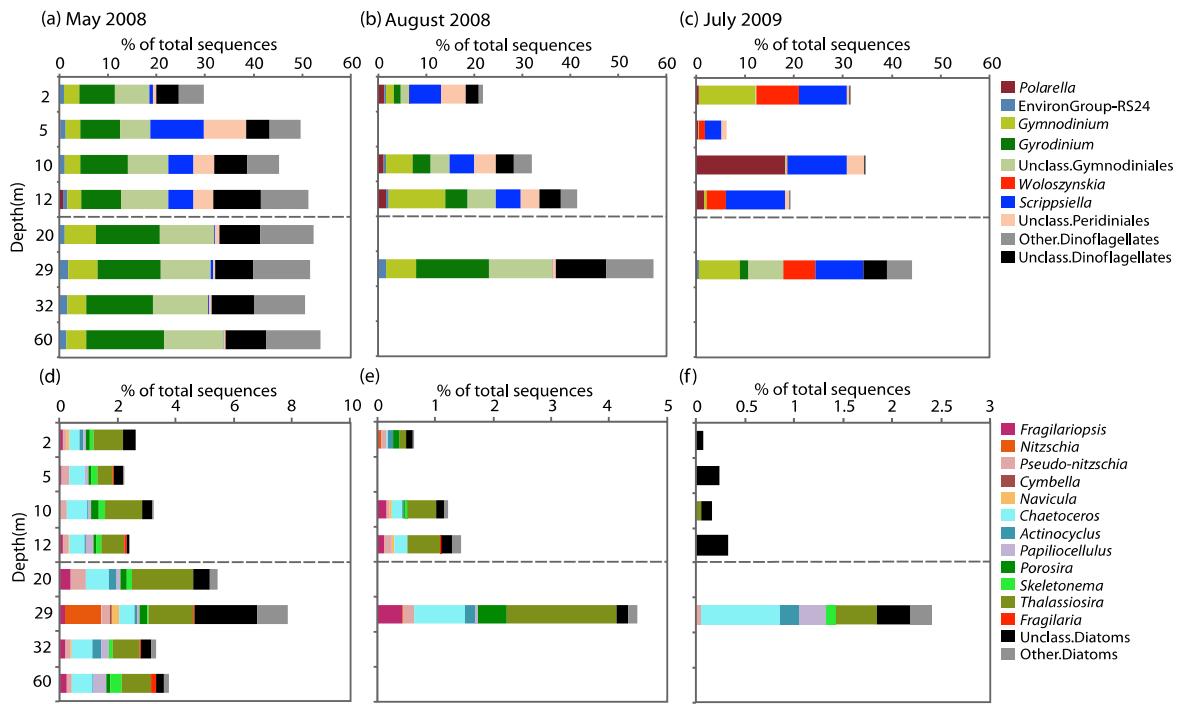


Figure 3.4 Sequences of genera showing seasonal stability within Lake A in May 2008, August 2008 and July 2009. The bar graphs represent the dinoflagellate (a-c) and diatom genera (d-f). Mixolimnion depths are above the dashed line and monimolimnion depths below. The proportions are based on total number of sequences (note differences in the x-axis scale for the different sampling times and groups).

Within the overall May 2008 protist community, stramenopile sequences represented 5-12%, of which 7-40% were assigned to chrysophytes (Figure 3.3). These chrysophyte sequences (Figure 3.5d) were mostly either novel or related to uncultured environmental 18S rRNA clones (Richards et al., 2005; Behnke et al., 2006; Scarcella, 2009; Charvet et al., 2012b). In May, the majority of chrysophyte taxa were restricted to either the mixolimnion or monimolimnion (Figure 3.5d), with a few exceptions, such as those classified with clones FV18_1B10 (Behnke et al., 2006) and Ar1663d47 (Scarcella, 2009). From May to August, the proportion of chrysophyte sequences in the mixolimnion increased from 1% to 24-29% of the total (Figure 3.3) with a taxonomic change including the appearance of sequences that matched several clones previously retrieved from Lake A, such as LA8E2G5 (Figure 3.5e). The July 2009 relative proportions of chrysophytes were

comparable to those of August 2008 (Figure 3.3) but with some differences in the community, as other chrysophyte taxa were recovered in addition to LA8E2G5, such as *Ochromonas*-related sequences (Figure 3.5f).

Table 3.3 BLAST search results for unclassified diatom sequences in July 2009 samples. The first name listed was the closest BLAST match the second name listed was the closest cultured match. Depth in meters; Seq, number of sequences; %, percent similarity; Acc.#, GenBank accession number; Origin, location from which the sequence was obtained; Ref., reference; Unc., uncultured. (1) Richards et al. 2005. Environ Microbiol (2) Yang et al. 2012. Protist (3) Theriot et al. 2010. Plant Ecol Evol (4) Sato et al. Unpublished (5) Balzano et al. 2012. ISME J (6) Newbold et al. 2012. Environ Microbiol

Depth	Seq	Closest match	%	Acc. #	Origin	Ref.
2	3	Unc. freshwater clone LG22-09	96	AY919761	Adirondack Park, USA. Lake Georges	(1)
		<i>Bolidomonas mediterranea</i> CCMP:1867	89	HQ710555	Culture	(2)
5	3	Unc. freshwater clone LG22-09	97	AY919761	Adirondack Park, USA. Lake Georges	(1)
		<i>Bolidomonas mediterranea</i> CCMP:1867	89	HQ710555	Culture	(2)
10		Unc. freshwater clone LG22-09	96	AY919761	Adirondack Park, USA. Lake Georges	(1)
		<i>Biddulphia alternans</i> ECT3856	91	HQ912677	Culture	(3)
12	1	Unc. freshwater clone LG22-09	95	AY919761	Adirondack Park, USA. Lake Georges	(1)
		<i>Biddulphia alternans</i> ECT3856	90	HQ912677	Culture	(3)
7		Unc. freshwater clone LG22-09	96	AY919761	Adirondack Park, USA. Lake Georges	(1)
		<i>Bolidomonas mediterranea</i> CCMP 1867	89	HQ710555	Culture	(2)
2		Unc. freshwater clone LG22-09	97	AY919761	Adirondack Park, USA. Lake Georges	(1)
		<i>Bolidomonas pacifica</i>	90	AB430618	Culture	(4)
29	1	Unc. <i>Chaetoceros</i> clone	99	JF698751	Beaufort Sea, Canada. 3 m depth	(5)
		MALINA_St390_3m_Pico_ES020_P1 H10				
		<i>Chaetoceros decipiens</i> strain RCC1997	93	JF794044	Culture	(5)
9		Unc. marine picoplankton ws_101, clone 1807E08	97	FR874617	Norwegian fjord. Marine coastal water	(6)
		<i>Chaetoceros decipiens</i> strain RCC1997	91	JF794044	Culture	(5)

From May to August 2008, and to July 2009, as the proportion of dinoflagellates and ciliates decreased, the protist community was also marked by increases in the proportion of sequences associated with other strict heterotrophic groups (Figure 3.3). The Cercozoa represented <2% of total protist sequences in the mixolimnion in May 2008, but in August 2008 and July 2009 this group represented 13 and 17% respectively at 2 m. The proportions of *Telomema* sequences also

increased, rising from <1% in May to 1-4% in August, and reaching *ca.* 40% at 5 m in July 2009. At the later date, *Telonema* actually dominated the heterotroph community at 5 m, while Coccozoa, ciliates and dinoflagellate sequences were reduced to 0.7, 1.5 and 6%, respectively.

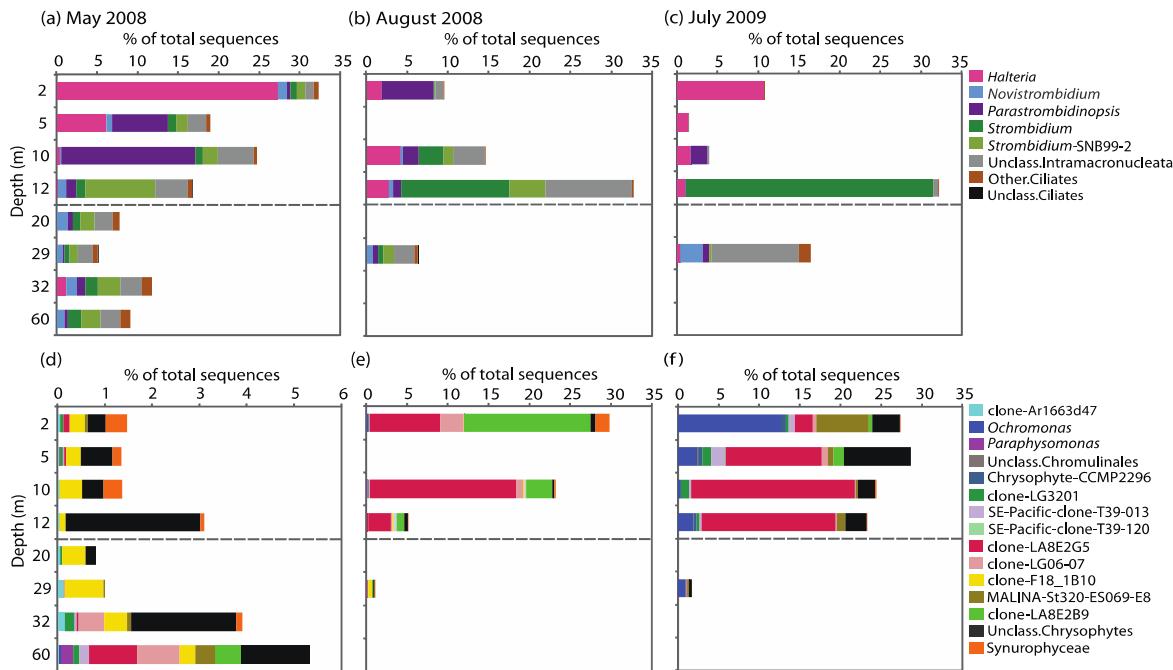


Figure 3.5 Sequences of genera showing seasonal changes within Lake A in May 2008, August 2008 and July 2009. The bar graphs represent the ciliate (a-c) and chrysophyte genera (d-f). Mixolimnion depths are above the dashed line and monimolimnion depths below. The proportions are based on total number of sequences (note differences in the x-axis scale for the different sampling times and groups).

3.3.3 Statistical and ordination analyses

At the OTU level, the May mixolimnion (2, 5, 10, 12 m) and monimolimnion (20, 29, 32, 60 m) were not significantly different (AMOVA, $F_s = 1.12, p = 0.285$). In contrast, the August and July communities in the mixolimnion and those at 29 m were highly significantly different from each other ($F_s = 2.56, p < 0.001$ and $F_s = 1.52, p < 0.001$, respectively) and the mixolimnion communities of May were also significantly different from those at 29 m at the same date (AMOVA, $F_s = 1.92, p = 0.048$). The mixolimnion communities from each date had significantly distinct OTU compositions (AMOVA, $F_{s(May/Aug)} = 2.12, F_{s(May/July)} = 4.7, F_{s(Aug/July)} = 3.7, p < 0.001$) which was also reflected in the community structure at the genus level. Compared with the mixolimnion in May 2008 the communities in August 2008 and July 2009 were significantly (Metastats, $p < 0.05$) enriched in some genera.

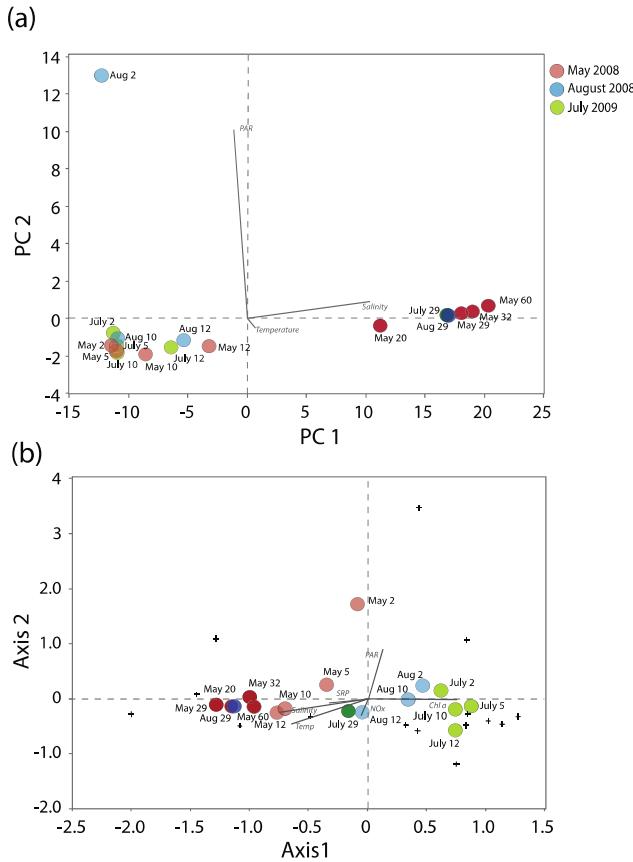


Figure 3.6 Principal component analysis (a) based on non-transformed environmental variables (temperature, salinity, PAR, NO_x, SRP, chlorophyll *a*), and canonical correspondence analysis (b) using sequence proportions of abundant genera and the same environmental variables. Samples from the mixolimnion are represented in light hues and from the monimolimnion in dark hues. Samples of May 2008 are in red, August 2008 in blue and July 2009 in green. Note the loadings for the nutrient and chlorophyll *a* variables are not indicated in the tri-plot (a) because they were not significantly different from zero.

A principal component analysis (PCA) with the environmental variables including temperature, salinity, NO_x, SRP, Chl *a* and PAR (eigenvalues 73.9% for PC1 and 25.5% for PC2) showed the abiotic segregation of samples (Figure 3.6a). This PCA showed that samples mostly grouped according to depths and water column strata, along the gradients of salinity (loading of 0.99 along Axis 1) and PAR (loading of 0.99 along Axis 2). A canonical correspondence analysis (CCA) with the abundance data of the dominant genera of the most variable groups (dinoflagellates, ciliates, chrysophytes, diatoms, Cercozoa, *Teloneema*, chlorophytes) of May, August and July, using the same environmental variables, revealed that adding biological parameters caused a different pattern of segregation in ordination space (Figure 3.6b; eigenvalues of Axis 1 and 2 were 49.48% and 28.8% respectively). The communities were distributed according to date and salinity, Chl *a*,

temperature and PAR seemed to be the most influential factors in structuring the DNA-inferred protist composition in Lake A. A CCA was also conducted based on a presence-absence matrix (not shown), and provided similar results. The separation of samples according to date was even more accentuated, with stronger similarity between May samples from the mixolimnion and the monimolimnion.

3.4 Discussion:

3.4.1 DNA preservation and constancy

Lake A is strongly meromictic with anoxic bottom waters likely persisting since it was formed several thousand years ago. The presence of banded iron deposits in the sediments of the lake indicates that only brief intervals of oxycline erosion have occurred in the past (Tomkins et al., 2009). The large differences between the mixolimnion and monimolimnion nutrient, oxygen and salinity conditions in Lake A are typical of meromictic lakes (Lauro et al., 2011). The major ion content of the water column (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , SO_4^{2-} , CO_3^{2-} , HCO_3^-) was analysed in Gibson et al. (2002). The authors found that the surface waters (mixolimnion) contained a higher proportion of Ca^{2+} and Mg^{2+} in the cations compared to the deeper waters (monimolimnion), which were enriched in Na^+ , indicative of their marine origins. In May 2008, Lake A temperature and salinity profiles were very similar to those previously published (Hattersley-Smith et al., 1970; Belzile et al., 2001; Van Hove et al., 2006) reflecting the physical stability of the system and lack of wind mixing (Vincent et al., 2008a). In August 2008 the surface of the lake was exposed to wind driven mixing and there was evidence that the halocline had been slightly eroded (Veillette et al., 2011). Despite these persistent vertical gradients in habitat properties, the proportional abundance of protist DNA sequences showed only muted shifts down the water column, particularly at the phylum level.

The relative constancy with depth of most protists identified from the DNA contrasts markedly with a parallel study of the prokaryotes in Lake A, also using high-throughput sequencing. This latter analysis of 16S rRNA genes indicated that both archaeal and bacterial communities in Lake A are very different even at the level of phylum in the mixolimnion and monimolimnion, with typical anaerobic groups in the deeper waters (Comeau et al., 2012). At a finer taxonomic level based on operational taxonomic units (OTUs) defined at a level of 97%, seasonal changes in the bacterial community were also evident in the mixolimnion between May and August. The changes in the bacterial communities suggest strong environmental selection and community turnover at least in the surface waters.

Table 3.4 BLAST search results for unclassified chrysophyte sequences in the 12 m sample from May 2008. The first name listed is the closest BLAST match, the second name listed is the closest cultured match. Depth, in meters; Seq, number of sequences; %, percent similarity; Acc.#, GenBank accession number; Origin, location from which the sequence was obtained; Ref., reference; Unc., uncultured. (1) Fujimoto Unpublished (2) Rice et al. 1997. Microbiol (3) Newbold et al. 2012. Environ Microbiol (4) Takishita et al. 2007. Extremophiles (5) Cavalier-Smith & Chao 2006. J Mol Evol (6) Majaneva et al. 2011. Polar Biol (7) Boenigk et al. 2005. Environ Microbiol (8) Richards et al. 2005. Environ Microbiol (9) Behnke et al. 2010. FEMS Microbiol Ecol (10) Chatzinota et al. Unpublished.

Seq	Closest match	%	Acc. #	Origin	Ref.
14	Unc. freshwater eukaryote K5MAR2010	97	AB622322	Gunma, Japan. Freshwater lake Kusaki	(1)
	<i>Paraphysomonas foraminifera</i>	91	Z38025	Culture	(2)
17	Marine picoeukaryote ws_159, clone 1815H10	98	FR874767	Marine biome, fjord, coastal water	(3)
7	Unc. Eukaryote clone CYSGM-8	97	AB275091	Sagami Bay, Japan. Methane cold seep sediment	(4)
	<i>Oikomonas</i> sp. SA-2.1	91	AY520450	South Africa	(5)
9	Unc. stramenopile clone 5c-F12	97	FN690679	Bothnian Bay, Sweden. Sea ice	(6)
	<i>Oikomonas</i> sp. SA-2.1	87	AY520450	South Africa	(5)
5	Unc. freshwater eukaryote K7MAY2010	96	AB622338	Gunma, Japan. Freshwater lake Kusaki	(1)
	<i>Spumella</i> -like flagellate JBM08	95	AY651098	Austria. Lake Mondsee	(7)
5	Unc. freshwater eukaryote K7MAY2010	97	AB622338	Gunma, Japan Freshwater lake Kusaki	(1)
	<i>Paraphysomonas foraminifera</i>	92	Z38025	Culture	(2)
1	Unc. eukaryote clone CYSGM-8	97	AB275091	Sagami Bay, Japan. Methane cold seep sediment	(4)
	<i>Oikomonas</i> sp. SA-2.1	88	AY520450	South Africa	(5)
1	Unc. freshwater eukaryote clone LG26-08	97	AY919776	Adirondack Park, USA. Lake George	(8)
	<i>Paraphysomonas foraminifera</i>	90	Z38025	Culture	(2)
1	Unc. marine eukaryote clone MIF_CilE6	96	EF526986	Framvaren Fjord, Norway	(9)
	<i>Spumella</i> sp. 9-12-B3	90	EU787418	Culture	(10)
1	Unc. freshwater eukaryote clone LG26-08	95	AY919776	Adirondack Park, USA. Lake George	(8)
	<i>Paraphysomonas foraminifera</i>	91	Z38025	Culture	(2)

The lack of marked variation of the eukaryotic community sequences implies a high background of either inactive encysted cells or recalcitrant DNA. The combination of cold, saline and anoxic conditions in the water column likely ensure a certain level of preservation of extracellular DNA, both autochthonous and allochthonous. Of particular note, diatom communities even at the level of genera were similar down the Lake A water column, and they represented a larger proportion of the total protist sequences in the monimolimnion. It is doubtful that the diatoms were active in the suboxic and sulfidic zones, but rather that sequences detected with DNA were from sedimented dead or dormant cells, accumulated and preserved in the lake's cold salty waters, as has been found in analogous cold, saline habitats elsewhere (Danovaro et al., 2005; Nielsen et al., 2007; Borin et al., 2008; Terrado et al., 2012). Similarly, the dinoflagellate genera found in the May and August 2008 profiles changed little with depth, with the exception of *Scrippsiella* and unclassified Peridiniales sequences that were only present in the mixolimnion, where they would be expected to be active. The remaining dinoflagellate genera were recovered irrespective of depth, suggesting that most sequences detected were not from an active community since genera composition would be expected to change with vertical and temporal shifts in light, nutrients and oxygen levels. Dinoflagellates have high copy-numbers of ribosomal genes (Zhu et al., 2005) and were likely over-represented. Therefore the relative proportion of dinoflagellate sequences does not directly reflect the proportion of cells or their level of activity. In addition there was no report of diatoms and few dinoflagellate counts ($\leq 2 \times 10^3$ cells L⁻¹) in a microscopy study carried out in parallel with our study (Veillette et al., 2011). The following year there were marked differences in the diatom and dinoflagellate genera detected in all samples, relative to 2008. Interestingly, many of the diatom genera are considered polar marine species (Lovejoy et al., 2002) suggesting, firstly, that the marine particles reach the lake either by way of transport in snow or directly by air (Harding et al., 2011) and, second, that there are interannual differences in the input of material into the lake. To correct for the detection of non-active or passively transported phylotypes that are inherent to DNA-based sequencing (Pawlowski et al., 2011) and as discussed above, we have limited the remaining discussion to genera or groups that varied over depths and seasons. Among these were the ciliates, chrysophytes, Cercozoa and Telonemia. Another caveat is that the number of copies of the 18S ribosomal RNA gene per cell varies greatly among taxa, and we can therefore only compare the relative contributions of each group to the total number of sequences among depths and dates.

3.4.2 Water Column Distributions in May 2008

Ciliates in May 2008 surface waters were related to the freshwater genus *Halteria* and in the lower mixolimnion (10 m) were related to *Parastrombidinopsis*. The small ciliate *Halteria grandinella* is

an efficient grazer on picoplankton (Pestová et al., 2008). *Halteria* was detected in the top 5 meters of the mixolimnion in the fresher and colder waters directly under the ice (Veillette et al., 2011) where photosynthetic picocyanobacteria are most abundant (Van Hove et al., 2008). Chrysophytes made up a small proportion of the May sequences and were characterized by the presence of Synurophyceae especially *Mallomonas*. This silica-scaled chrysophyte is reported to have low potential for dispersal (Kristiansen, 2007) and was mostly in the freshwater surface from 2 to 10 m. In May 2008, the mixolimnion was limited to the upper 10 m and the 12 m halocline communities were physically isolated from those above. The chrysophyte communities at 12 m contained significant proportions of unclassified sequences. A nBLAST search of the GenBank database (Table 3.4) indicated that the majority of these sequences grouped with environmental clone 1815H10 from a coastal Norwegian fjord (FR874767; Newbold et al., 2012), suggesting a halotolerant species consistent with the brackish conditions at 12 m in May. Among ciliates, the genus *Strombidium* is a diverse genus with over 100 described species and is found in a variety of habitats (Wylezich & Jürgens, 2011), an uncultivated species with broad ecological tolerances could account for its presence at 12 m as well as in the deeper monimolimnion waters.

The chemocline of Lake A is suboxic and extends from approximately 13 to 20 m where the water becomes anaerobic and the sulfidic zone starts at 32 m (Gibson et al., 2002). Protist DNA is often recovered from such extreme habitats (Edgcomb et al., 2009) including chrysophytes and ciliates (Wylezich & Jürgens, 2011; Orsi et al., 2012; Stock et al., 2012). In the present study, there were relatively fewer ciliate sequences in the chemocline and monimolimnion, compared to upper waters. Chrysophyte sequences were found throughout the chemocline, including sequences related to the clone FV18_1B10, from the super-sulfidic anoxic Framvaren Fjord (Behnke et al., 2010).

Ciliates prey on both protists (Pedrós-Alió et al., 1995) and bacteria (Saccà et al., 2009) in anoxic monimolimnia. Maximum bacterial pigment concentrations in Lake A are between 25 and 30 m, with the highest bacterial densities between 27.5 and 29 m (Antoniades et al., 2009). Photosynthetic sulfur bacteria live on the sulphides diffusing from the sulfidic zone under 32 m (Sakurai et al., 2010). The purple-sulfur bacteria are found in deeper layers than the green-sulfur bacteria (Comeau et al., 2012) and both could be grazed by ciliates (Wylezich & Jürgens, 2011) able to live under anaerobic conditions (Muller, 1993; Edgcomb et al., 2011b). The significant unclassified Intramacronucleata ciliates from 29 m in May had a best BLAST match (97% similarity) to an Alveolate clone 5b-D8 from Baltic Sea ice in Bothnian Bay, Sweden (Majaneva et al., 2011); the closest match to an anoxic sourced clone (95% similarity) was to the eukaryote clone cLA12B10 (EU446380) from the halocline of the anoxic hypersaline l'Atalante basin (Alexander et al., 2009)

suggesting that it belongs to either a cold adapted or anoxic species. Additional sequences from more environments and cultivated anoxic strains are required to clarify these affinities.

3.4.3 Temporal variation

The ciliate and chrysophyte communities showed clear changes among the three dates. There was a pronounced shift from *Halteria* to *Parastrombidinopsis* in the mixolimnion between May and August, and a reoccurrence of *Halteria* in July 2009, especially at 2 m. *Halteria* is a small, fast-swimming (Ueyama et al., 2005) bacterivorus ciliate (Simek et al., 2000), while *Parastrombidinopsis* is a large marine brackish choreotrich (Agatha, 2011) that feeds on large protists such as dinoflagellates and diatoms (Kim et al., 2005; Tsai et al., 2008). These results suggest the availability of larger prey under the August conditions compared to May or July when the lake was ice-covered. Interestingly, the maximum proportion of ciliate sequences was found immediately under the ice at 2 m in May 2008, while in August 2008 and July 2009 the peaks were at 12 m. Consistent with the ciliate co-occurring with their favoured food sources, chlorophyll *a* concentrations followed the same pattern with maxima at 2 m in May 2008 and at 12 m in August 2008 (Veillette et al., 2011) although they were uniform from 2 to 12 m in July (this study).

The chrysophyte community changed between May, August 2008 and July 2009, with greater vertical differences in August and July compared to May. Although chrysophytes accounted for a low proportion of total sequences in May, they were sensitive to environmental changes and accounted for higher proportions of the total community in August 2008 and July 2009. The August surface water community was previously investigated with microscopy, pigment analysis and clone libraries (Veillette et al., 2011; Charvet et al., 2012b). The pronounced increase in chrysophytes seemed to be most closely related to the increased PAR availability, as was shown in the CCA. The sequences that classified with Clone LA8E2G5 (Charvet et al., 2012b) represented the dominant chrysophyte group of the mixolimnion of August and July in the present study. Phylogenetic analysis indicated that this clone grouped within a clade represented by *Kephrynion* (Charvet et al., 2012b), which was consistent with the microscopy. The other dominant chrysophyte sequence in August 2008 was matched to clone LA8E2B9, which grouped at the base of the chrysophyte phylogenetic tree (Cluster I in Charvet et al., 2012b). In July 2009, the chrysophytes were dominated by *Ochromonas* sequences, at 2 m, while the rest of the mixolimnion still contained sequences of the putative *Kephrynion* sp. clone LA8E2G5. Chrysophytes are nanoflagellates with the capacity for motility and can therefore maintain their position in the water column (Pick & Lean, 1984), such as in the surface waters where PAR would be most available.

The comparison of community composition over the three dates revealed differences in the presence of other bacterivores. Many of these heterotrophs were found throughout the water column in May and assumed to be mostly non-active or background DNA. However they represented higher proportions of the sequence totals in August and July suggesting active growth. They occurred mostly in surface waters but not in deeper waters on those dates. In particular, Cercozoa were well represented at 2 m in August and July, but not in May, while *Telonema* dominated the protist sequences in July at 5 m, mostly replacing the dinoflagellate and ciliate sequences. Nonetheless, the dinoflagellate communities of July 2009 were less homogenous throughout the water column than in 2008, indicating that the sequences were more likely from active cells reacting to the environmental conditions at each depth. For example *Polarella* was found at 10 m in July, and this genus was originally isolated from sea ice (Montresor et al., 1999) but has been reported from marine influenced, meromictic lakes in Antarctica (Rengefors et al., 2008). The salinity within sea ice can vary greatly and sea ice associated species are often euryhaline, therefore *Polarella* may well have been growing in the mixolimnion in July 2009. Interestingly, we have been able to maintain a culture of Arctic *Polarella* in low salinity water over several weeks (Charvet & Lovejoy, unpublished data).

The changes in relative sequence abundance of protist taxa in the Lake A mixolimnion between May and August contrast with reports from Lake Fryxell. This perennial ice covered lake is located at a similar latitude in Antarctica (77.37°S for Lake Fryxell; 83.00°N for Lake A), yet the authors report no pattern of seasonal succession, but rather an increase in population densities of all species that were present at the beginning of the growing season (Spaulding et al., 1994). This contrasting response would be consistent with the large scale shifts in the mixolimnion environment of Lake A associated with ice-out between May and August 2008, for example the 40-fold increase in light availability and direct wind-induced mixing (Veillette et al., 2011). Furthermore, the PCA and CCA indicated that PAR was a determining factor for the biological changes between May, August and July. The 29 m community showed little change during this period, consistent with this being a zone of dead protist accumulation rather than growth. Even after ice-out there would be little light, much less than 1% surface irradiance, for phototrophic protists at this depth (Belzile et al., 2001).

The two ice-covered sampling dates showed evidence of interannual variability in overall protist composition, although this may also reflect seasonal changes between May and July. In a previous study based on pigment data (Antoniades et al., 2009), the monimolimnion phototrophic communities (dominated by green sulfur bacteria) were similar from year to year, whereas the mixolimnion communities were more variable. Our results showed that the 29 m protist

communities separated according to year, implying greater interannual variability in the anoxic zone for eukaryotes than for photosynthetic bacteria. Extreme variability between years has been reported for Lake Fryxell, where each year over 5 years a different phototroph dominated the phytoplankton (Spaulding et al., 1994). This variability has been attributed in part to differences in overwintering populations and to differences in stream flow inputs of nutrients, DOC and algal inocula between years (McKnight et al., 2000). Similar factors may influence the year-to-year differences in protist community structure in Lake A.

3.5 Conclusions

Our high throughput analysis of the Lake A protist community indicated the preservation of DNA throughout the water column. This relatively constant background included taxa derived from external sources such as diatoms and marine dinoflagellates that are unlikely to be active in the lake, particularly in the anoxic monimolimnion. There were pronounced changes in the upper water column that were superimposed upon this background, particularly in the mixolimnion between the late winter ice-covered period in May 2008 and the unusual open water conditions in late summer, August 2008. These results underscore the need for discrimination between active and inactive components of protist communities, for example direct sequencing of ribosomal RNA as cDNA or targeted mRNA sequencing to detect gene expression. Nevertheless our approach was sufficiently sensitive to detect change and provides a baseline to gauge the potentially larger changes in protist community structure that may occur with accelerated warming and ice loss in the High Arctic.

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Chapitre 4 - Effects of light and prey availability on High Arctic protist communities measured by high-throughput DNA and RNA sequencing

Résumé

Les contraintes environnementales que subissent les protistes phototrophes dans les lacs du haut Arctique incluent les températures froides, les faibles concentrations de nutriments inorganiques et la faible luminosité durant la majorité de l'année en raison du couvert de glace et de l'obscurité hivernale. Les flagellés mixotrophes, principalement chrysophytes, dominent les communautés de protistes de nombreux lacs arctiques en fin de saison de croissance à la fin de l'été. Les périodes d'absence de glace s'allongent en raison du réchauffement climatique et augmentent la disponibilité de la lumière pour le phytoplancton, mais l'ensemble des impacts sur le phytoplancton et les autres protistes reste inconnu. Nous avons examiné les réponses des communautés de protistes aux changements de combinaisons de disponibilité de lumière et de proies avec une expérience de lumière/dilution dans le lac Ward Hunt (lat. 83°05' N). En fin d'expérience, la structure des communautés a été investiguée par séquençage haut-débit de l'ARN ribosomal converti en ADNc et du gène de l'ARNr 18S, les deux molécules fournissant des informations complémentaires. Les séquences d'ADNc de dinoflagellés dominaient à faible luminosité, excepté à la plus forte dilution où les séquences de Cercozoa hétérotrophes étaient plus abondantes. Par contraste, l'ADNc communautaire des incubations à forte luminosité était dominé par des séquences de chrysophytes. Ces différences taxonomiques sous les deux conditions de luminosité suggèrent une divergence des stratégies de mixotrophie, les dinoflagellés étant essentiellement des brouteurs et les chrysophytes ayant une propension à la bactéritorophie. Ces résultats impliquent que la présence ou l'absence du couvert de glace et son impact sur l'irradiance de la colonne d'eau pourraient déterminer quelle type de mixotrophe domine, et par conséquent le fonctionnement des réseaux trophiques microbiens et l'efficacité de leur couplage avec les échelons trophiques supérieurs.

Abstract

Photosynthetic protists in high latitude lakes are constrained by cold temperatures, low inorganic nutrient supply, and low light availability for much of the year due to ice cover and polar darkness. Potentially mixotrophic flagellates, especially chrysophytes, dominate the late summer protist communities of many Arctic lakes. The lengthening ice-free periods in these freshwater ecosystems due to a warming climate is resulting in increased light availability, but the overall impacts on phytoplankton and other protists are unknown. We investigated protist community responses to changes in combinations of light and prey availability via a light-dilution experiment in Ward Hunt Lake (lat. 83°05' N), in the Canadian High Arctic. The community at the end of the experiment was identified using high-throughput pyrosequencing of the V4 region of 18S rRNA (converted to cDNA) and of the 18S rRNA gene. At the end of the experiment, reads from cDNA were dominated by dinoflagellates under low irradiance, except at the greatest dilution where heterotrophic Cercozoa were most abundant. In contrast, the cDNA communities incubated at high light were dominated by reads matching chrysophytes. These taxonomic differences under the two light regimes suggest a divergence in the prevailing type of mixotrophy at the end of 4 days; many dinoflagellates prefer to graze on other microbial eukaryotes, while chrysophytes are primarily bacterivores. These results suggest that underwater irradiance selects the dominant type of mixotrophy that will occur. Since ice cover is the most important factor regulating underwater light in high Arctic lakes, its presence or absence in a given year would be projected to influence the functioning of microbial food webs with implications for higher trophic levels.

4.1 Introduction

Arctic freshwater ecosystems are increasingly viewed as sentinels of global change (Schindler & Smol, 2006; Mueller et al., 2009). The Arctic is experiencing accelerated warming at rates well above the global average (Anisimov et al., 2007), and this rapid climate change has already resulted in visible perturbations, including ice shelf break up (Mueller et al., 2003; Copland et al., 2007) and irreversible loss of cryospheric habitats (Vincent, 2010). High Arctic perennially ice covered lakes are presently crossing thresholds, with more frequent loss of their summer ice covers (Mueller et al., 2009). The onset of summer open water in these once-perennially ice-covered lakes leads to an unusual exposure to wind-induced mixing, extreme increases in underwater irradiance and changes in the water inflow dynamics (Veillette et al., 2011).

Chrysophytes often dominate the late summer phytoplankton community of Arctic lakes (Charvet et al., 2012b) when 24 h sunlight is available for photosynthesis. However, the biological productivity of these ecosystems is low due to low nutrient input from the catchment and little or no deep-water mixing over most of the year, resulting in strong nutrient limitation (Vincent et al., 2008b). Chrysophytes have several strategies for surviving in low nutrient environments, including small cell size, which provides a higher surface to volume ratio favouring efficient dissolved inorganic nutrient uptake (Nicholls, 2009), and high affinity transporters for certain nutrients (Raven, 2009). The capacity of most photosynthetic chrysophytes to graze on bacteria or other protists is another efficient way to obtain nutrients in oligotrophic waters (Bird & Kalf, 1986; Maranger et al., 1998; Raven, 2009 and references therein). Such mixotrophic species would be expected to dominate under the high light and low nutrient conditions during Arctic summers.

In contrast, in spring and under normal ice cover in summer, Arctic lakes are light-limited. Such conditions may also favour mixotrophic species capable of obtaining both energy and nutrients from bacteria or protist prey when light is limiting. The protist communities under the spring ice cover have a different composition than in late summer (Charvet et al., 2012a; Veillette et al., 2011), suggesting an ice cover influence on protist communities. As the multiannual ice-cover melts more frequently, the seasonal succession of these protist communities could be modified, depending on the importance of light in species selection. The mixotrophs that are favoured under present conditions could lose their competitive advantage over other mixotrophs or be displaced by a mix of strict phototrophs and heterotrophs.

The potential for mixotrophs to use two trophic strategies confers an advantage over specialists, and implies a particular niche for these organisms, where both energy and nutrient resources are limiting

(Nygaard & Tobiesen, 1993; Rothhaupt, 1996). However, there is an additional energetic cost to maintaining cellular machinery for phagocytosis and digestion, along with chloroplasts for photosynthesis (Raven, 1997). Therefore, it is assumed that mixotrophs are less efficient than the specialist phototrophs and heterotrophs in the exclusive use of either resource (Rothhaupt, 1996; Tittel et al., 2003). Several studies have described effects of different environmental factors on mixotrophic communities (Hitchman & Jones, 2000; Bell & Laybourn-Parry, 2003; Tittel et al., 2003; Du Yoo et al., 2010), but a thorough knowledge of the combined effect of light and prey density is still lacking (Calbet et al., 2012). Furthermore, few studies have taken into account the multitude of mixotrophic behaviours (Jones, 1997; Stoecker, 1998; Granéli & Carlsson, 1998) when investigating the impacts of environmental factors on communities of mixotrophs (Stickney et al., 2000; Hammer & Pitchford, 2005) or considering the potential ecological impact of these organisms on lower (Tittel et al., 2003) and higher (Weithoff & Wacker, 2007) trophic levels.

In the present study we investigated the response of protist communities from an Arctic lake to high and low irradiance levels over a gradient of prey concentrations. The intention was to create a resource matrix of light and prey to investigate protist responses over a trophic continuum (Granéli & Carlsson, 1998). In the context of disappearing summer ice cover, we evaluated the effect of light, imitating dim ice-covered or bright ice-free conditions, which were expected to challenge the primarily phototrophic members in the initial communities. With the dilutions we expected to cause prey-limiting conditions that would affect the primarily phagotrophic protists. We hypothesized that, at low nutrient concentrations, irradiance and prey density would control the balance between phototrophs, heterotrophs and mixotrophs. We evaluated this hypothesis by way of an *in situ* incubation experiment in a High Arctic lake. The changes in the protist communities were tracked using the taxonomically informative V4 region of 18S rRNA sequences from both cDNA and DNA templates.

4.2 Materials and Methods

4.2.1 Study sites

Ward Hunt Lake is located on Ward Hunt Island, at latitude 83°05' N and longitude 74°10' W off the northern coast of Ellesmere Island. The lake's maximum measured depth is 8 m with a total area of 0.37 km². Ward Hunt Lake is ultra-oligotrophic and typically completely covered by thick perennial ice throughout the summer; limnological, catchment and climate details are given in Vincent et al. (2011).

4.2.2 Nutrients

Aliquots of 120 mL of sampled water were stored in glass bottles with polypropylene caps. These samples were later analysed at the Institut National de Recherche Scientifique-Centre Eau-Terre-Environnement (INRS-ETE; Quebec, Quebec) after being transported in the dark at *ca.* 4°C. Concentrations of nitrate and nitrite ($\text{NO}_x\text{-N}$), total nitrogen (TN), ammonia ($\text{NH}_3\text{-N}$) and soluble reactive phosphorus (SRP) were determined using standard colorimetric techniques (Gibson et al., 2002). Total phosphorus was determined from a separate 125 mL aliquot by the continuous flow analyser stannous chloride method. The detection limit for $\text{NO}_x\text{-N}$ was 0.005 mg N L⁻¹ and for SRP was 0.001 mg P L⁻¹.

4.2.3 Experimental set-up

Ward Hunt Lake was sampled on July 4, 2009, immediately below a 40 cm hole in the ice, which was 2 m away from the north-western shoreline. Water depth at the sampling site was one meter and at the time of sampling there was a 20-30 cm ice cover. Approximately 100 L were collected, of which 50 L was sequentially filtered through 47 mm diameter 3.0 and 0.8 µm polycarbonate filters (PC) then through 0.2 µm Sterivex units (Millipore). This filtrate was used to dilute the experimental series (Landry & Hassett, 1982). Dilutions were made by mixing the unfiltered lake water (LW) with the 0.2-µm filtered water (FW) in 10-L cubitainers (polyethylene collapsible containers; Reliance Products, Winnipeg, Canada), to obtain the following dilution series : dilution factor 1 (LW only), 0.5 (half LW, half FW), 0.2 (2 L of LW, 8 L of FW) and 0.1 (1 L of LW, 9L of FW). For each dilution, one cubitainer was exposed to ambient surface irradiance (High Light) and the other was kept in a doubled black translucent plastic bag (Low Light). The dilutions and light treatments were expected to create a matrix of resources along which would develop a continuum of potential feeding strategies (Granéli & Carlsson, 1998).

Phosphorus (K_2HPO_4) and nitrogen (NaNO_3) were added to each cubitainer with final concentrations of 3.5 µg L⁻¹ of P and 18 µg L⁻¹ of NO_3^- -N. The nutrient additions were meant to prevent or decrease nutrient limitation for bacteria (Landry & Hassett, 1982), while maintaining oligotrophic conditions. Hence, P was added in excess of its natural concentrations in the lake to avoid phosphorus limitation, but the added nitrate concentration was just above ambient concentrations for Ward Hunt Lake (Charvet et al., 2012b). The cubitainers were then left for 4 days, anchored in place and allowed to float half-submerged at the surface in an ice-free region of the lake, to maintain *in situ* temperature and exposure to natural irradiance levels.

4.2.4 Sampling

To determine cell abundance and growth rates, subsamples of 10 mL for prokaryotes and 40 mL for eukaryotes were collected at the start of experiment (T_0), after two days (T_2), and after four days (T_4) at the end of the experiment. These were fixed in 1% glutaraldehyde for 1 h, then incubated with 5 $\mu\text{g mL}^{-1}$ of 4'-6-diamino-2-phenylindol (DAPI) for 5 min (Porter & Feig, 1980) and filtered onto 25 mm diameter black polycarbonate filters (Millipore); 0.2- and 0.8- μm pore size filters were used for prokaryotes and eukaryotes, respectively. The filters were mounted onto microscope slides with a drop of non-fluorescing immersion oil, and stored in the dark, at -20°C. Samples for Chl *a* were collected by filtering 1 L of water onto 25-mm GF/F filters (Fisher Scientific) and stored at -80°C.

For eukaryote DNA, 2 L of water was sequentially filtered onto 3.0 and 0.8 μm pore size 47 mm polycarbonate (PC) filters, which were placed into lysis buffer (50 mM Tris, 40 mM EDTA, 0.75 M sucrose) and stored at -80°C. For eukaryote RNA, 2 L were filtered as for DNA, but filters were placed into a mix of 1:100 mix of β -mercaptoethanol and RNAlater (Sigma-Life Sciences), then stored at -80°C.

4.2.5 Microscopy and growth rate calculations

DAPI stained cells were enumerated at 1000 \times magnification under ultraviolet (UV) excitation and to visualize chlorophyll *a* autofluorescence, the same fields were examined under blue (420 nm) excitation, with a Zeiss Axiovert inverted epifluorescence microscope. Cells, for which the nucleus was apparent under UV and autofluoresced red under blue excitation, were classified as capable of photosynthesis and cells that only appeared using DAPI were considered heterotrophs. Cells were counted across 40 fields, representing a cross-section of the filter.

The cell concentrations in each cubitainer were estimated at the start of the experiment (N_0), after two days (N_2) and at the end of the experiment (N_4). Growth rates (K) were calculated as generations per day, for each time interval, as:

$$K = (\log N_t - \log N_0) / (\log 2 \times t),$$

where N_0 is the number of cells in the initial population, N_t is the number of cells at time t (Prescott et al., 2002). The average of the growth rates of the first two days and the last two days was used as the mean growth rate for the experiment.

4.2.6 Pigment analyses

Chlorophyll *a* (Chl *a*) was extracted following sonication in 95% methanol and concentrations were measured by high performance liquid chromatography (ProStar HPLC; Varian, Palo Alto, CA, USA), as in Bonilla et al. (2005).

4.2.7 Nucleic acid extractions

Community DNA was extracted using a salt (NaCl) based method modified from Aljanabi & Martinez (1997) with lysozyme and proteinase K steps (Diez et al., 2001) as detailed in Charvet et al. (2012b). The final ethanol-rinsed DNA pellets were dried and resuspended in 100 µL of 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at -80°C. The community RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Toronto, Canada) as per the manufacturer's indications, and included a DNase (RNase-free DNase kit, Qiagen) step. A PCR, using eukaryote primers Euk336F and EukR, (Sogin & Gunderson, 1987; Medlin et al., 1988) was used to verify the DNase step. Directly after extraction, the total community RNA was reverse transcribed into the more stable form of complementary DNA (cDNA) using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies). As most samples had very low concentration of cDNA, random amplification of all cDNA samples was conducted by multiple displacement amplification (MDA; Illustra GenomiPhi V2 DNA Amplification Kit, GE Healthcare, England). Negative controls from the MDA were preserved and subjected to the same downstream tag-PCRs as the samples, to verify that there was no contamination.

4.2.8 Tag- PCR amplifications

The hypervariable V4 region of the 18S rRNA gene was amplified using the primers from Comeau et al. (2011). The resulting approximately 500nt amplicons were quantified using a Nanodrop ND-1000 spectrometer. At this point PCR products for each treatment from the 3.0- and 0.8-µm extracts were combined in equal proportions to ensure representation of the entire community. Final sequencing was on a 454 GS FLX-Titanium Platform, at the Plateforme de Séquençage de l'Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, Canada. The raw reads were deposited in the NCBI Sequence Read Archive with the BioSample accession number SAMN02258453.

4.2.9 Pre-processing, quality control and taxonomy analyses

The quality filters, resampling of sequences and alignment protocols used in this study are fully described in Comeau et al. (2011). The alignments were further checked for chimeras using the Uchime chimera check function (Edgar et al., 2011) against the Silva reference alignment in Mothur

(Schloss et al., 2009). The final aligned reads were clustered into Operational Taxonomic Units (OTUs) at the $\geq 98\%$ similarity level using Mothur (furthest-neighbour clustering). This first clustering step of the pooled sequences allowed identification of singletons (OTUs represented by a single sequence in the total dataset), and these were removed as potential errors (Huse et al., 2010; Kunin et al., 2010).

Taxonomic assignation (using a 50% bootstrap cut-off) of the remaining sequences was made against our curated sequence database, which is based on NCBI taxonomy (Comeau et al., 2011). This version of the database included sequences from 18S rRNA gene clone libraries of Arctic lakes published in Charvet et al (2012b) and is available upon request. Taxa in this database were binned by likely trophic status; unambiguous phototrophs, mixotrophs and heterotrophs, based on literature searches. This reference sequence database was trimmed to the V4 region, as recommended by Werner et al. (2012). Common “unclassified sequences” were further investigated using BLASTn against the GenBank nr database (NCBI).

4.2.10 OTU-based analyses

Bray–Curtis clustering of communities from the experimental treatments was based on relative abundance of OTUs generated in Mothur. The phylogenetic diversity of cDNA OTUs between the different treatments was tested by computing net-relatedness (NRI) and nearest taxon (NTI) indexes (Webb et al., 2002). Briefly, representative OTU reads were placed in an approximate maximum-likelihood phylogenetic tree using FastTree v2.1 (Price et al., 2010) based on a multiple sequence alignment generated with pyNAST (Caporaso et al., 2010a) as implemented in Quantitative Insights Into Microbial Ecology (QIIME v1.5; Caporaso et al., 2010b). The phylogenetic tree was refined in the R package “Picante” (Kembel et al., 2010) to compute mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) against a null model comprised of 999 randomized trees with taxa shuffling, leading to standardized metrics (SES_{MPD} and SES_{MNTD} , corresponding to $-1 \times NRI$ and $-1 \times NTI$, respectively).

4.2.11 Statistical analyses

Significance of differences at the genus level, along the resource continuum was tested with Metastats (White et al., 2009). A canonical correspondence analysis (CCA) was used to visualize variability among communities, and to analyze this variability according to the experimental treatments, based on the experimental variables: light, dilution factors, final bacteria cell concentrations, Chl *a*. The sequence abundance data were transformed to relative proportions of the

major groups detected (alveolates, stramenopiles, Cercozoa, Cryptophyta, Chlorophyta, Haptophyta, Telonemia).

4.2.12 Estimation of photosynthetically active radiation (PAR)

The incident irradiance at Ward Hunt Lake during the experiment between June 4 and 8 was calculated based on data from the Ward Hunt Island meteorological station less than 1 km from the lake. The proportion of PAR transmitted through the black polyethylene bags was estimated using a QSL100/101 Quantum Scalar Irradiance meter (Biospherical Instruments Inc., San Diego, USA).

4.3 Results

At the start of the experiment, phosphate and nitrate concentrations in the lake water were low and the addition of nutrients decreased the N:P ratio in all treatments (Table 4.1). The PAR values over the experimental run, ranged from 572 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at midnight to 1052 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at noon, with a mean (\pm SD) daily incident PAR in the High Light treatment of 769 (\pm 19) $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Approximately 0.1% of the incident PAR was transmitted through the Low Light polyethylene bags, corresponding to a mean daily PAR of 0.77 (\pm 0.02) $\mu\text{mol photon m}^{-2} \text{s}^{-1}$.

Table 4.1 Nutrients ($\mu\text{g N or P L}^{-1}$) and N:P ratios at the beginning of the experiment (T_0), and Chlorophyll *a* (Chl *a*, in $\mu\text{g L}^{-1}$) and bacterial and protist abundances ($10^4 \text{ cells mL}^{-1}$) at T_0 and the end of the experiment.

	T_0	Low Light (T_4)				High Light (T_4)			
		0.1	0.2	0.5	1	1	0.5	0.2	0.1
NOx-N	23	20.3*	20.8*	30.0*	41.0*	41.0*	30.0*	20.8*	20.3*
SRP	0.35	3.53*	3.54*	3.67*	3.85*	3.85*	3.67*	3.54*	3.53*
N:P	66:1	6:1*	6:1*	8:1*	10:1*	10:1*	8:1*	6:1*	6:1*
Chl <i>a</i>	0.39	0.09	0.22	0.25	0.32	0.13	0.12	0.05	0.07
Bacteria	7.21	ND	2.63	5.56	7.00	8.17	5.84	4.33	4.94
Heterotrophs	0.51	0.19	0.37	0.47	0.53	0.31	0.30	0.13	0.07
Phototrophs	2.44	0.36	0.53	0.72	0.85	1.08	0.96	0.66	0.35

*Estimates of concentrations at the start of the experiment are calculated from the dilution of the T_0 concentrations and the subsequently added nutrients.

ND: no data

The final bacterial concentrations from microscopy were lower at high dilutions than at low dilutions (Table 4.1). In contrast, the bacterial growth rates were greater at high dilutions (Figure 4.1a). Microbial eukaryotic abundance was also greatest at low dilutions (Table 4.1), but growth rates varied among functional groups (Figure 4.1b). For the heterotrophic eukaryotes, the growth rates were greater in the Low Light treatments at highest dilutions. Under High Light, the growth rate trend of heterotrophs was similar to that of phototrophs (Figure 4.1b), although growth rates of phototrophs were less in the lowest dilutions (dilutions 1 and 0.5).

The community profiles from DNA and cDNA templates differed, with a maximum of 22% of OTUs shared between the two templates for any one treatment. The Bray-Curtis community analysis showed that DNA communities clustered together, apart from the cDNA communities (Figure 4.2). There was no clear clustering in the DNA derived communities with respect to either light or dilution (Figure 4.2). Among the cDNA-derived communities, the treatments clustered into High Light and Low Light communities (Figure 4.2), with the exception of the Low Light dilution 0.1, which branched apart. The net-relatedness index and nearest-taxon index were positive for most communities, but values were mostly greater for cDNA communities compared to DNA (Figure 4.3). Both phylogenetic metrics obtained from DNA communities were similar among the different treatments, while the cDNA communities showed greater variability (Figure 4.3).

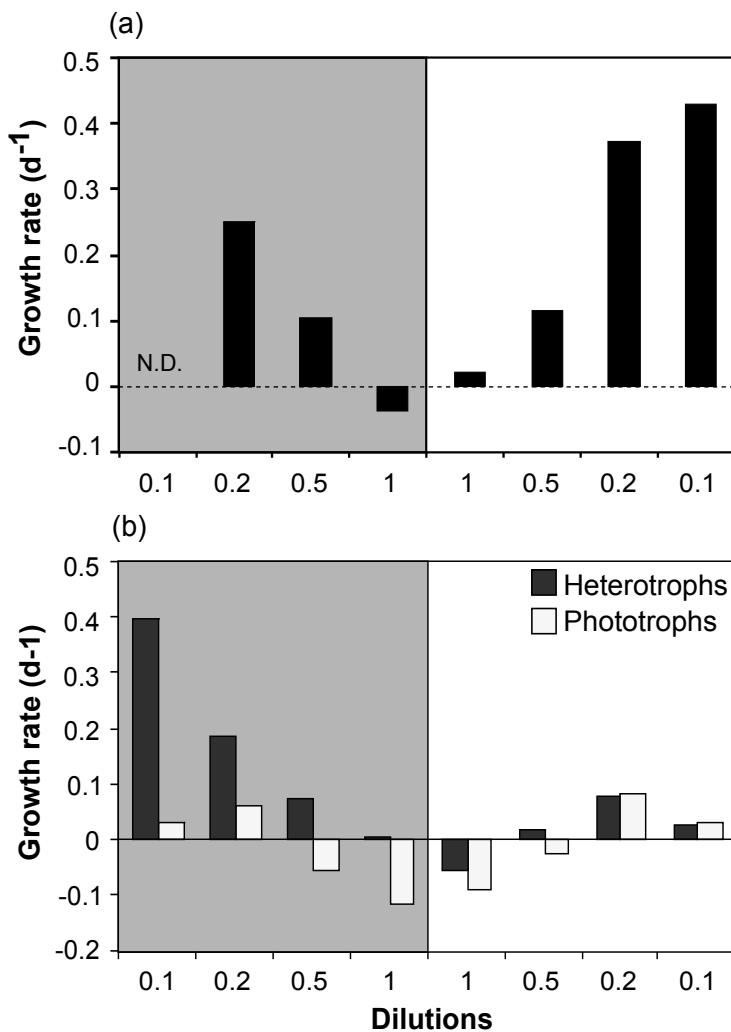


Figure 4.1 Growth rates of prokaryotes (a) and eukaryote heterotrophs and phototrophs (b) along the resource continuum.

Initial 18S rRNA gene reads consisted of 61% alveolates, 20% stramenopiles and 19% Cercozoa, with very few chlorophytes or cryptophytes (Figure 4.4a). At the end of the incubations, the DNA-derived reads from all of the treatments were still mostly alveolates with 50-80% of the total (Figure 4.4b), the majority of these belonged to dinoflagellates and ciliates (Table 4.2). The only exception was in dilution 0.2 under High Light, where stramenopiles accounted for 60% of the total reads, mostly corresponding to chrysophytes (Table 4.3). In contrast, reads obtained from the cDNA template showed more variability among the different light and dilution treatments (Figure 4.4c). Reads corresponding to Cercozoa related to *Protaspis* dominated the highest dilution (dilution 0.1), with some Oomycete and Synurophyceae reads (Table 4.3), while the other Low Light treatments were dominated by dinoflagellate reads (Table 4.2). In the High Light treatments, chrysophytes dominated the cDNA reads (Table 4.3).

Table 4.2 Proportions of ciliates and dinoflagellates for each treatment, obtained with DNA or cDNA templates, at the beginning (T_0) and end of the experiment; ND, no data.

Dilution	Molecule	T_0	Low Light				High Light			
			0.1	0.2	0.5	1	1	0.5	0.2	0.1
Dinoflagellates	cDNA	ND	20.17	65.83	44.82	63.19	29.61	54.64	6.64	20.20
	DNA	47.37	38.89	45.51	37.73	31.06	21.80	10.29	12.90	40.70
Ciliates	cDNA	ND	0.03	3.20	3.03	9.14	2.37	0.15	0.03	0.36
	DNA	13.91	7.74	32.01	28.09	51.44	34.83	38.03	5.93	16.88
Total	cDNA	ND	20.20	69.03	47.85	72.32	31.98	54.79	6.67	20.56
	DNA	61.29	46.63	77.51	65.83	82.50	56.63	48.32	18.84	57.58

To compare the results from the two nucleotide-templates used for sequencing, the number of reads from DNA was plotted against the number of reads from cDNA for each different taxonomic entity reported above (Figure 4.5). The Cercozoa had highest cDNA levels compared to DNA in dilution 0.1 at Low Light, but in the rest of the incubations had similar low numbers of reads for both DNA and cDNA. The Oomycetes and Synurophyceae were barely represented in terms of cDNA reads at High Light, but reached relatively high numbers of cDNA at Low Light, although DNA read numbers remained low. Dinoflagellates and chrysophyte DNA to cDNA ratios were relatively

constant (no significant difference) between the different treatments (Figure 4.5). However, the total number of both DNA and cDNA reads for dinoflagellates were greater in the Low Light incubations, while chrysophyte cDNA reads were significantly higher ($p < 0.05$) at High Light.

Dinoflagellate cDNA reads were mostly *Scrippsiella* and *Woloszynskia* with a lower contribution of *Gymnodinium* (Figure 4.6a). The *Woloszynskia* reads were more abundant at Low Light than under High Light, in which *Scrippsiella* reads were more frequent. The chrysophytes showed a greater diversity at Low Light, with best matches to the cultivated chrysophyte CCMP2296, a Beaufort Sea clone ST320-ES069_E8, *Ochromonas* and *Chrysolepidomonas* (Figure 4.6b). In contrast, under the High Light treatments the chrysophyte reads were largely composed of the Beaufort Sea clone ST320-ES069_E8 (80% of the chrysophytes and 40-70% of total reads) and some *Ochromonas* (15-20% of the chrysophyte reads).

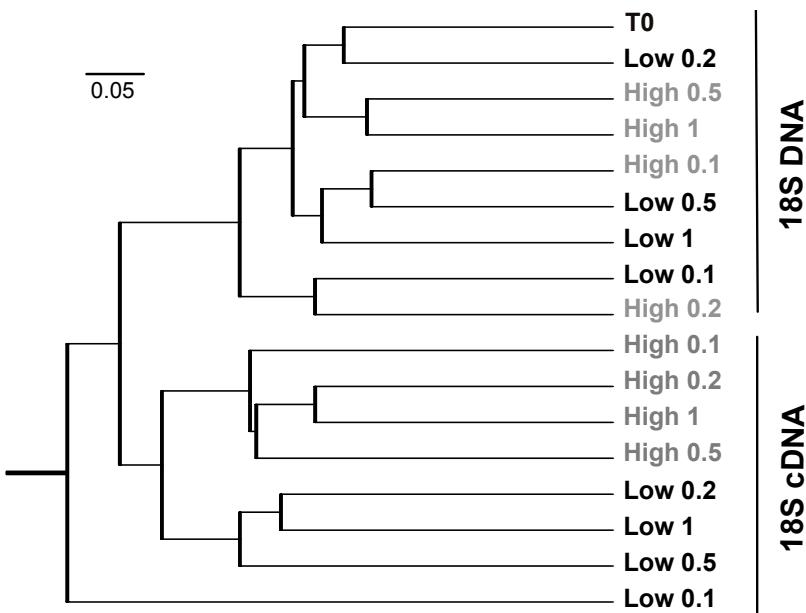


Figure 4.2 Bray Curtis dendrogram based on OTU (98% similarity) abundance data.

A CCA based on reads of major phyla from cDNA showed a separation of the communities according to the light treatment along Axis 1 (Figure 4.7). The Low Light communities were separated following higher Chl a concentrations, and were characterized by the presence of alveolates, except for dilution 0.1, where Cercozoa were a major factor. The High Light communities were influenced by light and bacterial concentrations, and were characterized by the stramenopiles. The Metastats analyses (Table 4.4) showed that the significant differences between

the High and Low Light treatments were attributable to the dinoflagellate *Woloszynskia*, which was significantly (p -value = 0.005) more abundant at Low Light, and the chrysophytes *Ochromonas* and clone St320_ES069_E8, which were significantly (p -values < 0.005) more abundant at High Light.

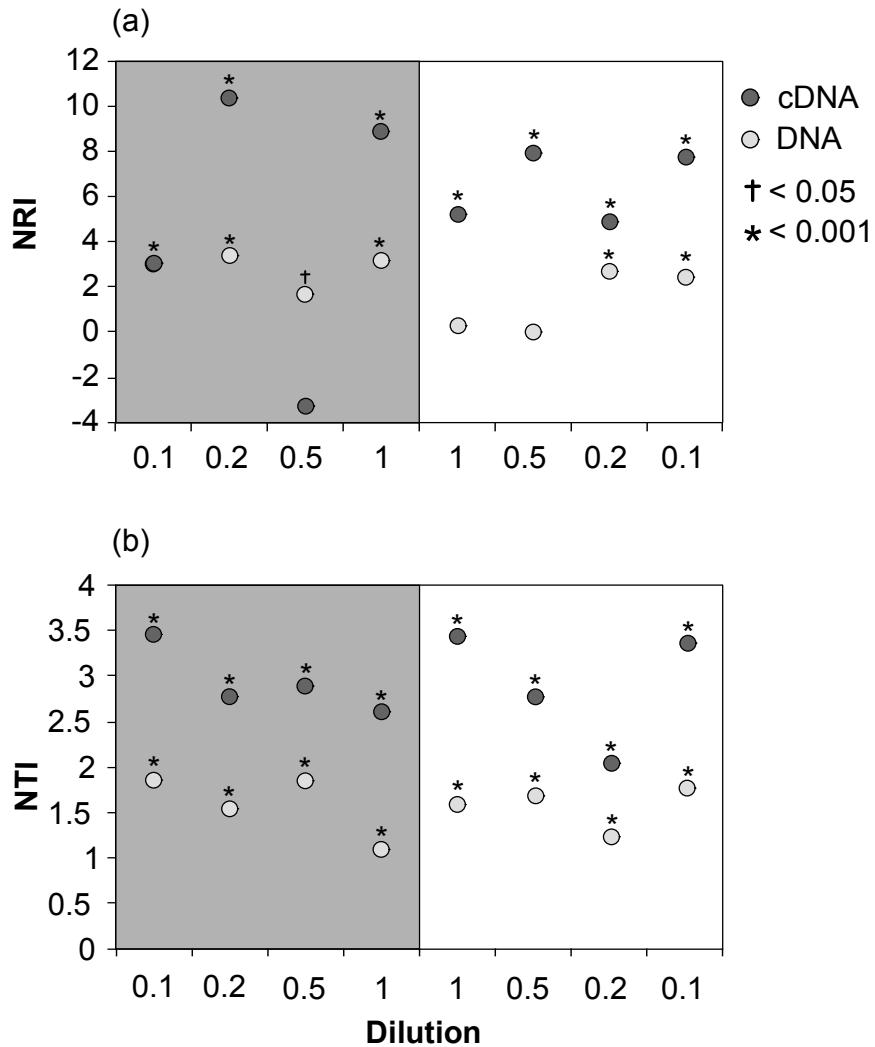


Figure 4.3 Net relatedness index (NRI, a) and nearest taxon index (NTI, b) of each treatment combination, based on cDNA (dark circles) and DNA (light circles) along the resource continuum. The gray zone corresponds to Low Light treatment and the white zone corresponds to High Light treatment.

4.4 Discussion

The dilution treatments resulted in changes in net growth rates for the bacteria and Eukarya. Bacterial growth rates increased with increasing dilution, consistent with lowered encounter rates and reduced predation pressure at higher dilutions (Landry & Hassett 1982). Chloroplast-containing

eukaryotes behaved similarly, as reported in other systems (Dolan et al., 2000; Landry & Hassett, 1982). Heterotrophic (colourless) eukaryotes showed the same trend, which differed from previous reports (Dolan et al., 2000). There was a high proportion of taxa nominally classified as nanoflagellates among the heterotrophic eukaryotes, and the results imply that this size class was subject to the same predation pressure as the small autotrophs. Negative growth rates (net losses) were observed for the pigmented protists, consistent with low growth of either mixotrophs, as a result of reduced prey availability (Dolan et al., 2000), or phototrophs due to inorganic nutrient limitation.

Table 4.3 Proportions of DNA or cDNA read of stramenopile groups along the resource continuum at the beginning (T_0) and end of the experiment; ND, no data.

Dilution	Molecule	T_0	Low Light				High Light			
			0.1	0.2	0.5	1	1	0.5	0.2	0.1
Chrysophyceae	cDNA	ND	2.22	8.72	9.34	8.72	55.09	41.83	81.07	72.83
	DNA	22.37	18.81	27.68	9.70	14.09	6.29	23.82	31.92	59.36
Diatoms	cDNA	ND	0.03	0.12	0.00	0.62	0.03	0.03	0.00	0.00
	DNA	0.00	0.00	0.03	0.12	0.00	0.00	0.09	0.24	0.03
Oomycetes	cDNA	ND	8.42	3.98	1.51	0.39	0.03	0.03	0.15	0.06
	DNA	0.06	0.12	0.30	0.09	0.06	0.00	0.00	0.00	0.00
Pelagophyceae	cDNA	ND	0.12	0.56	0.65	0.36	0.83	0.33	0.03	0.47
	DNA	0.21	0.36	0.71	0.30	0.59	0.71	0.21	0.56	0.15
Synurophyceae	cDNA	ND	0.00	5.28	1.42	2.20	0.03	0.27	0.00	0.00
	DNA	0.30	0.09	0.24	0.06	0.27	0.03	0.21	1.10	0.50
Unclass Stram	cDNA	ND	0.00	0.21	1.60	0.15	0.00	0.00	0.00	0.09
	DNA	0.00	0.00	0.06	0.00	0.00	0.00	0.03	0.00	0.03
Total	cDNA	ND	10.80	18.87	14.54	12.43	56.01	42.48	81.25	73.45
	DNA	22.93	19.37	29.01	10.26	15.01	7.03	24.35	33.82	60.07

The taxonomic composition of the initial community, collected from under the ice, differed from that reported in August 2008 (Charvet et al., 2012b). In 2009, dinoflagellates represented a greater proportion of the community compared to chrysophytes, similar to another ice-covered lake, Lake A, in May 2008 (Charvet et al., 2012a). The Bray-Curtis clustering indicated that the initial *in situ* community from DNA was similar to the DNA-based results at the end of the experiment, suggesting persistence of the community and no response to either experimental treatments or containment (Kim et al., 2011). However, lower phylogenetic similarity was evident from the cDNA template reads and changes occurred among treatments, perturbations of the system (Figure 4.2). The net-relatedness index (NRI) and nearest-taxon index (NTI) can be used to infer the

ecological processes involved in shaping a phylogenetic community structure. Positive values for both phylogenetic metrics indicated significant clustering in communities of most treatments, suggesting a strong influence of habitat selection on community structures (Webb et al., 2002). Recently disturbed systems are relatively more “clustered” than undisturbed communities (Ding et al., 2012). The higher NRI and NTI values found for cDNA-communities compared to the DNA suggest that the experimental treatments had a greater clustering effect on the cDNA OTUs. This was also reflected at the taxonomic level of both phyla and classes, which changed markedly among treatments in contrast to the communities inferred from DNA.

Nuclear DNA including the 18S rRNA gene is present in both active and dormant cells and can also persist as extracellular DNA (Charvet et al., 2012a; and references therein). 18S rRNA reads recovered from the cDNA template indicate the presence of more ribosomes, suggesting that the protein synthesis needed for growth is active (Not et al 2009; Poulsen et al 1993). Therefore, cDNA likely better depicts the metabolically active portion of the community (Not et al 2009). The phylogenetic metrics based on cDNA suggested strong influence of habitat selection on the structure of the active community. The proportion of DNA reads versus cDNA reads provided an indication of which taxa were represented in the active portion of the community. Although the variability of 18S rRNA gene copy numbers among eukaryote taxa makes it difficult to estimate actual abundances, the graphical relation between DNA and cDNA number of reads in Figure 4.5 was an indication of relative activity levels for the same genus in the different treatments. The persistence of the starting community as inferred from the DNA template, over the 4-day incubation would suggest insufficient time for degradation of DNA from dead or lysed cells (Danovaro et al., 2005; Stoeck et al., 2007; Terrado et al., 2011; Charvet et al., 2012a). Furthermore, given the slow generation times, of one doubling over the 4 days, the experiment may have been too short to detect differences. As exposure to new environmental conditions can cause mortality or trigger a metabolic shut down with low protein production and less need for ribosomes (Stoeck et al. 2007), cDNA is a more sensitive tool to detect changes in active communities, compared to DNA.

The high throughput sequencing of the 18S rRNA genes and cDNA from experimental communities showed that most taxa were from mixotrophic groups. However, the style of mixotrophy is difficult to discern since species, even within the same genus, may have markedly different mixotrophic strategies (Jones, 1997; Stoecker, 1998), and could therefore react differently to changed environmental conditions. The experiment was conducted on the premise that the resulting functional groups dominating community structure (Thingstad et al., 1996; Stickney et al., 2000) would reflect the resource matrix created by the different dilutions and light treatments.

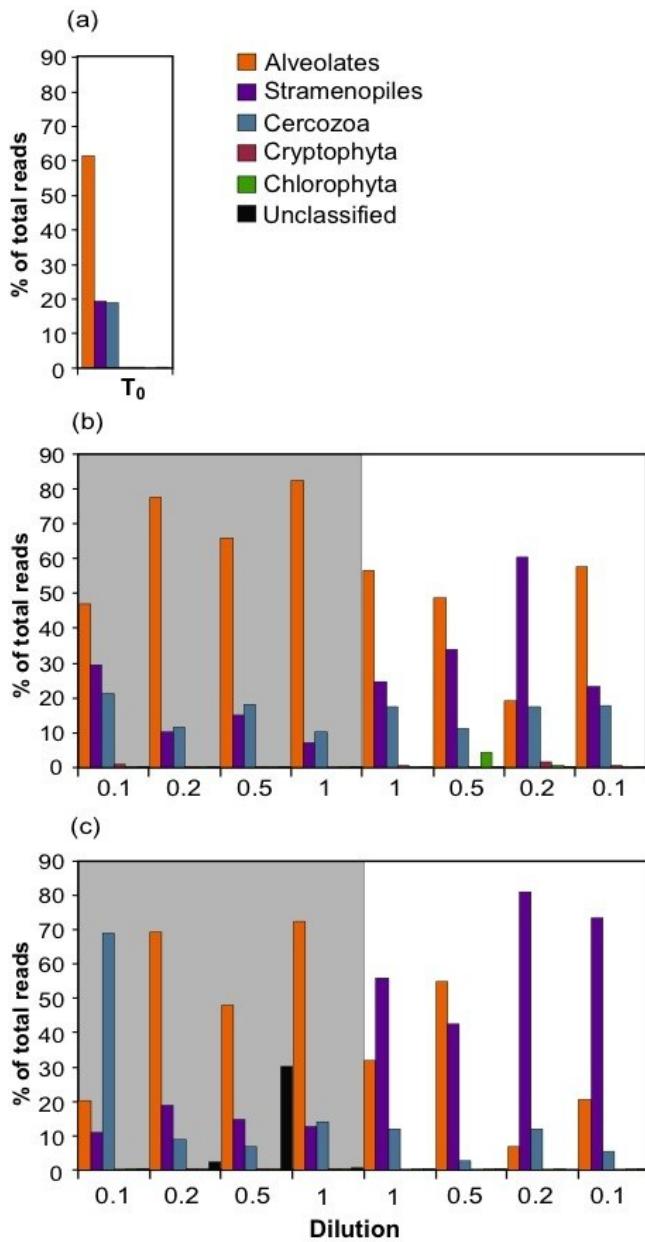


Figure 4.4 Phylum composition of the initial lake community DNA (a) and along the resource continuum at the end of the experiment, obtained with DNA (b) and cDNA (c) templates. The gray zone corresponds to Low Light treatment and the white zone corresponds to High Light treatment.

By focusing on the taxa identified from cDNA and on the ratios between DNA and cDNA reads, the outcome of each experimental treatment can be interpreted in terms of competitive interactions, based on Stoecker's conceptual models of mixotrophic behaviour (Stoecker, 1998) and Rothhaupt's resource-plane diagrams (Rothhaupt, 1996). Primarily heterotrophic mixotrophs, classified as model

III A mixotrophs, rely on photosynthesis only at times when prey become limiting and can have a low light threshold if the prey are sufficiently abundant to sustain cellular carbon requirements (Stoecker, 1998). Conversely, primarily phototrophic mixotrophs, or model II mixotrophs, can use phagotrophy for nutritional requirements but are dependent on photosynthetic activity for growth (Stoecker, 1998). Within this category, type IIB mixotrophs will require sufficient prey for growth, type IIA mixotrophs will have much lower prey concentration thresholds as long as light is available, and type IIC can withstand low light availability by turning to phagotrophy for their carbon and energy requirements. These particular traits are likely to influence the outcome of competition between mixotrophs and specialists, but also between the different categories of mixotrophs under different conditions of light and prey availability.

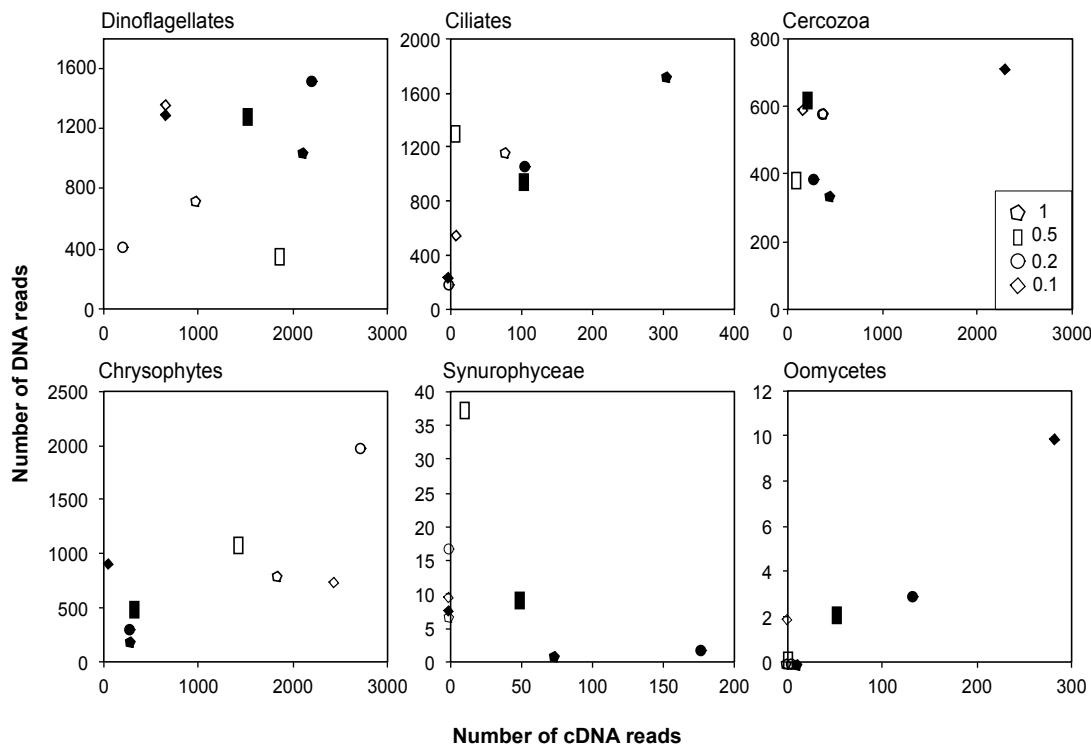


Figure 4.5 Number of DNA reads plotted against number of cDNA reads, for dinoflagellates, ciliates, and Cercozoa (top panel) and chrysophytes, Synurophyceae and oomycetes (lower panel). Open symbols indicate High Light treatments and closed symbols indicate Low Light. Dilution symbols are given as an insert in the Cercozoa panel. Note the different scales for each taxonomic class.

4.4.1 Impacts of light on protist communities

Under the Low Light treatments, both IIC and IIIA mixotrophs could be in competition with the strict heterotrophs. We found that dinoflagellates dominated the cDNA reads under these

conditions, indicating an active community. Mixotrophic behaviours have been reported for species of *Scrippsiella*, *Woloszynskia* and *Gymnodinium*, all of which reportedly feed on a similar range of small prey (< 12 µm) implying possible competition among the three when they coexist in the environment (Kang et al., 2011; Jeong et al., 2005). The *Scrippsiella* cDNA reads showed close similarity (99% in GenBank) to the cold-ecotype *Scrippsiella* aff. *hangoeii*, reported to co-exist with *Gymnodinium* and *Polarella glacialis*, in saline lakes in the Vestfold Hills of Antarctica (Rengefors et al. 2008). The *Woloszynskia* cDNA reads were 99% similar to the freshwater species *W. paschieri* (Logares et al., 2007), and, in a maximum-likelihood tree, grouped close to *Woloszynskia* reads from a meromictic lake near Ward Hunt Lake (Charvet et al., 2012a), suggesting a polar-ecotype of the genus. *Woloszynskia* reads were significantly (Metastats p-value < 0.05) more represented in the Low Light bottles, and appeared to be outcompeting *Gymnodinium*. The lower proportion of *Scrippsiella* reads detected by cDNA in the Low Light incubations was consistent with reports of encystment at low irradiances (Lundgren & Granéli, 2011; Rintala et al., 2007). In the High Light incubations, however, *Scrippsiella* was well represented by cDNA reads, relative to the other dinoflagellate genera. These profiles suggest that different strategies were used by the dinoflagellates, with *Woloszynskia* having a lower light threshold than *Scrippsiella*, possibly corresponding to type III or IIC mixotrophy. The presence of reads from both genera at various dilutions suggests that co-existence of the different strategists can occur, although specific light conditions tend to favour one dinoflagellate more than another.

High Light treatments could be expected to be favourable for photosynthetic protists, both strict autotrophs and mixotrophs (Jones, 2000). However, nutrients in Ward Hunt Lake are generally limiting (Bonilla et al., 2005) and it seems that despite addition of NO₃ and PO₄ at the start of the experiment, inorganic nutrients would have been quickly taken up and additions were insufficient to maintain growth of bacteria and phytoplankton, (Dolan et al., 2000) over the 4-day incubation. Therefore, competition for inorganic nutrients between phytoplankton and bacterioplankton was probably strong and the primarily phototrophic model IIA mixotrophs (Stoecker, 1998) outcompeted the strict autotrophs, due to their ability to prey on bacteria.

The proportion of chrysophyte cDNA reads, compared to DNA, was higher in the High Light treatments and the relatively similar distributions of taxa at all dilutions suggest that their activity was influenced by light, more than prey availability. Numerous chrysophytes have been identified as bacterivores (Bird & Kalff, 1986; Jones & Rees, 1994; Caron et al., 1993; Kimura & Ishida 1985), while others are considered phytoplankton grazers (Tittel et al., 2003). Most chrysophyte reads at High Light were related to the uncultured Beaufort Sea clone St320-ES069_E8 (Balzano et

al., 2012) or *Ochromonas*. The Beaufort Sea clone groups with a *Dinobryon* clade in a GenBank distance-tree, while the *Ochromonas* reads had BLAST results close to 98-99% similarity to an *Ochromonas* CCMP 1899 isolated from sea-ice. These two dominant taxa are likely primarily photosynthetic mixotrophs, which could use phagotrophy to supplement photosynthesis as a source of organic nutrients when the dissolved inorganic nutrients are limiting (Stoecker, 1998). Different species of *Ochromonas* have been used as model organisms for the study of mixotrophy in chrysophytes and compete with both strict autotrophs and strict heterotrophs (Rothhaupt, 1996; Rothhaupt, 1997). However, an unambiguous interpretation of the ecological function of these *Ochromonas* reads is difficult because the genus is polyphyletic (Andersen et al., 1999) and mixotrophic behaviours are diverse among the different species of this genus. For example, *O. minima* reportedly feeds in response to limiting inorganic nutrients, but requires a certain irradiance for growth (Nygaard & Tobiesen, 1993; Flöder et al., 2006) while *Ochromonas* sp. isolated from the north Baltic Sea shows heterotrophic growth independent from light (Andersson et al., 1989).

In the Low Light dilution series, the genus *Poterioochromonas* in the Synurophyceae had relatively high proportions of cDNA reads compared to DNA, in contrast to the High Light treatment. Species from this genus have been identified as belonging to the model IIIA mixotrophs (Stoecker, 1998) due to their mainly heterotrophic behaviour. *Poterioochromonas malhamensis* can use photosynthesis when prey concentrations fall below a threshold (Caron et al. 1990). In addition to bacterivory, *P. malhamensis* can also feed on larger phytoplankton cells such as chlorophytes (Zhang & Watanabe, 2001; Tittel et al., 2003). Among chrysophytes, the cDNA reads at Low Light, in dilutions 0.2, 0.5 and 1 included strain CCMP 2296, isolated originally from sea-ice in Baffin Bay. Its activity under Low Light suggests that it is also likely to be a phagotrophic phototroph. While the genera mentioned above dominated stramenopile reads, overall they represented a small proportion of the total protist community (<12%). The more abundant reads of other predators such as the strict heterotroph *Protaspis* or the larger mixotrophic dinoflagellates *Woloszynskia* and *Scrippsiella*, suggest that these model IIIA flagellates were outcompeted and perhaps under predation pressure from these competitors.

4.4.2 Impacts of prey concentrations on protist communities

Under Low Light and possibly darkness, model IIIA mixotrophs would thrive at high prey concentrations (Stoecker, 1998), and when photosynthesis was possible they would maintain growth at lower prey concentrations than strict heterotrophs (Jones, 1997; Tittel et al., 2003). However, under light-limiting conditions, heterotrophs have lower minimum prey concentration thresholds compared to mixotrophs (Tittel et al., 2003). In the present experiment, dilutions

artificially caused these low prey levels, forcing the mixotrophs below their minimum prey concentrations, between dilutions 0.2 and 0.1, with insufficient light to use photosynthesis to make up the energy shortfall.

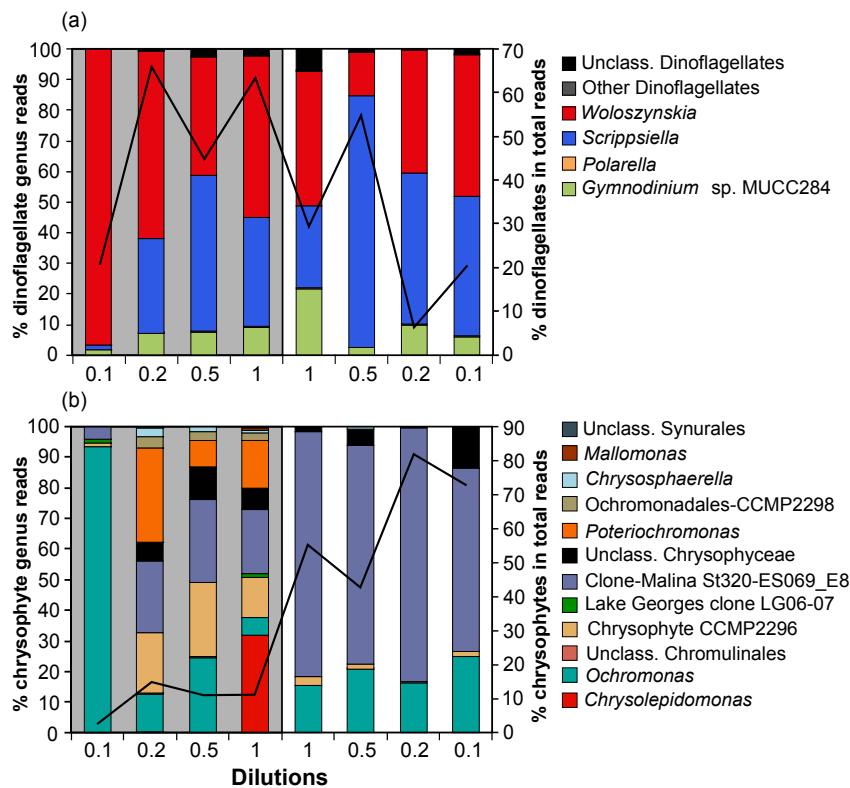


Figure 4.6 Details of relative dinoflagellate genera (a) and chrysophyte genera (b) community compositions along the resource continuum, based on cDNA sequences. The left y-axes indicate the reads for each genus as a percentage of the total reads for dinoflagellates (a) or chrysophytes (b). The right y-axes correspond to the phylum reads as a percentage of the total protist reads. The gray zone corresponds to Low Light treatment and the white zone corresponds to High Light treatment.

Correspondingly, the Cercozoa dominated as specialist heterotrophs in the Low Light at the lowest prey concentrations (dilution 0.1). The main cercozoan genus among cDNA reads was *Protaspis*, a large Cryomonadida that uses pseudopodia for prey capture and ingestion (Hoppenrath & Leander, 2006; Adl et al., 2012). Cercozoa were not strongly represented by either DNA or cDNA reads in the rest of the experimental communities and *Protaspis*-like organisms seemed to take advantage of the very specific conditions where both light and prey resources were low. Similarly, among the heterotrophic stramenopiles, Oomycetes, although rarely represented by the DNA reads throughout the experiment, increased in the Low Light treatment, when prey was scarce. The Oomycete reads detected by cDNA were related to *Aplanopsis* and *Leptolegnia*, both belonging to the saprotrophic

water moulds, Saprolegniales (Spencer et al., 2002; Beakes & Sekimoto, 2009). In the Low Light and at unfavourable prey concentrations for mixotrophs, it is possible that the Oomycetes were saprophytically feeding on dying plankton.

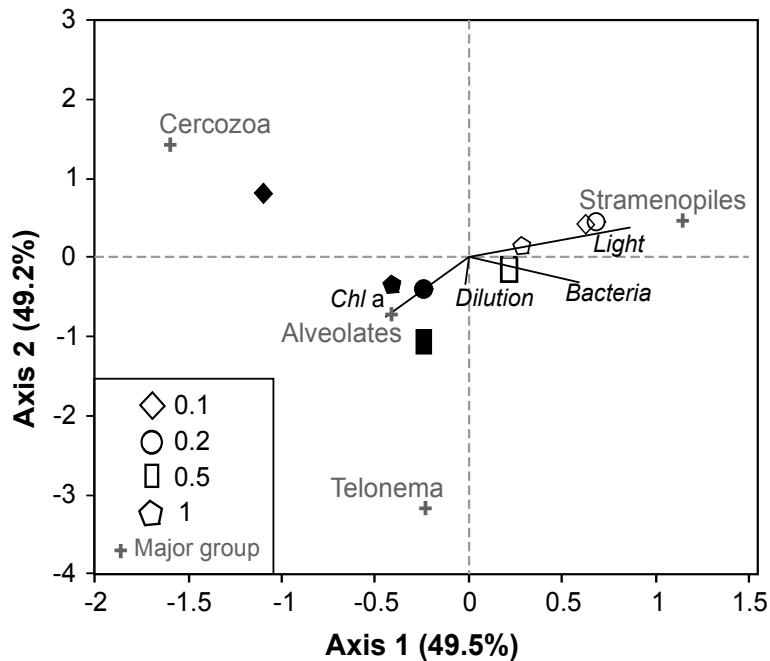


Figure 4.7 Canonical correspondence analysis of cDNA community structure from each treatment combination. Open symbols indicate High Light, closed symbols indicated Low Light treatments. Dilution symbols are in the insert.

In the High Light treatment, prey concentrations had little effect on community structure but still influenced the active populations. The chrysophytes remained active as long as light was favourable, irrespective of prey concentrations. *Scrippsiella*, however, was restricted to the relatively high prey concentrations (dilutions 1 and 0.5). At higher dilutions, the chrysophyte Malina clone ST320-ES069_E8 and *Ochromonas* represented a greater proportion of the cDNA reads. This would be consistent with a lower requirement for nutrients among chrysophytes compared to the energetic requirements of dinoflagellates, resulting in the stronger dependence of the latter on phagotrophy. Overall, generally we found that chrysophytes were favoured relative to dinoflagellates at High Light, especially at higher dilutions, while under Low Light dinoflagellates were more advantaged.

Table 4.4 Results from a Metastats analysis, based on a genus data matrix. Values indicate significant differences between High and Low Light treatments. All dilutions for each treatment were pooled for replication (White et al., 2009). "+", significantly more reads; "-", significantly less reads.

Taxon	High Light	Low Light	p-value
unclassified.intramacronucleata	-	+	0.047
<i>Woloszynskia</i>	-	+	0.005
unclassified.Cercozoa	-	+	0.008
unclassified.Trebouxiophyceae	-	+	0.014
<i>Ochromonas</i>	+	-	0.002
Chrysophyte_clone_MALINA_St320_ES069_E8	+	-	0.001
<i>Poteriochromonas</i>	-	+	0.047
unclassified.ochromonadales	-	+	0.018

4.4.3 Impacts of mixotrophs on microbial communities

During the Arctic winter, the polar night that lasts for four months likely causes the collapse of mixotroph communities, outcompeted by strict heterotrophs such as *Protaspis*. Conversely, during spring, summer and autumn, the lack of inorganic nutrients limits a strictly phototrophic primary production. In most Arctic lakes, picocyanobacteria contribute to the primary production and most likely provide a food source for mixotrophs, as postulated for nearby Lake A (Charvet et al., 2012b). In Ward Hunt Lake, where picocyanobacteria have not been reported, summer primary production is likely carried out almost exclusively by mixotrophic phytoplankton. As implied by the results of the present study, the presence or absence of ice cover will influence which type of mixotrophy dominates (III or II), with potential consequences for the lake.

Selection of chrysophytes might result in increased overall primary production in Arctic lakes over the summer when lakes are ice-free. In oligotrophic Ward Hunt Lake, primary production and phytoplankton biomass are restricted by competition with bacteria for the low nutrients (Bonilla et al., 2005). Mixotrophs with strategies such as those used by the chrysophytes could sustain food webs by their primary production that is less limited by nutrient supply. Their capacity to feed on their bacterial competitors for nutrients would allow them to be more numerous and active than strict phototrophs would be in these conditions. Furthermore, by transforming heterotrophic bacterial biomass into phytoplankton biomass the C:N ratio of the seston in mixotrophic flagellate dominated systems would likely be greater than those in which heterotrophic flagellates are the main grazers (Ptacnik et al., 2004). This may allow for more biomass build-up per limiting nutrient, thereby increasing energy transfer efficiency in planktonic food webs (Ptacnik et al., 2004).

Following a change in light regime and dinoflagellates giving way to chrysophytes, specific prey populations might in turn be affected. The large, primarily heterotrophic dinoflagellates, *Woloszynskia* and *Scrippsiella*, possess versatile predation strategies and prey on a wide range of cell sizes (Kang et al., 2011; Jeong et al., 2010). Nanoflagellates such as chrysophytes have the potential to significantly impact bacterial populations through predation (Sanders & Gast, 2012; Unrein et al., 2007) but could also facilitate bacteria and strict autotrophs through excretion of nutrients (Rothhaupt, 1996; Maranger et al., 1998; Wang et al., 2009). The shift from dinoflagellate to chrysophyte dominance in the lake could displace grazing pressure from larger cells to bacteria, and potentially influence the size, morphology and taxonomic composition of the planktonic bacterial or cyanobacterial communities (Hahn & Höfle, 1999).

In conclusion, this study implies that protist community structure may change in response to light regime and thereby to changes in the frequency and extent of ice cover loss. Specifically, light-limiting conditions appeared to be advantageous to dinoflagellates, while the direct exposure to full sunlight favoured chrysophytes. The generality of these responses requires further investigation; however they are consistent with a study of nearby Lake A, where an usual ice-out event in 2008 was accompanied by an increased abundance of chrysophytes (Charvet et al, 2012). Such changes imply that ongoing warming of the Arctic may induce a shift in grazing pressure on bacterial communities, and modify the pathway of carbon flow to higher trophic levels.

4.5 Acknowledgments

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Chapitre 5 Conclusions Générales

En réponse à la question principale de cette thèse, les mixotrophes semblent favorisés par les conditions environnementales des lacs arctiques. En effet, les chrysophytes mixotrophes dominent les communautés de protistes d'une large gamme de lacs arctiques aux propriétés limnologiques différentes en fin de saison de croissance (chapitre 2). La composition de ces communautés n'est pas la même au début de l'été et change au cours de la saison de croissance entraînant une variabilité spatiale plus forte au fur et à mesure que la saison progresse (chapitre 3). Plus particulièrement, il semblerait que l'éclairement détermine quel type de mixotrophie (Stoecker, 1998) prédomine, ce qui explique la succession de différentes catégories de mixotrophes lors de la transition entre la présence et l'absence d'un couvert de glace (chapitre 4).

5.1 Synthèse

Cette thèse contribue à l'avancement de la recherche en microbiologie environnementale en milieu aquatique polaire. Elle répertorie les premières investigations par biologie moléculaire des protistes de lacs du haut Arctique canadien. De plus, alors que la plupart des études de compétition sur les mixotrophes traitent de la compétition existant entre ceux-ci et leurs concurrents spécialistes, nous présentons ici des résultats qui évoquent pour la première fois une compétition entre mixotrophes de différentes catégories. Voici un bref résumé des résultats clés et des réalisations de chaque chapitre de cette thèse.

Le chapitre 2 est une étude de la biodiversité des protistes dans trois lacs arctiques aux propriétés limnologiques différentes. Les trois approches analytiques utilisées concordent sur la dominance des chrysophytes à la fin de l'été. L'abondance de chrysophytes résulte des techniques d'adaptation allant de la formation de kystes à la capacité d'ingérer les bactéries compétitrices pour les nutriments. Le fait que les chrysophytes soient fréquemment signalés dans les lacs arctiques (Laybourn-Parry & Marshall, 2003; Christoffersen et al., 2008) implique qu'ils aient un rôle conséquent dans la productivité de ces systèmes. De plus, la proportion jusque là ignorée des hétérotrophes tels que *Telonema*, les ciliés et les fungi permet d'envisager un réseau trophique impliquant des interactions subtiles et complexes (prédatation, compétition, facilitation) entre les mixotrophes, les protistes phagotrophes et les populations de procaryotes.

Le chapitre 3 documente l'étude de la variabilité spatiale et temporelle des protistes dans un lac méromictique perdant son couvert de glace, habituellement permanent, de plus en plus fréquemment (Mueller et al., 2009; Veillette et al., 2011). En début de saison de croissance (mai

2008) la composition des séquences du gène de l'ARNr 18S semble peu varier avec la profondeur contrairement à ce que le gradient physico-chimique permettait d'anticiper. La variation temporelle est plus évidente avec des changements de certains groupes phylogénétiques plus marqués dans le mixolimnion que dans le monimolimnion, établissant ainsi une variation spatiale qui n'existe pas au début de l'été. Ce changement plus marqué dans la couche de surface entre mai et août 2008 suggère que la disparition du couvert de glace provoque une forte augmentation de l'éclairement dans la colonne d'eau, surtout dans la zone euphotique. Cependant, la comparaison des communautés entre mai 2008 et juillet 2009, toutes deux sous la glace, implique que le retour printanier de la lumière solaire après un hiver dans l'obscurité ait une influence plus importante que celle de la perte du couvert de glace. Le mois de mai 2008 est vraisemblablement caractérisé par des vestiges de communautés d'organismes n'ayant pas pu maintenir leur métabolisme au cours de l'hiver. À ces températures où la dégradation se fait lentement, les organismes latents ou morts sont détectés par séquençage de l'ADN communautaire. Par comparaison, sous un couvert de glace classique en juillet 2009, les communautés varient en fonction de la profondeur indiquant que suffisamment de lumière s'est accumulée, même sous la glace, pour permettre aux communautés d'être métaboliquement actives et sélectionnées par les conditions physico-chimiques.

L'expérience de lumière/dilution rapportée au chapitre 4 souligne l'importance des mixotrophes parmi les communautés de protistes en été dans les lacs arctiques. L'éclairement influence le type de mixotrophes qui dominent ces communautés. Selon les résultats obtenus, sous une faible luminosité les dinoflagellés mixotrophes de modèle IIIA dominent. Cependant, pour maintenir ces communautés de dinoflagellés il faut une certaine abondance de proies au-dessous de laquelle les prédateurs hétérotrophes plus compétitifs dominent. Sous une forte et constante luminosité, les chrysophytes dominent, tel qu'observé aux chapitres 2 et 3. Ces nanoflagellés mixotrophes de modèle IIA peuvent utiliser la bactéritorophie pour contourner le manque de nutriments inorganiques nécessaires à la photosynthèse. Étant donné la différence de comportement de prédation et de valeur nutritive pour les niveaux trophiques supérieurs entre les chrysophytes et les dinoflagellés, des variations de l'éclairement dans la colonne d'eau pourraient avoir des conséquences sur les réseaux trophiques aquatiques.

En résumé, ces lacs arctiques présentent des communautés similairement dominées par des chrysophytes en été, durant les périodes d'éclairement maximal. L'interprétation la plus parcimonieuse au chapitre 2 suggérait un assemblage de type 1, (i.e. une communauté dominée par des chrysophytes durant toute la saison de croissance) à la fin de l'été (Holmgren, 1984). Par la suite, l'étude saisonnière du lac A et l'expérience de lumière/dilution ont permis de parachever cette

première conclusion. Les résultats suggèrent qu'au printemps et sous des conditions de très faible éclairement, les dinoflagellés sont plus représentés que les chrysophytes, du moins en terme de séquences du gène et de l'ARN de la petite sous-unité ribosomale 18S. Par conséquent, il se peut que les communautés décrites ici soient plus caractéristiques des assemblages de type 4 définies par une succession de dinoflagellés au printemps, d'une dominance de chrysophytes accompagnée de chlorophytes ou de diatomées en été, suivi de dinoflagellés en automne (Holmgren, 1984 ; Table 1.3).

5.2 Commentaires sur les méthodes

Au cours de cette thèse, les méthodologies utilisées rassemblent une panoplie de procédés allant des observations microscopiques au séquençage à haut-débit. En commençant par la microscopie, le HPLC et les banques de clones couplées au séquençage Sanger, nous avons comparé entre elles ces trois techniques, contrastant les plus « classiques » avec les méthodes de biologie moléculaire (Sanger et al., 1977). Nous sommes ensuite passés à la fine pointe de la technologie avec le séquençage haut-débit du gène de l'ARNr 18S. Puis, après s'être heurtés aux limites de l'information contenue dans les séquences d'ADN du gène de la petite sous-unité ribosomale (chapitre 3), nous avons exploité l'information contenue dans l'ARN ribosomal afin d'obtenir une image plus fidèle de la portion active des communautés (chapitre 4). Chaque chapitre présente donc sa particularité méthodologique avec son lot de désavantages, mais également d'informations pertinentes et parfois complémentaires.

Les analyses basées sur le gène codant pour l'ARN de la petite sous-unité ribosomale (ADNr 18S) confèrent divers avantages par rapport à la microscopie et aux pigments. L'identification phylogénétique des séquences détectées permet une plus grande précision taxonomique que la microscopie. Le séquençage permet également de déceler et d'identifier les organismes hétérotrophes dont la détection par la microscopie et le HPLC est limitée par le manque de pigmentation. Les trois techniques corroborent le résultat principal, soit la dominance les lacs arctiques par les chrysophytes. Par contre, le manque de fidélité entre les genres trouvés avec la microscopie et ceux trouvés avec les banques de clones est révélateur des limites des bases de données des séquences du gène de l'ARNr 18S qui sont encore incomplètes. Beaucoup d'organismes n'ont pas encore été répertoriés et certaines lignées ne possèdent pas de représentant cultivés ce qui rend difficile l'interprétation de leurs fonctions écologiques. De plus, certains groupes phylogénétiques possèdent de multiples copies du gène de l'ARNr 18S dans leurs cellules, entraînant un biais dans l'interprétation quantitative des résultats de séquençage. La détection de ces organismes est favorisée par le nombre de copies du gène comparativement aux organismes

possédant moins de copies. Par exemple, les dinoflagellés contiennent jusqu'à 1000 fois plus de copies que les Mamiellales (Zhu et al., 2005). Au contraire, les techniques de HPLC et les observations microscopiques représentent des approches plus quantitatives que le séquençage, permettant d'obtenir des concentrations de pigments ou de cellules. Ainsi, ces trois techniques fournissent des informations complémentaires sur la structure des communautés de protistes dans les lacs étudiés (chapitre 2).

Le séquençage par la méthode de Sanger (Sanger et al., 1977) offre des avantages différents par rapport au pyroséquençage-454 (Margulies et al., 2005). Grâce à son débit élevé qui fournit 200 000 séquences par analyse (Huse et al., 2007), le pyroséquençage-454 permet d'obtenir un échantillonnage en profondeur des séquences contenues dans un échantillon (Sogin et al., 2006; Medinger et al., 2010). Au contraire, les séquences obtenues par la méthode de Sanger sont limitées par la quantité de clones obtenus au préalable dans les banques de clones. Ainsi, avec le pyroséquençage les investigateurs ont la possibilité de déceler les organismes moins abondants de la communauté (Sogin et al., 2006). De plus, bien que le pyroséquençage soit encore assez onéreux, le rendement est nettement supérieur à celui de Sanger et le prix par séquence est donc moins élevé (Gharizadeh et al., 2006). Néanmoins, le séquençage par la méthode de Sanger permet l'obtention de séquences plus longues (1 000 pb) que le pyroséquençage, bien que ce dernier soit en voie de développement pour obtenir des séquences plus longues (*Roche 454-Sequencing*; <http://454.com/products/gs-flx-system/index.asp>). Ainsi, au chapitre 2, nous avons pu séquencer le gène de l'ARNr 18S presqu'au complet (1750 pb) en séquençant dans un sens avec l'amorce sens (*forward*) et dans l'autre avec l'amorce anti-sens (*reverse*), rendant accessible les reconstructions phylogénétiques. Nous avons ainsi pu identifier plus précisément nos séquences environnementales de chrysophytes non-répertoriées avec les séquences des banques de données telles que GenBank/NCBI (National Center for Biotechnology Information), EMBL (European Molecular Biology Laboratory) et DDBJ (DNA Data Bank of Japan). Bien que Roche améliore les performances du GS FLX régulièrement depuis quelques années, les séquences (*reads*) issues du pyroséquençage sont encore trop courtes (400-500 pb) pour permettre une résolution phylogénétique comparable au gène entier. Cependant, la région V4 que nous avons ciblée pour le séquençage peut s'avérer une bonne alternative, car c'est une région hypervariable dans laquelle s'enregistre le plus de changements entre les taxons ce qui permet une bonne résolution taxonomique (Stoeck et al., 2010; Behnke et al., 2011; Luddington et al., 2012). D'autres études séquentent la région V9, également très variable et plus courte (en moyenne 130 pb) avec des régions adjacentes (*flanking regions*) très conservées (Amaral-Zettler et al., 2009) et moins

d'homopolymères (Stoeck et al., 2010). Des travaux récents commencent à utiliser les deux techniques de séquençage de façon complémentaire pour déterminer les nouveautés phylogénétiques qui se cachent dans la biosphère rare (Lynch et al., 2012). Ainsi, les séquences non-identifiables par BLASTn issues du pyroséquençage peuvent être utilisées pour créer des amorces spécifiques permettant l'amplification d'une portion presque complète du gène qui est ensuite séquencée par la méthode de Sanger et utilisable pour caractériser phylogénétiquement l'organisme détecté (Lynch et al., 2012).

Par ailleurs, il existe plusieurs biais intrinsèques au pyroséquençage-454 compliquant l'utilisation de cette technique pour l'analyse d'échantillons environnementaux. Les erreurs peuvent provenir de différentes étapes, soit durant la PCR (e.g. insertion du mauvais nucléotide, formation de séquences chimériques), au cours du séquençage lui-même (e.g. extension de séquences d'homopolymères, plusieurs séquences sur une bille) ou lors de l'interprétation du flowgramme par le programme de Roche GS FLX (Huse et al., 2007; Huse et al., 2010). Ces biais peuvent produire des séquences erronées qui diffèrent de plus de 2-3% par rapport à la séquence modèle (*template*) et ont le potentiel de causer une surestimation du nombre d'OTUs (Kunin et al., 2010). Il est donc important de suivre un protocole de filtration des séquences afin d'assurer la qualité du jeu de données utilisé par la suite (Huse et al., 2007; Brockman et al., 2008; Quince et al., 2009; Kunin et al., 2010; Edgar et al., 2011). Malgré une filtration stricte pour la qualité des séquences, il reste d'autres sources d'erreurs causant une surestimation de la diversité d'OTUs. En particulier, l'algorithme utilisé pour regrouper les séquences en OTU est important. Par exemple, il est suggéré d'utiliser l'algorithme de « *average neighbour* » qui résulte en moins de faux OTUs que la méthode de « *furthest neighbour* » (Quince et al., 2009; Huse et al., 2010).

Aux chapitres 3 et 4 nous avons utilisé la méthode de « *furthest neighbour* » car c'était l'algorithme le plus fréquemment utilisé dans Mothur au début de nos analyses. Une fois qu'il est devenu évident à la société scientifique que l'algorithme « *average neighbour* » était plus adéquat, nous avons décidé de continuer avec « *furthest neighbour* » dans une optique de consistance et de garder le même protocole d'une analyse à l'autre. Par conséquent, les OTUs « *singletons* » ont été supprimés des jeux de données suivant le protocole de Comeau et al. (2011). Il se peut qu'ainsi nous perdions des informations sur la biosphère rare (Sogin et al., 2006) contenue dans ces lacs arctiques, mais puisque ce n'était pas l'objectif principal de cette thèse nous avons préféré éviter le risque de confondre biosphère rare et erreurs de séquençage.

En indiquant que les séquences d'ADN gène de l'ARNr 18S varient moins que prévu, les résultats du chapitre 3 illustrent une autre problématique liée au séquençage dans la reconstruction des communautés microbiennes. La stabilité des molécules d'ADN combinée avec le haut rendement du pyroséquençage-454 implique que beaucoup de séquences obtenues proviennent probablement d'organismes morts, dont l'ADN n'est pas encore dégradé, ou peuvent être issus d'ADN extracellulaire conservé dans la colonne d'eau froide (Stoeck et al., 2007). En somme, il n'y a pas un assez haut renouvellement des populations de protistes dans les lacs arctiques, soit par la préation, soit par la sédimentation ou la dégradation de la matière organique, pour pouvoir faire un suivi temporel ou spatial avec le gène de l'ARNr 18S. Le chapitre 3 démontre ainsi l'importance de discriminer les organismes actifs de ceux en dormance en utilisant l'ARN ribosomal de la petite sous-unité 18S pour suivre le nombre de ribosomes en fonction, qui est plus élevé chez les organismes métaboliquement actifs (Binder & Liu, 1998; Nicklisch & Steinberg, 2009). Par conséquent, au chapitre 4 nous avons choisi d'analyser les communautés de protistes avec la combinaison du gène et de l'ARN ribosomal 18S.

L'expérience de lumière/dilution utilisée au chapitre 4 est un outil simple pour déterminer l'importance de la luminosité et des proies sur la compétition entre mixotrophes et spécialistes. Cette expérience est basée sur la méthodologie détaillée par Landry et Hassett (1982) et reprise par d'autres (Dolan et al., 2000; Dolan & McKeon, 2005; Taniguchi et al., 2012) pour étudier les taux de croissance et de broutage du phytoplancton. Les effets du confinement et de l'enrichissement qui ont lieu lors d'incubations en bouteille (*bottle effect*) impactent souvent la structure des communautés à l'étude (Agis et al., 2007; Kim et al., 2011), que ce soit des communautés de bactéries (Massana et al., 2001) ou de protistes (Countway et al., 2005). Ainsi, l'expérience de dilution telle qu'utilisée traditionnellement pour déterminer des taux *in situ* de croissance et de broutage risque d'insérer des biais dans ces valeurs, car les communautés dans les bouteilles ne sont plus les mêmes que dans l'échantillon initial. Cependant, l'expérience de lumière/dilution détaillée dans cette thèse n'a pas pour but de quantifier des processus écologiques et nous avons adopté une courte durée d'incubation pour minimiser les effets de confinement. Nous cherchons simplement à témoigner des changements de composition des communautés de protistes suite à une modification de certaines conditions environnementales. Il se peut que les changements observés soient causés par l'effet de confinement, mais il est plus probable que l'effet des traitements de lumière et de disponibilité de proies ait eu plus d'impact sur la structure des communautés observées. Les dilutions ont pour but de créer un manque de proies pour les protistes phagotrophes, ce qui causerait

une adaptation de la communauté de phagotrophes observable par un changement dans la composition taxonomique.

Avec le recul, certaines lacunes apparaissent dans le protocole utilisé sur le terrain pour préparer l'expérience, malgré le travail de mise au point préalable. La lacune principale de cette expérience provient du manque des réplicas pour chaque traitement/combinaison de traitement. Des réplicas auraient permis de faire des analyses statistiques plus poussées, menant à des conclusions plus fiables et concrètes. Nous étions conscient lors de l'établissement de l'expérience, cependant la logistique du terrain en Arctique est complexe et la filtration pour obtenir de l'eau de dilution est particulièrement longue, nécessitant de faire un choix entre les traitements et les réplicas. Il aurait également été souhaitable d'effectuer un suivi précis de la température et de la radiation photosynthétiquement disponible (PAR) dans les bouteilles lors de l'incubation *in situ*. De plus, une des conclusions de l'expérience étant que les chrysophytes sont avantagés par le manque de nutriments inorganiques dissous, les concentrations de nutriments auraient également dû faire partie des mesures effectuées au cours de l'incubation.

5.3 Perspectives

Pour les lacs arctiques autant que pour d'autres écosystèmes, les perspectives de recherche au sujet des mixotrophes sont nombreuses. En effet, cette thèse soulève plusieurs questions quand à l'impact des changements climatiques sur les écosystèmes arctiques mais aussi sur le comportement des mixotrophes en général. Ainsi il est important d'accroître le nombre de lacs étudiés en augmentant l'éventail de conditions limnologiques, notamment en effectuant plus d'études comparatives entre les pôles et avec les régions tempérées. De plus, dans une perspective d'évolution temporelle, il est nécessaire de continuer à échantillonner les lacs décrits dans cette étude pour raffiner les connaissances sur les variations saisonnières et interannuelles, et ainsi mieux appréhender les dynamiques de ces communautés microbienne sur le long terme. Par exemple, il se peut que les changements climatiques aient d'autres impacts que celle de l'augmentation de l'irradiance sur les lacs arctiques. Entre autres, l'augmentation de la végétation dans les bassins versants pourrait avoir un effet sur les apports de nutriments inorganiques, stimulant ainsi les communautés bactériennes et le phytoplancton autotrophe. Il y a donc un intérêt à étudier l'impact d'une augmentation en nutriments inorganiques ainsi qu'une modification de leurs proportions respectives sur les communautés de mixotrophes.

Les résultats présentés dans cette thèse pourraient être approfondis par une série d'expériences alternatives. En effet, ayant déterminé que le niveau d'éclairement influence quel type de

mixotrophes domine les protistes en été, il serait bon de pouvoir confirmer qu'ils sont effectivement des prédateurs actifs au sein de la communauté et de déterminer l'identité de leurs proies. En ayant recours à des incubations avec des bactéries ou des algues marquées par la fluorescence (*fluorescently labeled bacteria*, ou *live-fluorescently labeled algae*) les prédateurs de ces proies seraient identifiables par microscopie à épifluorescence ou isolables par cytométrie en flux et pourraient ensuite être séquencés cellule par cellule (*single-cell sequencing*) pour confirmer l'identification taxonomique. Cette méthodologie permettrait également de déterminer les liens prédateurs/proies entre les organismes de niveaux trophiques différents. Par exemple, pour vérifier les différentes stratégies de prédation ou de choix de proies entre les dinoflagellés et les chrysophytes.

Par ailleurs, la recherche sur les mixotrophes dans les échantillons environnementaux serait facilitée et renforcée par le développement une méthode d'identification directe des organismes activement mixotrophes. Le gène et l'ARN ribosomal de la petite sous-unité 18S constitue un proxy médiocre pour déterminer la présence d'organismes mixotrophes. Premièrement, la fiabilité des assignations taxonomiques est limitée par la longueur des séquences (*reads*) et par l'étendue des informations dans les banques de données. Deuxièmement, les stratégies adoptées par les mixotrophes sont variables au sein même d'un genre (e.g. *Dinobryon*; Jones, 1997), rendant l'assignation taxonomique un outil encore plus contestable pour l'identification des mixotrophes et de leur impact écologique. L'identification d'un gène marqueur de la mixotrophie serait une grande avancée dans le domaine et permettrait, comme le gène de l'ARNr 18S, un suivi par pyroséquençage des communautés de protistes. En séquençant le transcriptome de différents mixotrophes provenant de cultures exposées à différentes combinaisons d'éclairement, de nutriments et de proies, il serait possible d'établir un profile des gènes exprimés sous chaque condition. Une telle étude effectuée en présence ou absence de bactéries indique une régulation négative de certains gènes impliqués dans la photosynthèse et la fixation du carbone (Moustafa et al., 2010). Avec une assez grande banque de données sur l'expression différentielle des gènes dans une gamme de conditions environnementales, il sera éventuellement possible d'isoler le gène responsable de l'activité mixotrophe, certainement un régulateur des gènes codant pour des activités photosynthétiques ou phagotrophes.

5.4 Mot de la fin

C'est avec une rapidité fulgurante que l'Arctique change sous le joug d'un réchauffement plus accentué que partout ailleurs sur la planète. En effet, les anomalies de température sont de plus en plus fréquentes et modifient le couvert de glace. L'étendue de la banquise polaire a connu un record

minimum en septembre 2012 (National Snow and Ice Data Centre ; NSIDC) avec 18% en moins que lors du précédent record de septembre 2007 (Zhang et al., 2013). Ces évènements suggèrent que l'océan Arctique sera entièrement libre de glace plus tôt que prévu par le GIEC (Anisimov et al., 2007), possiblement au cours des années 2030 (Wang & Overland, 2012). Plusieurs plateformes de glace ont été perdues à jamais dans l'archipel Arctique canadien (Mueller et al., 2003) entraînant avec elles des habitats microbiens et toute une diversité génétique et écologique (Mueller et al., 2006; Bottos et al., 2008; Vincent, 2010). Dans un tel contexte, les lacs arctiques sont également en changement, en raison de l'augmentation de la fréquence de la perte du couvert de glace (Mueller et al., 2009; Veillette et al., 2011). Les outils de paléolimnologie et leurs particularités physico-chimiques intrinsèques font de ces lacs des systèmes propices à un suivi de l'évolution du climat (Pienitz et al., 2004; Vincent et al., 2008a; Mueller et al., 2009). De plus, ces lacs oligotrophes aux températures froides et isolés des activités anthropiques constituent des systèmes simples pour l'étude des réseaux trophiques microbiens. Le suivi de leur transformation actuelle face aux changements climatiques permet d'étudier « en temps réel » l'impact de la variation des facteurs environnementaux.

Les travaux de cette thèse soulignent la dynamique des communautés de protistes dans les lacs arctiques. Les trois articles de recherche présentés dans cette thèse décrivent des communautés de protistes caractéristiques de ces lacs et offrent des perspectives sur la dynamique des mixotrophes au sein de ces communautés en fonction des conditions environnementales. Les lacs arctiques sont particulièrement propices aux mixotrophes, surtout en été lorsque soit la lumière, soit les nutriments limitent la photosynthèse. Nous pouvons conclure que les lacs arctiques constituent des systèmes modèles pour poursuivre les recherches sur le rôle des mixotrophes dans les réseaux trophiques et leur contribution aux cycles biogéochimiques. Les changements des propriétés fondamentales de ces lacs vont imposer un stress aux communautés microbiennes résidentes. Bien que les prédictions soient difficiles à faire, les résultats recueillis lors de cette thèse laissent présager des changements de communautés qui suivront une perte plus fréquente du couvert de glace. Il se peut que dans un arctique libre de glace durant les mois d'été, les chrysophytes mixotrophes soient tout d'abord favorisés par un accès accru à la lumière solaire tel que démontré par l'expérience du chapitre 4. Par la suite, un enrichissement du couvert de végétation dans les bassins versants (Anisimov et al., 2007), augmentera l'apport de matière organique et de nutriments dissous dans les lacs, avec pour conséquence un apport accru en ressources pour les phototrophes strictes (*bottom-up effect*). En parallèle, avec l'augmentation des températures et la disponibilité de la matière organique l'hétérotrophie pourrait devenir plus importante chez les mixotrophes que la phototrophie (Wilken

et al., 2013), réduisant encore plus la contribution des mixotrophes à la production primaire des lacs arctiques.

Bibliographie

- Adl S M, Simpson A G B, Lane C E et al. 2012. The revised classification of eukaryotes. *J Eukaryot Microbiol*, 59: 429–514.
- Agatha S. 2011. Global diversity of loricate Oligotrichaea (Protista, Ciliophora, Spirotricha) in marine and brackish sea water. *PLoS One*, 6: e22466.
- Agis M, Granda A & Dolan J R. 2007. A cautionary note: Examples of possible microbial community dynamics in dilution grazing experiments. *J Exp Mar Biol Ecol*, 341: 176–183.
- Alexander E, Stock A, Breiner H-W, Behnke A, Bunge J, Yakimov M M & Stoeck T. 2009. Microbial eukaryotes in the hypersaline anoxic L'Atalante deep-sea basin. *Environ Microbiol*, 11: 360–381.
- Aljanabi S & Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res*, 25: 4692–4693.
- Altschul S, Gish W, Miller W, Myers E W & Lipman D J. 1990. Basic local alignment search tool. *J Mol Biol*, 215: 403–410.
- Amaral-Zettler L A, McCliment E A, Ducklow H W & Huse S M. 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PloS One*, 4: e6372.
- Amaral-Zettler L A. 2013. Eukaryotic diversity at pH extremes. *Front Microbiol*, 3: 1–17.
- Andersen R, Van de Peer Y, Potter D, Sexton J P, Kawachi M & LaJeunesse T. 1999. Phylogenetic analysis of the SSU rRNA from members of the Chrysophyceae. *Protist*, 150: 71–84.
- Andersson A, Falk S, Samuelsson G & Hagström A. 1989. Nutritional characteristics of a mixotrophic nanoflagellate, *Ochromonas* sp. *Microb Ecol*, 17: 251–262.
- Anisimov O A, Vaughan D G, Callaghan T, et al. 2007. Polar regions (Arctic and Antarctic). In Parry M L, Canziani, O F, Palutikof, J P, van der Linden, P J, Hanson, C E (eds.) *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press, pp. 653–685.
- Antoniades D, Crawley C, Douglas M S V, Pienitz R, Andersen,D, Doran P T, Hawes I, Pollard W & Vincent W F. 2007. Abrupt environmental change in Canada's northernmost lake inferred from fossil diatom and pigment stratigraphy. *Geophys Res Lett*, 34: DOI 10.1029/2007GL030947
- Antoniades D, Veillette J, Martineau M-J, Belzile C, Tomkins J, Pienitz R, Lamoureux S & Vincent WF. 2009. Bacterial dominance of phototrophic communities in a High Arctic lake and its implications for paleoclimate analysis. *Polar Sci*, 3: 147–161.

- Azam F, Fenchel T, Field J G, Gray J S, Meyer-Reil L A & Thingstad F. 1983. The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser*, 10: 257–263.
- Baldauf S L, Roger A J, Wenk-Siefert I & Doolittle F W. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science*, 290: 972–977.
- Baldauf S L. 2003. The deep roots of eukaryotes. *Science*, 300: 1703–1706.
- Balzano S, Marie D, Gourvil P & Vaulot D. 2012. Composition of the summer photosynthetic pico and nanoplankton communities in the Beaufort Sea assessed by T-RFLP and sequences of the 18S rRNA gene from flow cytometry sorted samples. *ISME J*, 6: 1480–1498.
- Beakes G W & Sekimoto S. 2009. The evolutionary phylogeny of Oomycetes- insights gained from studies of holocarpic parasites of algae and invertebrates. In Lamour K & Kamoun S (eds.) *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools*. John Wiley & Sons, Inc., pp. 1–24.
- Behnke A, Bunge J, Barger K, Alla V & Stoeck T. 2006. Microeukaryote community patterns along an O₂/H₂S gradient in a supersulfidic anoxic fjord (Framvaren, Norway). *Appl Environ Microbiol*, 72: 3626–3636.
- Behnke A, Barger K J, Bunge J & Stoeck T. 2010. Spatio-temporal variations in protistan communities along an O₂/H₂S gradient in the anoxic Framvaren Fjord (Norway). *FEMS Microbiol Ecol*, 72: 89–102.
- Behnke A, Engel M, Christen R, Nebel M, Klein R R & Stoeck T. 2011. Depicting more accurate pictures of protistan community complexity using pyrosequencing of hypervariable SSU rRNA gene regions. *Environ Microbiol*, 13: 340–349.
- Bell E M & Laybourn-Parry J. 2003. Mixotrophy in the Antarctic phytoflagellate, *Pyramimonas gelidicola* (Chlorophyta: Prasinophyceae). *J Phycol*, 39: 644–649.
- Belzile C, Vincent W F, Gibson J A & Van Hove P. 2001. Bio-optical characteristics of the snow, ice, and water column of a perennially ice-covered lake in the High Arctic. *Can J Fish Aquat Sci*, 58: 2405–2418.
- Bennett M E & Hobbie J E. 1972. The uptake of glucose by *Chlamydomonas* sp. *J Phycol*, 8: 392–398.
- Bhattacharya D, Yoon H S & Hackett J D. 2004. Photosynthetic eukaryotes unite: endosymbiosis connects the dots. *BioEssays*, 26: 50–60.
- Binder B J & Liu Y C. 1998. Growth rate regulation of rRNA content of a marine *Synechococcus* (Cyanobacterium) strain. *Appl Environ Microbiol*, 64: 3346–3351.
- Bird D F & Kalff J. 1986. Bacterial grazing by planktonic lake algae. *Science*, 231: 493–495.
- Boenigk J, Pfandl K, Stadler P & Chatzinotas A. 2005. High diversity of the “*Spumella*-like” flagellates: an investigation based on the SSU rRNA gene sequence of isolates from habitats located in six different geographic regions. *Environ Microbiol*, 7: 685–697.

- Bonilla S, Villeneuve V & Vincent W F. 2005. Benthic and planktonic algal communities in a High Arctic lake: pigment structure and contrasting responses to nutrient enrichment. *J Phycol*, 41: 1120–1130.
- Borin S, Crotti E, Mapelli F, Tamagnini I, Corselli C & Daffonchio D. 2008. DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea. *Saline Sys*, 4: DOI 10.1186/1746-1448-4-10.
- Bottos E M, Vincent W F, Greer C W & Whyte L G. 2008. Prokaryotic diversity of arctic ice shelf microbial mats. *Environ Microbiol*, 10: 950–966.
- Bourrelly P. 1968. *Les algues d'eau douce II: Les algues jaunes et brunes*, Paris: Société Nouvelle des Éditions Boubée.
- Bråte J, Klaveness D, Rygh T, Jakobsen K S & Shalchian-Tabrizi K. 2010. Telonemia-specific environmental 18S rDNA PCR reveals unknown diversity and multiple marine-freshwater colonizations. *BMC Microbiol*, 10: DOI 10.1186/1471-2180-10-168.
- Brockman W, Alvarez P, Young S, Garber M, Giannoukos G, Lee W L, Russ C, Lander E S, Nusbaum C, & Jaffe D B. 2008. Quality scores and SNP detection in sequencing-by-synthesis systems. *Genome Res*, 18: 763–770.
- Brutemark A & Granéli E. 2011. Role of mixotrophy and light for growth and survival of the toxic haptophyte *Prymnesium parvum*. *Harmful Algae*, 10: 388–394.
- Butler H G, Edworthy M G & Ellis-Evans J C. 2000. Temporal plankton dynamics in an oligotrophic maritime Antarctic lake. *Freshwater Biol*, 43: 215–230.
- Calbet A, Martínez R A, Isari S, Zervoudaki S, Nejstgaard J C, Pitta P, Sazhin A F, Sousoni D, Gomes A, Berger S A, Tsagaraki T M & Ptacnik R. 2012. Effects of light availability on mixotrophy and microzooplankton grazing in an oligotrophic plankton food web: Evidences from a mesocosm study in Eastern Mediterranean waters. *J Exp Mar Biol Ecol*, 424-425: 66–77.
- Canter-Lund H & Lund W G J. 1995. *Freshwater algae: Their microscopic world explored*, Bristol, England: Biopress Limited.
- Caporaso J G, Bittinger K, Bushman F D, DeSantis T Z, Andersen G L & Knight R. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*, 26: 266–267.
- Caporaso J G, Kuczynski J & Stombaugh J. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*, 7: 335–336.
- Caron, D A, Sanders R W, Lim E L, Marrasé C, Amaral L A, Whitney S, Aoki R B & Porter K G. 1993. Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microb Ecol*, 25: 93–111.

- Caron D A, Porter K G & Sanders R W. 1990. Carbon, nitrogen, and phosphorus budgets for the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. *Limnol Oceanogr*, 35: 433–443.
- Caron D A. 1983. Technique for enumeration of heterotrophic and phototrophic nanoplankton, using epifluorescence microscopy, and comparison with other procedures. *Appl Environ Microbiol*, 46: 491–498.
- Cavalier-Smith T & Chao E E-Y. 2006. Phylogeny and megasystematics of phagotrophic heterokonts (Kingdom Chromista). *J Mol Evol*, 62: 388–420.
- Centre d'Études Nordiques. 2013. Environmental data from Northern Ellesmere Island in Nunavut, Canada, v. 1.0 (2002-2012). Nordicana DI, doi : 10.5885/44985SL-8F203FD3ACCD4138.
- Charvet S, Vincent W F, Comeau A & Lovejoy C. 2012a. Pyrosequencing analysis of the protist communities in a High Arctic meromictic lake: DNA preservation and change. *Front Microbiol*, 3: DOI 10.3389/fmicb.2012.00422.
- Charvet S, Vincent W F & Lovejoy C. 2012b. Chrysophytes and other protists in High Arctic lakes: molecular gene surveys, pigment signatures and microscopy. *Polar Biol*, 35: 733–748.
- Christoffersen K S, Amsinck S L, Landkildehus F, Lauridsen T L & Jeppesen E. 2008. Lake flora and fauna in relation to ice-melt, water temperature and chemistry at Zackenberg. *Adv Ecol Res*, 40: 372–389.
- Comeau A M, Li W K W, Tremblay J-É, Carmack E C & Lovejoy C. 2011. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. *PloS One*, 11: e27492.
- Comeau, A M, Harding T, Galand P E, Vincent W F & Lovejoy, C. 2012. Vertical distribution of microbial communities in a perennially stratified Arctic lake with saline, anoxic bottom waters. *Nature Sci Rep*, 2: DOI 10.1038/srep00604.
- Copland L, Mueller D R & Weir L. 2007. Rapid loss of the Ayles Ice Shelf, Ellesmere Island, Canada. *Geophys Res Lett*, 34: L21501.
- Côté G, Pienitz R, Velle G & Wang X. 2010. Impact of geese on the limnology of lakes and ponds from Bylot Island (Nunavut, Canada). *Internat Rev Hydrobiol*, 95: 105–129.
- Countway P D, Gast R J, Savai P & Caron D A. 2005. Protistan diversity estimates based on 18S rDNA from seawater incubations in the Western North Atlantic. *J Euk Microbiol*, 52: 95–106.
- Crane K W & Grover J P. 2010. Coexistence of mixotrophs, autotrophs, and heterotrophs in planktonic microbial communities. *J Theor Biol*, 262: 517–527.
- Daley RJ & Hobbie JE. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. *Limnol Oceanogr*, 20: 875–882.
- Danovaro R, Corinaldesi C, Anno AD, Fabiano M & Corselli C. 2005. Viruses, prokaryotes and DNA in the sediments of a deep-hypersaline anoxic basin (DHAB) of the Mediterranean Sea. *Environ Microbiol*, 7: 586–592.

- del Giorgio P A & Peters R H. 1993. Balance between phytoplankton production and plankton respiration in lakes. *Can J Fish Aquat Sci*, 50: 282–289.
- De Rijk P, Neefs J M, Van de Peer Y & De Wachter R. 1992. Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Res*, 20: 2075–2089.
- Diez B, Pedròs-Aliò C & Massana R. 2001. Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. *Appl Env Microbiol*, 67: 2932–2941.
- Ding Y, Zang R, Letcher S G, Liu S & He F. 2012. Disturbance regime changes the trait distribution, phylogenetic structure and community assembly of tropical rain forests. *Oikos*, 121: 1263–1270.
- Dolan J, Gallegos C & Moigis A. 2000. Dilution effects on microzooplankton in dilution grazing experiments. *Mar Ecol Prog Ser*, 200: 127–139.
- Dolan J R & McKeon K. 2005. The reliability of grazing rate estimates from dilution experiments: Have we over-estimated rates of organic carbon consumption by microzooplankton? *Ocean Sci*, 1: DOI 10.5194/os-1-1-2005.
- Doran P T, Wharton R A & Lyons W B. 1994. Paleolimnology of the McMurdo Dry Valleys, Antarctica. *J Paleolimnol*, 10: 85–114.
- Douglas M S V & Smol J P. 2000. Eutrophication and recovery in the high arctic: Meretta Lake (Cornwallis Island, Nunavut, Canada) revisited. *Hydrobiologia*, 431: 193–204.
- Dunthorn M, Klier J, Bunge J & Stoeck T. 2012. Comparing the hyper-variable V4 and V9 regions of the small subunit rDNA for assessment of ciliate environmental diversity. *J Eukaryot Microbiol*, 59: 185–187.
- Du Yoo Y, Jeong H J, Kang N S, Song J Y, Kim K Y, Lee G & Kim J. 2010. Feeding by the newly described mixotrophic dinoflagellate *Paragymnodinium shiwhaense*: feeding mechanism, prey species, and effect of prey concentration. *J Eukaryot Microbiol*, 57: 145–158.
- Edgar R C, Haas B J, Clemente J C, Quince C & Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27: 2194–2200.
- Edgcomb V, Orsi W, Leslin C, Epstein S S, Bunge J, Jeon S, Yakimov M M, Behnke A & Stoeck T. 2009. Protistan community patterns within the brine and halocline of deep hypersaline anoxic basins in the eastern Mediterranean Sea. *Extremophiles*, 13: 151–167.
- Edgcomb V P, Leadbetter E R, Bourland W, Beaudoin D & Bernhard J M. 2011a. Structured multiple endosymbiosis of bacteria and archaea in a ciliate from marine sulfidic sediments: a survival mechanism in low oxygen, sulfidic sediments? *Front Microbiol*, 2: DOI 10.3389/fmicb.2011.00055.
- Edgcomb V, Orsi W, Taylor G T, Vd'ačný P, Taylor C, Suarez P & Epstein S. 2011b. Accessing marine protists from the anoxic Cariaco Basin. *ISME J*, 5: 1237–1241.

- Epstein S S & Shiaris M P. 1992. Size-selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colorless flagellates, and ciliates. *Microb Ecol*, 23: 211–225.
- Esteban G F, Fenchel T & Finlay B J. 2010. Mixotrophy in ciliates. *Protist*, 161: 621–641.
- Falkowski P G & Knoll A H. 2007. *Evolution of Primary Producers in the Sea*, Academic Press, Elsevier Science.
- Findlay D & Kling H. 1979. *A Species List and Pictorial Reference to the Phytoplankton of Central and Northern Canada: Part I.*, Winnipeg, Manitoba: Department of Fisheries and the Environment.
- Finlay B J. 2002. Global dispersal of free-living microbial eukaryote species. *Science*, 296: 1061–1063.
- Flöder S, Hansen T & Ptacnik R. 2006. Energy-dependent bacterivory in *Ochromonas minima*- a strategy promoting the use of substitutable resources and survival at insufficient light supply. *Protist*, 157: 291–302.
- Flynn K J, Stoecker D K, Mitra A, Raven J A, Glibert P M, Hansen P J, Granelli E & Burkholder J M. 2012. Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *J Plankton Res*, 35: DOI 10.1093/plankt/fbs062.
- Foissner W, Müller H & Agatha S. 2007. A comparative fine structural and phylogenetic analysis of resting cysts in oligotrich and hypotrich Spirotrichea (Ciliophora). *Eur J Protistol*, 43: 295–314.
- Forsström L, Sorvari S, Korhola A & Rautio M. 2005. Seasonality of phytoplankton in subarctic Lake Saanajärvi in NW Finnish Lapland. *Polar Biol*, 28: 846–861.
- Gerhart D & Likens G. 1975. Enrichment experiments for determining nutrient limitation: four methods compared. *Limnol Oceanogr*, 20: 649–653.
- Gharizadeh B, Herman Z S, Eason R G, Jejelowo O & Pourmand N. 2006. Large-scale pyrosequencing of synthetic DNA: a comparison with results from Sanger dideoxy sequencing. *Electrophoresis*, 27: 3042–3047.
- Gibson J, Vincent W F, Van Hove P, Belzile C, Wang X & Muir D. 2002. Geochemistry of ice-covered, meromictic Lake A in the Canadian High Arctic. *Aquat Geochem*, 8: 97–119.
- González J M, Sherr B F & Sherr E. 1993. Digestive enzyme activity as a quantitative measure of protistan grazing: the acid lysozyme assay for bacterivory. *Mar Ecol Prog Ser*, 100:197–206.
- Granéli E & Carlsson P. 1998. The ecological significance of phagotrophy in photosynthetic flagellates. *NATO ASI Ser G Ecol Sci*, 41: 539–558.

- Hahn M W & Höfle M G. 1999. Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. *Appl Env Microbiol*, 65: 4863–4872.
- Hall T A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*, 41: 95–98.
- Hammer A & Pitchford J. 2005. The role of mixotrophy in plankton bloom dynamics, and the consequences for productivity. *ICES J Mar Sci*, 62: 833–840.
- Hammer Ø, Harper D & Ryan P. 2001. PAST : Paleontological statistics software package for education and data analysis. *Palaeontol Electron*, 4: 1-9.
- Hara S, Tanoue E, Zenimoto M, Komaki Y & Takahashi E. 1986. Morphology and distribution of heterotrophic protists along 75°E in the Southern Ocean. *Mem Natl Inst Polar Res*, 40: 69–80.
- Harding T, Jungblut A D, Lovejoy C & Vincent W F. 2011. Microbes in High Arctic snow and implications for the cold biosphere. *Appl Environ Microbiol*, 77: 3234–3243.
- Hartmann M, Grob C, Tarhan G A, Martin A P, Burkhill P H & Scanlan D J. 2012. Mixotrophic basis of Atlantic oligotrophic ecosystems. *PNAS*, 109: 5756–5760.
- Hasle G. 1978. Settling: the inverted microscope method. In Sournia A (ed.) *Phytoplankton Manual*. Paris: UNESCO Monographs on Oceanographic Methodology, pp.96.
- Hattersley-Smith G, Keys J E, Serson H & Mielke J E. 1970. Density stratified lakes in northern Ellesmere Island. *Nature*, 225: 55–56.
- Hendriks L, De Baere R, Van de Peer Y, Neefs J, Goris A & De Wachter R. 1991. The evolutionary position of the rhodophyte *Porphyra umbilicalis* and the Basidiomycete *Leucosporidium scottii* among other eukaryotes as deduced from complete sequences of small ribosomal subunit RNA. *J Mol Evol*, 32: 167–177.
- Hillebrand H, Dürselen C D, Kirschtel D, Pollingher U & Zohary T. 1999. Biovolume calculation for pelagic and benthic microalgae. *J Phycol*, 35: 403–424.
- Hitchman R B & Jones H L J. 2000. The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. *Freshwater Biol*, 43: 231–241.
- Hobbie J E, Bahr M, Bettez N & Rublee P A. 1999. Microbial food webs in oligotrophic Arctic Lakes. *Polar Microb Ecol*, 1–6.
- Hodson A, Anesio A M, Tranter M, Fountain A, Osborn M, Priscu J, Laybourn-Parry J & Sattler B. 2008. Glacial Ecosystems. *Ecol Monogr*, 78: 41–67.
- Holmgren S K. 1984. Experimental lake fertilization in the Kuokkel area, Northern Sweden: Phytoplankton biomass and algal composition in natural and fertilized subarctic lakes. *Int Revue ges Hydrobiol*, 69: 781–817.

- Hoppenrath M & Leander B S. 2006. Dinoflagellate, Euglenid, or Cercomonad? The ultrastructure and molecular phylogenetic position of *Protaspis grandis* n. sp. *J Eukaryot Microbiol*, 53:327–342.
- Huse S M, Huber J A, Morrison H G, Sogin M L & Welch D M. 2007. Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biol*, 8: R143.
- Huse S M, Welch D M, Morrison H G & Sogin M L. 2010. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environ Microbiol*, 12: 1889–1898.
- Jacquet S, Briand J, Leboulanger C, Avois-Jacquet C, Oberhaus L, Tassin B, Vinçon-Leite B, Paolini G, Druart J, Anneville O & Humbert, J-F. 2005. The proliferation of the toxic cyanobacterium following restoration of the largest natural French lake (Lac du Bourget). *Harmful Algae*, 4: 651–672.
- Jansson M, Blomqvist P, Jonsson A & Bergstrom A-K. 1996. Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Örträsket. *Limnol Oceanogr*, 41: 1552–1559.
- Jeffrey S W, Mantoura R F C & Wright S W. 1997. *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*, Paris: UNESCO.
- Jeffries M, Krouse H R, Shakur M A & Harris SA. 1984. Isotope geochemistry of stratified Lake “A”, Ellesmere Island, N.W.T., Canada. *Can J Earth Sciences*, 21: 1008–1017.
- Jeffries M & Krouse H. 1985. Isotopic and chemical investigation of two stratified lakes in the Canadian Arctic. *Zeit Glets Glazial*, 21: 71–78.
- Jeong H J, Du Yoo Y, Park J Y, Song J Y, Kim S T, Lee S H, Kim K Y & Yih W H. 2005. Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. *Aquat Microb Ecol*, 40: 133–150.
- Jeong H J, Du Yoo Y, Kang N S, Rho J R, Seong K A, Park J W, Nam G S & Yih W H. 2010. Ecology of *Gymnodinium aureolum*. I. Feeding in western Korean waters. *Aquat Microb Ecol*, 59: 239–255.
- Jones H J. 1997. A classification of mixotrophic protists based on their behaviour. *Freshwater Biol*, 37: 35–43.
- Jones H L J, Leadbeater B S C & Green J C. 1993. Mixotrophy in marine species of *Chrysocromulina* (Prymnesiophyceae): ingestion and digestion of a small green flagellate. *J Mar Biol Ass U.K.*, 73: 283–296.
- Jones H J, Cockell C S, Goodson C, Price N, Simpson A & Thomas B. 2009. Experiments on mixotrophic protists and catastrophic darkness. *Astrobiol*, 9: 563–571.
- Jones R. 2000. Mixotrophy in planktonic protists: an overview. *Freshwater Biol*, 45: 219–226.
- Jones R I & Rees S. 1994. Influence of temperature and light on particle ingestion by the freshwater phytoflagellate *Dinobryon*. *Archiv Hydrobiol*, 132: 203–211.

- Jungblut A, Lovejoy C & Vincent W F. 2010. Global distribution of cyanobacterial ecotypes in the cold biosphere. *ISME J*, 4: 191–202.
- Jurgens K, Wickham S A, Rothhaupt K O & Santer B. 1996. Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. *Limnol Oceanogr*, 41: 1833–1839.
- Kalff J, Kling H J, Holmgren S H & Welch H E. 1975. Phytoplankton, phytoplankton growth and biomass cycles in an unpolluted and in a polluted polar lake. *Verh Inter Ver Limnol*, 19: 487–495.
- Kamjunke N, Henrichs T & Gaedke U. 2007. Phosphorus gain by bacterivory promotes the mixotrophic flagellate *Dinobryon* spp. during re-oligotrophication. *J Plankton Res*, 29: 39–46.
- Kang N, Jeong H J, Du Yoo Y, Yoon E Y, Lee K H, Lee K & Kim G. 2011. Mixotrophy in the newly described phototrophic dinoflagellate *Woloszynska cincta* from western Korean waters: feeding mechanism, prey species and effect of prey concentration. *J Eukaryot Microbiol*, 58: 152–170.
- Katechakis A & Stibor H. 2006. The mixotroph *Ochromonas tuberculata* may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. *Oecologia*, 148: 692–701.
- Kembel S W, Cowan P D, Helmus M R, Cornwell W K, Morlon H, Ackerly D D, Blomberg S P & Webb C O. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26: 1463–1464.
- Kim D Y, Countway P D, Gast R J & Caron D A. 2011. Rapid shifts in the structure and composition of a protistan assemblage during bottle incubations affect estimates of total protistan species richness. *Microb Ecol*, 62: 383–398.
- Kim J S, Jeong H J, Strüeder-Kypke M C, Lynn D H, Kim S, Kim J H & Lee S H. 2005. *Parastrombidinopsis shimi* n. gen., n. sp. (Ciliophora: Choreotrichia) from the coastal waters of Korea: morphology and small subunit ribosomal DNA sequence. *J Eukaryot Microbiol*, 52: 514–522.
- Kimura B & Ishida Y. 1985. Photophagotrophy in *Uroglena americana*, Chrysophyceae. *Jap J Limnol*, 46: 315–318.
- Klaveness D, Shalchian-Tabrizi K, Thomsen H A, Eikrem W & Jakobsen K S. 2005. *Telonema antarcticum* sp. nov., a common marine phagotrophic flagellate. *Int J System Evol Microbiol*, 55: 2595–2604.
- Kooijman S A L M, Auger P, Poggiale J C & Kooi B W. 2003. Quantitative steps in symbiogenesis and the evolution of homeostasis. *Biol Rev*, 78: 435–463.
- Kozak A, 2005. Seasonal changes occurring over four years in a reservoir's phytoplankton composition. *Pol J Environ Stud*, 14: 451–465.

- Kremp A, Elbrächter M, Schweikert M, Wolny J L & Gottschling M. 2005. *Woloszynskia halophila* (Biecheler) comb. nov.: a bloom-forming cold-water dinoflagellate co-occurring with *Scrippsiella hangoei* (Dinophyceae) in the Baltic Sea. *J Phycol*, 41: 629–642.
- Kristiansen J. 2007. Dispersal and biogeography of silica-scaled chrysophytes. *Biodivers Conserv*, 17: 419–426.
- Kristiansen J, Wilken L R & Jürgensen T. 1995. A bloom of *Mallomonas acaroides*, a silica-scaled chrysophyte, in the crater pond of a pingo Northwest Greenland. *Polar Biol*, 15: 319–324.
- Kunin V, Engelbrektson A, Ochman H & Hugenholtz P. 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ Microbiol*, 12: 118–123.
- Lafarga-De la Cruz F, Valenzuela-Espinoza E, Millán-Núñez R, Trees C C, Santamaría-del-Ángel E & Núñez-Cembrero F. 2006. Nutrient uptake, chlorophyll *a* and carbon fixation by *Rhodomonas* sp. (Cryptophyceae) cultured at different irradiance and nutrient concentrations. *Aquacult Eng*, 35: 51–60.
- Laliberté G & De la Noüe J. 1993. Auto-, hetero-, and mixotrophic growth of *Chlamydomonas humicola* (Chlorophyceae) on acetate. *J Phycol*, 29: 612–620.
- Landry M R & Hassett R P. 1982. Estimating the grazing impact of marine micro-zooplankton. *Mar Biol*, 67: 283–288.
- Lauro F M, DeMaere M Z, Yau S, Brown M V, Ng C, Wilkins D, Raftery M J, Gibson J A E, Andrews-Pfannkoch C, Lewis M, Hoffman J M, Thomas T & Cavicchioli R. 2011. An integrative study of a meromictic lake ecosystem in Antarctica. *ISME J*, 5: 879–895.
- Laybourn-Parry J. 2002. Survival mechanisms in Antarctic lakes. *Philos T Roy Soc B*, 357: 863–869.
- Laybourn-Parry J & Marshall W A. 2003. Photosynthesis, mixotrophy and microbial plankton dynamics in two high Arctic lakes during summer. *Polar Biol*, 26: 517–524.
- Laybourn-Parry J, Marshall W A & Marchant H J. 2005. Flagellate nutritional versatility as a key to survival in two contrasting Antarctic saline lakes. *Freshwater Biol*, 50: 830–838.
- Lazarevic V, Whiteson K, Huse S, Hernandez D, Farinelli, L, Østerås M, Schrenzel J & François P. 2009. Metagenomic study of the oral microbiota by Illumina high-throughput sequencing. *J Microbiol Meth*, 79: 266–271.
- Lizotte M P. 2008. Phytoplankton and primary production. In Vincent W F & Laybourn-Parry J (eds.) *Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems*. London: Oxford University Press, pp.157–178.
- Logares R, Shalchian-Tabrizi K, Boltovskoy A & Rengefors K. 2007. Extensive dinoflagellate phylogenies indicate infrequent marine-freshwater transitions. *Mol Phylogenet Evol*, 45: 887–903.

- Lovejoy C, Legendre L, Martineau M-J, Bâcle J & von Quillfeldt C H. 2002. Distribution of phytoplankton and other protists in the North Water. *Deep-Sea Res II*, 49: 5027–5047.
- Lovejoy C, Vincent W F, Bonilla S, Roy S, Martineau M-J, Terrado R, Potvin M, Massana R & Pedrós-Alió C. 2007. Distribution, phylogeny, and growth of cold-adapted picoprasinophytes in Arctic seas. *J Phycol*, 43: 78–89.
- Lovejoy C, Massana R & Pedrós-Alió C. 2006. Diversity and distribution of marine microbial eukaryotes in the Arctic Ocean and adjacent seas. *Appl Env Microbiol*, 72: DOI 10.1128/AEM.72.5.3085.
- Lovejoy C & Potvin M. 2011. Microbial eukaryotic distribution in a dynamic Beaufort Sea and the Arctic Ocean. *J Plankton Res*, 33: 431–444.
- Lovejoy C, Vincent W F, Frenette J J & Dodson J J. 1993. Microbial gradients in a turbid estuary: application of a new method for protozoan community analysis. *Limnol Oceanogr*, 38: 1295–1303.
- Lozupone C & Knight R. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol*, 71: 8228–8235.
- Luddington I A, Kaczmarcza I & Lovejoy C. 2012. Distance and character-based evaluation of the V4 region of the 18S rRNA gene for the identification of diatoms (Bacillariophyceae). *PLoS One*, 7: e45664.
- Lundgren V & Granéli E. 2011. Influence of altered light conditions and grazers on *Scrippsiella trochoidea* (Dinophyceae) cyst formation. *Aquat Microb Ecol*, 63: 231–243.
- Luo W, Bock C, Li H R, Padisák J & Krienitz L. 2011. Molecular and microscopic diversity of planktonic eukaryotes in the oligotrophic Lake Stechlin (Germany). *Hydrobiologia*, 661: 133–143.
- Lynch M D J, Bartram A K & Neufeld J D. 2012. Targeted recovery of novel phylogenetic diversity from next-generation sequence data. *ISME J*, 6: 2067–2077.
- Majaneva M, Rintala J-M, Piisilä M, Fewer D P & Blomster J. 2011. Comparison of wintertime eukaryotic community from sea ice and open water in the Baltic Sea, based on sequencing of the 18S rRNA gene. *Polar Biol*, 35: 875–889.
- Maranger R, Bird D F & Price N M. 1998. Iron acquisition by photosynthetic marine phytoplankton from ingested bacteria. *Lett Nature*, 396: 248–251.
- Margulies M, Egholm M, Altman W E, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, 437: 376–380.
- Margulis L. 1993a. Serial endosymbiotic theory (SET) and composite individuality: transition from bacterial to eukaryotic genomes. *Microbiol Today*, 31: 172–174.
- Margulis L. 1993b. *Symbiosis in Cell Evolution* 2nd ed., New York: Freeman.

- Markager S & Sand-Jensen K. 1990. Heterotrophic growth of *Ulva lactuca* (Chlorophyceae). *J Phycol*, 26: 670–673.
- Markager S, Vincent W F & Tang E P Y, 1999. Carbon fixation by phytoplankton in High Arctic lakes : implications of low temperature for photosynthesis. *Limnol Oceanogr*, 44: 597–607.
- Marshall W & Laybourn-Parry J. 2002. The balance between photosynthesis and grazing in Antarctic mixotrophic cryptophytes during summer. *Freshwater Biol*, 47: 2060–2070.
- Massana R, Pedrós-Alio C, Casamayor E O & Gasol J M. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. *Limnol Oceanogr*, 46: 1181–1188.
- McFadden G. 2002. Primary and secondary endosymbiosis and the origin of plastids. *J Phycol*, 959: 951–959.
- Mcknight D M, Howes B L, Taylor C D & Goehringer D D. 2000. Phytoplankton dynamics in a stably stratified Antarctic lake during winter darkness. *J Phycol*, 36: 852–861.
- Medinger R, Nolte V, Pandey R V, Jost S, Ottenwälder B, Schlötterer C & Boenigk J. 2010. Diversity in a hidden world: potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Mol Ecol*, 19: 32–40.
- Medlin L, Elwood H J, Stickel S & Sogin M L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71: 491–499.
- Menden-Deuer S & Lessard E J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol Oceanogr*, 45: 569–579.
- Michelutti N, Douglas M S V & Smol J P. 2003. Diatom response to recent climatic change in a high arctic lake (Char Lake, Cornwallis Island, Nunavut). *Global Planet Change*, 38: 257–271.
- Miller C B. 2004. *Biological Oceanography*, Oxford: Blackwell Publishing.
- Møller E F, Thor P & Nielsen T G. 2003. Production of DOC by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* through sloppy feeding and leakage from fecal pellets. *Mar Ecol Prog Ser*, 262: 185–191.
- Montagnes D J S, Barbosa A B, Boenigk J, Davidson K, Jürgens K, Macek M, Parry J D, Roberts E C & Imek K. 2008. Selective feeding behaviour of key free-living protists: avenues for continued study. *Aquat Microb Ecol*, 53: 83–98.
- Montresor M, Lovejoy C, Orsini L, Procaccini G & Roy S. 2003. Bipolar distribution of the cyst-forming dinoflagellate *Polarella glacialis*. *Polar Biol*, 26: 186–194.
- Montresor M, Procaccini G & Stoecker D K. 1999. *Polarella glacialis*, Gen. Nov., Sp. Nov. (Dinophyceae): Suessiaceae are still alive! *J Phycol*, 35: 186–197.
- Moreira D & López-Garcia P. 2002. The molecular ecology of microbial eukaryotes unveils a hidden world. *TRENDS Microbiol*, 10: 31–38.

- Moustafa A, Evans A N, Kulis D M, Hackett J D, Erdner D L, Anderson D M & Bhattacharya D. 2010. Transcriptome profiling of a toxic dinoflagellate reveals a gene-rich protist and a potential impact on gene expression due to bacterial presence. *PLoS One*, 5: e9688.
- Mueller D R, Vincent W F & Jeffries M O. 2003. Break-up of the largest Arctic ice shelf and associated loss of an epishelf lake. *Geophys Res Lett*, 30: e2031.
- Mueller D R, Vincent W F & Jeffries M O. 2006. Environmental gradients, fragmented habitats, and microbiota of a northern ice shelf cryoecosystem, Ellesmere Island, Canada. *Arct Antarct Alp Res*, 38: L21501.
- Mueller D, Vincent W F, Bonilla S & Laurion I. 2005. Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol Ecol*, 53: 73–87.
- Mueller D, Van Hove P, Antoniades D, Jeffries M O & Vincent W F. 2009. High Arctic lakes as sentinel ecosystems: cascading regime shifts in climate, ice cover, and mixing. *Limnol Oceanogr*, 54: 2371–2385.
- Mullen P C & Warren S G. 1988. Theory of the optical properties of lake ice. *J Geophys Res*, 93: 8403–8414.
- Muller M. 1993. The hydrogenosome. *J Gen Microbiol*, 139: 2879–2889.
- Newbold L, Oliver A E, Booth T, Tiwari B, Desantis T, Maguire M, Andersen G, van der Gast C J & Whiteley A S. 2012. The response of marine picoplankton to ocean acidification. *Environ Microbiol*, 44: DOI 10.1111/j.1462-2920.2012.02762.x.
- Nicholls K H. 2009. Chrysophyte blooms in the plankton and neuston of marine and freshwater systems. In Sandgren C, Smol JP, & Kristiansen J, (eds.) *Chrysophyte Algae: Ecology, Phylogeny and Development*. New York: Cambridge University Press, pp. 181–213.
- Nicklisch A & Steinberg C E W. 2009. RNA/protein and RNA/DNA ratios determined by flow cytometry and their relationship to growth limitation of selected planktonic algae in culture. *Eur J Phycol*, 44: 297–308.
- Nielsen K M, Johnsen P J, Bensasson D, Daffonchio D. 2007. Release and persistence of extracellular DNA in the environment. *Environ Biosafety Res*, 6: 37–53.
- Nygaard K & Tobiesen A. 1993. Bacterivory in algae: A survival strategy during nutrient limitation. *Limnol Oceanogr*, 38: 273–279.
- Orsi W, Edgcomb V, Faria J, Foissner W, Fowle W H, Hohmann T, Suarez P, Taylor C, Taylor G T, Vd'acny P & Epstein S S. 2012. Class Cariacotrichea, a novel ciliate taxon from the anoxic Cariaco Basin, Venezuela. *IJSEM*, 62: 1425–1433.
- Pace N R, Olsen G J & Woese C R. 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. *Cell*, 45: 325–326.

- Pace N R, Sapp J & Goldenfeld N. 2012. Phylogeny and beyond: Scientific, historical, and conceptual significance of the first tree of life. *PNAS*, 109: 1011–1018.
- Packroff G & Woelfl S. 2000. A review on the occurrence and taxonomy of heterotrophic protists in extreme acidic environments of pH values ≤ 3 . *Hydrobiologia*, 433: 153–156.
- Panzenböck M, Möbes-Hansen B, Albert R & Herndl G J. 2000. Dynamics of phyto- and bacterioplankton in a high Arctic lake on Franz Joseph Land archipelago. *Aquat Microb Ecol*, 21: 265–273.
- Pawlowski J, Christen R, Lecroq B, Bachar D, Shahbazkia H R, Amaral-Zettler L & Guillou L. 2011. Eukaryotic richness in the abyss: insights from pyrotag sequencing. *PloS One*, 6: e18169.
- Pedrós-Alió C, Massana R, Latasa M, García-Cantizano J & Gasol J M. 1995. Predation by ciliates on a metalimnetic Cryptomonas population: feeding rates, impact and effects of vertical migration. *J Plankton Res*, 17: 2131–2154.
- Perrière G & Gouy M. 1996. WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie*, 78: 364–369.
- Pestová D, Macek M & Martínez Pérez M. 2008. Ciliates and their picophytoplankton-feeding activity in a high-altitude warm-monomictic saline lake. *Eur J Protistol*, 44: 13–25.
- Pfandl K, Chatzinotas A, Dyal P & Boenigk J. 2009. SSU rRNA gene variation resolves population heterogeneity and ecophysiological differentiation within a morphospecies (Stramenopiles, Chrysophyceae). *Limnol Oceanogr*, 54: 171–181.
- Pick F R & Lean D R S. 1984. Diurnal movements of metalimnetic phytoplankton. *J Phycol*, 20: 430–436.
- Pielou E. 1966. The measurement of diversity in different types of biological collections. *J Theor Biol*, 13: 131–144.
- Pienitz R, Douglas M S V & Smol J P. 2004. *Long-term Environmental Changes in Arctic and Antarctic lakes. Developments in Paleoenvironmental Research (DPER) Series, volume 8*, Dordrecht/Berlin/Heidelberg/New York: Springer-Berlag.
- Pollingher U. 1981. The structure and dynamics of the phytoplankton assemblages in Lake Kinneret, Israel. *J Plankton Res*, 3: 93–105.
- Pomeroy L R, Williams P J, Azam F & Hobbie J E. 1998. The microbial loop. *Oceanography*, 20: 28–33.
- Porter K G & Feig Y S. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr*, 25: 943–948.
- Potvin M & Lovejoy C. 2009. PCR-based diversity estimates of artificial and environmental 18S rRNA gene libraries. *J Eukaryot Microbiol*, 56: 174–181.

- Prescott L M, Harley J P & Klein D A. 2002. *Microbiology* 5th ed., McGraw-Hill.
- Price M N, Dehal P S & Arkin A P. 2010. FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS One*, 5: e9490.
- Ptacnik R, Sommer U, Hansen T & Martens V. 2004. Effects of microzooplankton and mixotrophy in an experimental planktonic food web. *Limnol Oceanogr*, 49: 1435–1445.
- Quesada A, Vincent W F & Lean D R S. 1999. Community and pigment structure of Arctic cyanobacterial assemblages: the occurrence and distribution of UV-absorbing compounds. *FEMS Microbiol Ecol*, 28: 315–323.
- Quince C, Lanzén A, Curtis T P, Davenport R J, Hall N, Head I M, Read L F & Sloan W T. 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. *Nature Methods*, 6: DOI 10.1038/NMETH.1361.
- Raven J. 2009. Comparative aspects of chrysophyte nutrition with emphasis on carbon, phosphorus and nitrogen. In Sandgren C, Smol J P, & Kristiansen J, (eds.) *Chrysophyte Algae: Ecology, Phylogeny and Development*. New York: Cambridge University Press, pp. 95–118.
- Raven J. 1997. Phagotrophy in phototrophs. *Limnol Oceanogr*, 42: 198–205.
- Rehman A, Shakoori F R & Shakoori A R. 2007. Heavy metal resistant *Distigma proteus* (Euglenophyta) isolated from industrial effluents and its possible role in bioremediation of contaminated wastewaters. *World J Microb Biot*, 23: 753–758.
- Reid F M H. 1983. Biomass estimation of components of the marine nanoplankton and picoplankton by the Utermöhl settling technique. *J Plankton Res*, 5: 235–252.
- Rengefors K, Laybourn-Parry J, Logares R, Marshall W A & Hansen G. 2008. Marine-derived dinoflagellates in Antarctic saline lakes: community composition and annual dynamics. *J Phycol*, 44: 592–604.
- Rice J, O'Connor C D, Sleigh M A, Burkhill P H, Giles I G & Zubkov M V. 1997. Fluorescent oligonucleotide rDNA probes that specifically bind to a common nanoflagellate, *Paraphysomonas vestita*. *Microbiology*, 143: 1717–1727.
- Richards T A, Vepritskiy A A, Gouliamova D E & Nierwicki-Bauer S A. 2005. The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake reveals diverse, distinctive and globally dispersed lineages. *Environ Microbiol*, 7: 1413–1425.
- Rigler F H. 1972. The Char Lake Project: a study of energy flow in a high Arctic lake. In Kajak Z & Hillbrecht-Illkowska A (eds.) *Productivity Problems of Freshwaters*. Poland Warszawa, Kraków: Kazimierz Dolny, pp. 287–300.
- Rigler F H. 1974. *The Char Lake Project PF-2, Final Report*. Canadian Committee for the International Biological Programme, Toronto.
- Rintala J-M, Spilling K & Blomster J. 2007. Temporary cyst enables long-term dark survival of *Scrippsiella hangoei* (Dinophyceae). *Mar Biol*, 152: 57–62.

- Roberts E C & Laybourn-Parry J. 1999. Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. *Freshwater Biol*, 41: 737–746.
- Ronaghi M, Karamohamed S, Pettersson B, Uhlén M & Nyrén P. 1996. Real-time DNA sequencing using detection of pyrophosphate release. *Anal Biochem*, 242: 84–89.
- Ronaghi M, Uhlén M & Nyrén P. 1998. A sequencing method based on real-time pyrophosphate. *Science*, 281: 363–365.
- Rothhaupt K O. 1996a. Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. *Ecology*, 77: 716–724.
- Rothhaupt K O. 1996b. Utilization of substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp. *Ecology*, 77: 706–715.
- Rothhaupt K O. 1997. Nutrient turnover by freshwater bacterivorous flagellates: differences between a heterotrophic and a mixotrophic chrysophyte. *Aquat Microb Ecol*, 12: 65–70.
- Round F E, Crawford R M & Mann D G. 1990. *The Diatoms: Biology and Morphology of the Genera*, Cambridge: Cambridge University Press.
- Roy S, Llewellyn, C A, Egeland E S & Johnsen G. 2011. *Phytoplankton Pigments : Characterization, Chemotaxonomy and Applications in Oceanography*, Cambridge University Press.
- Saccà A, Borrego C M, Renda R, Triadó-Margarit X, Bruni V & Guglielmo L. 2009. Predation impact of ciliated and flagellated protozoa during a summer bloom of brown sulfur bacteria in a meromictic coastal lake. *FEMS Microbiol Ecol*, 70: 42–53.
- Sakurai H, Ogawa T, Shiga M & Inoue K. 2010. Inorganic sulfur oxidizing system in green sulfur bacteria. *Photosynth Res*, 104: 163–76.
- Sanders R W. 2011. Alternative nutritional strategies in protists: symposium introduction and a review of freshwater protists that combine photosynthesis and heterotrophy. *J Eukaryot Microbiol*, 58: 181–184.
- Sanders R W & Gast R J. 2012. Bacterivory by phototrophic picoplankton and nanoplankton in Arctic waters. *FEMS Microbiol Ecol*, 82: 242–253.
- Sandgren C, Smol J P & Kristiansen J. 2009. *Chrysophyte Algae: Ecology, Phylogeny and Development*, New York: Cambridge University Press.
- Sanger F, Nicklen S & Coulson A R. 1977. DNA sequencing with chain-terminating inhibitors. *PNAS*, 74: 5463–5467.
- Sarmento H & Gasol J M. 2012. Use of phytoplankton-derived dissolved organic carbon by different types of bacterioplankton. *Environ Microbiol*, 14: 2348–2360.

- Scarella K. 2009. *La diversité de communautés microbiennes eucaryotes actives dans les océans canadiens: Analyses moléculaires de la diversité du gène d'ARNr 18S et de la nitrate réductase assimilatrice*. Thesis. Université Laval.
- Scheckenbach F, Wylezich C, Mylnikov A P, Weitere M & Arndt H. 2006. Molecular comparisons of freshwater and marine isolates of the same morphospecies of heterotrophic flagellates. *Appl Environ Microbiol*, 72: 6638–6643.
- Schindler DW & Smol JP. 2006. Cumulative effects of climate warming and other human activities on freshwaters of Arctic and Subarctic North America. *Ambio*, 35:160–168.
- Schindler D, Welch H E, Kalff J, Brunskill G J & Kritsch N. 1974. Physical and chemical limnology of Char lake, Cornwallis Island (75°N lat.). *J Fish Res Board Can*, 31: 585–607.
- Schloss P, Westcott S L, Ryabin, T *et al.* 2009. Introducing Mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*, 75: 7537–7541.
- Schnepf E & Kühn S F. 2000. Food uptake and fine structure of *Cryothecomonas longipes* sp. nov., a marine nanoflagellate *incertae sedis* feeding phagotrophically on large diatoms. *Helgol Mar Res*, 54: 18–32.
- Shalchian-Tabrizi K, Eikrem W, Klaveness D, Vaulot D, Minge M A, Le Gall F, Romari K, Throndsen J, Botnen A, Massana R, Thomsen H A & Jakobsen K S. 2006. Telonemia, a new protist phylum with affinity to chromist lineages. *Proc R Soc B*, 273: 1833–1842.
- Sheath RG. 1986. Seasonality of phytoplankton in northern tundra ponds. *Hydrobiologia*, 138: 75–83.
- Sherr E B & Sherr B F. 2002. Significance of predation by protists in aquatic microbial food webs. *Anton Leeuw*, 81: 293–308.
- Sieburth J M, Smetacek V & Lenz J. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol Oceanogr*, 23: 1256–1263.
- Simek K, Jürgens K, Nedoma J, Comerma M & Armengol J. 2000. Ecological role and bacterial grazing of *Halteria* spp.: small freshwater oligotrichs as dominant pelagic ciliate bacterivores. *Aquat Microb Ecol*, 22: 43–56.
- Simpson E H. 1949. Measurement of diversity. *Nature*, 163: 688.
- Skovgaard A, Hansen P J & Stoecker D K. 2000. Physiology of the mixotrophic dinoflagellate *Fragilidium subglobosum*. I. Effects of phagotrophy and irradiance on photosynthesis and carbon content. *Mari Ecol Prog Ser*, 201: 129–136.
- Skovgaard A, Legrand C, Hansen P J & Granéli E. 2003. Effects of nutrient limitation on food uptake in the toxic haptophyte *Prymnesium parvum*. *Aquat Microb Ecol*, 31: 259–265.

- Skuja H. 1948. Taxonomie des phytoplankton einiger seen in Uppland, Schweden. *Symbolae Botanicae Upsalienses*, 9: 1–399.
- Slapeta J, Moreira D & López-García P. 2005. The extent of protist diversity: insights from molecular ecology of freshwater eukaryotes. *Proc R Soc B*, 272: 2073–2081.
- Smith L M, Sander J Z, Kaiser R J, Hughes P, Dodd C, Connell C R, Heiner C, Kent S B H & Hood L E. 1986. Fluorescence detection in automated DNA sequence analysis. *Nature*, 321: 674–679.
- Smol J. 1988. Chrysophycean microfossils in paleolimnological studies. *Palaeogeogr Palaeoclimatol Palaeoecol*, 62: 287–297.
- Sogin M L & Gunderson J H. 1987. Structural diversity of eukaryotic small subunit ribosomal RNAs. *Ann NY Acad Sci*, 503: 125–139.
- Sogin M L, Morrison H G, Huber J A, Welch D M, Huse S M, Neal P R, Arrieta J M & Herndl G. 2006. Microbial diversity in the deep sea and the underexplored “rare biosphere”. *PNAS*, 103: 12115–12120.
- Spaulding S, McKnight D M, Smith R L & Dufford R. 1994. Phytoplankton population dynamics in perennially ice-covered Lake Fryxell, Antarctica. *J Plankton Res*, 16: 527–541.
- Spencer M A, Vick M C & Dick M W. 2002. Revision of *Aplanopsis*, *Pythiopsis*, and “subcentric” *Achlya* species (Saprolegniaceae) using 18S rDNA and morphological data. *Mycol Res*, 106: 549–560.
- Stickney H L, Hood R R & Stoecker D K. 2000. The impact of mixotrophy on planktonic marine ecosystems. *Ecol Model*, 125: 203 – 230.
- Stock A, Breiner H-W, Pachiadaki M, Edgcomb V, Filker S, La Cono V, Yakimov M M & Stoeck T. 2012. Microbial eukaryote life in the new hypersaline deep-sea basin Thetis. *Extremophiles*, 16: 21–34.
- Stoeck T, Zuendorf A, Breiner H-W & Behnke A. 2007. A molecular approach to identify active microbes in environmental eukaryote clone libraries. *Microb Ecol*, 53: 328–339.
- Stoeck T, Bass D, Nebel M, Christen R, Jones M D M, Breiner H-W & Richards T A. 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol Ecol*, 19: 21–31.
- Stoecker D K. 1998. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur J Protistol*, 34: 281–290.
- Stoecker D K. 1999. Mixotrophy among dinoflagellates. *J Eukaryot Microbiol*, 46: 397–401.
- Strom S L, Benner R, Ziegler S & Dagg M J. 1997. Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnol Oceanogr*, 42: 1364–1374.

- Takishita K, Yubuki N, Kakizoe N, Inagaki Y & Maruyama T. 2007. Diversity of microbial eukaryotes in sediment at a deep-sea methane cold seep: surveys of ribosomal DNA libraries from raw sediment samples and two enrichment cultures. *Extremophiles*, 11: 563–576.
- Tang Y Z, Egerton T A, Kong L & Marshall H G. 2008. Morphological variation and phylogenetic analysis of the dinoflagellate *Gymnodinium aureolum* from a tributary of Chesapeake Bay. *J Eukaryot Microbiol*, 55: 91–99.
- Taniguchi D A A, Franks P J S & Landry M R. 2012. Estimating size-dependent growth and grazing rates and their associated errors using the dilution method. *Limnol Oceanogr Meth*, 10: 868–881.
- Terrado R, Medrinal E, Dasilva C, Thaler M, Vincent W F & Lovejoy C. 2011. Protist community composition during spring in an Arctic flaw lead polynya. *Polar Biol*, 34: 1901–1914.
- Terrado R, Scarcella K, Thaler M, Vincent W F & Lovejoy C. 2012. Small phytoplankton in Arctic seas: vulnerability to climate change. *Biodiversity*, 13: 1–17.
- Theriot E C, Ashworth M, Ruck E, Nakov T & Jansen R K. 2010. A preliminary multigene phylogeny of the diatoms (Bacillariophyta): challenges for future research. *Plant Ecol Evol*, 143: 278–296.
- Thingstad T F, Havskum H, Garde K & Riemann B. 1996. On the strategy of “eating your competitor”: a mathematical analysis of algal mixotrophy. *Ecology*, 77: 2108–2118.
- Thomas D N, Fogg G E, Convey P, Fritsen C H, Gili J-M, Gradinger R, Laybourn-Parry J, Reid K & Walton D W H. 2012. *The Biology of Polar Regions*, Oxford University Press.
- Thompson J D, Gibson T J, Plewniak F, Jeanmougin F & Higgins D G. 1997. CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*, 25: 4876–4882.
- Tilman D. 1990. Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos*, 58: 3–15.
- Tittel J, Bissinger V, Zippel B, Gaedke U, Bell E, Lorke A & Kamjunke N. 2003. Mixotrophs combine resource use to outcompete specialists: implications for aquatic food webs. *PNAS*, 100: 12776–12781.
- Tomkins J D, Lamoureux S F, Antoniades D & Vincent W F. 2009. Sedimentology of perennial ice-covered, meromictic Lake A, Ellesmere Island, at the northern extreme of Canada. *Can J Earth Sci*, 46: 83–100.
- Troost T A, Kooi B W & Kooijman S A L M. 2005. Ecological specialization of mixotrophic plankton in a mixed water column. *Am Nat*, 166: E45–61.
- Tsai S-F, Xu D, Chung C-C & Chiang K-P. 2008. *Parastrombidinopsis minima* n. sp. (Ciliophora: Oligotrichia) from the coastal waters of northeastern Taiwan: morphology and small subunit ribosomal DNA sequence. *J Eukaryot Microbiol*, 55: 567–573.

- Tsuji T & Yanagita T. 1981. Improved fluorescent microscopy for measuring the standing stock of phytoplankton including fragile components. *Mar Biol*, 64: 207–211.
- Ueyama S, Katsumaru H, Suzaki T & Nakaoka Y. 2005. *Halteria grandinella*: a rapid swimming ciliate with a high frequency of ciliary beating. *Cell Motil Cytoskel*, 60: 214–221.
- Unrein F, Izaguirre I, Massana R, Balagué V & Gasol J M. 2005. Nanoplankton assemblages in maritime Antarctic lakes: characterisation and molecular fingerprinting comparison. *Aquat Microb Ecol*, 40: 269–282.
- Unrein F, Massana R, Alonso-Saez L & Gasol J M. 2007. Year-round effect of small mixotrophic flagellates on bacterioplankton in an ologitrophic coastal system. *Limnol Oceanogr*, 52: 456–469.
- Urabe J, Gurung TB, Yoshida T, Sekino T, Nakanishi M, Maruo M & Nakayama E. 2000. Diel changes in phagotrophy by *Cryptomonas* in Lake Biwa. *Limnol Oceanogr*, 45: 1558–1563.
- Utermöhl H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Angew Limnol*, 9: 1–38.
- Vallières C, Retamal L, Ramlal P, Osburn C & Vincent W F. 2008. Bacterial production and microbial food web structure in a large arctic river and the coastal Arctic Ocean. *J Mar Syst*, 74: 756–773.
- Van Hove P, Belzile C, Gibson J A E & Vincent W F. 2006. Coupled landscape-lake evolution in High Arctic Canada. *Can J Earth Sci*, 43: 533–546.
- Van Hove P, Vincent W F, Galand P E & Wilmette A. 2008. Abundance and diversity of picocyanobacteria in High Arctic lakes and fjords. *Algol Stud*, 126: 209–227.
- Vaulot D, Eikrem W, Viprey M & Moreau H. 2008. The diversity of small eukaryotic phytoplankton ($\leq 3 \mu\text{m}$) in marine ecosystems. *FEMS Microbiol Rev*, 32: 795–820.
- Veillette J, Mueller D R, Antoniades D & Vincent W F. 2008. Arctic epishelf lakes as sentinel ecosystems: Past, present and future. *J Geophys Res*, 113: DOI 10.1029/2008JG000730.
- Veillette J, Martineau M-J, Antoniades D, Sarrazin D & Vincent W F. 2011. Effects of loss of perennial lake ice on mixing and phytoplankton dynamics: insights from High Arctic Canada. *Ann Glaciol*, 51: 56–70.
- Veillette J, Muir D G, Antoniades D, Small J M, Spencer C, Loewen T N, Babaluk J A, Reist J D & Vincent W F. 2012. Perfluorinated chemicals in meromictic lakes on the northern coast of Ellesmere Island, High Arctic Canada. *Arctic*, 65: 245–256.
- Villeneuve V, Vincent W F & Komárek J. 2001. Community structure and microhabitat characteristics of cyanobacterial mats in an extreme high Arctic environment: Ward Hunt Lake. *Nova Hedwigia*, 123: 199–224.
- Vincent W F, Bowman J P, Rankin L M & McMeekin T A. 2000. Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems. In Brylinsky M, Bell C, & Johnson-

Green P (eds.) *Microbial Biosystems: New Frontiers. Proceedings of the Eighth International Symposium on Microbial Ecology*. Halifax: Atlantic Canada Society for Microbial Ecology, pp. 317–322.

Vincent A C, Mueller D R & Vincent W F. 2008a. Simulated heat storage in a perennially ice-covered high Arctic lake: Sensitivity to climate change. *J Geophys Res*, 113: DOI 10.1029/2007JC004360.

Vincent W F, Hobbie J E & Laybourn-Parry J. 2008b. Introduction to the limnology of high-latitude lake and river ecosystems. In Vincent W F & Laybourn-Parry J (eds.) *Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems*. London: Oxford University Press, pp. 1–24.

Vincent W F, Whyte L G, Lovejoy C, Greer C W, Laurion I, Suttle C A, Corbeil & Mueller D R. 2009. Arctic microbial ecosystems and impacts of extreme warming during the International Polar Year. *Polar Sci*, 3: 171–180.

Vincent W F. 2010. Microbial ecosystem responses to rapid climate change in the Arctic. *ISME J*, 4: 1087–1090.

Vincent W F, Fortier D, Lévesque E, Boulanger-Lapointe N, Tremblay B, Sarrazin D, Antoniades D & Mueller D R. 2011. Extreme ecosystems and geosystems in the Canadian High Arctic: Ward Hunt Island and vicinity. *Ecoscience*, 18: 236–261.

Voronin L V. 1997. Aquatic and aero-aquatic hyphomycetes in small lakes from Vorkuta vicinities. *Mikol Fitopatol*, 31: 9–17.

Vors N, Buck K R, Chavez F P, Eikrem W, Hansen L E, Ostergaard J B & Thomsen H A. 1995. Nanoplankton of the equatorial Pacific with emphasis on the heterotrophic protists. *Deep-Sea Res. II*, 42: 585–602.

Wacker A & Weithoff G. 2009. Carbon assimilation mode in mixotrophs and the fatty acid composition of their rotifer consumers. *Freshwater Biol*, 54: 2189–2199.

Wang H, Jiang L & Weitz J S. 2009. Bacterivorous grazers facilitate organic matter decomposition: a stoichiometric modeling approach. *FEMS Microb Ecol*, 69: 170–179.

Wang M & Overland J E. 2012. A sea ice free summer Arctic within 30 years: An update from CMIP5 models. *Geophys Res Lett*, 39: DOI 10.1029/2012GL052868.

Webb C O. 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. *Am Nat*, 156: 145–155.

Webb C O, Ackerly S D, McPeek M A & Donoghue M J. 2002. Phylogenies and community ecology. *Ann Rev Ecol System*, 33: 475–505.

Wehr J & Sheath R. 2003. *Freshwater Algae of North America: Ecology and Classification*, Academic Press, Elsevier Science.

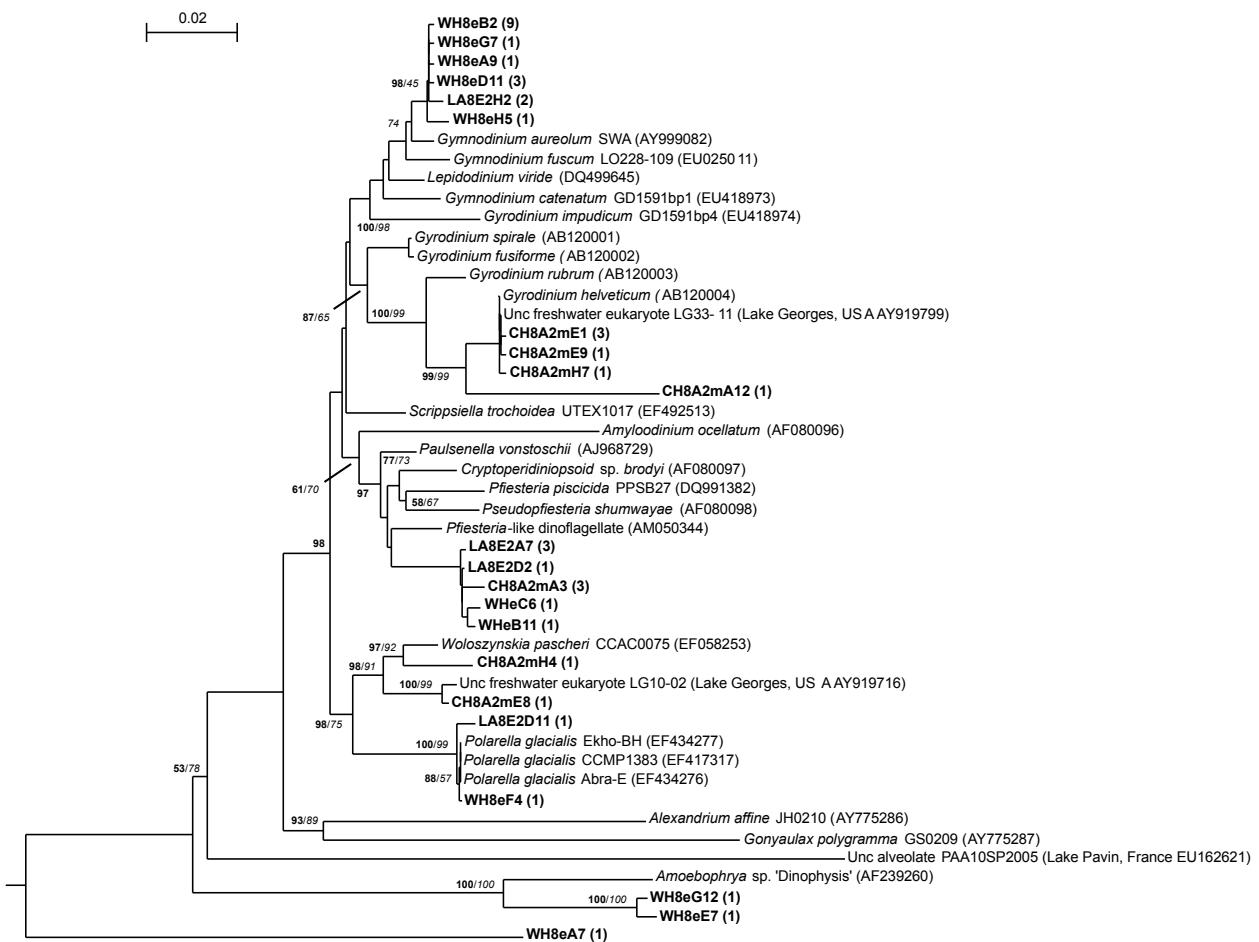
- Weithoff G & Wacker A. 2007. The mode of nutrition of mixotrophic flagellates determines the food quality for their consumers. *Funct Ecol*, 21: 1092–1098.
- Welch H J. 1973. Emergence of *Chironomidae* (Diptera) from Char Lake, Resolute, Northwest Territories. *Can J Zool*, 51: 1113-1123.
- Werner J J, Koren O, Hugenholtz P, DeSantis T Z, Walters W A, Caporaso J G, Angenent L T, Knight R & Ley R E. 2012. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *ISME J*, 6: 94–103.
- White J, Nagarajan N & Pop M. 2009. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Comput Biol*, 5: e1000352.
- Wilhelm S W & Suttle C A. 1999. Viruses and nutrient cycles in the sea. *BioScience*, 49: 781–788.
- Wilken L, Kristiansen J & Jürgensen T. 1995. Silica-scaled chrysophytes from the peninsula of Nuusuaq/Nûugssuaq. *Nova Hedwigia*, 61: 355–366.
- Wilken S, Huisman J, Naus-Wiezer S & Van Donk E. 2013. Mixotrophic organisms become more heterotrophic with rising temperature. *Ecol Lett*, 16: 225–233.
- Williamson C E, Saros J E, Vincent W F & Smol J P. 2009. Lakes and reservoirs as sentinels, integrators, and regulators of climate change. *Limnol Oceanogr*, 54: 2273–2282.
- Willoughby L G. 2001. The activity of *Rhizophlyctis rosea* in soil: some deductions from laboratory observations. *Mycologist*, 15: 113–117.
- Woese C R & Fox G E. 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *PNAS*, 74: 5088–5090.
- Woese C R, Kandler O & Wheelis M L. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *PNAS*, 87: 4576–4579.
- Wylezich C & Jürgens K. 2011. Protist diversity in suboxic and sulfidic waters of the Black Sea. *Environ Microbiol*, 13: 2939–2956.
- Yang E C, Boo G H, Kim H J, Cho S M, Boo S M, Andersen R A & Yoon H S. 2012. Supermatrix data highlight the phylogenetic relationships of photosynthetic stramenopiles. *Protist*, 163: 217–231.
- Yubuki N, Nakayama T & Inouye I. 2008. A unique life cycle and perennation in a colorless chrysophyte *Spumella* sp. *J Phycol*, 44: 164–172.
- Zapata M, Rodriguez F & Garrido J L. 2000. Separation of chlorophylls and carotenoids from marine phytoplankton : a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. *Mar. Ecol. Prog. Ser.*, 195: 29–45.
- Zhang J, Lindsay R, Schweiger A & Steele M. 2013. The impact of an intense summer cyclone on 2012 Arctic sea ice retreat. *Geophys Res Lett*. DOI 10.1002/grl.50190.

Zhang X & Watanabe M M. 2001. Grazing and growth of the mixotrophic chrysomonad *Poterioochromonas malhamensis* (Chrysophyceae) feeding on algae. *J Phycol*, 37: 738–743.

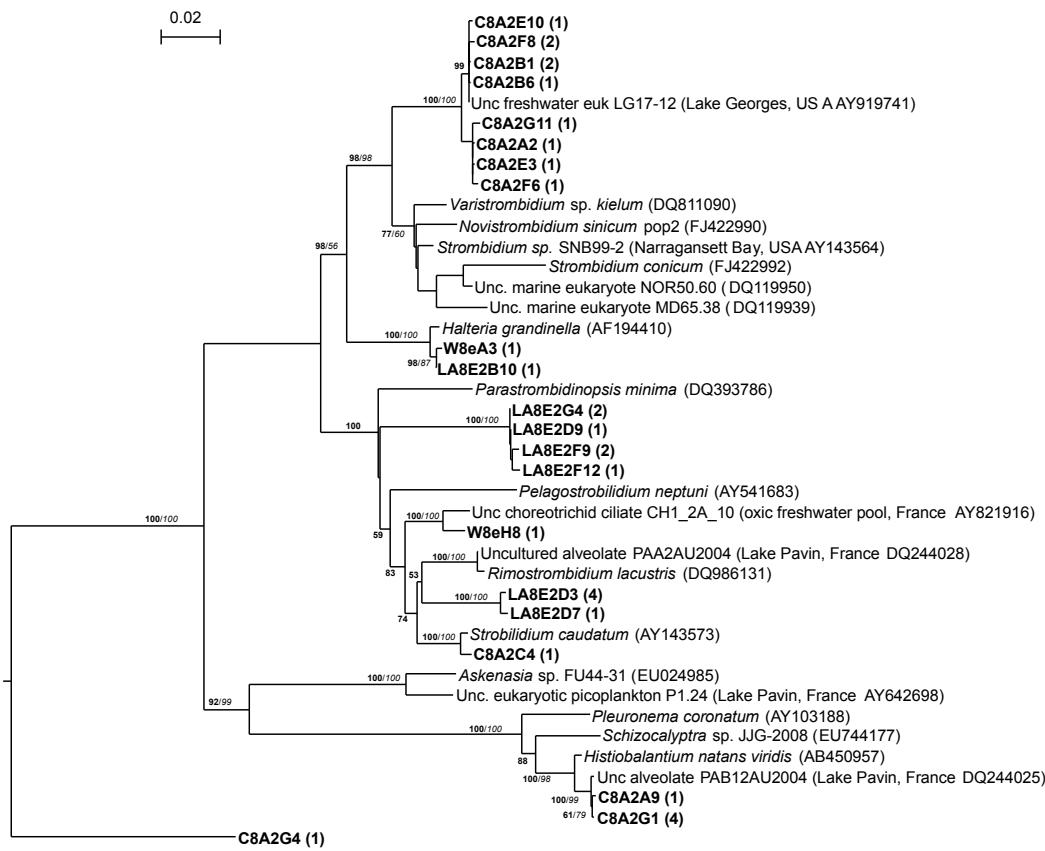
Zhu F, Massana R, Not F, Marie D & Vaulot D. 2005. Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiol Ecol*, 52: 79–92.

Annexes

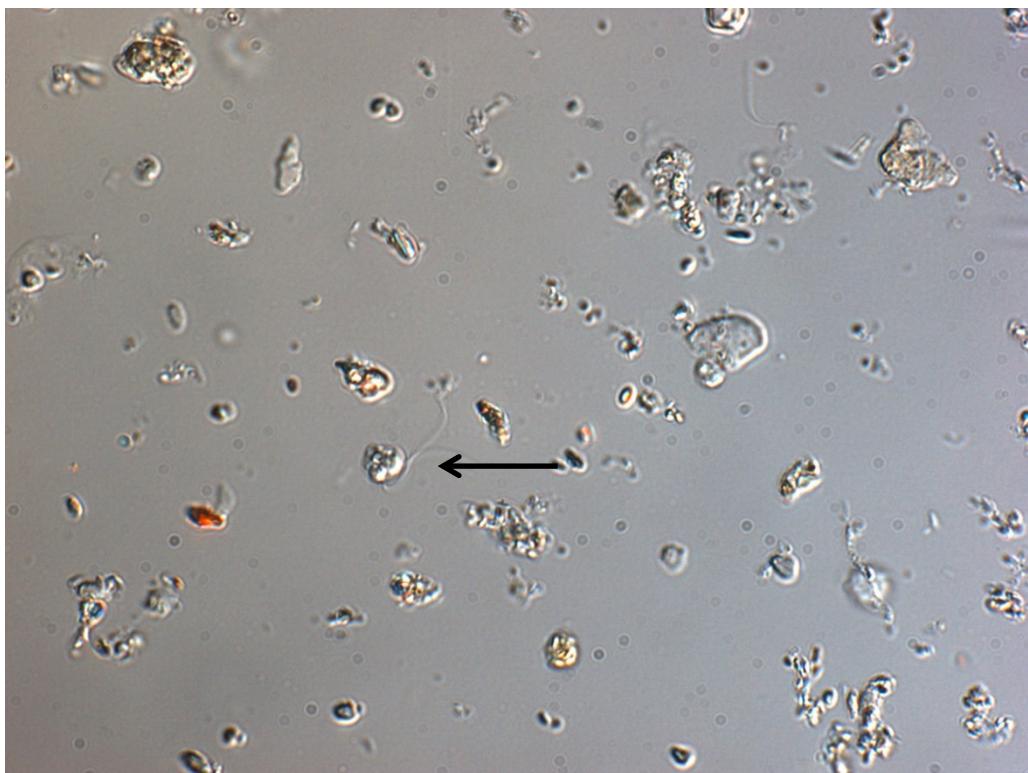
Annexe 1 Dinoflagellate 18S rRNA gene neighbour-joining phylogenetic tree. The outgroup (not shown) used to root this tree was the choanoflagellate *Monosiga brevicollis*. Bootstrap values (>50%) from the neighbour-joining tree are in bold, and those from the maximum-likelihood tree are in italics. Sequences from Char Lake (CH), Lake A (LA) and Ward Hunt Lake (WH) are in bold, with the number of clones for each sequence indicated in parentheses



Annexe 2 Ciliate 18S rRNA gene neighbour-joining phylogenetic tree. The outgroup (not shown) used to root this tree was the choanoflagellate *Monosiga brevicollis*. Bootstrap values (>50%) from the neighbour-joining tree are in bold, and those from the maximum-likelihood tree are in italics. Sequences from Char Lake (CH), Lake A (LA) and Ward Hunt Lake (WH) are in bold, with the number of clones for each sequence indicated in parentheses



Annexe 3. Planche photographique de *Erkenia subaequiciliata* observé par microscopie optique à 1000x dans le lac Ward Hunt, le 15 Août 2008.



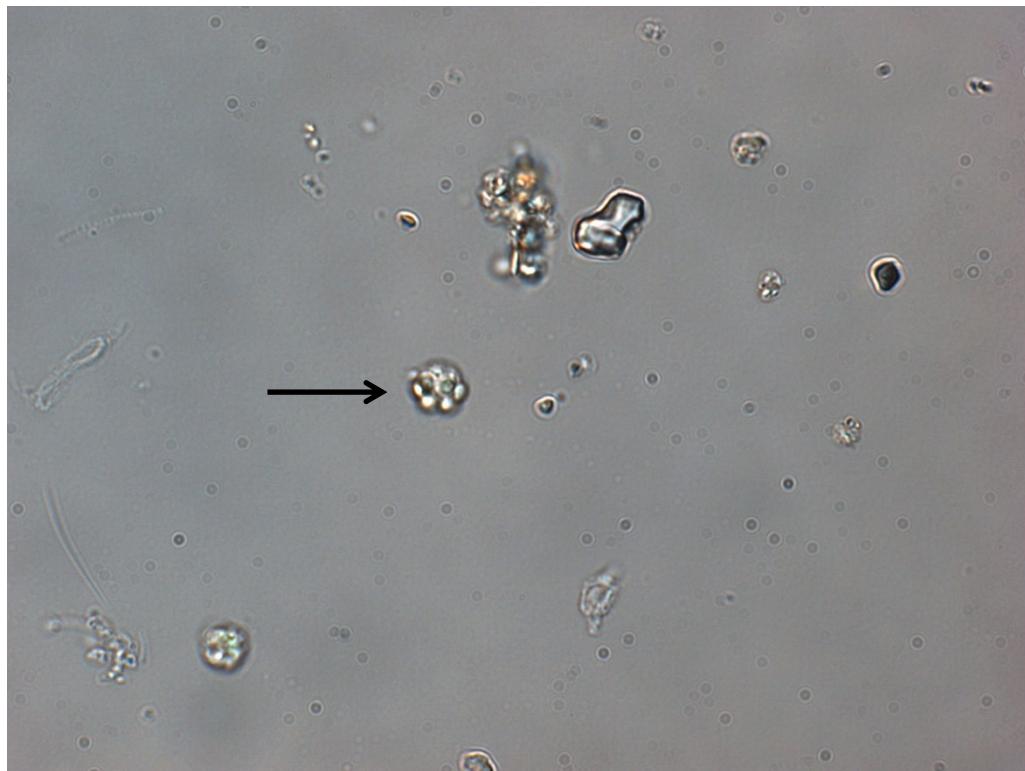
Annexe 4. Planche photographique de *Dinobryon sociale* observé par microscopie optique à 1000x dans le lac Char, le 11 Août 2008.



Annexe 5. Planche photographique de *Kephrion* observé par microscopie optique à 1000x dans le lac Ward Hunt, le 15 Août 2008.



Annexe 6. Planche photographique de *Pseudopedinella* observé par microscopie optique à 1000x dans le lac Char, le 11 Août 2008.



Annexe 7. Dispositif expérimental de l'expérience de lumière/dilution et continuum de ressources correspondant.

