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DIVERSITÉ MICROBIENNE DES MARES GÉNÉRÉES PAR LA FONTE DU PERGÉLISOL EN RÉGIONS ARCTIQUE ET SUBARCTIQUE

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Mémoire présenté
pour l'obtention
du grade de Maître ès sciences (M.Sc.)
en Sciences de l'eau

Jury d'évaluation

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RÉSUMÉ

Les mares de fonte sont formées par la dégradation locale du pergélisol en hautes latitudes. Les bouleversements climatiques actuels favorisent leur apparition dans les zones nordiques autour du globe. Les mares subarctiques en zone de pergélisol discontinu ont été échantillonnées au Nunavik au nord du Québec et résultent de l'affaissement de buttes pergélisolées. Elles sont caractérisées par une grande turbidité et une stratification thermique importante. Les mares arctiques en zone de pergélisol continu sont situées au Nunavut sur l'Île Bylot et sont des mares au creux de polygones à coin de glace et des canaux adjacents. Les mares arctiques sont moins profondes, plus transparentes et donc plus illuminées, entraînant la formation de tapis microbiens. Ces deux types de mare nordiques émettent des gaz à effet de serre (principalement du CO_2 et du CH_4) vers l'atmosphère et attirent de plus en plus l'attention des scientifiques. La diversité microbienne de ces plans d'eau reste par contre grandement méconnue, de même que les variables limnologiques qui la régissent. Trois approches complémentaires ont été déployées pour caractériser la flore microbienne des mares de fonte : la taxinomie du phytoplancton, l'analyse de pigments diagnostiques et l'analyse moléculaire des picoeukaryotes via le DGGE (*denaturing gradient gel electrophoresis*). Les mares se sont avérées être très différentes au niveau physique, optique et chimique, tant au sein d'un même site qu'au niveau inter-site. Les mares subarctiques sont souvent dominées par les Chlorophyceae et les Chrysophyceae alors que les Cyanophyceae sont prépondérantes dans les mares arctiques. La diversité microbienne est sensiblement plus faible en zone arctique, tant au niveau phytoplanctonique que pigmentaire. Les assemblages picoeukaryotes (avec le DGGE) varient au sein d'une même mare subarctique entre la surface et le fond puisque les conditions limnologiques y sont très différentes. Des analyses statistiques révèlent que parmi les variables mesurées, le K_dPAR (la lumière) et la pente spectrale (indice de qualité de la matière organique) seraient les deux facteurs influençant les patrons de répartition phytoplanctoniques. La diversité microbienne des mares de fonte s'est donc un peu dévoilée et des suggestions d'études futures sont finalement abordées.

AVANT-PROPOS

Le présent mémoire est du type « par article ». Il est constitué d'abord d'un premier chapitre-synthèse présentant l'état des connaissances de la problématique abordée. Les hypothèses et objectifs sont ensuite présentés, suivis d'un bref aperçu de l'approche méthodologique choisie. Les résultats sont également discutés succinctement. La seconde partie du mémoire (le chapitre 2) est constituée de l'article scientifique qui sera soumis à la revue Aquatic Microbial Ecology (facteur d'impact = 2.385). L'article dans sa forme actuelle comprend certainement plus d'information qu'il n'y en aura dans sa version finale soumise, mais j'ai préféré conserver certains détails (par ex. le tableau des pigments) afin de stimuler la discussion parmi les réviseurs du mémoire. Pour plus d'informations sur l'aspect « gaz à effet de serre des mares», en annexe se trouve l'article traitant de la variabilité des émissions de méthane et gaz carbonique des mares nordiques. Cet article est accepté et sera publié dans la revue Limnology and Oceanography.

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ADN: Acide désoxyribonucléique

ARN_r : acide ribonulcéique ribosomique

CA: Canonical analysis

CCA: Correspondence canonical analysis

CDOM : Chromophoric dissolved organic matter

Chl *a* : Chlorophylle *a*

DGGE : Denaturing gradient gel electrophoresis

DOC : Dissolved organic carbon

DOM: Dissolved organic matter

GES : Gaz à effet de serre

HPLC : High performance liquid chromatography

K_dPAR: Coefficient d'atténuation diffus

PAR: Photosynthetically available radiation

PCA: Principal component analysis

PCR: Polymerase chain reaction

POC : Particulate organic carbon

PPC: Photoprotective carotenoid

PSC: Photosynthetic carotenoid

S: Spectral slope

TP : Total phosphorus

TSS : Total suspended solids

CHAPITRE 1 : ÉTAT DES CONNAISSANCES

1 RÉCHAUFFEMENT CLIMATIQUE GLOBAL

L'apport anthropique de l'accumulation de gaz à effet de serre dans l'atmosphère n'est désormais plus à prouver et ce phénomène est devenu une préoccupation de premier ordre et ce, partout sur le globe. Il est désormais clair que l'activité humaine joue un rôle majeur quant à l'augmentation du carbone dans l'atmosphère (IPCC 2007). Le gaz carbonique (CO_2) et le méthane (CH_4) sont les principaux gaz émis, ce dernier étant environ 25 fois plus efficace pour retenir la chaleur par rapport au CO_2 (Wuebbles & Hayhoe 2002). La plupart des modèles climatiques globaux prédisent un réchauffement du climat de 3 à 5°C au cours des 60 prochaines années. De par leur nature, les terres se réchauffent plus rapidement que les océans et seront donc grandement affectées. De nombreuses études le soulignent : les pôles seront touchés sévèrement par les bouleversements climatiques et pourraient se réchauffer jusqu'à deux fois plus vite qu'ailleurs sur le globe (IPCC 2007) (ACIA 2005).

1.1 Fonte du pergélisol

Le pergélisol se définit comme étant un sol où la température ne dépasse pas 0 °C pour une période minimale de 2 ans (Washburn 1979). Le processus de fonte et de gel de la couche supérieure, appelée mollisol, du sol pergélisolé des régions polaires et subpolaires est un phénomène normal et saisonnier. Or, les récents changements climatiques accentuent et accélèrent l'altération et l'érosion des sols gelés en permanence. Une hausse de la température du pergélisol a été rapportée entre autres en Alaska (Osterkamp & Romanovsky 1999, Osterkamp 2005), au nord du Canada (Camill 2005, Walvoord & Striegl 2007), en

Sibérie (Pavlov 1994, Romanovsky et al. 2007) et en Europe du nord (Luoto & Seppala 2003, Malmer et al. 2005, Farbrot et al. 2007, Isaksen et al. 2007). Les températures des couches de pergélisol ont augmenté parfois jusqu'à 3°C depuis les années 1980 et une réduction de 7% de la zone de pergélisol saisonnière de l'hémisphère nord a été observé (IPCC 2007). Les zones marginales du pergélisol discontinu et continu sont particulièrement affectées, ce qui explique une plus grande présence de lacs et mares de thermokarst[1] en ces régions (Yoshikawa & Hinzman 2003, Smith et al. 2005, Osterkamp & Romanovsky 1999). Au Québec, en zone subarctique, une fonte accélérée du pergélisol a été observée au cours des 50 dernières années et il est avancé qu'au rythme actuel, le pergélisol pourrait disparaître de certaines zones d'ici 20 ans (Payette et al. 2004). Puisque le pergélisol occupe 20% de la surface de la planète (ACIA 2005), 25% de l'hémisphère nord (Zhang et al. 2003) et 25% du Québec, celui-ci constitue une composante majeure de notre environnement planétaire. Les paysages nordiques dépendent de la présence ou l'absence de pergélisol et sont façonnés par l'épaisseur de la couche qui gèle et dégèle selon les saisons dite « couche active ». Il est clair

[1] **Note**: Dans la littérature, plusieurs expressions sont utilisées pour désigner une mare, incluant de simples petites étendues d'eau dans la toundra et les mares thermokarstiques: *tundra pond, peat pond, tundra lake, shallow lake, thermokarst pond*, etc. Le sens du terme « thermokarst » est plus ou moins large selon les auteurs. Dans le cas du présent mémoire, pour plus de simplicité, les termes « mare thermokarstique », « mare nordique » et surtout « mare de fonte » et en anglais, « thaw pond » s'appliquent tant pour les mares subarctiques que les mares arctiques. Nous sommes conscients des différences géomorphologiques de ces deux écosystèmes mais l'essence de cette étude n'étant pas la caractérisation géomorphologique, nous avons opté pour une désignation commune afin d'en faciliter la compréhension: mares de fonte ou mare nordique.

qu'une régression du pergélisol affectera grandement les composantes hydrologiques terrestres telles l'accumulation et l'abondance de l'eau douce de même que la répartition de la végétation toundrique (Peterson et al. 2002).

1.1.2 Apparition de mares de fonte

La dégradation locale du pergélisol, qu'elle soit d'origine naturelle ou anthropique, stimule le processus de formation des mares de fonte. Celles-ci sont des dépressions remplies d'eau causées par l'affaissement de la structure du sol (souvent des palses) lors de la fonte (Figure 1). Le sol qui reste gelé en profondeur permet ainsi une rétention de l'eau en l'empêchant de s'infiltrer, telle une couche imperméable (Yoshikawa & Hinzman 2003 , Brouchkov et al. 2004). (Payette et al. 2004) souligne que la fonte accélérée du pergélisol induit une occupation croissante des mares de thermokarst dans la zone de pergélisol discontinu. Un réchauffement plus intense peut entraîner une dégradation plus prononcée des mares par un drainage et causer ultimement, leur disparition (Yoshikawa & Hinzman 2003). Des comparaisons d'images satellites récentes et datant de 1970 par (Smith et al. 2005) révèlent un important déclin de l'abondance des lacs et mares en Sibérie lorsque la fonte du sol s'intensifie et ce, malgré une augmentation des précipitations. Les plans d'eau sont lentement envahis par la végétation et ne se reforment plus par la suite. Les patrons de fonte suggèrent fortement que c'est la disparition du pergélisol qui induit le drainage ultime de tous ces lacs. Malgré l'intérêt grandissant pour ces écosystèmes, le cycle de formation des mares de fonte reste encore fort méconnu.

1.1.1.1 Les mares en zone de pergélisol discontinu

Les mares de fonte en régions subarctiques se développent en zone de pergélisol discontinu. Au Québec, la zone de pergélisol discontinu se situe entre les latitudes 53°N et 58°N et est caractérisée par un mollisol d'une épaisseur maximale d'environ 1m dans les tourbières (Couillard & Payette 1985, Allard & Séguin 1987). Les mares de fonte échantillonnées lors de la présente étude (55°N) se situent dans une zone où moins de 50% de la surface est sous régime pergélisolique (Allard & Séguin 1987). Sans entrer dans les détails des processus géomorphologiques, celles-ci peuvent se former sur deux grands types de substrats : une assise tourbeuse ou un limon argileux. Notre site d'étude en zone subarctique près de la municipalité de Whapmagoostui-Kuujjuarapik présente des mares au fond argileux. En effet, selon (Vincent 1989), les plaines côtières près de la Baie d'Hudson sont caractérisées par une prédominance des dépôts marins et glaciolacustres laissés par l'ancienne Mer de Tyrrell. On y retrouve donc généralement des limons et argiles d'origine marines. Ces mares subarctiques peu profondes (< 3 m) sont sujettes à l'exposition éolienne et à une turbidité élevée ((Breton 2007) et résultats non-publiés). Conséquemment, la lumière ne pénètre pas profondément et il en résulte une stratification thermique qui entraîne à son tour une stratification oxique de par le manque de mélange dans toute la colonne d'eau et l'absence de photosynthèse en profondeur. Les sédiments au fond des mares en zone de pergélisol discontinu n'offrent donc pas un substrat adéquat pour l'établissement de macrophytes et/ou tapis microbiens.

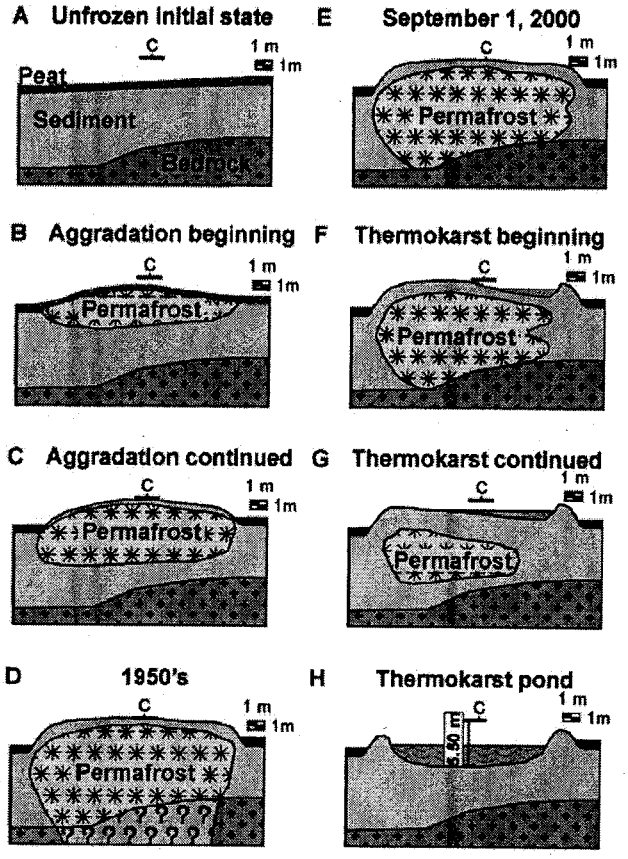

Figure 1 Processus de formation et dégradation d'une palse en zone de pergélisol discontinu, créant du coup une mare thermokarstique. Tiré de (Seppala 1986, Calmels et al. 2008).

1.1.1.2 Les mares en zone de pergélisol continu

Le pergélisol dit continu est défini par un pourcentage de 90 à 100% de sol gelé (Ressources Naturelles Canada 2007). En régions arctiques, la surface des tourbières peut se soulever par le gel. Ce phénomène s'explique en partie par l'occurrence de « polygones à coin de glace ». Un coin de glace correspond à un triangle de glace renversé enfoncé dans la tourbe et le sol minéral gelé environnant (Figure 2). Ces fentes de glaces sont gelées pendant la saison hivernale et se gorgent d'eau au printemps lors de la fonte de la neige en surface.

Conséquemment, les coins de glace ont tendance à s'élargir avec les années. L'apparition de plusieurs fentes de glace entraîne la formation de tout un réseau de polygones et de sillons à l'aspect visuel très caractéristique et singulier (Payette 2001). Ces polygones présentent parfois une dépression en leur centre, on les désigne alors comme étant des « polygones à centre déprimé » (Figure 3). Ces creux peuvent être soit remplis d'eau stagnante soit drainés et laissant place à une végétation graminoïde hydrophile ou mésique selon la hauteur du centre du polygone (Fortier et al. 2007). Notre équipe a échantillonné les mares situées dans les creux des polygones de même que dans les sillons environnants.

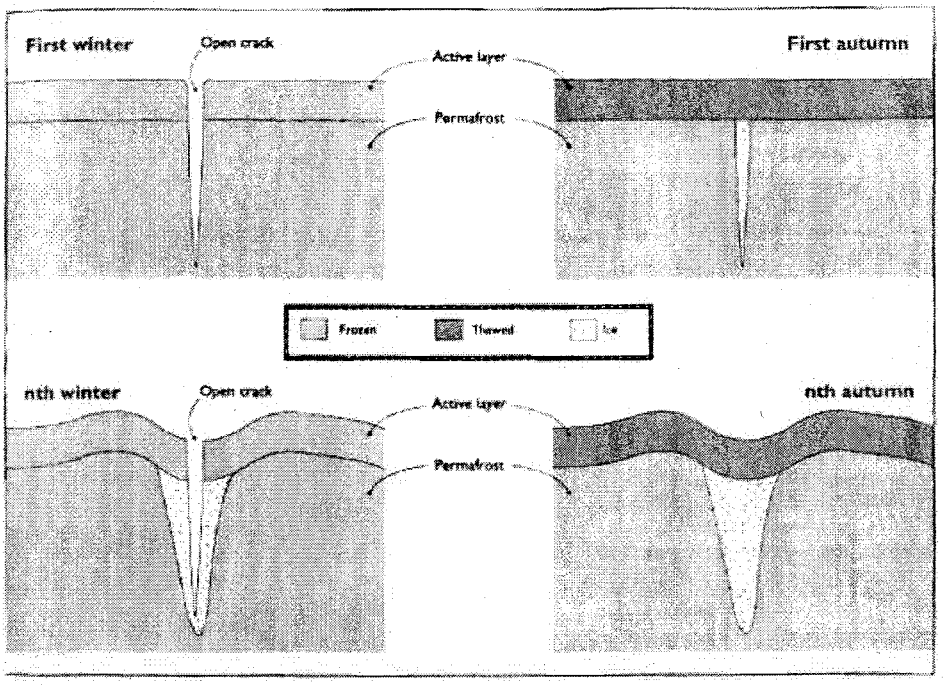

Figure 2 Processus simplifié de l'apparition des « coins de glace » (*ice wedges*). Tiré de (Trenhaile 1998).

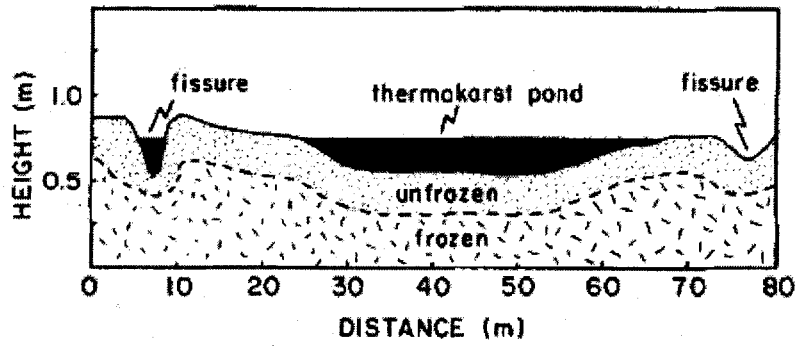

Figure 3 Mare de fonte sur un polygone à centre déprimé. Tiré de (Sheath 1986).

Les mares formées sur les polygones à coin de glace et dans les sillons sont très peu profondes (< 0.5 m) et la turbidité est relativement comparable à celle de lacs rocheux environnants (données non publiées, I. Laurion). La lumière pénètre facilement jusqu'au fond et il ne semble pas y avoir de stratification significative qui empêcherait le mélange de toute la colonne d'eau. Ces conditions particulières facilitent l'apparition de tapis microbiens (phytobenthos) et de macrophytes. L'étude de (Vezina & Vincent 1997) souligne d'ailleurs que 90% des mares échantillonnées sur l'Île Bylot contenaient un épais tapis microbien mucilagineux avec une coloration de surface allant de l'orangé au brun. Les cyanobactéries dominent (77% de la communauté lors de leurs analyses à cette période) l'assemblage de ces tapis benthiques. Avec une si petite colonne d'eau, la biomasse benthique s'avère dramatiquement plus élevée que celle pélagique et joue un rôle de premier plan dans la boucle microbienne. En effet, le phytobenthos peut s'avérer être une source majeure de carbone organique pour les communautés pélagiques (Sand-Jensen & Borum 1991). De plus, dans les lacs très peu profonds où la lumière atteint facilement les sédiments, l'abondance d'algues périphytiques peut affecter négativement la biomasse phytoplanctonique pélagique puisque les tapis absorbent une grande fraction des nutriments disponibles dans l'eau et ceux

minéralisés dans les sédiments. À l'opposé, le phytoplancton n'aurait qu'un impact négligeable sur le phytobenthos (Hansson 1988). Selon les études de Hobbie (1980) sur des mares toundriques en Alaska, une grande partie du carbone total aquatique est sous forme de DOC (carbone organique dissous; *dissolved organic carbon*) et la production benthique compte pour presque la moitié de toute la production primaire lacustre alors que les macrophytes sont responsables pour l'autre fraction. Seulement 3% de la production primaire serait l'apanage du phytoplancton pélagique. Une étude dans dix mares toundriques dans le Québec subarctique et en Haut-Arctique canadien indique d'ailleurs que la communauté benthique représenterait entre 60 et 96% de la productivité primaire par unité de masse selon le site (Rautio & Vincent 2006). La Figure 4 démontre bien l'apport important au flux de carbone des détritus de macrophytes accumulés dans les sédiments (*particulate organic carbon* ; POC). La majeure partie du POC est ensuite respirée par la faune et flore benthique, créant ainsi un flux de CO_2, soit capté par les végétaux aquatiques émergents ou libéré dans l'atmosphère.

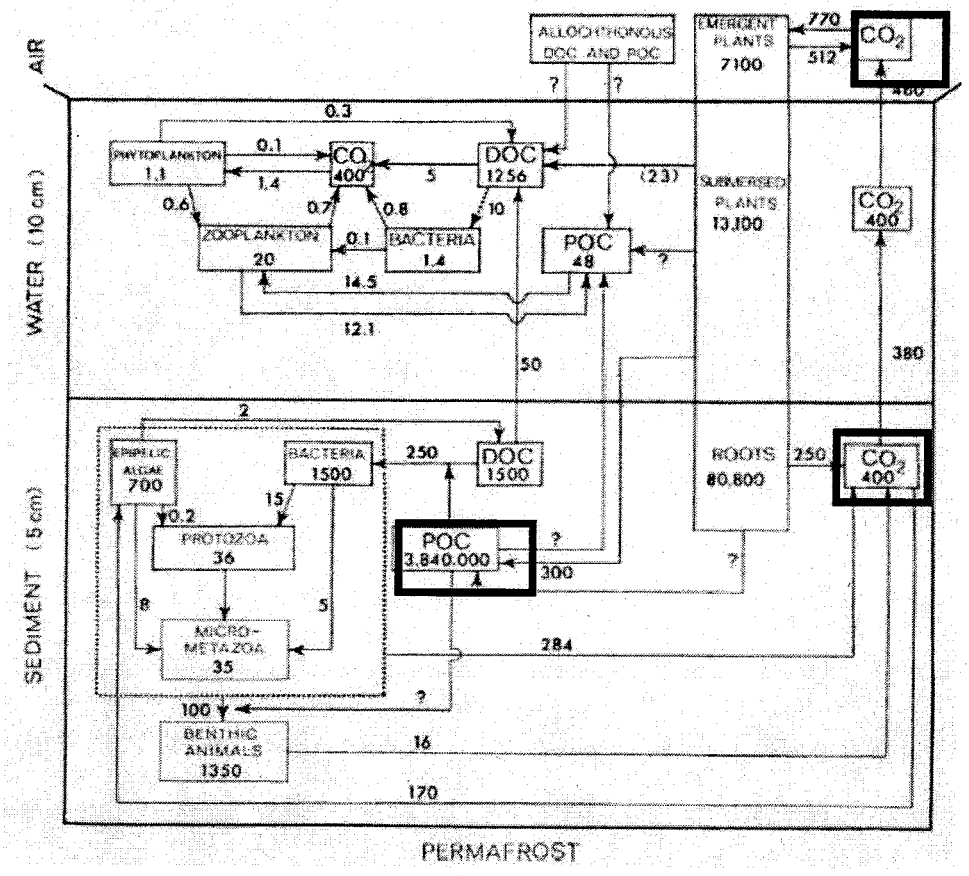

Figure 4 Flux de carbone entre les différentes composantes d'une mare arctique peu profonde en Alaska. Tiré de (Hobbie 1980) dans (Wetzel 2001).

1.2 Un écosystème hors du commun

Tant aux latitudes subarctiques qu'arctiques, les mares formées par la fonte du pergélisol ne se comparent pas à aucun autre écosystème nordique. Les mares subissent de grandes variabilités thermiques et lumineuses. La période de croissance est courte mais intense grâce à l'été arctique où le soleil brille pratiquement 24h/24. Aussi, plus particulièrement dans les mares arctiques peu profondes et limpides, la communauté algale s'expose à de hauts niveaux de PAR (*photosynthetically available radiation*) et de rayons UV (Roos & Vincent 1998), créant un amalgame particulier de pigments photoprotecteurs et photosynthétiques.

Le coefficient d'atténuation diffus (*diffuse attenuation coefficient* ou *vertical attenuation coefficient*, K_d PAR) est un indice utilisé pour décrire la pénétration de la lumière dans l'eau (Kirk 2000) et fait partie de ce que l'on appelle « les propriétés apparentes de l'eau ». Ce coefficient permet de décrire la turbidité d'un plan d'eau (Arst et al. 2008) et est fréquent en limnologie. Puisque les mares de fonte présentent de grandes variabilités tant au niveau de la matière particulaire que dissoute, une estimation de l'atténuation de la lumière pourrait s'avérer être une composante environnementale importante quant à la régulation de la communauté microbienne.

Il n'existe pas de définitions claires dans la littérature quant à la nomenclature des mares et lacs de fonte. Dans plusieurs cas, il est stipulé que les mares gèlent complètement en hiver (Hobbie 1980, Van Geest et al. 2007b). Or, si c'est probablement le cas pour les mares situées dans les creux des polygones à coin de glace, les mares subarctiques du site KWK ne gèlent pas jusqu'au fond, créant une zone anoxique hivernale (Laurion et al., soumis). De plus, contrairement aux lacs, les mares ne contiennent pas de poissons et cet élément affecte la structure et la complexité de toute la chaîne trophique (le zooplancton est au sommet de la chaîne). En effet, les niveaux trophiques se résument ici aux producteurs primaires et aux herbivores planctivores. Le genre zooplanctonique *Daphnia* peut atteindre de hautes concentrations dans ces milieux nordiques (O'Brien et al. 2004). Il s'agit ici d'un cas de relation trophique « bottom up » puisqu'en l'absence de prédateurs, c'est principalement la quantité et la qualité du phytoplancton qui affecte les populations d'herbivores, malgré que les conditions environnementales aient également leur importance (Van Geest et al. 2007b). Une étude de (Van Geest et al. 2007a) sur l'impact de l'ajout de nutriments par les excréments d'oies sur les brouteurs de phytoplancton sur l'île de Svaldbard en Norvège

révèle une absence de corrélation positive entre la concentration de chl *a*, le TP et l'azote inorganique dissous (alors que c'est habituellement le cas dans les lacs de plus basses latitudes). Ceci s'expliquerait en grande partie par la grande efficacité de broutage de *Daphnia* : l'ajout de nutriments active bel et bien la production algale mais stimule du coup le broutage et conséquemment, la production primaire est « canalisée » vers *Daphnia* sans augmentation de la biomasse algale. Une limitation en azote pourrait aussi expliquer la faible croissance algale.

1.3 Émissions de gaz à effet de serre par les milieux nordiques

L'essence même du présent mémoire ne réside pas dans la quantification des émissions de GES (gaz à effet de serre) par les mares arctiques et subarctiques mais il est important de souligner l'apport de ce milieu au cycle global du carbone. De plus, le gaz carbonique et le méthane sont, après tout, formés en partie par la flore microbienne des mares et deviennent de surcroît une conséquence et un témoignage de l'activité biologique qui prend place dans cet écosystème.

La toundra est un environnement caractérisé par des conditions climatiques extrêmes : froids intenses, pluie rare et vents violents. Les arbres y sont quasiment absents et ce sont les lichens, mousses et graminées de faible taille qui dominent le paysage. Au Canada, sa limite méridionale s'étend du delta du Mackenzie jusqu'au sud de la baie d'Hudson et, dans le Nord-Est, jusqu'au Labrador (Terasme 2007). La toundra arctique a longtemps été considérée comme un puits pour le carbone atmosphérique mais ces calculs n'incluaient pas l'apport des milieux aquatiques. Il est appréhendé que cet apport au cycle du carbone accentue la boucle de rétroaction positive du climat. En effet, les énormes quantités de carbone séquestrées dans

les sols des zones en hautes latitudes impliquent qu'un changement régional du stockage ou des flux de carbone peut avoir des répercussions substantielles sur le bilan global de carbone (Chapin et al. 2000).

Le CO_2 et le CH_4 sont les deux principaux gaz à effet de serre (avec l'eau) émis par les écosystèmes nordiques. Le catabolisme microbien explique une grande proportion de l'oxydation du carbone organique dans les environnements aquatiques (Canfield et al. 2005) et terrestres. À l'heure actuelle, il n'existe pas de consensus quant à l'impact réel du réchauffement climatique sur l'environnement toundrique : Certains soutiennent qu'une augmentation de température entraînent des sols plus secs et plus chauds, favorisant la respiration du carbone stocké dans le sol (Keyser et al. 2000, McGuire et al. 2000) et d'autres affirment que ces conditions stimuleraient la production végétale et favoriseraient donc la séquestration supplémentaire de carbone (McKane et al. 1997).

1.3.1 Les tourbières

Les tourbières où se forment les mares de thermokarst occupent 30% du paysage circumboréal et celles-ci stockent environ 30% du carbone total contenu dans les sols de la planète (Gorham 1991). Cet écosystème s'est révélé être un puits de carbone considérable au cours de l'Holocène jusqu'au Petit Âge glaciaire, période entre 1550 et 1860, où un refroidissement climatique a permis l'expansion du pergélisol et a gelé presque complètement les tourbières (Worsley et al. 1995). Actuellement, les tourbières sont encore considérées comme un puits de carbone pour l'atmosphère mais leurs capacités peuvent varier grandement selon les paramètres environnementaux, géographiques et climatiques (Waddington & Roulet 2000). Les estimations de la quantité de carbone enfouie dans les

tourbières varient d'une étude à l'autre mais sont du même ordre de grandeur : Maltby & Immirzi (1993) estiment le pool total de carbone tourbique à environ 462 Gt de carbone et Gorham, (1991) à 455Gt.

1.3.2 Les lacs boréaux

Les écosystèmes aquatiques constituent un pool significatif de carbone organique au même titre que les tourbières (Molot & Dillon 1996). Dean & Gorham (1998) ont estimé que les lacs au niveau planétaire accumuleraient 42Gt de carbone par an. Au niveau des zones boréales, l'accumulation des lacs serait de l'ordre de 18 à 31 Gt de carbone par an (Molot & Dillon 1996). On peut avancer qu'en général, environ 90% des lacs boréaux sont sursaturés en CO_2 ex.(Cole et al. 1994). Lors d'une de leurs études, (Kling et al. 1991) ont démontré que 27 des 29 mares et lacs échantillonnés en Alaska présentaient une sursaturation en CO_2 et sont donc considérés comme une source de GES pour l'atmosphère. Sobek et al. (2003) constatent le même phénomène avec 33 lacs boréaux en Suède et concluent que la quantité de CO_2 dégagée est liée à la concentration du carbone organique dissous de chaque lac. Puisque la plupart des lacs boréaux sont peu productifs, une grande part du carbone qu'ils reçoivent est d'origine allochtone et proviendrait de l'érosion terrestre (Dean & Gorham 1998, Algesten et al. 2003). Une altération de la structure terrestre par la fonte peut donc modifier l'apport de DOC et conséquemment celui du CO_2 émit dans l'atmosphère. Lors d'une étude sur 16 petits lacs peu profonds en Suède, Jonsson et al. (2003) ont noté que les lacs thermo-stratifiés présentaient une plus grande production de gaz carbonique en profondeur. Il est avancé qu'une grande fraction du CO_2 retrouvé dans l'eau des lacs humiques proviendrait non pas de la colonne d'eau mais bien des sédiments par la respiration benthique, et peut-être également des apports souterrains. Aussi, la stratification des lacs (King et al. 1999) et

l'apport de DOC aux milieux aquatiques peuvent être affectés par les changements climatiques (Kling et al. 1991, Clair et al. 1999).

Les sédiments de subsurface au fond des lacs présentent souvent des conditions anoxiques, favorisant ainsi la production et la diffusion de CH_4 à l'interface sédiments-eau. Dans le cas des lacs profonds, plus de 95% du méthane issu des sédiments est oxydé dans la colonne d'eau (Frenzel et al. 1990, King 1990). Cependant, dans certaines zones peu profondes, il peut y avoir formation de bulles de CH_4 qui échappent à l'oxydation et vont vers l'atmosphère (Keller & Stallard 1994, Walter et al. 2007). De plus, lorsque la profondeur est < 1 m, le CH_4 émit à l'interface eau-air peut représenter jusqu'à 45% du CH_4 produit dans les sédiments (Duchemin et al. 1995).

1.3.3 Les mares de fonte

Le catabolisme bactérien et plus particulièrement la méthanogénèse en zone anoxique sont la principale source de GES des mares de fonte. Les organismes méthanogènes (les archéobactéries) et les méthanotrophes jouent un rôle important dans le cycle du carbone dans les sédiments lacustres (Dagurova et al. 2004). Malheureusement, très peu d'études ont porté spécialement sur le rôle que les mares de thermokarst peuvent jouer dans le cycle global du carbone et sur les émissions de carbone atmosphérique. D'ailleurs, les bilans des flux de GES des milieux humides ne tiennent habituellement pas compte des mares et étangs présents (Kling et al. 1991, Roulet et al. 1994, Walter et al. 2007) rapporte que les mares de fonte en Sibérie représentent une source significative et grandissante de CH_4 atmosphérique; 95% de l'évasion du CH_4 s'y fait par ébullition et le reste, par diffusion. Selon leurs calculs, une hausse de 16.4% de la surface des mares induirait une hausse de 58% des émissions de CH_4

(pour une analyse plus détaillée de l'aspect des gaz émis par des mares de fonte, consulter le mémoire de Julie Breton (2007)). Les émissions de GES par les lacs et mares sont produites par différents assemblages microbiens présents en zone anoxique des sédiments lacustres. Les archéobactéries méthanogènes sont les productrices de CH_4 et peuvent se diviser en plusieurs classes selon leur substrat idéal (acétate, formate ou H_2+ CO_2) (Whitman et al. 2006). Il existe des bactéries méthanotrophes, souvent établies à l'interface oxique/anoxique, qui utilisent le CH_4 comme source d'énergie, celles-ci font donc partie intégrante des écosystèmes nordiques où il y a production de méthane. Ces bactéries jouent un rôle majeur en tant que « biofiltre » pour le CH_4, diminuant ainsi les quantités et les flux de ce gaz à effet de serre dans l'atmosphère (Trotsenko & Valentina 2005).

1.4 La diversité phytoplanctonique des eaux douces en milieu nordique

Les mares de fonte tardent encore à nous révéler toute l'ampleur de leur diversité microbienne. Quelques études traitent de différents aspects de la caractérisation biologique de cet écosystème mais il est rarement précisé si les mares en questions sont bel et bien de nature thermokarstique. Sheath (1986) s'est attardé aux variations saisonnières de la communauté phytoplanctonique de mares toundriques en Alaska, Vezina & Vincent (1997) ont caractérisé les assemblages des cyanobactéries dans des mares en arctique dont certaines sur notre site d'étude, Rautio & Vincent (2006) se sont plutôt attardé aux liens entre la nourriture benthique vs. pélagique et le zooplancton des mares arctiques et subarctiques alors que Maciolek (1989) a porté son intérêt sur la faune des macro-invertébrés dans les mares de toundra au Yukon.

La biodiversité se définit comme étant la richesse en éléments biologiques (c'est-à-dire. gènes, espèces ou genres) d'une communauté (Estrada et al. 2004). La communauté pélagique aquatique microbienne est constituée de trois groupes majeurs : le phytoplancton, le bactérioplancton et le microzooplancton. Pour les besoins de la présente étude, notre intérêt sera porté principalement sur le phytoplancton pélagique. Celui-ci comprend principalement des cyanobactéries procaryotes de même que plusieurs groupes d'algues eucaryotes de dimension variant entre 0.2 µm et 500 µm (Sorokin 1999). Le picoplancton se définit comme étant la fraction du plancton où les cellules mesurent entre 0.2 et 2 µm. L'étude du picoplancton au sein des écosystèmes a été souvent négligée ou son rôle, diminué (Richardson & Jackson 2007). Pourtant, il est maintenant reconnu que le picoplancton (plus spécialement le picophytoplancton) occupe une proportion importante de la production de biomasse dans les lacs et mares (Drakare et al. 2003). Le suivi des communautés phytoplanctoniques lacustres est utilisé comme outil d'avertissement pour les floraisons de cyanobactéries (Dahl & Johannessen 1998) et peut être indicateur de changements climatiques en zones polaires (Moline & Prezelin 1996).

1.4.1 Exemples d'assemblages microbiens lacustres nordiques

Sheath (1986) a dressé un portrait de l'aspect phytoplanctonique de mares de fonte situées en Alaska et dans les Territoires du Nord-Ouest et démontre que les Chlorophyceae, Chrysophyceae, Bacillariophyceae et Cyanobactéries sont les familles dominantes et comptent pour 79% de toutes les espèces recensées dans les mares arctiques. En zone subarctique, il peut y avoir deux pics de production phytoplanctonique au cours de la saison, souvent dominés par les Chrysophyceae et Cryptophyceae (Figure 5). En région arctique, ces mêmes familles dominent et plus particulièrement l'espèce *Rhodomonas minuta*, ubiquiste

dans les lacs et mares des hautes-latitudes. Des mares de fonte situées en Alaska et semblables aux mares arctiques faisant l'objet de notre échantillonnage ont été étudiées exhaustivement par Alexander et al. (1980). Certes, il existe une variabilité inter-mares mais le patron de dominance phytoplanctonique saisonnier reste le même : une prépondérance de Chrysophyceae au printemps suivi d'une transition vers les Cryptophyceae plus tard en saison. *Rhodomonas minuta* était le cryptophyte le plus abondant. D'autres études citées dans Alexander et al. (1980) soulignent également la présence marquée de diatomées dans certains lacs plus profonds en Alaska.

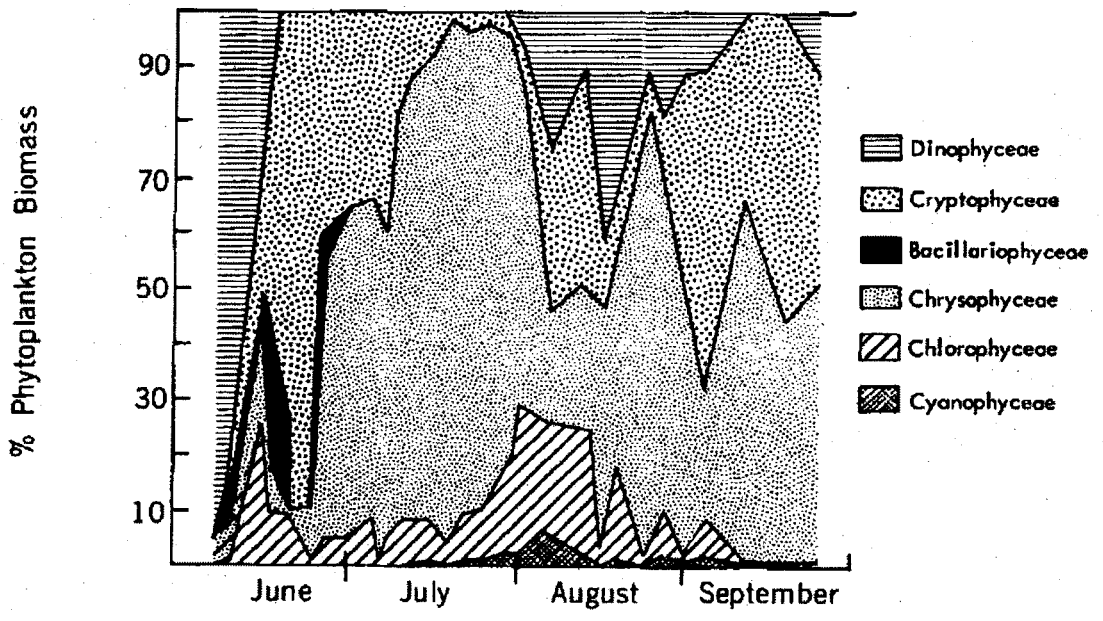

Figure 5 Proportion des principales familles phytoplanctoniques échantillonnées durant l'été dans une mare de fonte des Territoires du Nord-Ouest. (Sheath 1986).

1.4.2 Impacts des paramètres environnementaux

La majorité des études portant sur la dynamique phytoplanctonique ne sont pas seulement descriptives mais cherchent à corréler la structure des communautés aux paramètres environnementaux. Ainsi, un lac oligotrophique subarctique situé en Finlande a fait l'objet d'une étude par Forsstrom et al. (2005) et ils y ont détecté 148 taxons de phytoplancton. Les trois groupes dominants se sont avérés être les Chrysophytes, les Diatomées et les Dinoflagellés. La température de l'eau, la fréquence du cycle de mélange et la stabilité thermique ont été les principales variables de contrôle de la communauté algale. Tolotti et al. (2003) ont pour leur part évalué la taxonomie et la biodiversité des algues flagellées (c'est-à-dire. Chrysophyceae, Dinophyceae et Cryptophyceae) dans 48 lacs des Alpes Orientales. La physico-chimie lacustre changeant significativement d'un lac à l'autre, les patrons de diversité phytoplanctonique sont tout aussi variés. Chaque taxon algal n'est pas influencé par les mêmes facteurs mais les conditions thermiques, la concentration en azote, l'alcalinité et la profondeur du lac restent les principaux paramètres influents. L'étude des communautés phytoplanctoniques et de leur persistance dans quatre bassins peu profonds de stades trophiques différents en Finlande par Soininen et al. (2005) souligne que les assemblages varient significativement d'une année à l'autre et que les conditions environnementales seraient le facteur modulateur le plus important (surtout la température puisqu'elle affecte la vitesse de croissance). La présence de cladocères brouteurs, et plus spécialement de *Daphnia*, expliquerait aussi certaines différences d'assemblages spécifiques. Finalement, une étude de la saisonnalité du picoplancton autotrophe dans quatre lacs boréaux de stades trophiques différents par (Jasser & Arvola 2003) montre une dominance des cyanobactéries pour trois lacs mais le plus humique est plutôt dominé par des picoalgues eucaryotes. Encore ici, la

température et la disponibilité de la lumière sont les principaux facteurs abiotiques modulateurs. Toutes ces études soulignent l'aspect primordial des facteurs environnementaux pour expliquer les diversifications et fluctuations au sein des communautés phytoplanctoniques des eaux douces.

1.5 Méthodes utilisées pour qualifier et quantifier la diversité microbienne

1.5.1 Approche taxinomique

L'énumération du phytoplancton au microscope est pratiquée depuis plusieurs décennies et la méthode selon Utermöhl (1958) reste un standard peu modifié. Cependant, des techniques plus simples et rapides ont graduellement fait leur apparition (Willen 1976, Paxinos & Mitchell 2000). Malheureusement, l'identification des individus se fait par l'analyse des caractéristiques morphologiques et il devient difficile de distinguer les autotrophes des hétérotrophes puisque la chl *a* se dégrade lors des procédures (Havskum et al. 2004). La microscopie à épifluorescence permet de conserver l'intégrité de la chl *a* facilitant ainsi l'identification et ce, sans ajout d'agents colorants (Havskum & Riemann 1996). Par contre, la distribution spatiale inégale du phytoplancton demande de nombreux échantillons de même qu'une grande expertise de la part de l'observateur. Ces opérations demandent beaucoup de temps et impliquent une variabilité d'une analyse à l'autre de même qu'entre laboratoires (Havskum & Riemann 1996). Malgré tout, selon Havskum et al. (2004), la microscopie, de paire avec le HPLC (*High Performance Liquid Chromatography*) et/ou la cytométrie en flux, est essentielle pour déterminer les espèces dominantes et sous-dominantes d'un milieu. Garibotti et al. (2003) ajoute que la microscopie fournit de l'information de « grande valeur »

comme les changements d'abondance et de dimensions des cellules que le HPLC ne peut révéler.

1.5.2 Approche pigmentaire

Un pigment se définit comme étant une substance capable d'absorber la lumière (Raven et al. 2000). Les pigments photosynthétiques se divisent en trois grandes catégories : les chlorophylles, les caroténoïdes et les phycobilines. Les caroténoïdes sont des pigments accessoires qui permettent d'élargir la gamme de lumière utilisable en photosynthèse mais ils jouent principalement le rôle d'antioxydant et de protecteur solaire (Edge et al. 1997, Havaux & Niyogi 1999). Les carotènes et les xanthophylles constituent deux sous-groupes des caroténoïdes. Les xanthophylles sont dominants chez le phytoplancton et sont utilisé comme marqueurs taxonomiques (Ston et al. 2002). Selon Ston et al. (2002), la fucoxanthine, la péridinine et la α-carotène appartiennent aux caroténoïdes photosynthétiques alors que la diadinoxanthine, l'alloxanthine, la zéaxanthine, la lutéine, la néoxanthine, la violaxanthine et la β-carotène sont plutôt des caroténoïdes aux vertus photo-protectrices. On trouve principalement les phycobilines chez les cyanophytes et cryptophytes (Raven et al. 2000). La fucoxanthine est associée aux Diatomées, Dhrysophytes et certains Dinoflagellés ; la violaxanthine aux Chlorophytes ; l'astaxanthine au zooplancton (crustacés); diatoxanthine et diadinoxanthine aux Diatomées et Euglenophytes (seulement diadinoxanthine); l'anthéraxanthine aux Chlorophytes; l'alloxanthine aux cryptophytes; la zéaxanthine et la lutéine aux Chlorophytes et Cyanophytes; la canthaxanthine et l'échinenone aux cyanophytes et au zooplancton (cladocères). La chlorophylle *a* est présente chez tous les organismes photosynthétiques, la chlorophylle *b* chez les algues vertes, la chlorophylle *c* chez les diatomées et la β,β-carotène est ubiquiste chez toutes les algues. Ston et al. (2002)

rappelle toutefois qu'un pigment diagnostique n'est pas nécessairement exclusif à un seul groupe de phytoplancton. Des inventaires plus exhaustifs des pigments diagnostiques sont disponibles dans de nombreuses publications dont le livre Phytoplankton pigments in oceanography (Jeffrey 1997) et dans Millie et al. (1993).

La chromatographie liquide à haute performance/pression (HPLC) est une technique applicable pour l'analyse quantitative des pigments photosynthétiques phytoplanctoniques (Wilhelm et al. 1995). Celle-ci permet une caractérisation chemotaxique des pigments photosynthétiques de même qu'une estimation de la biomasse phytoplanctonique grâce à la concentration en chl *a* (Ediger et al. 2006). Le HPLC peut représenter une alternative intéressante à la microscopie conventionnelle qui requiert beaucoup de temps et d'expertise. L'utilisation du HPLC est automatisée et toutes les cellules sont quantifiables, peu importe leur taille (Wilhelm et al.). De plus, cette méthode s'avère très sensible et hautement reproductible (Schluter et al. 2000). Bien que le HPLC ne permette pas d'extrapoler les taxons présents plus précisément qu'à la classe, plusieurs études témoignent de la corrélation positive entre l'analyse de la biomasse phytoplanctonique au microscope et à l'aide de la chromatographie liquide à haute performance/pression (Wilhelm et al. 1991, Roy et al. 1996). Malgré tous ces avantages, le HPLC seul ne permet pas une détermination précise et quantifiable de la communauté planctonique et cet outil doit être utilisé de concert avec la microscopie afin d'obtenir un portrait juste et global d'un environnement (Schmid et al. 1998).

Le ratio pigment diagnostique/ chl *a* est un indice fréquemment utilisé pour connaître la contribution de chaque classe à la chlorophylle totale (Wilhelm et al. 1991, Roy et al. 1996, Ansotegui et al. 2001) puisque la concentration des pigments photosynthétiques covarie avec

celle de la chl *a* (Schluter et al. 2000). Toutefois, certains chercheurs préfèrent ne pas utiliser cet indice. Par exemple, Schmid et collaborateurs (Schmid et al. 1998) soulignent que la concentration en chl *a* peut varier dramatiquement d'une cellule algale à l'autre, biaisant ainsi les données. Ils ont préféré corréler la concentration des pigments diagnostiques au biovolume phytoplanctonique obtenu avec le décompte par microscopie. Leurs résultats démontrent une meilleure corrélation entre les pigments diagnostiques et la classe dominante d'algue présente de même qu'avec le biovolume total qu'avec la chl *a* et le biovolume total. À l'opposé, Marinho & Rodrigues (2003) ont observé une forte corrélation entre la biomasse totale de carbone (proportionnel au biovolume) et la chl *a* totale. Bref, ces conclusions inverses nous démontrent l'urgent besoin de nombreuses autres études dans ce domaine.

1.5.3 Approche moléculaire

Les picoeucaryotes autotrophes et hétérotrophes sont présents dans la plupart des écosystèmes mais leur manque de caractéristiques taxonomiques distinctes rend leur identification et classification plutôt ardue (Aguilera et al. 2006). La mise au point de techniques de biologie moléculaire a permis de nombreuses avancées scientifiques tant au niveau de l'identification d'individus qu'au niveau de la structure d'une communauté biologique (Zehr 1999). Plus récemment, l'avènement des techniques de « fingerprinting », notamment le « denaturing gradient gel electrophoresis, DGGE » (Figure 6) permettent l'analyse de la biodiversité bactérienne, archaebactérienne, eucaryotique et conséquemment, picoplanctonique (Muyzer & Smalla 1998, Diez et al. 2004, Yan et al. 2007). Cette méthode est communément utilisée pour étudier la dynamique spatiale et temporelle des communautés (Yan et al. 2006) et est qualifiée de « robuste » pour analyser la communauté picoeucaryote (Diez et al. 2004). Brièvement, le DGGE consiste à faire migrer l'ADN (acide

désoxyribonucléique) à travers un gel de polyacrylamide, qui joue en quelque sorte le rôle des mailles d'un filet pour les brins d'acides nucléiques. La migration se produit grâce à un courant électrique et puisque l'ADN est chargé négativement il migre donc vers la cathode positive. En se déplaçant dans le gel, l'ADN rencontre des conditions dénaturantes croissantes (urée et formamide) et se déforme partiellement, exposant ainsi certaines parties où les brins d'ADN sont solitaires et non doubles. Au fur et à mesure de la migration, l'ADN devient de plus en plus déformé et ralenti dans les mailles du gel. Puisque chaque communauté microbienne n'a pas les mêmes alignements de paires de bases, chaque fragment réagit différemment et arrête sa migration à différents points. En théorie, chaque bande représente une espèce microbienne spécifique.Yan et al. (2007) ont utilisé la microscopie et le DGGE pour recenser la communauté procaryote et eucaryote d'un lac en Chine et ils concluent que les méthodes moléculaires fournissent des informations plus poussées, précises et reproductibles. Ils ajoutent que ces méthodes sont rapides, économiques et fiables tout en permettant l'analyse de plusieurs échantillons à la fois. Malheureusement, encore peu d'études se sont penchées sur les assemblages microbiens eucaryotes et picoeucaryotes des eaux douces (Richards et al. 2005, Aguilera et al. 2006, Yan et al. 2007).

Malgré les avantages incontestés du DGGE, des bémols restent toutefois à souligner : des biais sont possibles lors de l'extraction de l'ADN et de l'amplification par PCR (*polymerase chain reaction*). En effet, l'ADN des différents organismes ne s'amplifie pas toujours aussi facilement et le choix des amorces reste crucial (Savin et al. 2004). De plus, il peut y avoir co-migration de plusieurs fragments d'ADN lors du DGGE, ce qui empêche la distinction des bandes sur le gel (Muyzer & Smalla 1998).

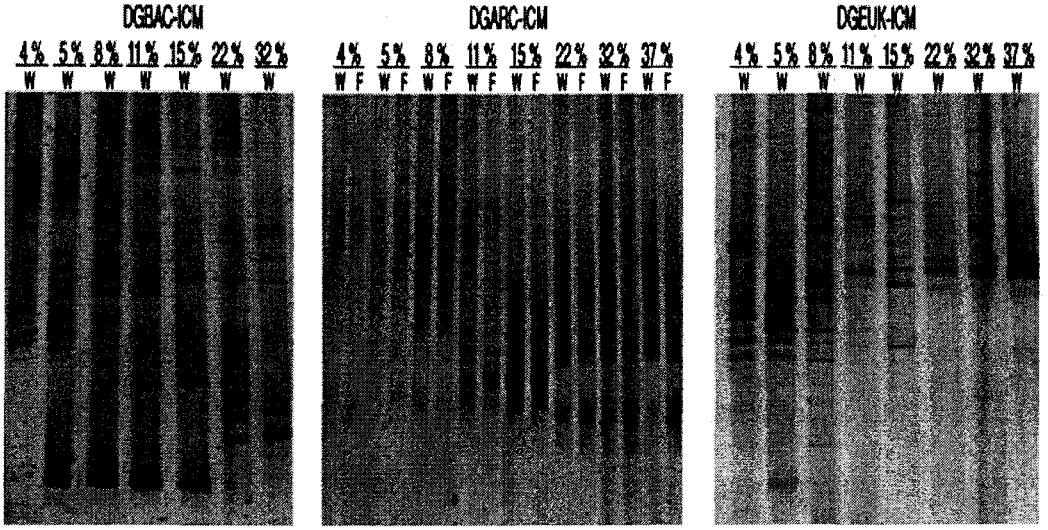

Figure 6 Exemple de DGGE de bactéries, archéobactéries et eucaryotes, ici selon un gradient de salinité. Tiré de (Casamayor et al. 2002).

1.5.4 Utilisation en parallèle des trois techniques

Comme mentionné précédemment, la caractérisation de la diversité microbienne n'est pas chose facile et requiert plusieurs approches complémentaires afin de cerner un maximum d'espèces et de répartition de populations. Estrada et al. (2004) a utilisé la microscopie, la cytométrie en flux, l'analyse pigmentaire via HPLC et le DGGE en parallèle afin de caractériser la diversité planctonique photo-autotrophe le long d'un gradient de salinité. Ils ont conclu que les différents indicateurs de diversité (à savoir le phytoplancton, les pigments et les différences génétiques du ARNr 16S et 18S (acide ribonucléique) sont corrélés positivement entre eux et qu'ils diminuent avec une augmentation de la salinité. Les quatre indicateurs présentaient des tendances de diversité comparables. La quantification du phytoplancton marin en eaux pauvres et enrichies en azote, phosphore, silice, glucose et

autres sources de carbone par Havskum et al. (2004) a permis de recommander l'usage conjoint du HPLC (via le programme CHEMTAX) avec la microscopie et la cytométrie en flux pour la quantification des picocyanobactéries, des flagellés et des diatomées. Nubel et al. (1999) ont également observé une corrélation positive entre la richesse spécifique obtenue par microscopie, HPLC et ARNr 16s pour les phototrophes oxiques dans des tapis microbiens de mares évaporées et marais salants en Californie. Ces quelques exemples démontrent la pertinence d'opter pour un consortium d'approches en parallèle afin de dresser un portrait le plus réaliste possible de la diversité microbienne d'un écosystème en particulier.

1.5.5 Indice de diversité Shannon-Weaver

L'indice de diversité Shannon-Weaver est fréquemment utilisé pour estimer et comparer la diversité microbienne tant en milieu aquatique qu'océanique (Romo & Miracle 1995, Estrada et al. 2004, Graham et al. 2004, Savin et al. 2004). Celui-ci tient en compte de la richesse spécifique et de l'uniformité de la communauté microbienne (Graham et al. 2004) et est le plus utilisé par les écologistes (Washington 1984). Globalement, plus l'indice de diversité de Shannon *H'* est élevé, plus la biodiversité est grande et l'écosystème en santé et vice versa (Wilhm 1970). C'est l'indice qui a été privilégié dans le cadre de notre étude.

2 OBJECTIFS ET HYPOTHÈSES

2.1 Objectifs

L'objectif global de cette étude est de caractériser l'habitat microbien que constituent les mares de fonte. Puisqu'il n'existe quasiment aucune information sur cet aspect dans la littérature, le premier sous-objectif réside donc dans la qualification et la quantification de la communauté microbienne des mares à l'aide de divers outils taxinomiques et moléculaires. Puisque les mares de fonte incarnent un microcosme particulier et varié à petite échelle, le second sous-objectif consiste à étudier les divers facteurs physiques, chimiques et optiques qui influencent les assemblages microbiens aquatiques.

2.2 Hypothèses

- Les mares thermokarstiques d'un même site sont dissemblables entre elles pour de nombreuses variables physico-chimiques, conséquemment, les patrons de dominance microbiens divergent d'une mare à l'autre. Les caractéristiques optiques des mares étant particulièrement diverses, la lumière serait le facteur le plus important dans la répartition spatiale des microorganismes.

- La présence d'une stratification estivale thermique et oxique des mares subarctiques crée des zones d'habitats distincts au sein d'une même mare modulant ainsi les assemblages de microorganismes selon la profondeur.

- La flore microbienne diffère entre les mares arctiques et subarctiques puisque celle-ci divergent tant au niveau latitudinal, géomorphologique que physico-chimique.

3 MÉTHODES

3.1 Approche générale

Deux sites différents ont été visités au cours des mois de juin et juillet de l'été 2006 et 2007 au Nunavik et au Nunavut sur l'Île Bylot dans le parc national de Sirmilik. Les mares de fonte subarctiques en zone de pergélisol discontinu sont situées en périphérie nord-est du village de Whapmagoostui-Kuujjuarapik (55°N 17' 77°O 46'). En zone de pergélisol continu, les mares de fonte ont été échantillonnées dans la région sud-est de l'Île Bylot (73°N 09' 79°W 59') située dans l'Arctique canadien. En juin et juillet 2006, 36 mares de fonte ainsi que neuf mares et lacs rocheux (pour fin de comparaison) ont été échantillonnés à Whapmagoostui-Kuujjuarapik afin d'en connaître les caractéristiques physiques, chimiques et biologiques (mares ayant le préfixe KWK). Il est à noter que les mares thermokarstiques choisies en région subarctique sont apparues suite à l'affaissement de buttes pergélisolées (lithalses dites palses minérales). Suites à l'étude des résultats, un sous-groupe de 12 mares a été établi afin de bien représenter la variabilité possible de ce milieu aquatique distinct et en approfondir l'étude. Ces mares ont été étudiées en détail lors la campagne 2007. Les critères de sélections considérés : le DOC, le TSS (*total suspended solids*), la concentration bactérienne et picoplanctonique, la couleur, la teneur en gaz dissous et l'état de stratification thermique et oxique. Vingt-et-unes mares de fonte ont fait l'objet d'études lors de notre passage sur l'Île Bylot (mares ayant pour préfixe BYL) en 2007. Les variables mesurées sont sensiblement les mêmes que pour les mares KWK sauf pour les profils de stratification puisque la profondeur moyenne des ces mares arctiques ne dépasse pas 0,4 m. Toutes les prises de mesure sont de nature ponctuelle.

3.2 Paramètres physiques, chimiques et optiques

Un portait physicochimique a été dressé systématiquement pour les 12 mares KWK choisies du sous-groupe de même que pour les mares BYL. Les données obtenues avec le profileur étant : la température, le pH, la conductivité et l'oxygène dissous des mares. La caractérisation limnologique comprenait également :

En surface :

- DOC
- TSS
- Phosphore total (TP)
- Anions et cations majeurs

Toute la colonne d'eau :

- CO_2 et CH_4 dissous à intervalles de 0.25 m à 0.5 m

3.3 Paramètres biologiques

3.3.1 Phytoplancton bactéries et picophytoplancton

La quantification par microscopie des bactéries et du picophytoplancton et du phytoplancton total a été possible pour la strate de sous-surface pour toutes les mares, tous les sites confondus. De plus, de par leur plus grande profondeur, les mares KWK (sauf KWK 35) ont également été échantillonnées pour les bactéries et le picophytoplancton au fond, près des sédiments.

3.4 Pigments diagnostiques

Entre 0,03 et 0,25 L passent à travers des filtres 0.2 µm jusqu'à colmatage. Les pigments ont été subséquemment extraits, analysés et quantifiés par chromatographie liquide à haute performance/pression (HPLC) Pour les besoins de la présente étude, seuls les pigments en quantité suffisante et détectables sont tenus en compte.

3.4.1 Analyse moléculaire de la diversité microbienne

3.4.1.1 Récolte d'ADN

La caractérisation de la flore microbienne nécessite la récolte d'ADN. De l'eau a été amassée et filtrée à l'aide d'une pompe péristaltique. L'eau passe d'abord à travers un filtre de 3 µm puis dans un Sterivex^{TM} de 0,2 µm pour conserver la fraction plus petite que 3 µm. L'ADN microbien a été subséquemment extrait avec la méthode dite au sel.

3.4.1.2 Denaturing gradient gel electrophoresis (DGGE) Eucaryotes

En premier lieu, les amorces EUK1F et Euk516r-GCR ont été choisies pour l'amplification des eucaryotes par PCR. Le gradient dénaturant d'urée du gel est de 35% et 55%. Un volume de 20 µl de produit de PCR est ajouté dans chaque puits vertical et les échantillons migrent pendant 16h à 100 Volts. Les bandes d'ADN sont ensuite révélées et une photo est prise de chaque gel afin d'ultimement calculer l'intensité relative des bandes des gels.

3.5 Approche statistique

L'indice de Shannon-Weaver est utilisé pour comparer la diversité des différents échantillons :

$$H' = - \sum (n_i / N) \ln (n_i / N)$$

Où n_i est le nombre de descripteurs de la communauté (nombre de cellules phytoplanctoniques, nombre de bandes obtenues avec le DGGE et nombre de pigments), La richesse spécifique est le nombre total d'espèces d'un échantillon (ici N). L'indice de Sorensen (ici nommé C_s) permet de comparer la similarité entre deux échantillons (utilisé dans ce cas-ci pour comparer les échantillons de DGGE) :

$$C_s = 2j / (a+b)$$

Où, j est le nombre de bandes communes aux deux échantillons et a et b, le nombre de bandes dans les échantillons A et B. Les analyses canoniques de correspondances (CCA en anglais) et les analyses en composantes principales (PCA en anglais) sont couramment utilisées pour mettre en lumière les corrélations entre les variables physico-chimiques et les communautés microbiennes. Selon le cas, elles permettent de regrouper visuellement les sites semblables et de voir quelles variables sont les plus significatives.

4 DISCUSSION GÉNÉRALE ET CONCLUSION

La présente étude avait pour but d'évaluer et d'estimer la diversité microbienne des mares de fontes en zones arctiques et subarctiques et d'explorer l'influence des paramètres physico-chimiques sur celle-ci. Un consortium de trois différentes approches méthodologiques a été choisi afin de dresser un portrait le plus global possible de la flore microbienne, plus particulièrement, le phytoplancton, les picoeukaryotes et les bactéries.

Les deux sites d'échantillonnage présentent de nombreuses différences, tant au niveau géomorphologique que limnologique (Table 1). Les mares subarctiques montrent une turbidité (due aux argiles) très élevée qui atténue rapidement la pénétration des rayons lumineux. Il en résulte une stratification thermique et oxique remarquable pour des plans d'eau aussi peu profonds (Figure 9). Le modèle d'estimation du coefficient d'atténuation diffus (K_dPAR) appliqué aux mares subarctiques a permis de classer les mares selon un gradient de transparence : les mares noirâtres étaient les plus transparentes et les beiges, les plus opaques. L'atténuation s'explique par l'absorption des rayons par le CDOM (matière organique dissoute chromophorique) et les solides en suspension. Les analyses taxinomiques phytoplanctoniques révèlent une grande diversité algale, relativement comparable aux lacs tempérés (Table 2, 3 et Table 5). La concentration en phosphore total des mares subarctiques est très élevée (mares considérées eutrophes) mais les espèces dominantes phytoplanctoniques ne reflètent pas nécessairement cet état : les Chrysophyceae étaient très présentes alors que ceux-ci témoignent en théorie d'un milieu plus oligotrophe. Une explication serait que le phosphore biodisponible est beaucoup plus faible que le phosphore total mesuré. Une fraction du P serait adsorbée sur les fines particules d'argile en suspension,

ce qui le rend inaccessible aux microorganismes. Les analyses pigmentaires ont révélé une biomasse (estimée via la chl *a*) plus faible qu'anticipée par les concentrations de P total. Encore ici, la faible disponibilité du P peut être en cause mais un broutage intense par le zooplancton pourrait expliquer également ce taux. En zone subarctique, la concentration de caroténoïdes photoprotecteurs était élevée, malgré la limitation en lumière causée par l'intense turbidité (Table 4). L'échantillonnage de surface pourrait expliquer ce phénomène puisque les cellules pourraient rester « prisonnières » de la strate supérieure et donc se protéger. La quasi absence d'oxygène au fond des mares subarctiques crée en quelque sorte deux habitats microbiens au sein d'une seule et même mare. Les analyses moléculaires picoeukaryotiques à l'aide du DGGE (Figure 13 et Table 5) avec des échantillons en surface et en profondeur des mares subarctiques corroborent cette hypothèse puisque les le patron de répartition des bandes diffère entre la surface et le fond. La concentration bactérienne semble également être plus grande en zone anoxique et plus spécialement dans les mares très stratifiées.

Les polygones à centre déprimé arctiques présentent un profil assez différent des mares subarctiques. Leur très faible profondeur empêche la stratification et indique un aspect plus temporaire (des mares visitées en 2005 n'existaient plus en 2007). La lumière peut pénétrer jusqu'au fond et favorise l'apparition de tapis microbiens (constitués surtout de cyanobactéries) (Figure 2). Malgré nos comparaisons, aucune différence physico-chimique significative n'a été trouvée entre les polygones à centre déprimé et les sillons adjacents sauf pour le $CDOM_{a320}$ et le DOC. Les mares arctiques étaient plus transparentes avec un K_dPAR plus bas et la majeure fraction de l'atténuation s'explique par le carbone organique dissous. Les communautés phytoplanctoniques arctiques sont moins diversifiées que les subarctiques

et ces deux écosystèmes présentent des espèces typiques absentes sur l'autre site (*Ochromonas* sp., *Rhodomonas minuta* en arctique et *Dinobryon* en subarctique) (Table 2). Une différence marquée entre les mares arctiques et subarctiques est l'omniprésence des cyanobactéries en zone arctique. Leur résistance particulière aux conditions extrêmes (luminosité intense, dessiccation, gel-dégel etc.) pourrait expliquer leur dominance.

Les caroténoïdes étaient en moins grande proportion par rapport à la chl *a* dans les polygones et sillons arctiques (Table 4). N'étant pas limité en lumière, les cellules algales n'auraient pas besoin de synthétiser autant de pigments accessoires qu'en milieu plus turbide. Le DGGE appliqué à certains échantillons de mares a permis de constater des différences de dominance picoeukaryotes d'une mare à l'autre, le patron des bandes étant changeant selon la mare. L'usage du DGGE aurait pu permettre d'approfondir l'aspect génétique de la communauté microbienne mais étant donné la nature même de l'étude, le DGGE a été utilisé afin d'avoir un portrait plus global des différences possibles entre les deux strates d'une mare, entre les mares d'un même site et entre les mares de deux sites différents. Une revue de littérature a permis de s'apercevoir que très peu d'études se sont penchées sur la diversité picoeukaryotes en milieu lacustre, la majorité prenant place en milieu océanique.

Les résultats du présent mémoire suggèrent que la diversité microbienne des mares de fonte est comparable à d'autres écosystèmes lacustres plus tempérés. Les mares sont considérées comme productives et riches en espèces. Grâce aux analyses de correspondance canoniques et de composantes principales, il est possible d'affirmer que les conditions physico-chimiques diffèrent entre les deux sites et que leur variabilité ne s'explique pas par les mêmes facteurs (Figure 10). La lumière (K_dPAR), le carbone organique dissout (DOC), le ratio *a*320/DOC et la qualité de la matière organique (*S*) (*S* étant la pente spectrale obtenue lors de l'analyse du

spectre d'absorption du carbone organique dissout, elle aide à indiquer son origine et sa biodisponibilité) se sont avérés être les facteurs les plus importants quant à la répartition et la dominance des espèces phytoplanctoniques (Figure 12). Une hypothèse avancée pour l'impact de la qualité de la matière organique serait la présence de taxons mixotrophes au sein des communautés. Les mares subarctiques voisines sont distinctes et ne présentent pas les mêmes patrons de dominance et/ou d'espèces. Un échantillonnage phytoplanctonique et pigmentaire sur toute une saison et à plusieurs profondeurs permettrait probablement d'approfondir la connaissance des liens entre le biote et les conditions limnologiques.

En conclusion, l'utilisation en parallèle de trois indicateurs biologiques (phytoplancton, pigments et ADN picoeukaryote) a permis de dresser un premier portrait global de la diversité algale des mares de fonte arctiques et subarctiques. Les communautés algales sont différentes entre les mares d'un même site et entre les deux sites échantillonnés tel qu'anticipé dans nos hypothèses. De plus, la lumière et la nature et la quantité de la matière organique semblent être des facteurs qui influencent la structure de la communauté des mares de fonte. Ces résultats corroborent avec l'hypothèse de départ où il était question que la lumière serait le facteur le plus important dans la répartition spatiale des microorganismes.

Évidemment, plusieurs autres facteurs affectant les communautés microbiennes n'ont pu être pris en compte au cours de la présente étude tels la pression de broutage du zooplancton, l'âge des mares de fonte et leur topographie, les conditions météorologiques et climatiques, les divers nutriments (tel l'azote) etc. D'autres études établies sur une plus longue période temporelle permettrons d'approfondir encore davantage les encore maigres connaissances de la diversité microbienne des mares de fonte.

CHAPITRE 2 : L'ARTICLE

Microbial diversity of arctic and subarctic thaw ponds: characterization using different biological indicators

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Running head: microbial diversity, thaw pond, biological indicator, arctic, subarctic

Acknowledgments

We thank M.-È. Bédard, F. Bouchard, J. Breton, G. Gauthier, T. Harding, I. Lavoie, M.-J. Martineau, M. Potvin, L. Retamal, N. Rolland, A. Rouillard, M. Rousseau, D. Sarrazin, R. Terrado, C. Vallières and S. Watanabe for their essential support in the field and laboratory, FQRNT, NSERC, ArcticNet, Polar Continental Shelf Project, Northern Affairs, Parks Canada, Federal IPY program, Centre d'études nordiques and U.S. NSF grants DEB-0640953 and ARC-0714085 for logistic and financial support. This is PCSP contribution 02509.

Abstract

Thaw ponds are formed when permafrost melts in arctic and subarctic regions. These small shallow depressions filled with water are productive ecosystems and display unique features such as high turbidity, thermal stratification and supersaturation of greenhouse gases. However, their microbial assemblages are practically unknown. During July 2006 and 2007, 53 thaw ponds were sampled in Whapmagoostui-Kuujjuarapik (Nunavik, Canada) and on Bylot Island (Nunavut, Canadian High Arctic). Microbial diversity was characterized using different community descriptors: phytoplankton taxonomy via microscopy, pigment composition with HPLC, bacteria and picoautotroph biomass estimated with microscopy or flow cytometry and picoeukaryote assemblages (18S rRNA gene) using denaturing gradient gel electrophoresis (DGGE). Thaw pond physicochemical and optical characteristics differed between the two latitudes and were quite variable within one site. Subarctic ponds were mostly dominated by Chlorophyceae, Chrysophyceae and to a lesser extent, Cyanobacteria, while Cyanobacteria were preponderant in arctic ponds, along with Chlorophyceae. Photosynthetic and photoprotective carotenoids normalised to chlorophyll *a* were significantly lower in arctic ponds, which are not light-limited compared to turbid subarctic ponds. Phytoplankton and pigment diversity were significantly lower in arctic thaw ponds. Steep thermal and oxic stratification of subarctic ponds created two distinct water masses and DGGE indicated different picoeukaryote communities within single ponds. Canonical correspondence analysis (CCA) performed with phytoplankton and limnological data revealed that the diffuse attenuation coefficient of visible light (K_dPAR), spectral slope of absorption by dissolved organic matter (*S*), dissolved organic carbon (DOC) and absorption of chromophoric fraction of dissolved organic matter at 320 nm (a_{320})/ DOC ratio were the significant variables explaining the diversity observed in phytoplankton communities.

These results suggest that the light availability and the type of dissolved organic matter are partially driving the composition of microbial assemblages in thaw ponds.

Introduction

Thermokarst occurs in continuous and discontinuous permafrost regions when ground ice melts, leading to peaty soil structure collapse and creating thaw lakes and ponds (Schuur et al. 2008). This phenomenon is a growing trend worldwide as a result of global warming (Osterkamp & Romanovsky 1999, Payette et al. 2004, Smith et al. 2005). These small, fishless shallow bodies of water exhibit unique features such as a combination of high turbidity, steep thermal and oxic stratification, intense microbial activity and high greenhouse gas emissions (Breton 2007). Furthermore, despite close spatial proximity, these ponds offer a wide range of varying optical and limnological conditions thus creating diverse microbial habitats. At higher latitudes, in arctic continuous permafrost zones ice-wedges, a phenomenon in peatlands, lead to the formation of polygons and runnels (Payette 2001). Low-centered polygons and runnels that are not colonized by hydrophilic graminoïds can fill with water (Fortier et al. 2007). They freeze to the bottom during winter and are exposed 24 hours a day to sunlight during summer, creating harsh but unique habitats. Because they are shallower and less turbid than subarctic thaw ponds, low-centered polygon ponds allow light penetration to the bottom, which promotes the growth of benthic microbial mats (Vezina & Vincent 1997). With such a shallow water column, pelagic primary production is low compared to benthic and macrophytes production (Hobbie 1980, Karlsson et al. 2008). The thaw pond simple food web is typically "bottom-up" and consists mainly of primary producers and herbivorous planctivores. The zooplankton genus *Daphnia* sp. is often the main grazer and can reach high concentrations in these ecosystems (Alexander et al. 1980, O'Brien et al. 2004, Rautio & Vincent 2006).

To our knowledge, this is the first study of microbial diversity comparing arctic and subarctic thaw ponds. Yet, microbial catabolism plays a major role in the carbon cycle of lakes and ponds

(Dagurova et al. 2004). Likewise, the role of picoplankton has often been neglected in aquatic ecosystems (Richardson & Jackson 2007) but is now recognized as an important biomass component in lakes and ponds (Drakare et al. 2003). Since neighbouring water bodies have strikingly different limnological characteristics as is the contrast between arctic and subarctic, these ponds make ideal study sites for investigation of microbial diversity patterns.

There are a wide array of methods that have been developed to assess microbial diversity. Classic microscopic approaches for phytoplankton diversity evaluation is of great value since it can be used to determine of abundance and biovolume of each taxon (Garibotti et al. 2003) but it is time consuming and may generate analytical errors (Havskum & Riemann 1996). Moreover, phytoplankton taxonomy based on morphological features often results in taxonomical groups of different hierarchical ranks which make analysis more difficult and less precise. Taxonomy based on cell morphology is a particular problem for the smallest species (e.g., picoeukaryotes). High performance liquid chromatography (HPLC) allows phytoplanktonic pigment quantification (Wilhelm et al. 1995) and has proven to be both sensitive and reproducible (Schluter et al. 2000) providing insight on the phytoplankton pigment class contribution to total chlorophyll *a* (chl *a*). Molecular tools are becoming common to determine microbial diversity in both oceanic and freshwater ecosystems (Diez et al. 2004, Richards et al. 2005, Aguilera et al. 2006, Lefevre et al. 2007, Galand et al. 2008). Furthermore, fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) may be used for spatial and temporal microbial community analysis (Massana & Jurgens 2003, Yan et al. 2006, Hamilton et al. 2008) and have been applied with success in picoeukaryotes studies (Diez et al. 2004). A combination of these three techniques can

provide an overview of microbial diversity, community structure and seasonality at a given site (Estrada et al. 2004, Savin et al. 2004, Not et al. 2007).

The aim of this study was to characterize the microbial diversity of thaw ponds. The specific objectives were to investigate subarctic and arctic thaw pond microbial diversity via the use of three biological indicators: larger phytoplankton via microscopy, photosynthetic classes via HPLC pigments, and picoeukaryotes via DGGE, and to determine whether environmental characteristics of these particular systems influenced the microbiota. We hypothesized: (1) microbial patterns vary among ponds because of the variation in physical and chemical factors, (2) light limitation affects pigment patterns and therefore drives microbial repartition, (3) seasonal thermal and oxic stratification creates two distinct microbial communities within a single pond, and (4) arctic and subarctic regions have distinct microbial assemblages and diversity patterns.

Methods

Study sites - Two geographical zones were visited during June and July in 2006 and 2007: firstly the subarctic discontinuous permafrost region in northern Quebec, and second the continuous permafrost region in the Canadian High Arctic (Figure 7). The subarctic thaw ponds northeast of Whapmagoostui-Kuujjuarapik village on the eastern coast of Hudson Bay (55°17'N, 77°46'W) are referred to as KWK (from the nearby Kwakwatanikapistikw River). The arctic thaw ponds in the western part of Bylot Island in Sirmilik National Park (73°09'N, 79°59'W) are referred to as BYL.

The mean annual air temperature (1971-2000) in Whapmagoostui-Kuujjuarapik is -4.4 °C. The subarctic ponds lie astride the forested tundra and spruce lichen zones (Ministère des

Ressources naturelles du Québec 2003) and are on sporadic discontinuous permafrost. The surrounding vegetation is mostly lichens, mosses and shrubs such as *Picea mariana, P. glauca, Betula glandulosa* and *Salix* spp. The subarctic thaw ponds are formed following the collapse of permafrost mounds (lithalsas or mineral palsas). In mid-summer of 2006, 34 thaw ponds were sampled (Figures 8 b and c). Based on 2006 results, a subgroup of 12 ponds were chosen and analysed more extensively in 2007. Ponds were classified from aerial photos into four color categories: black, brown, beige and green. Water temperature monitoring of one of the subarctic ponds (KWK16) from July 2006 to July 2007 indicated ice-cover from November to May and revealed two main mixing events: one long episode beginning in September and ending in October and a second short episode in May (Laurion et al. submitted on January 2009; under revision). Other isolated mixing events of the whole water column caused by strong wind and cold temperature events were also noted on two occasions in July.

The mean annual air temperature (1971-2000) at the nearest community Pond Inlet, about 85 km southeast of the Arctic site on Bylot Island, is -15.1 °C (Environment Canada 2002). The short arctic summer is also characterized by constant daylight at this latitude. Summer of 2007 was especially dry in this region, with no precipitation in June and only 5.4 mm of rain in July. A large proportion of Bylot Island is covered by glaciers but the south-western coastal areas have sparse vegetation dominated by lichens, mosses and grasses. Detailed descriptions of the area are available in Fortier et al. (2007) and Vezina & Vincent (1997). Eighteen thaw ponds were sampled on Bylot Island in 2007. The same measurements were taken at both arctic and subarctic sites except for the stratification profiles, which were only done in the subarctic ponds.

Physicochemistry - Temperature (± 0.15°C), pH (± 0.2), and dissolved oxygen (± 0.2 mg L^{-1}) were measured with a multiparametric probe profiler (model 600R, YSI Inc.). For total suspended solids (TSS), 125-1250 ml of water was filtered onto glass fiber filters (MFS Advantec) pre-combusted at 500 °C for 2 h and pre-weighed; the filters were stored at -20 °C until analysis. Filters were subsequently dried for 24 h at 60 °C and re-weighed. For total phosphorus (TP), 30% H_2SO_4 was added (0.15% final concentration) in pre-washed Teflon-capped glass bottles. Samples were kept in the dark at 4°C until analysis. TP concentrations were obtained via perchlorate digestion and spectrophotometric measurement using a 10 cm-cuvette (Stainton et al. 1977).

K_dPAR of the subarctic ponds was estimated with the following equation obtained via multiple linear regression: $K_d\text{PAR} = 0.253 + (0.0685 * a_{320}) + (0.102 * \text{TSS})$ ($n = 15$, $R^2 = 0.977$, $p > 0.001$; unpubl. results). For the arctic ponds, the relationship was not as strong but it was still used as a rough estimate of water column transparency:

$K_d\text{PAR} = -0.0138 + (1.592 * \log a_{320}) + (0.0635 * \text{TSS})$ ($n = 11$, $R^2 = 0.681$, $p = 0.01$; unpubl. results).

Dissolved organic matter (DOM) characterization - Water samples were filtered through pre-rinsed (with pond's water) cellulose acetate filters (0.2 µm pore size, MFS Advantec) and stored in pre-washed Teflon-capped glass bottles in the dark at 4°C. Dissolved organic carbon concentrations were estimated using a Shimadzu TOC-5000A carbon analyzer calibrated with potassium biphthalate. To determine the chromophoric fraction of DOM (CDOM, quantified with the absorption coefficient at 320 nm, a_{320}), absorbance scans were performed on a spectrophotometer (Cary 100, Varian) from 250 to 850 nm at the natural pH.

Absorption coefficients were calculated following Mitchell et al. (2002) and spectral slopes *S* were obtained using an exponential fit between 275 and 295 nm and 350 and 400 nm (Helms et al. 2008).

Bacterioplankton and picoautotrophs – Procaryotes (bacteria and archaea) abundance was estimated in subsurface water samples for all ponds, at both sites. Samples from bottom waters were also taken in subarctic ponds (except KWK35). Samples were fixed with a 0.2 µm filtered solution of paraformaldehyde (0.1% final concentration) and glutaraldehyde (1% final concentration) after adding a protease inhibitor (phenylmethanesulphonylfluoride, 1 µM final concentration) as in Gundersen et al. (1996), and were stored frozen at -20 °C in the field and subsequently at -80 °C in the laboratory. Procaryotes, referred to as bacteria for convinience, and picoautotrophs were stained with 4',6-diamidino-2-phenylindole (DAPI, DNA probe, Sigma; 5 µg L^{-1} final concentration). Samples were filtered onto black polycarbonate filters (0.22 µm, Nucleopore) and counted using epifluorescence microscopy (Zeiss Axiovert 200), using blue and green excitation to distinguish between bacteria and picoautotrophs. In 2006, bacteria and picoautotrophs were counted using flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA). Fluorescent beads (0.94 mm, Polysciences Inc., Warrington, PA, USA) were systematically added to each sample. Bacteria were labelled with SYBR green I (Sigma Aldrich) and counted during 2 min at a low flow rate (12-15 µl sec^{-1}) and picoautotrophs were counted during 3-4 min at a high flow rate (50-60 µl sec^{-1}).

Plankton identification - The 2006 phytoplankton community (and planktonic heterotrophs) was analysed in 24 subarctic and four arctic thaw ponds. Water samples were fixed with the same solution described above for the bacteria and stored in HDPE bottles at 4 °C. Between

10 to 50 ml of pond water were concentrated in Utermöhl sedimentation chambers (Utermöhl 1958), depending on cell abundance. Phytoplankton identification and quantification were performed using an inverted microscope (Zeiss Axiovert 200) under differential-interference contrast illumination. Abundance and identity of rarer and larger species were estimated at a magnification of 200X, from to 25% of the total chamber area. Smaller and more common species concentrations were estimated by transects of vertical and horizontal diameters of the chamber at 400X. Very abundant species were quantified from two transects of the chamber (length depending of the quantity of cells) at 400X. For each sample, a total of more than 400 cells were counted.

Planktonic pigments - Pigments were analysed for 34 subarctic thaw ponds in 2006 (in association to the taxonomic analyses), 12 ponds in 2007 and 17 arctic thaw ponds. Between 30 and 250 ml of water were filtered onto MFS glass fiber filters under dim light Filters were immiediately frozen at -20 °C in the field and at -80 °C once back in the laboratory, until analysis. Pigments were analyzed by high performance liquid chromatography (HPLC) within eight months of sampling. Pigments were extracted in cold aqueous methanol (95%) in an ice bath and using ultrasonication (3 × 10 seconds at 19 W). Extracts were cleared by filtration (Acrodisc PTFE 0.2 μm membrane, Cole-Palmer Inc.) vials were purged with argon to prevent pigment oxidation. The pigment separation followed the method of Zapata et al. (2000). Briefly, 100 μl of the extracts were injected in the HPLC (Varian, Palo Alto, CA, USA), equipped with a pre-column Symmetry C_8 (5 μm, 3.9 × 20 mm) and a column Symmetry C_8 (3.5 μm, 4.6 × 150 mm, Waters Corporation, Milford, MA, USA), and connected to a diode-array spectrophotometer (2 nm slit width, scan from 390 to 790 nm). Chlorophylls were also detected by fluorescence (excitation 440 nm; emission, 650 nm). The

chromatograms were analyzed using Star Chromatography Workstation and Polyview2000 (program version 6.20, Varian) and the following standard pigments (Sigma Inc., St. Louis, MO, USA; DHI Water & Environment, Hørsholm, Denmark): chl *a*, b and c2, alloxanthine, antheraxanthine, β-carotene, canthaxanthin, cis-neoxanthine, diadinoxanthine, echinenone, fucoxanthine, lutein, myxoxantophyl, peridine, violaxanthine and zeaxanthin. For the calculations of diversity index, the unidentified pigments were excluded.

DNA collection and extraction - Environmental DNA of eight subarctic and four arctic thaw ponds was collected in 2007. Pond water was filtered using a peristaltic pump (Masterflex L/S with Easy-Load II heads) onto 3 µm pore size polycarbonate filters and subsequently onto 0.22 µm Sterivex units (Millipore, USA) to obtain the 3 to 0.2 µm fraction (picoplankton). The filters were stored in lysis buffer (40 mM EDTA; 50 mM Tris pH 8.5; 0.75 M sucrose) and frozen at -20 °C in the field and at -80 °C at the laboratory. DNA was extracted using a salt extraction method (Aljanabi and Martinez 1997) in a lysozyme solution (1 mg ml^{-1} final concentration) and incubated at 37 °C for 45 min. After, a solution of proteinase K (0.2 mg ml^{-1} final concentration) and sodium dodecyl sulfate (1% final concentration) was added for 1h at 55 °C. Then, 1 ml of the above lysis buffer was added to incubate 15 min at 55 °C. Subsequently, 1.9 ml of NaCl 6 M was added followed by 10 min centrifugation at 10 000 RPM. The supernatant was recovered in another tube with 5 ml of cold ethanol and set on ice for 10 min. Solutions were separated by 10 min centrifugation at 13 000 RPM and supernatant was discarded. A final washing was done by adding 200 µl of cold ethanol and followed by a final centrifugation of 5 min. Finally, the DNA was solubilised in 200 µl TE (10 mM Tris • HCl; 1 mM EDTA) and frozen at -80 °C until further analysis.

DGGE fingerprints - The extracted DNA was amplified by polymerase chain reaction (PCR) using the eukaryotic 18S rRNA gene primers Euk1F and Euk516r-GC (Diez et al. 2001). The PCR mixture (25 µl) contained 1 µl of extracted DNA, 200 µM of deoxynucleotide triphosphates (dNTPs), 0.3 µM of each primer, 2.5 units of Taq DNA polymerase (New England Biolab, (NEB)), with NEB buffer.. The thermocycling protocol was as follow: 94 °C for 120 s, 30 amplification cycles of 30 s at 94 °C, annealing 45 s at 56 °C, 120 s extension at 72 °C, additional 6 min extension at 72 °C and final cooling at 4 °C. PCR reactions were validated by agarose gel electrophoresis. DGGE were run on a 0.75 mm thick 6% polyacrylamide gel on a linear 35-55% denaturing gradient (100% denaturant is 7 M urea and 40% deionized formamide). Twenty µl of PCR product was loaded into each lane and the samples migrated for 16 h at 100 Volts. Gels were then stained with SYBRGold (Invitrogen) and images were acquired via Bio-Rad Gel Doc imaging system and analysed with Quantity One software (Bio-Rad v. 4.6.0).

Data analysis and statistical treatment - Only thaw ponds where taxonomy was performed are presented in Table 1, but comparisons using averages take into account all sampled ponds. For temperature and oxygen profiles, picoautotrophs and DGGE, only data collected in 2007 were considered. For phytoplankton and pigments, the 2006 dataset was used (phytoplankton taxonomic analysis was only performed in 2006).

The Shannon-Weaver index (H') was used to assess the microbial diversity:

$$H' = - \sum (n_i / N) \ln (n_i / N)$$

where n_i is the number of descriptors of the community (abundance of each taxon in cells

ml^{-1}, or the concentration of each pigment or the intensity of each DGGE band), N is the total number of individuals (or pigments or bands) and n_i / N is the relative abundance for each class. The Sorenson's index (C_s) was used as in (Konopka et al. 1999) in order to compare the similarity between two DGGE samples:

$$C_s = 2j / (a+b)$$

where j is the number of bands obtained in both samples and a and b are the number of bands in samples A and B.

Basic statistics were run with SigmaStats (version 3.0.1 Systat Software Inc.). A detrended correspondence analysis (DCA) with detrending by linear segments and using nonlinear rescaling of axes was first conducted to determine the maximum amount of variation in the phytoplankton data. The resulting gradient length was greater than 2 standard deviations, which suggested a unimodal response for diatoms (Birks 1995). Canonical correspondence analysis (CCA) was used to further explore the phytoplankton-environmental variable relationships. Variables were tested for significance ($p \leq 0.05$) using Monte Carlo permutation tests (with 499 unrestricted permutations). Only the variables that were not highly correlated together (variance inflation factor less than 10) were included in the CCA. Rare taxa were downweighted. Principal component analysis (PCA) and canonical analysis (CA) were also conducted with environmental and phytoplanktonic variables respectively. All ordinations were performed using CANOCO (Version 4.5, Biometris – Plant Research International, Netherlands).

Results

Physicochemical characteristics - The subarctic thaw ponds from the discontinuous permafrost zone are round or oval-shaped with estimated depths between 0.6 and 3 m (average = 1.8 m; N = 28). Diverse optical conditions were evident among ponds, forming a range of color categories: black, brown, beige and green (Figure 8 a). Except for DOM optical properties (higher a_{320}/DOC and lower S in 2007; $p < 0.046$), there were no significant differences in the limnological properties of subarctic ponds which were sampled in both years, the 12 ponds sampled in 2007 were selected from a sub-set of the 2006 series for this comparison, Pond water tended to be pH neutral with pH values ranging from 5.8 to 7.4, except KWK2, which had a pH of 8.5 at the surface in 2007 (Table 1). Most of the subarctic ponds were thermally stratified, some showing a marked thermal stratification and hypo/anoxic hypolimnions, on average, surface O_2 was 10.6 ± 0.4 mg L^{-1} and bottom O_2 was 2.4 ± 3.3 mg L^{-1} in 2007 (Figure 9). Pond KWK6, the only green pond, had an O_2 peak at ~2 m depth in 2007. TSS in the subarctic was generally high, (average 20 mg L^{-1}). According to the standard trophic lake classification using total phosphorus (Wetzel 2001), the subarctic ponds would be considered eutrophic, with TP values averaging 61.5 ± 24.6 µg L^{-1}. Estimated values of K_dPAR in subarctic ponds using the above model averaged 4.7 ± 2.1 m^{-1}. Pond KWK6 was the most transparent subarctic pond and also had the lowest DOC concentration.

The arctic ponds, generally less than a meter deep, were shallow compared to the subarctic ponds. The arctic ponds were also mostly alkaline, with pH values ranging from 7.2 to 9.7. Several ponds contained benthic microbial mats, especially the polygon ponds. In some cases, submerged *Sphagnum* spp. mosses were also abundant. TSS was significantly lower (t-

test, $p < 0.001$) in the arctic ponds (average 4.6 mg L^{-1}) compared to the subarctic. Arctic ponds would be considered meso-eutrophic according to TP (26.6 ± 9.7 µg L^{-1}). DOC content of the arctic ponds (average 11.1 ± 3.0 mg L^{-1}) was significantly higher (t-test, $p < 0.001$) than in subarctic ponds (8.1 ± 2.1 µg L^{-1}). The opposite trend was noted for CDOM (a_{320}), with higher values in subarctic ponds (t-test, $p < 0.001$). Therefore, the absorptivity (a_{320}/DOC) was significantly higher in subarctic ponds (4.1 compared to 1.7 L mg^{-1} m^{-1}, for subarctic and arctic ponds; t-test, $p < 0.001$). Spectral slope of DOM absorption (*S*) at 275-295 nm range was significantly lower in subarctic ponds (average 0.0149 ± 0.0008) than in arctic ponds (average 0.0174 ± 0.0009; Mann-Whitney, $p < 0.001$). The same trend was found with *S* at 350-400 nm (Mann-Whitney, $p < 0.001$; subarctic ponds average 0.0163 ± 0.0006, arctic ponds average 0.0181 ± 0.0008).

PCA showed that the physical and chemical characteristics of the two regions differed with a clear clustering of ponds from each region. The eigenvalues for the PCA performed with arctic and subarctic physicochemical data were 0.509 for the first axis and 0.210 for second axis (Figure 10). The two first axes explained 71.9 % of the total variance. While the sites separated into two clusters, subarctic ponds were more spread out compared to arctic ponds, indicating greater differences among the subarctic ponds. Notably the subarctic ponds sampled in 2006 and 2007 did not exhibit different patterns. Arctic ponds were characterized by high pH, DOC, *S* 350-400 nm et *S* 275-295 nm, while many subarctic ponds were characterized either by high TP, a_{320}, TSS and K_dPAR or low pH, DOC and or *S* 350-400 nm et *S* 275-295 nm.

Bacteria and picoeukaryotes - Surface bacterial concentrations in 2007 were similar in subarctic and arctic ponds (average 11.1 ± 4.0 × 10^6 cells ml^{-1}) although the range was wider

in the subarctic ponds (Table 1). Bacterial densities in bottom waters tended to be greater than at the surface (average 25.2 ± 18.3 × 10^6 cells ml^{-1}), although the difference was not significant. Interestingly, the four most stratified ponds KWK1, KWK6, KWK21 and KWK23 showed the highest bottom bacterial concentrations. Autotrophic picoplankton density observed in thaw ponds ranged over two orders of magnitude (Table 1).

Phytoplanktonic community - Overall, 198 phytoplankton categories, distinct taxa at the level of species, genera, or as distinct morphologies, were separated in the 24 subarctic and 14 arctic thaw ponds (Table 2). The average phytoplankton abundance in the subarctic ponds in 2006, 0.23 ± 0.16 × 10^6 cells L^{-1}, was much lower than in the arctic ponds, 11.3 ± 13.7 × 10^6 cells L^{-1}. Ponds BYL24 and BYL22 had the highest overall phytoplankton abundance (Table 3). Twelve phytoplankton (algal) classes were identified (Chrysophyceae, Chlorophyceae, Ulvophyceae, Prasinophyceae, Charophyceae, Xanthophyceae/Tribophyceae, Cryptophyceae, Dinophyceae, Bacillariophyceae, Euglenophyceae, Cyanobacteria and Raphidophyceae). Ten classes were identified in all 24 subarctic ponds. The Ulvophyceae and Prasinophyceae were completely absent, while the Xanthophyceae were absent from five ponds and Raphidophyceae absent from 12 ponds. Subarctic ponds had on average 50 taxa. Half of the subarctic thaw ponds were dominated by the Chlorophyceae, seven by the Chrysophyceae, and the Cyanobacteria prevailed in five ponds. The Chlorophyceae were most diverse (average of 19 taxa per pond), with *Ankistrodesmus/Monoraphidium* spp. one of the major contributors, followed by the Chrysophyceae (average of ten taxa per pond), mainly cf. *Chrysococcus* sp. and *Dinobryon* sp. Although some dominant Cyanobacteria were not identified, *Anabaena* spp. were found in the subarctic ponds. The Shannon diversity index calculated for the subarctic ponds ranged between 0.66 and 3.08 (Table 5). KWK10 had the

lowest diversity index, with a community dominated by the Chrysophyte *Uroglena* sp. which represented 93% of total cell abundance.

The arctic ponds (N = 14) had on average 48 taxa, from 11 algal classes. Cyanobacteria, mainly *Anabaena* spp and cf. *Chroococcus* sp., were preponderant in eight ponds. Whereas the other ponds were more dominated by Chrysophyceae. Only BYL29 with *Rhodomoas minuta* was dominated by Cryptophyceae. The Chlorophyceae were the most diverse class found in the arctic ponds with 17 taxa on average per pond, followed by the Charophyceae with nine taxa and the Cyanobacteria with eight taxa. Arctic ponds were less diverse with significantly lower (t-test, $p < 0.001$) Shannon index values, that rangee from 0.21-1.31; BYL22 dominantd by *Uroglena/Ochromonas* sp. had the lowest index value. Phytoplankton Shannon index increases with a_{320}/DOC ratio when both sites are combined ($R^2 = 0.647$, $p < 0.001$).

The CA ordination of plankton density for autotrophs only at both study sites is shown in Figure 11. Eigenvalues were 0.453 and 0.414 for the first and second axes, respectively. The two first axes captured 71.0 % of the variance. The ordination based on phytoplankton data showed a clear distinction between the two study regions. This pattern was also evident from the ordination based solely on the physicochemical data (see PCA results).

The CCA ordination of phytoplankton autotrophs communities expressed in cell abundance and physicochemical variables for subarctic and arctic ponds is shown in Figure 12. The CCA included DOC, a_{320}/DOC, K_dPAR and S 275-295 nm. These results showed that a large portion of the total phytoplankton variance on the first 2 axes was explained by environmental variables. These two first axes explained 49.8 % of the total variance in

phytoplankton communities and 99.1% of the canonical variance (the percent of the phytoplankton community variance explained by the first 2 axes). Each environmental variable included in the CCA explained a significant ($p < 0.05$) and independent (VIF < 10) direction of the variance in phytoplankton data. The figure indicates that *S* 275-295 nm was variable most strongly associated with the first axis and that DOC was closely associated with the second axis.

A second CCA performed with phytoplankton communities expressed as relative abundance (ordination not shown) revealed that K_dPAR, DOC and *S* 275-295 nm were the three significant variables. Although the ordination varied slightly depending on the metric used, absolute abundance or relative abundance, arctic and subarctic ponds were still well separated. A third CCA conducted on both planktonic communities (not shown) suggested that the metric based on both phytoplankton and heterotrophs did not provide additional information compared with the metric composed of phytoplankton only. Finally, when a CCA was performed within subarctic site only (not shown), variability was lower, and although it did not yield any pattern, DOC and a_{320} were found significant. A CCA for arctic ponds only was also performed and no environmental variable was significant.

HPLC pigments - No significant differences were found between 2006 and 2007 pigments assemblages, therefore only 2006 results will be discussed, to allow comparatisons with the planktonic assemblages from microscope data. A wide range of chl *a* concentrations were found in thaw ponds of both regions. The chl *a* concentration was significantly higher in subarctic ponds (average 5.4 ± 4.3 compared to 3.6 ± 4.6 µg L^{-1} in arctic ponds; Mann-Whitney, $p = 0.008$; Table 4). Overall, an average of 11 ± 1 pigments per subarctic pond (N = 34) and 8 ± 3 pigments per arctic pond (N = 16) were distinguished. The only significant

relationship obtained between the pigment abundance and the phytoplankton composition was between the Euglenophyceae and the diadinoxanthin (R^2 = 0.641; n = 23; $p < 0.001$). Within the 14 arctic ponds where phytoplankton composition was determined, there was a high fucoxanthin ratio in pond BYL22 and BYL30 with exceptionally high abundance of Chrysophytes (mainly *Uroglena/Ochromonas* sp).

Total carotenoids/chl *a* ratio was lower in the arctic (average 0.5 ± 0.2) than in the subarctic (1.1 ± 0.4) ponds. The carotenoids were classified into two functional groups: the photosynthetic carotenoids (PSC; fucoxanthin, antheraxanthin, violaxanthin) and the photoprotective carotenoids (PPC; canthaxanthin, astaxanthin, diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin, lutein, echinenone, β-carotene). In half of the arctic ponds, no PSC were detected but they were present in all subarctic ponds. Both functional groups (normalised to chl *a*) were significantly less abundant in arctic ponds (average PSC/chl *a* = 0.15 ± 0.18 and PPC/chl *a* = 0.44 ± 0.28 compared to 0.31 ± 0.13 and 0.78 ± 0.27 respectively for subarctic ponds; $p < 0.015$). The Shannon diversity index values from pigments did not vary widely among ponds and averaged 1.7 and 1.3 in subarctic and arctic ponds, respectively.

DGGE - The 18s rRNA DGGE targeted all picoeukaryotes (cells < 3 μm). DGGE fingerprints indicated different picoeukaryote communities in surface waters of the six subarctic and the four arctic ponds analysed. Subarctic samples were run on two separate gels (named Gel#1 and Gel#2; Gel#1 is shown in Figure 13). The subarctic ponds were moderate (KWK2, KWK3, KWK7, KWK11, KWK33) to very stratified (KWK1, KWK6, KWK21) at time of sampling. DGGE performed on the stratified subarctic ponds showed clear differences between surface and bottom communities. The number of DGGE bands

representing separted taxa ranged from 3 and 18. The highest Shannon diversity index vaules, from using band number and intensity were from the bottom waters of ponds KWK11 and KWK3, while the lowest index values were from surface waters of KWK1, KWK3 and BYL29. Overall, picoeukaryote diversity was greatest in the bottom waters of the subarctic ponds (t-test, $p = 0.016$). Surface and bottom DGGE fingerprints from KWK11 were quite different (Sorensen's index of 0.34) but were more similar in KWK3 and KWK6 (0.53 and 0.61 respectively).

Discussion

Thaw ponds are widespread at high latitudes (Osterkamp 2005, Walter et al. 2007, White et al. 2007) and integral part of northern landscapes, and are strikingly different compared to other water bodies. Their unique formation and subsistence processes (French 1996, Brouchkov et al. 2004), shallowness, high nutrient concentrations, productivity (Breton et al. submitted), widely variable optical conditions (Watanabe et al. 2006) and simple trophic food chains (Rautio & Vincent 2006) characterize these small ecosystems. Neighbouring ponds can exhibit very different physicochemical characteristics providing an opportunity to study the influence of environmental variables on microbial diversity

Unique limnological features - Thaw ponds have relatively unique physicochemical characteristics compared to the oligotrophic lakes at similar northern latitudes (Vezina & Vincent 1997, Lim et al. 2001, Rautio & Vincent 2006, Breton 2007). The subarctic thaw ponds had comparable or even higher TP levels than high latitude meso-eutrophic and eutrophic lakes (Liikanen et al. 2003, Lepisto et al. 2006), but a significant fraction of this

phosphorus is likely associated with the high densities of suspended inorganic particles found in these ponds (mostly clays and silts) (Vincent 1989, Calmels & Allard 2004, Calmels et al. 2008). Therefore, the bioavailability of this phosphorus adsorbed to particles (Ekholm 1994) may be reduced. For example, (Breton under revision) showed that the bacterial production was phosphorus limited in a thaw pond from the same area as in the present study, despite relatively high TP concentrations (26.7 μg P L^{-1}). Most subarctic thaw ponds were thermally stratified several days up to several weeks over summer, creating two distinct microbial habitats, one oxic and the other hypo/anoxic. PondKWK6 was the most transparent pond sampled in the subarctic and the only one with an oxygen peak, which was around 2 m. Surface chl *a* concentrations in KWK6 were relatively low (2.5 μg L^{-1}) but reached about 32 μg L^{-1} at 2 m. Such 'deep' oxygen maxima are encountered when the water is transparent enough to let the light reach the thermocline and stimulate photosynthesis in this zone (Boehrer & Schultze 2008). Although these subarctic thaw ponds were quite shallow, they exhibit features of larger seasonally stratified lakes, but vertically compressed.

The arctic thaw ponds were shallower, had lower phosphorus content, were more alkaline and had lower turbidity (with more organic particles) than subarctic ponds. All factors favouring the development of benthic microbial mats. These mats are dominated by filamentous cyanobacteria (mostly Oscillatorians), but also contain rich communities of protists and bacteria (Rautio & Vincent 2006). Thick orange mats were only observed in the low-centered polygon ponds, while the runnels had either thin green mats or no apparent mats. Although the two pond types had similar DOC concentrations, DOM was more chromophoric in the runnels ponds (a_{320} and a_{320}/DOC significantly higher; $p < 0.001$) decreasing the light availability and changing the quality of carbon available to heterotrophic microbes. Another

variable which can be used to characterise DOM is the spectral slope *S*, which was significantly higher in arctic ponds (but not different between polygon and runnel ponds). Spectral slopes give better insight of CDOM characteristics than absorption alone (Helms et al. 2008). Slopes of shorter wavelength (275-295 nm) can be measured with precision and are considered very sensitive to DOM source shifts in aquatic environment (Helms et al. 2008). Photodegradation of DOM, known to increase *S* (Morris & Hargreaves 1997), was likely an important factor in this northern ponds during the arctic summer. Breton et al. (submitted) pointed out that low molecular weight compounds also significantly contributed to the DOM pool of arctic ponds, indicating recently produced organic matter, likely coming from the microbial mats.

The PCA performed on limnological characteristics clearly showed how arctic ponds were clustered tightly in the upper left quadrant (driven by high DOC, pH and both *S* slopes,), while subarctic ponds were spread over all three other quadrants. Limnological characteristics of these strikingly different systems most likely shaped their microbial assemblages. The high turbidity of the subarctic ponds limits light availability and their vertical stratification limits photosynthesis to the upper surface. In contrast, photosynthesis can take place in the whole water column of the shallower arctic ponds exposed to 24 hours of sunlight.

Bacteria and picoautotrophs - The thaw ponds had comparably high bacterial abundance (2.2-17.8 $\times$ 10^6 ml^{-1}) as reported in other high-latitude ponds on the island of Svalbard, Norway (Van Geest et al. 2007a). Other studies on subarctic and arctic ponds and lakes reporte slightly lower bacterial abundances (Hobbie et al. 1980, Granéli et al. 2004, Sawstrom et al. 2008). Although not statistically significant, the bacterial abundance in the hypolimnion of stratified ponds was greater than in surface waters. This trend could be

explained by the higher nutrient and organic matter concentrations observed in the anoxic hypolimnion (Ochs et al. 1995, Simon et al. 1998). It should be noted however that epifluorescence microscopy possibly underestimated the bacterial abundance since 2-6% of bacteria can pass through 0.2 µm filters (Gasol & Moràn 1999) while an additional unknown fraction could be overlooked when using fading fluorophores such as DAPI.

The picoautotroph abundance was higher and more variable in subarctic thaw ponds than in arctic ponds, with values of the same magnitude as for Andean (Callieri et al. 2007) and Danish lakes (Sondergaard 1991). Arctic pond picoautotroph abundances were comparable with boreal lakes in northern Sweden (Drakare et al. 2003). Picoautrophs have igher surface/volume ratios (Ning et al. 2000) and an important portion of biomass and production in lakes comes from picoautotrophs (Drakare et al. 2003), especially in nutrient-poor habitats.. On the other hand, allochtonous DOC input is known to stimulate planktonic heterotrophic bacteria (Jones 1992, Bergstrom & Jansson 2000, Drakare et al. 2002) which compete with picoautotrophs for phosphorus and other nutrients (Jansson et al. 1996, Drakare et al. 2002). Bacterial abundance increased with TP levels (both sites and years combined; R^2 = 0.293, n = 61, $p < 0.001$) but was not correlated to DOC. However, bacteria concentrations were inversely correlated with S (R = -0.336, p = 0.007) which is an index of DOM quality. Nevertheless, the biological production in this environment is more likely controlled by the availability of light (see below).

Phytoplankton communities - The total number of phytoplankton taxa observed in both sampling sites was comparable with data from other northern regions (77-117 taxa in two semi-forested Finnish lakes, (Holopainen et al. 2003); 148 taxa in a large Finnish lake, (Forsstrom et al. 2005); 152 taxa in 25 boreal wetland ponds in Norway, (Soininen et al.

2007); 251 phytoplankton species in tundra ponds across Northern Canada, (Sheath 1986). As Trifonova (1998) pointed out, the taxonomic diversity of boreal lakes can be as high as in the tropical regions, with a global average around 50-150 taxa per lake.

Since thaw ponds were classified as meso-eutrophic or eutrophic according to TP concentration, typical high nutrient phytoplankton communities may have been expected, these are usually dominated by Chlorophyceae, Bacillariophyceae, Euglenophyceae and Cyanobacteria (Wetzel 2001, Reynolds 2006). Subarctic and arctic thaw ponds were indeed dominated by Chlorophyceae and Cyanobacteria, but also by Chrysophyceae. The Bacillariophyceae (diatoms) were not abundant in thaw ponds, as opposed to peatland lakes in the subarctic Quebec (Magnin 1977) or in Alaskan tundra ponds (Sheath 1986). Silica concentrations, which may limit diatom growth (Prentki et al. 1980) were not measured in the present study and we cannot comment. However, the thermally stratified water columns of thaw ponds, particularly the subarctic ponds, would not favour diatoms. The phytoplankton data summarized by (Sheath 1986) from tundra ponds in Alaska and Northwest Territories revealed a preponderance of Chrysophyceae, especially *Dinobryon* and *Uroglena* spp.,which are normally ubiquitous and often dominant in oligotrophic lakes (Lepisto & Rosenström 1998, Wetzel 2001). *Dinobryon* was omnipresence in the subarctic thaw ponds and is frequently recorded from Canadian Shield lakes (Ostrofsky & Duthie 1975). Such high Chrysophyceae abundance in these TP-rich ponds may suggest a limited availability of this phosphorus. Many Chrysophyceae are mixotrophic and can obtain their carbon sources via both photosynthesis and phagotrophy, providing a competitive advantage for these species (Olrik 1998). Moreover, chrysophyceaen flagellates can dominate when DOC is high (Drakare et al. 2003) and phagotrophy provides supplemental phosphorus

(Rothhaupt 1996) via their consumption of bacteria. Many subarctic ponds were dominated by Chlorophyceae, which are also diverse and abundant in *Sphagnum* sp. bogs in cool temperate regions (Wetzel 2001).

One striking difference between both regions was the preponderance of cyanobacteria in arctic ponds,first reported by Vezina & Vincent (1997), who showed a wide range of growth-irradiance capacities in these cyanobacterial communities, a powerful trait enabling growth under 24h sunlight. Environmental factors such as winter freezing, frequent freeze-thaw cycles (Davey 1989), desiccation (Potts 1994) and strong ultraviolet radiation (Vincent & Quesada 1994) could explain why cyanobacteria are so abundant in these harsh habitats. Once established, this preponderance of cyanobacteria reduces CO_2 levels by their intense photosynthesis, increasing pH and reducing the ability for other species to expand (Shapiro 1997).

Cryptophyceae were present in both regions. Nonetheless, while *Rhodomonas minuta* is widespread in tundra ponds (Sheath 1986) we nly found this specien in the arctic ponds. A study by Hickman (1974) revealed that *R. minuta* was restricted tothe upper 2 m of the water column of a small thermo-stratified pond in UK, mainly during spring and to a lesser extent in autumn. The author suggested that nutrients released from the hypolimnion could explain such a high Cryptomonad abundance, since this taxon has high nutrient requirements and can migrate in the water column. It is therefore possible that Cryptomonads were present in subarctic ponds but within a narrow depth range and not sampled. Overall the protist community, including unidentified taxa, of arctic ponds was dominated by autotrophs (> 97.5%), but some of the subarctic ponds had up to one third of their protist community

composed by heterotrophs, with most > 5% heterotrophs, a large proportion of the photosynthetic taxa wer also potentially mixotrophic.

The Shannon diversity index values (*H'*) varied widely among subarctic thaw ponds (Table 5) and fell within the range often reported for eukaryote microbes (usually between 1.5 and 3.5; Graham et al. 2004). Graham et al. (2004) noted that pond microbial diversity can also vary widely over the summer (*H'* from 0.5-3.0). Logistic constraints meant we only sampled phytoplankton once and only in surface waters. Despite that, we found rather high phytoplanktonic diversity at mid-summer. Several factors can influence autotrophic communities such as water temperatures and the seasonal mixing regime, irradiance, nutrients and grazing pressure (Richardson et al. 2000, Tolotti et al. 2003). Linear relationship was found between Shannon index and a_{320}/DOC when both sites were combined. This finding leads to the hypothesis that the proportion of chromophoric DOC in total DOC might affect phyotplankton diversity. At a shorter time scale, wind-induced mixing can also alter phytoplankton diversity by bringing up nutrients from the hypolimnion or decreasing the light availability from sediment resuspension (Weithoff et al. 2000). Shallow thaw ponds are prone to partial mixing during the summer (Breton 2007), likely affecting the microbial community distribution within the water column through time (Alexander et al. 1980).

PCA of limnological characteristics showed that arctic ponds were less diverse than the subarctic ponds. This might explain why phytoplankton diversity was not as great as at the subarctic site. The CA ordination (Figure 11) clearly demonstrates that communities of both sites were different which was expected. Interestingly, only K_dPAR, DOC, a_{320}/DOC ratio and *S* 275-295 nm were significant in the CCA combining limnological data and

phytoplankton (Figure 12). Since *S* can be used as an indicator of DOM quality, this suggests that the organic matter quality could partially drive phytoplankton assemblages since many taxa were mixotrophic, but there are possibly indirect and more complex effects on the whole planktonic community. The light availability (K_dPAR) is also a driving factor. A combination of optical characteristics, along with organic matter origin and quality would influence phytoplankton assemblages.

Pigments assemblages - The pigment signatures found in the subarctic ponds indicated the presence of Chrysophyceae (fucoxanthin, diatoxanthin), Chlorophyceae (chl *b*, lutein, zeaxanthin, violaxanthin, antheraxanthin), Cyanobacteria (echinenone, zeaxanthin, canthaxanthin), Cryptophyceae (alloxanthin) and Bacillariophyceae or Euglenophyceae (diatoxanthin, fucoxanthin and diadinoxanthin). The pigment diversity was also lower in the arctic ponds, with mainly pigements consistant with Chrysophyceae (fucoxanthin, but no diadinoxanthin and diatoxanthin), Chlorophyceae (only one pond with antheraxanthin), Cryptophyceae and Cyanobacteria.

Total chl *a* concentrations increased with TP, but the r value was low ($r = 0.441$; $n = 61$; $p < 0.001$), possibly because photosynthesic production was light limited, at least in the subarctic ponds. Potential low phosphorus bioavailability, discussed above, but also high zooplankton grazing pressure could also explain such results (Alexander, 1980, Van Geest et al. 2007b.). On average, total carotenoid concentrations (normalised to chl *a*) in subarctic ponds (around 0.5) were similar to concentrations measured in the turbid sections of the Mackenzie River in northern Canada (Retamal et al. 2008). Moreover, the measured K_dPAR in these sections were close to our estimates (5.2 ± 0.1) (Retamal et al. 2008). Light limitation in the turbid subarctic ponds was associated to a higher proportion of accessory photosynthetic pigments

(PSC/chl *a*), but also a higher proportion of photoprotective pigments (PPC/chl *a*) as compared to the more exposed arctic ponds. Such low PSC/PPC ratio was rather unexpected in such turbid systems where high photosynthetic performance would be needed. One possible explanation could be linked to the strong stratification at time of sampling. Since only surface waters were sampled for pigment analysis, they represent cells likely trapped in the epilimnion long enough to stimulate photoacclimation and the production of PPC against excessive light and UV damage (Falkowski & Laroche 1991).

Arctic pond pigment patterns were clearly different, with total carotenoids/chl *a* about half of those in the subarctic. Arctic pond communities were likely not light-limited, therefore they did not need to produce as many accessory pigments. PSC were only present in polygon ponds, but when detected, the PSC/chl *a* ratio was higher than the PPC/chl *a*. The study by Bonilla et al. (2005) in a Canadian high arctic lake revealed high concentrations of fucoxanthin associated to the presence of *Dinobryon* sp. in pelagic waters In the present study, *Dinobryon* was not reported, but the pigment assemblage can also be explained by a high prevalence of *Ochromonas* sp another Chrysophyte.. The presence of PPC, mainly lutein, zeaxanthin, alloxanthin, β-caroten and echinenone, in all arctic ponds is to be expected primarily because of the high UV radiation during summer, although PPC/chl *a* was about half of the ratio measured at the surface of subarctic ponds. The Shannon diversity index values for pigments was lower in the arctic compared to the subarctic ponds, and overall did not vary widely.

Picoeukaryote diversity - DGGE is a molecular technique which allows separation of similar-size DNA fragments via the exploitation of nucleotides base differences (Muyzer & Smalla 1998). Bacterial and archeal communities have been studied using this fingerprinting

technique in many freshwater environments (Ovreas et al. 1997, Lindstrom 1998, Haukka et al. 2005, Van der Gucht et al. 2005, Yan et al. 2007). DGGE targeting eukaryotes, especially picoeukaryotes, is also frequently reported for marine waters (Diez et al. 2004, Hamilton et al. 2008) but very few studies have focused on lacustrine picoeukaryotes. In the present study, the aim of using DGGE was to estimate picoeukaryote diversity among ponds and within stratified ponds comparing epilimnetic and hypolimnetic assemblages.

Between 12 and 27 bands were seen in the three DGGE gels, a richness that falls within that of other studies. For example, (Diez et al. 2004, 2001) reported 20 to 45 bands in samples from the south-west Mediterranean Sea and 11 to 14 bands from the North Atlantic, while Gast et al. (2004) found 10 to 30 bands Southern Ocean samples. Konopka et al. (1999) studied bacterioplankton community diversity in the epilimnion and metalimnion of ten small stratified lakes in Indiana, USA. They reported that the anoxic metalimnetic communities were less similar to epilimnetic communities, suggesting that different conditions and selection pressure yielded different bacterial communities. Ovreas et al. (1997) also reported a bacterial community shift in the anoxic part of Lake Saelenvannet in Norway, with a general reduction in diversity with depth. In light of these studies and even if prokaryotes and eukaryotes cannot be systematically compared, the same trend could be expected. On the contrary, the stratified subarctic ponds bottom samples (anoxic/hypoxic) were more diverse than surface sample (t-test, $p = 0.016$) and contained different assemblages (Figure 13 and Table 5). Sorensens' similarity index values were comparable with those of Konopka et al. (1999) who also had moderate indices with bacterial communities in stratified lakes. Similarity indices for surface and bottom waters indicate that some taxa are shared by both depths, but each displayed unique patterns, thus most likely different species and phylotypes.

The very shallow and weakly stratified pond KWK3 had the highest similarity index between surface and bottom. The very stratified pond KWK21 contained less similar picoeukaryote assemblages. Overall, these results indicate the presence of distinct picoeukaryote communities within a single pond. Thermal stratification produces heterogeneous conditions in terms of temperature, oxygen, nutrients and light availability, which likely control the observed patterns.

Varied band patterns were also found for the arctic ponds. No significant differences were found between Shannon diversity index vaules of arctic and subarctic ponds. Still, there were fewer bands in the arctic compared to the subarctic, with BYL25 and BYL27 having single bands. This result suggests the possible dominance of fewer picoeukaryote taxa per pond in such extreme systems. However, DGGE fingerprinting needs to be optimized for such humic and turbid systems. We did not necessarily optimize the DNA extraction protocol. PCR itself may leads to biased because of primer composition (Wintzingerode 1997) and since primers may not target all micro-organisms in an environment even though they are labelled "universal" (Schmalenberger et al. 2001). Co-migration of different sequences with the same denaturing characteristics on a gel can also occur.

Comparison of biological indicators - Three biological indicators were used to describe the microbial diversity of thaw ponds. The aim was to compare ponds within a specific site and between thaw ponds at two latitudes with discontinuous versus continuous permafrost. These indicators revealed varied phytoplankton communities, pigment patterns and picoeukaryote assemblages among ponds. Neighbouring ponds exhibited noticeable differences in phytoplankton and pigment dominance. Some subarctic ponds were nearly completely dominated by Chlorophyceae while Chrysophyceae were dominant in other ponds. For most

of the ponds, one group was dominant, leading to lower diversity. Yet the opposite was noted with ponds KWK15 and KWK36 with high diversity and almost no dominant group. PSC/PPC ratio also varied broadly among subarctic ponds, with KWK9, KWK10 and KWK11 having exceptionally more PSC than PPC (concurrently with high *Uroglena* sp. abundances). Arctic ponds were either dominated by Chrysophyceae or Cyanobacteria and, as for pigments, they were less diverse than subarctic ponds. This trend could be explained by the extreme conditions at higher latitudes. From year to year, polygon ponds could completely dry up depending on precipitation, such as observed between 2005 and 2007. Subarctic thaw ponds are also harsh habitats, but in several cases they apparently may not freeze completely (Laurion et al. submitted on January 2009; under revision) and turbidity offers protection from UV radiation, allowing the establishment of more diverse communities. CCA revealed that pond assemblages were not all driven by the same physical and chemical variables, and that light availability, K_dPAR, which depends on TSS and CDOM, and DOM quality (*S*) were the only significant controlling variables among those was measured.

No correlations were found between phytoplankton and pigment diversity indexes, unlike a study by Estrada et al. (2004) on phototrophic communities along a salinity gradient, or by Nubel et al. (1999) on oxygenic phototrophs in microbial mats. In our study, many phytoplankton taxa were not identified at the level of species and only referred as morphological types, which underestimates the number of species. Microscopy can determine the presence of specific organisms within a community, but may be biased towards larger cells (Nubel et al. 1999). The HPLC approach is fast, reproducible and sensitive (Schluter et al. 2000) but cannot reach species level. In the present study, the only direct correlation

between phytoplankton abundance and pigment concentration was with the Euglenophyceae and diadinoxanthin concentration, which is a diagnostic pigment for this class. This lack of direct concordance is because several different algal classes have the same dominant pigments, and a single class may have several pigment signatures (Vaulot et al. 2008). Furthermore, cellular pigment concentrations depend on the photoadaptive status of the cell and varies in the aquatic environment. Choosing pigment analysis as the only biological indicator can lead to a wrong impression of phytoplankton community, if for example abundant species have low concentrations of diagnostic pigments (Havskum et al. 2004). Microscopy performed along with HPLC and flow cytometry is therefore recommended (Ansotegui et al. 2001, Ston et al. 2002). In the present study, DGGE analyses on picoeukaryotes (2007) were not done on the same samples as the microscopy and HPLC analyses (2006), but still can be used to compare communities in different ponds and water depths.

Our study results suggest that the irradience and related light conditions along with DOM quality were at least partially driving the pigment assemblages, with K_dPAR, DOC, a_{320}/DOC ratio and *S* 275-295 nm being the variable that explained most the variability in phytoplankton distribution in the CCA analysis. DOM quality impact on phytoplankton needs to be investigated to determine whether its influence is direct or indirect, for example via zooplankton grazing. Also, the subarctic thaw ponds were characterized by their stable water column in summer, creating two microbial habitats that harboured very different assemblages. Finally, clear differences in phytoplankton diversity and composition were observed between arctic and subarctic ponds.

Table 1 Physicochemical and biological characteristics of thaw ponds sampled in 2006 and 2007, including dissolved organic carbon (DOC), absorption coefficient of dissolved organic matter at 320 nm (a_{320}), slope of the spectral absorption by dissolved organic matter (*S*), estimated diffuse attenuation coefficient (K_dPAR), total suspended solids (TSS), total phosphorus (TP), pH, oxygen concentration (O_2) and abundance of bacteria (at the surface and bottom of some ponds) and picoautotrophs.

Pond name	Type or color	DOC	TP	TSS	a_{320}	*S 275-295nm*	*S 350-400nm*	K_dPAR	pH	O_2	Surface bacterial abundance (bottom)*	Picoauto*
		mg l^{-1}	ug l^{-1}	mg l^{-1}	m^{-1}	nm^{-1}	nm^{-1}	m^{-1}		mg l^{-1}	10^6cells ml^{-1}	10^6cells ml^{-1}
Whapmagoostui-Kuujjuarapik												
2006												
KWK1	BRO	7.6	39.7	6.3	32.7	0.015	0.017	3.1	6.63	9.6	35	0.22
KWK2	BLA	5.9	36.3	2.8	21.1	0.015	0.018	2	6.31	8.1	30.4	0.33
KWK3	BEI	8.8	67.6	21.9	36.8	0.015	0.016	5	7.37	9.1	15.8	0.17
KWK4	BEI	8.4	65.9	22.3	32.4	0.015	0.017	4.7	6.13	9.1	17.5	0.36
KWK5	BLA	9.5	46.5	4.3	44.2	0.014	0.017	3.7	-	-	17.07	0.364
KWK6	GRE	3.8	25.9	3.7	8.3	0.018	0.016	1.2	6.26	9.2	14.4	0.76
KWK7	BRO	8	60.2	9.6	33.7	0.015	0.017	3.5	7.28	9.1	46.3	2.89
KWK8	BRO	9.9	61.1	30.6	38.5	0.015	0.017	6	-	-	16.26	0.073
KWK9	BEI	5	69.1	21.8	14.9	0.016	0.015	3.5	6.6	10	41.3	0.19
KWK10	BEI	5.7	52.5	16.2	15.7	0.017	0.016	3	-	-	40	0.47
KWK11	BLA	10.4	49.3	1.8	44.6	0.014	0.016	3.5	-	-	22.7	0.179
KWK12	BLA	6.9	24.7	2.5	27.17	0.014	0.017	2.4	6.3	9.1	4.72	0.18
KWK13	BRO	8.1	64.4	19.9	35.1	0.015	0.016	4.7	-	-	20.33	0.367
KWK14	BEI	7.3	49.3	46.4	22.32	0.015	0.017	6.5	7.2	10.6	23.78	0.182
KWK15	BEI	9.1	66.5	50.4	33.1	0.015	0.017	5.2	6.96	9.7	18.77	0.087
KWK16	BEI	8.7	-	24.3	35.7	0.015	0.016	6.2	7.24	9.3	48.11	0.49
KWK17	BEI	9.2	89.2	30.1	41.3	0.014	0.017	9	6.21	8.7	61.58	0.314
KWK18	BRO	11.5	83.7	43.7	63.1	0.013	0.016	6.6	6.32	8.7	15.13	0.213
KWK19	BEI	8.8	69.9	39.4	34.2	0.015	0.016	4.8	6.48	9	17.95	0.456
KWK20	BEI	7.7	67.5	22.2	32.7	0.015	0.016	4	6.17	9.1	26.82	0.42
KWK21	BEI	8.3	107	55.2	33.4	0.015	0.016	8.2	10.1	6.98	23.37	-
KWK23	BEI	7	62.4	19.3	25.87	0.015	0.016	4	6	9.1	39.4	0.677
KWK24	BLA	9.3	54.5	4.7	46.6	0.014	0.016	3.9	9.61	6.74	27.63	0.69
KWK25	BRO	10.8	64.4	21.5	47.5	0.014	0.017	4	5.82	8.9	27.35	0.1
KWK27	BLA	10.7	31.4	3.8	49.75	0.014	0.017	4	6.2	8.8	32.04	0.204
KWK28	BEI	10	122.3	51	37.8	0.015	0.016	8	8.47	6.9	78.88	0.384
KWK31	BEI	6	42.4	8.9	18.3	0.016	0.016	2.4	8.04	6.03	21.1	2.631
KWK33	BRO	12.6	123.6	75.8	59.9	0.014	0.016	12.1	8.07	5.78	20.06	0.101
KWK34	BRO	7.6	80.3	20.4	28.6	0.015	0.016	4.3	6.5	7.4	28.06	0.073

KWK35	BRO	12.9	31.2	6	52.1	0.014	0.017	4	-	-	7.99	-
KWK36	BLA	9.9	39.5	7.1	44.9	0.014	0.017	6.3	6.84	10	14.63	0.488
KWK37	BRO	8.7	70.9	36.9	32.9	0.015	0.017	6.3	6.9	9.1	35.98	0.954
KWK38	BEI	10.7	-	4.8	29.3	0.014	0.015	2.8	7.49	7.18	111.43	0.028
KWK39	BEI	8.2	-	6.2	14.8	0.019	0.017	1.9	-	-	70.18	-
2007												
KWK1	BRO	7.6	48.2	11.7	39.5	0.014	0.016	4.2	7.36	10.92	6.73 (46.91)	0.06
KWK2	BLA	5.2	37.6	5.1	31	0.013	0.017	2.9	8.46	10.49	9.57 (5.15)	0.29
KWK3	BEI	8.7	52.8	42	41.9	0.015	0.016	7.4	6.97	11.07	12.59 (8.53)	0.2
KWK6	GRE	3.1	33.6	6.9	9.9	0.016	0.016	1.6	6.89	11.09	12.84 (53.3)	0.09
KWK7	BRO	9.5	59.7	11.3	45.4	0.014	0.016	4.5	7.2	10.08	15.11 (13.71)	0.02
KWK11	BLA	9.4	95.6	9.5	38.3	0.014	0.016	3.8	6.77	11.21	15.22 (15)	0.02
KWK21	BEI	7.7	86	24	41	0.015	0.016	5.5	6.87	10.44	16.25 (28.58)	0.07
KWK23	BEI	6.6	69.5	16.8	33.8	0.015	0.016	4.3	7.18	10.52	15.58 (55.76)	0.25
KWK33	BRO	9.7	108.9	25.1	56.1	0.014	0.016	6.7	6.2	9.9	15.93 (13.55)	0.57
KWK35	BRO	10.5	41.2	10.6	42.3	0.015	0.017	4.2	7.25	10.49	9.48	0.05
KWK36	BLA	8.1	36.9	8	40.4	0.014	0.017	3.8	6.52	9.8	10.3 (19.41)	0.05
KWK38	BRO	6	54.2	5.5	19.5	0.016	0.016	2.15	7.33	10.9	10.09 (17.42)	0.43
Bylot Island												
BYL1	POL	10.4	26.6	3.3	13.48	0.022	0.017	2	9.6	11.42	13.83	0.009
BYL22	POL	8.9	34.2	4.4	15.32	0.020	0.018	2.2	9.49	11.69	17.77	0.009
BYL23	RUN	18.4	41.8	2.6	40.09	0.035	0.019	2.7	8.87	12.98	5.68	0.005
BYL24	RUN	11	43	6.2	24.21	0.018	0.019	2.6	8.99	11.98	6.63	0.066
BYL25	RUN	12.4	16.7	4	23.76	0.020	0.019	2.4	8.08	11.36	10.93	0.003
BYL26	POL	10.8	18	2.8	13.17	0.023	0.018	1.9	9.59	12.82	7.9	0.005
BYL27	RUN	14.4	28.6	4.5	45.99	0.020	0.018	2.9	8.54	9.14	14.68	0.005
BYL28	RUN	12.4	30.2	4.4	30.6	0.021	0.019	2.6	7.88	9.33	13.71	0.009
BYL29	POL	12.7	28	1.7	20.38	0.021	0.017	2.2	9.65	12.61	10.24	0.01
BYL30	POL	10.7	26.5	5.7	13.4	0.022	0.018	2.1	9.46	12.34	10.25	n.a
BYL31	POL	14.2	28.3	9	22.56	0.025	0.019	2.7	9.2	12.46	9.33	0.006
BYL32	POL	10.4	26.2	5	15.56	0.023	0.018	2.2	9.61	12.12	17.71	0.015
BYL33	RUN	8.2	14.3	1.5	14.86	0.022	0.018	1.9	9.38	12.56	4.67	0.003
BYL34	POL	11.8	35.1	13.7	15.66	0.021	0.017	2.8	9.53	12.8	12.7	0.01
BYL35	POL	8.7	35.4	3.3	14.36	0.018	0.017	2	9.4	12.84	9.93	0.022
BYL41	POL	8.9	20.8	n.a	13.2	0.023	0.018	1.8	7.19	11.59	8.35	0.027
BYL42	POL	11.3	4.2	n.a	15.88	0.024	0.019	1.9	8.11	12.36	2.21	0.026

BRO, Brown; BLA, Black; BEI, Beige; GRE, Green; POL, Polygon thaw pond; RUN, Runnel thaw pond; Picoauto, picoautotrophs.

*Bacteria and picoautotrophs were counted via flow cytometry in 2006 and epifluorescence microscopy in 2007.

Table 2 List of phytoplankton (and some heterotrophs) taxa identified in subarctic (2006) and arctic (2007) thaw ponds.

Class	Taxon
Chrysophyceae	Chrysophyceae 10-20um
	Bitrichia sp.
	cf. Chromulina sp.
	cf. Chrysococcus sp.
	Chrysolykos planctonicus
	Chrysosphaerella spp.
	Dinobryon bavaricum
	Dinobryon cylindricum
	Dinobryon divergens
	Dinobryon cf. sertularia
	Dinobryon cf. suecicum
	Dinobryon sp. 1
	Dinobryon spp.
	cf. Epixyxis sp.
	cf. Gleorochloris
	Kephyrion spp.- *like cells*
	Mallomonas cf. acaroides
	Mallomonas cf. akrokomos
	Mallomonas cf. tonsura
	Mallomonas spp. *10-20um*
	Mallomonas spp. *20-50um*
	Mallomonas spp. *50-100um*
	cf. Ochromonas spp.
	Pseudokephyrion cf. attenuatum
	Pseudopedinella sp.
	Synura sp.
	Uroglena sp. *and/or Ochromonas* sp.
Chlorophyceae	Flagellate 2-5 um
	Flagellate 5-10 um
	Flagellate 10-20 um
	Flagellate 20-50 um
Charophyceae (cont.)	*Mougeotia* sp.
	Spinocosmarium cf. quadridens
	Spondylosium sp.
	Staurastrum cf. vestitum
	Staurastrum spp. 10-20 µm
	Staurastrum spp. 20-50 µm
	Staurastrum spp. >50 µm
	Staurodesmus cf. dejectus
	Staurodesmus spp. 10-20 µm
	Staurodesmus spp. 20-50 µm
	Teilingia sp.
	Xanthidium spp.
	Zygnema sp.
Xanthophyceae (Tribophyceae)	cf. *Centritractus* sp.
	Ophiocytium sp.
	Pseudostaurastrum limneticum
	cf. Tetraedriella sp. (10-20 µm)
Cryptophyceae	Crypto spp. 5-10 µm
	Crypto spp. 10-20 µm
	Crypto spp. 20-50 µm
	cf. Cryptomonas sp.
	cf. *Rhodomonas* sp. 1 (16-20 µm)
	cf. *Rhodomonas* sp. 2 (9-10 µm)
	Crypto type 3 (medium with round tail)
	Crypto short (maybe heterotroph)
	cf. Rhodomonas minuta
Dinophyceae	Dino without theca 10-20 µm
	Dino with theca 20-50 µm
	Gymnodinium spp. 5-10 µm
	Gymnodinium spp. 10-20 µm
	Gymnodinium spp. 20-50 µm

Chlorophyceae (cont.)	Chloro 5-10 um
	Chloro 10-20 um
	Chloro 20-50 um
	Chloro colonial mucilage
	Chloro colonial mucilage
	Chloro colonial mucilage
	Chloro colonial mucilage
	Ankyra sp.
	Ankistrodesmus/Monoraphidium spp.
	cf. Asterococcus sp.
	cf. Carteria sp. 5-10 µm
	cf. Carteria sp. 10-20 µm
	cf. Chaetosphaeridium sp.
	cf. Chlamydomonas spp. 5-10 um
	cf. Chlamydomonas spp. 10-20 um
	cf. Chlamydomonas spp. 20-50 um
	Chodatella sp.
	cf. Chlorangiopsis sp.
	Chlorogonium sp.
	cf. Coccomonas sp. (or Dysmorphococcus)
	Coelastrum spp.
	Crucigenia cf. crucifera
	Crucigenia quadrata
	Crucigenia rectangularis
	Crucigenia tetrapedia
	Crucigenia spp. <10 um
	Desmatractum bipyramidatum
	Desmodesmus sp.
	cf. Dictyochlorella sp.
	Dictyosphaerium spp.
	Dimorphococcus cf. lunatus
	cf. Dispora sp.
	cf. Elakatothrix sp.

Dinophyceae (cont.)	*Peridinium* spp. 5-10 µm
	Peridinium spp. 10-20 µm
	Peridinium spp. 20-50 µm
	Peridinium spp. >50 µm
	Pennales 20-50 µm
	Pennales 50-100 µm
	Pennales >50 µm
	Centrales 10-20 µm
	Centrales 20-50 µm
	Centrales >50 µm
	Pennale sp. 3
	Achnantes sp.10-20 µm
	Amphora sp. 10-20 µm
	Amphora sp. 20-50 µm
	cf. Asterionella formosa
	Eunotia sp. 20-100 µm
	cf. Gomphonema sp.
	cf. Fragilaria sp.
	cf. Melosira sp.
	Navicula sp. 10-20 µm
	Navicula sp. 20-50 µm
	cf. Pinnularia sp. 20-50 µm
	Tabellaria fenestra
	Tabellaria flocculosa
	Urosolenia sp.
Euglenophyceae	Eugleno 10-20 µm
	Eugleno >50 µm
	Eugleno sp.1 (50-100 µm)
	Eugleno sp.2 (50-100 µm)
	Eugleno sp.3 (20-50 µm)
	Strombomonas sp. 20-50 µm
	Trachelomonas cf. superba
	Trachelomonas spp. 10-20 µm

Chlorophyceae (cont.)	*Euastropsis richteri*
	Eudorina sp.
	Franceia spp.
	Gonium sp.
	Kirchneriella spp.
	cf. Lagerheimia genevensis
	cf. Micractinium sp.
	cf. Monoraphidium minutum
	Oocystis spp. 5-10µm
	Oocystis spp. 10-20µm
	Oocystis spp. 20-50µm
	Pandorina sp. (small)
	Pandorina sp. (large)
	Paulschulzia sp.
	Pediastrum duplex var. clathratum
	Pediastrum cf. boryanum
	Pediastrum tetras
	Pediastrum sp.
	cf. Polychaetophorasp.
	cf. Quadrigula sp.
	Scenedesmus cf. arcuatus
	Scenedesmus spp.
	cf. Selenastrum sp.
	Spermatozopsis sp.
	cf. Sphaerellopsis sp.
	cf. Stylosphaerium sp.
	cf. Tetraselmis sp.
	Tetraedron spp.
	Volvox sp.
	cf. Westella sp.
Ulvophyceae	*cf. Binecularia* sp.
Prasinophyceae	*Pyramimonas* sp.
Charophyceae	*Arthrodesmus* spp. 20-50 µm (include *Octacanthium* sp.)
	Bambusina cf. brebissonii
	Closterium spp. 50-100 µm
Cyanobacteria	*Trachelomonas* spp. 20-50 µm
	Cyano 2-5 µm
	Cyano 5-10 µm
	Cyano 10-20 µm
	Trichome with tube and mucilage
	Trichome without tube or mucilage
	Anabaena spp.
	cf. Anabaenopsis sp.
	cf. Aphanocapsa sp.
	cf. Chroococcus sp.
	cf. Gomphosphaeria sp.
	Merismopedia sp. (2-5 µm)
	cf. Microcystis sp.
	Nodularia-like
	cf. Nostoc sp.
	Oscillatoriacae
	cf. Rhabdogloea sp.
	cf. Snowella sp.
Raphidophyceae	*Gonyostomum* sp.
	Closterium spp. 50-100 µm
Ciliates	cf. *Actinobolina* sp.
	cf. *Askenasia* sp.
	cf. *Campanella* sp.
	cf. *Vorticella* sp.
	Peritrich 10-20 µm
	Peritrich 20-50 µm
	Peritrich 50-100 µm
	Peritrich > 100 µm
	"Holotrich" 5-10 µm
	"Holotrich" 10-20 µm
	"Holotrich" 20-50 µm
Zooflagellates	*Aulomonas purdyi*
	Bicoeca sp.a
	Bicoeca sp.b
	Bicoeca sp.c

Charophyceae (cont.)	*Closterium* spp. >100 µm
	Closterium cf. kuetsingii
	Cosmarium spp. 5-10 µm
	Cosmarium spp. 10-20 µm
	Cosmarium spp. 20-50 µm
	Cosmarium spp. >50 µm
	Euastrum spp. 10-20 µm
	Euastrum spp. 20-50 µm
	Euastrum spp. >100 µm
	cf. Genicularia sp.
	cf. Gonatozygon spp.
	Hyalotheca sp.
Zooflagellates (cont.)	*Bicoeca* spp. *(include epiphytes)*
	Choanoflagellates sp. (include epiphytes)
	Monosiga spp.
	cf. *Paramastix* sp.
	cf. *Rhipidodendron* sp.
Heliozoa and Actinopoda	Amibea
	cf. *Difflugia* sp.
	Diplophrys sp.
	Heliozoa-like cells

Table 3 Abundance of phytoplankton groups in subarctic (2006) and arctic (2007) thaw ponds, with dominant groups, percentage of dominant groups and Shannon taxa diversity index.

				Phytoplankton concentration											
Pond name	Dominant group	% of dominance	Shannon index	Chryso (10^6cells L^{-1})	Chloro	Ulvo	Prasino	Charo	Xantho	Crypto	Dino	Bacillario	Eugleno	Cyano	Raphido
Whapmagoostui-Kuujjuarapik															
KWK1	CHLORO	54	3.05	0.054	0.127	0	0	0	0	0.022	0.008	0.001	0.006	0.016	0
KWK2	CHRYSO	49	2.65	0.076	0.068	0	0	0.001	0	0.004	0.001	0.001	0	0.005	0
KWK3	CHLORO	76	2.52	0.011	0.165	0	0	0	0	0.020	0.001	0.001	0.001	0.016	0
KWK4	CHLORO	70	2.62	0.014	0.072	0	0	0	0	0.010	0.002	0	0	0.003	0
KWK6	CHLORO	64	2.16	0.058	0.131	0	0	0	0	0.008	0.001	0.001	0	0.005	0
KWK7	CHLORO	70	2.81	0.018	0.098	0	0	0	0	0.007	0.010	0.001	0.002	0.002	0
KWK9	CHRYSO	88	1.92	0.443	0.046	0	0	0	0	0.004	0	0.004	0	0.008	0
KWK10	CHRYSO	93	0.66	0.366	0.010	0	0	0	0	0.013	0	0.001	0.001	0.003	0
KWK12	CYANO	40	2.83	0.066	0.124	0	0	0	0	0.008	0.005	0.002	0	0.136	0
KWK14	CHRYSO	86	2.07	0.453	0.044	0	0	0	0	0.002	0.011	0.002	0.003	0.008	0.005
KWK15	CYANO	31	2.91	0.068	0.056	0	0	0.001	0	0.008	0.003	0.001	0.015	0.072	0.005
KWK16	CHLORO	55	3.15	0.053	0.119	0	0	0	0	0.022	0.005	0.001	0.009	0.006	0
KWK17	CHLORO	53	2.73	0.015	0.066	0	0	0	0	0.005	0.002	0.010	0	0.024	0
KWK18	CHLORO	91	1.71	0.018	0.527	0	0	0.001	0	0.012	0.005	0.002	0.005	0.005	0
KWK19	CHLORO	62	2.80	0.028	0.075	0	0	0	0.001	0.001	0.001	0.009	0	0.005	0
KWK20	CHRYSO	59	2.17	0.286	0.172	0	0	0	0	0.016	0.004	0	0.001	0.004	0
KWK23	CHLORO	50	3.20	0.003	0.041	0	0	0.001	0	0.012	0.002	0.012	0	0.010	0
KWK25	CYANO	55	2.67	0.008	0.039	0	0	0	0	0.001	0	0.003	0	0.062	0
KWK27	CYANO	40	3.08	0.004	0.045	0	0	0.002	0	0.006	0.004	0.001	0	0.051	0
KWK34	CHLORO	75	2.11	0.010	0.078	0	0	0	0	0.007	0.001	0.001	0.001	0.007	0
KWK35	CYANO	53	2.62	0.014	0.024	0	0	0.002	0	0.013	0.002	0.001	0	0.064	0
KWK36	CHRYSO	24	2.95	0.025	0.018	0	0	0	0	0.021	0.001	0.001	0	0.009	0
KWK37	CHLORO	85	2.34	0.005	0.247	0	0	0.001	0	0.006	0.004	0.016	0.004	0.007	0
Bylot Island															
BYL22	CHRYSO	96	0.21	30.633	0.083	0	0	0.024	0	0.148	0.003	0.004	0	1.146	0
BYL23	CYANO	48	1.11	1.016	0.153	0.006	0	0.081	0.001	0.013	0.009	0.016	0.005	1.224	0
BYL24	CYANO	95	0.29	0.204	0.813	0.138	0	0.583	0	0.063	0.016	0.122	0.008	33.887	0
BYL25	CYANO	57	1.22	0.035	0.040	0.016	0	0.008	0	0.188	0.001	0.015	0	0.407	0
BYL26	CYANO	50	1.24	0.104	0.145	0	0	0.008	0	0.388	0	0.011	0.004	0.658	0
BYL27	CYANO	88	0.51	0.297	0.076	0	0	0.115	0	0.036	0.001	0.041	0	4.306	0
BYL28	CHRYSO	59	1.22	0.304	0.025	0.008	0	0.006	0	0.063	0.002	0.004	0	0.100	0
BYL29	CRYPTO	77	0.80	0.061	0.118	0	0	0.002	0	1.661	0	0.026	0.002	0.289	0
BYL30	CHRYSO	83	0.52	28.845	0.214	0.076	0	0.069	0	0.028	0.002	0.077	0	5.266	0

BYL31	CHRYSO	76	0.65	6.912	0.123	0	0	0.002	0	0.032	0	0.058	0	1.946	0
BYL32	CHRYSO	83	0.50	18.503	0.190	0	0	0.006	0	0.006	0	0.032	0.003	3.539	0
BYL33	CYANO	45	1.31	0.335	0.091	0.005	0.001	0.004	0	0.146	0.001	0.005	0.007	0.491	0
BYL34	CYANO	75	0.78	0.712	0.216	0	0	0.025	0	0.004	0.001	0.038	0.002	2.915	0
BYL35	CYANO	51	1.05	1.647	0.146	0.002	0	0.029	0	0.204	0.003	0.026	0	2.125	0

Chryso,Chrysophyceae; Chloro, Chlorophyceae; Ulvo, Ulvophyceae; Prasino, Prasinophyceae; Charo, Charophyceae;Xantho, Xanthophyceae; Crypto, Cryptophyceae; Dino, Dinophyceae; Bacillario, Bacillariophyceae; Eugleno, Englenophyceae; Cyano, Cyanobacteria; Raphido, Raphidophyceae.

Table 4 Pigment concentration (μg l^{-1}) in subarctic (2006) and arctic (2007) thaw ponds as determined by HPLC.

	Chlorophylls				Photosynthetic carotenoids					Photoprotective carotenoids										
Pond name	***chl a***	***chl b***	***chl c2***	**Tot. chl**	**Fuco**	**Anthera**	**Viola**	**Tot. PSC**	**PSC/chl *a***	**Cantha**	**Asta**	**Diadino**	**Allo**	**Diato**	**Zea**	**Lut**	**Echin**	**β-car**	**Tot. PPC**	**PPC/chl *a***
Whapmagoostui-Kuujjuarapik																				
KWK1	4.32	0.64	0.04	5.0	0.48	0.00	0.23	0.71	0.16	0.12	0.00	1.55	0.24	0.00	0.36	0.66	0.10	0.18	3.22	0.75
KWK2	3.20	0.41	0.02	3.6	0.40	0.08	0.42	0.89	0.28	0.41	0.10	0.99	0.23	0.03	1.72	1.54	0.05	0.16	5.22	1.63
KWK3	11.43	0.67	0.03	12.1	0.67	0.00	0.62	1.29	0.11	0.36	0.00	0.62	0.00	0.05	0.26	0.66	0.67	0.53	3.14	0.28
KWK4	2.45	0.16	0.01	2.6	0.30	0.00	0.44	0.74	0.30	0.00	0.00	0.28	0.16	0.00	0.39	0.99	0.11	0.13	2.06	0.84
KWK5	1.61	0.26	0.00	1.9	0.18	0.00	0.36	0.54	0.33	0.00	0.00	0.22	0.07	0.00	0.33	0.81	0.00	0.05	1.48	0.92
KWK6	3.00	0.13	0.03	3.2	0.70	0.00	0.33	1.03	0.34	0.06	0.00	0.33	0.10	0.00	1.14	0.27	0.23	0.24	2.36	0.78
KWK7	2.98	0.40	0.02	3.4	0.28	0.00	0.36	0.65	0.22	0.00	0.00	0.61	0.24	0.00	1.78	1.01	0.00	0.17	3.81	1.28
KWK8	3.26	0.75	0.01	4.0	0.33	0.00	0.24	0.57	0.17	0.00	0.00	1.29	0.12	0.00	0.30	0.67	0.00	0.13	2.51	0.77
KWK9	4.72	0.31	0.03	5.1	1.68	0.07	1.13	2.87	0.61	0.00	0.00	0.63	0.16	0.00	0.20	0.47	0.00	0.31	1.77	0.38
KWK10	3.52	0.46	0.02	4.0	1.32	0.03	0.90	2.25	0.64	0.00	0.00	0.45	0.15	0.00	0.26	0.82	0.00	0.16	1.84	0.52
KWK11	12.10	1.20	0.15	13.5	4.21	0.00	1.85	6.06	0.50	0.19	0.00	2.52	0.43	0.00	0.34	1.63	0.00	0.37	5.49	0.45
KWK12	3.32	0.34	0.03	3.7	0.48	0.00	0.62	1.11	0.33	0.12	0.00	0.27	0.24	0.00	0.21	0.54	0.00	0.16	1.54	0.46
KWK13	3.40	0.39	0.03	3.8	0.32	0.00	0.67	0.99	0.29	0.00	0.00	0.44	0.34	0.00	0.44	1.17	0.00	0.13	2.53	0.74
KWK14	12.23	0.78	0.07	13.1	0.84	0.00	0.68	1.52	0.12	0.00	0.00	7.08	2.14	0.09	0.30	0.82	0.00	0.44	10.88	0.89
KWK15	10.99	1.34	0.07	12.4	0.63	0.00	0.62	1.25	0.11	0.00	0.00	8.15	1.60	0.22	0.21	0.62	0.00	0.41	11.21	1.02
KWK16	5.22	0.85	0.03	6.1	0.52	0.00	0.55	1.07	0.21	0.12	0.00	2.40	0.29	0.10	0.81	0.98	0.10	0.25	5.04	0.97
KWK17	5.61	0.55	0.02	6.2	0.62	0.00	1.05	1.67	0.30	0.00	0.00	0.86	0.29	0.00	0.49	2.21	0.00	0.20	4.05	0.72
KWK18	9.73	1.41	0.14	11.3	0.72	0.00	0.96	1.68	0.17	0.27	0.00	2.97	0.50	0.00	1.89	2.68	0.00	0.44	8.75	0.90
KWK19	3.89	0.53	0.01	4.4	0.40	0.00	0.82	1.22	0.31	0.00	0.00	0.34	0.00	0.00	0.40	2.03	0.00	0.17	2.93	0.75
KWK20	4.99	0.47	0.05	5.5	1.54	0.00	0.77	2.32	0.46	0.11	0.00	0.98	0.32	0.00	0.43	0.76	0.05	0.33	2.98	0.60
KWK21	6.93	1.14	0.03	8.1	1.52	0.00	1.47	2.99	0.43	0.00	0.00	0.81	0.50	0.00	1.59	1.95	0.00	0.24	5.09	0.74
KWK23	13.11	0.75	0.08	13.9	1.07	0.00	0.96	2.03	0.16	0.42	0.00	2.40	0.49	0.04	0.43	1.04	0.96	0.82	6.59	0.50
KWK24	2.58	0.45	0.01	3.0	0.52	0.00	0.47	0.99	0.38	0.00	0.00	0.30	0.12	0.00	0.68	0.92	0.00	0.14	2.16	0.84
KWK25	3.48	0.87	0.01	4.4	0.70	0.00	0.43	1.13	0.32	0.00	0.00	0.81	0.00	0.04	0.17	0.97	0.00	0.17	2.16	0.62
KWK27	2.54	0.47	0.01	3.0	0.22	0.00	0.34	0.56	0.22	0.00	0.00	0.44	0.00	0.00	0.11	0.70	0.00	0.10	1.35	0.53
KWK28	3.74	0.66	0.04	4.4	0.78	0.00	0.56	1.34	0.36	0.00	0.00	1.40	0.54	0.08	0.21	0.85	0.00	0.16	3.24	0.87
KWK31	1.97	0.41	0.01	2.4	0.21	0.00	0.49	0.70	0.36	0.00	0.00	0.26	0.00	0.00	0.57	0.83	0.00	0.10	1.77	0.90
KWK33	20.58	3.45	0.15	24.2	4.88	0.00	1.77	6.64	0.32	0.00	0.00	3.20	0.41	0.00	0.73	4.64	0.00	0.54	9.52	0.46
KWK34	2.36	0.68	0.02	3.1	0.18	0.00	0.51	0.69	0.29	0.00	0.00	0.68	0.55	0.00	0.32	1.09	0.00	0.18	2.81	1.19
KWK36	2.26	0.40	0.02	2.7	0.50	0.00	0.49	0.99	0.44	0.00	0.00	0.68	0.27	0.00	0.27	0.63	0.00	0.10	1.95	0.86
KWK37	4.26	0.55	0.02	4.8	0.58	0.12	0.78	1.48	0.35	0.00	0.00	0.58	0.13	0.00	1.00	1.77	0.00	0.23	3.71	0.87
KWK38	2.65	0.43	0.06	3.1	0.38	0.04	0.30	0.72	0.27	0.00	0.00	0.39	1.03	0.00	0.19	0.83	0.00	0.09	2.53	0.96
KWK39	2.72	0.71	0.02	3.5	0.14	0.03	0.56	0.73	0.27	0.13	0.00	0.15	0.36	0.00	0.20	1.23	0.07	0.14	2.28	0.84
Bylot Island																				
BYL1	0.69	0.09	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.13	0.00	0.00	0.17	0.25

BYL22	19.25	1.06	0.00	20.30	7.36	0.31	2.48	7.67	0.40	0.00	0.00	0.00	0.00	0.00	0.56	1.07	0.00	0.48	4.59	0.24
BYL23	1.17	0.38	0.00	1.56	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.39	0.00	0.00	0.37	0.00	0.06	0.91	0.78
BYL24	4.02	0.82	0.00	4.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.78	0.20	0.12	1.34	0.33
BYL25	0.40	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BYL26	1.08	0.16	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.06	0.24	0.08	0.00	0.55	0.51
BYL27	1.60	0.32	0.00	1.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.00	0.48	0.30
BYL28	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.13	0.38
BYL29	0.61	0.00	0.00	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.07	0.11	0.00	0.00	0.52	0.86
BYL30	8.13	0.00	0.00	8.13	3.33	0.00	0.67	3.33	0.41	0.00	0.28	0.00	0.00	0.00	0.23	0.13	0.00	0.25	1.56	0.19
BYL31	3.91	0.00	0.00	3.91	1.33	0.00	0.70	1.33	0.34	0.00	0.00	0.00	0.00	0.00	0.06	0.13	0.00	0.08	0.96	0.24
BYL32	8.22	0.30	0.00	8.52	3.05	0.00	1.03	3.05	0.37	0.00	0.00	0.00	0.00	0.00	0.14	0.25	0.18	0.19	1.79	0.22
BYL33	1.07	0.06	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.03	0.10	0.05	0.00	0.81	0.76
BYL34	5.76	1.11	0.00	6.87	0.67	0.00	2.11	0.67	0.12	0.23	0.00	0.68	0.00	0.00	0.43	1.53	0.61	0.19	5.77	1.00
BYL41	3.23	0.27	0.00	3.50	1.02	0.00	0.53	1.02	0.31	0.04	0.00	0.33	0.25	0.00	0.10	0.35	0.00	0.11	1.72	0.53
BYL42	1.58	0.08	0.00	1.66	0.64	0.00	0.33	0.64	0.41	0.00	0.08	0.00	0.11	0.00	0.07	0.14	0.00	0.07	0.79	0.50

Fuco, fucoxanthin; Cantha, canthxanthin; Viola, violoxanthin; Asta, astaxanthin; Diadino, diadinoxanthin; Anthera, antheraxanthin; Allo, alloxanthin; Diato, diatoxanthin; Zea, zeaxanthin; Lut, lutein; Chl b, chlorophyll b; Echin, echinenone; β-car, β-carotene; Chl a, chlorophyll a.

Table 5 Shannon diversity index of arctic and subarctic ponds using three biological indicators.

Pond name	Shannon diversity index			
	Phytoplankton	Pigments	DGGE	
			surface	bottom
Whapmagoostui-Kuujjuarapik				
KWK1	3.05	1.73	0.90	-
KWK2	2.65	2.02	1.62	1.89
KWK3	2.52	1.31	0.84	1.74
KWK4	2.62	1.76	-	-
KWK6	2.16	1.78	1.76	1.44
KWK7	2.81	1.79	1.22	-
KWK9	1.92	1.65	-	-
KWK10	0.66	1.76	-	-
KWK11	-	1.66	-	2.54
KWK12	2.83	1.69	-	-
KWK14	2.07	1.50	-	-
KWK15	2.91	1.50	-	-
KWK16	3.15	1.83	-	-
KWK17	2.73	1.67	-	-
KWK18	1.71	1.78	-	-
KWK19	2.80	1.59	-	-
KWK20	2.17	1.80	-	-
KWK21	-	1.81	1.45	1.93
KWK23	3.20	1.61	-	-
KWK25	2.67	1.69	-	-
KWK27	3.08	1.56	-	-
KWK33	-	1.59	-	1.97
KWK34	2.11	1.90	-	-
KWK35	2.62	-	-	-
KWK36	2.95	1.87	-	-
KWK37	2.34	1.80	-	-
KWK38	-	1.83	-	-
Bylot Island				
BYL1	-	0.86	-	-
BYL22	0.21	1.24	-	-
BYL23	1.11	1.70	-	-
BYL24	0.29	1.31	-	-
BYL25	1.22	-	-	-
BYL26	1.24	1.29	1.13	-
BYL27	0.51	0.93	-	-
BYL28	1.22	1.00	-	-
BYL29	0.80	1.10	0.31	-
BYL30	0.52	1.13	1.22	-
BYL31	0.65	1.04	-	-
BYL32	0.50	1.18	-	-
BYL33	1.31	1.12	1.34	-
BYL34	0.78	1.79	-	-
BYL35	1.05	1.45	-	-
BYL41	-	1.60	-	-
BYL42	-	1.52	-	-

Figure 7 Location of the two study sites in subarctic Québec and arctic Nunavut

Figure 8 Photographs of some subarctic and arctic ponds. **a**. Aerial view of subarctic study site, **b**. subarctic pond KWK21, **c**. humic subarctic pond KWK11, **d**. aerial view of arctic study site, **e**. runnel pond BYL23, **f**. polygon pond BYL22, **g**. orange microbial mat of pond BYL30, **h**. greenish microbial mat of pond BYL34.

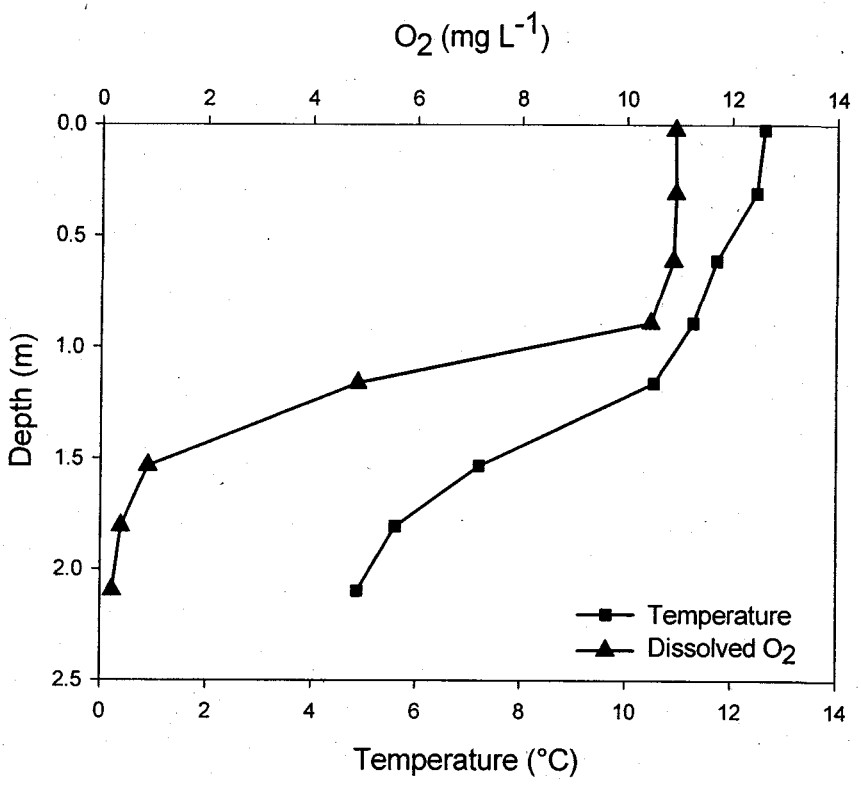

Figure 9 Vertical profile temperature and oxygen concentration in the subarctic pond KWK1 performed at time of the day on June 28, 2007.

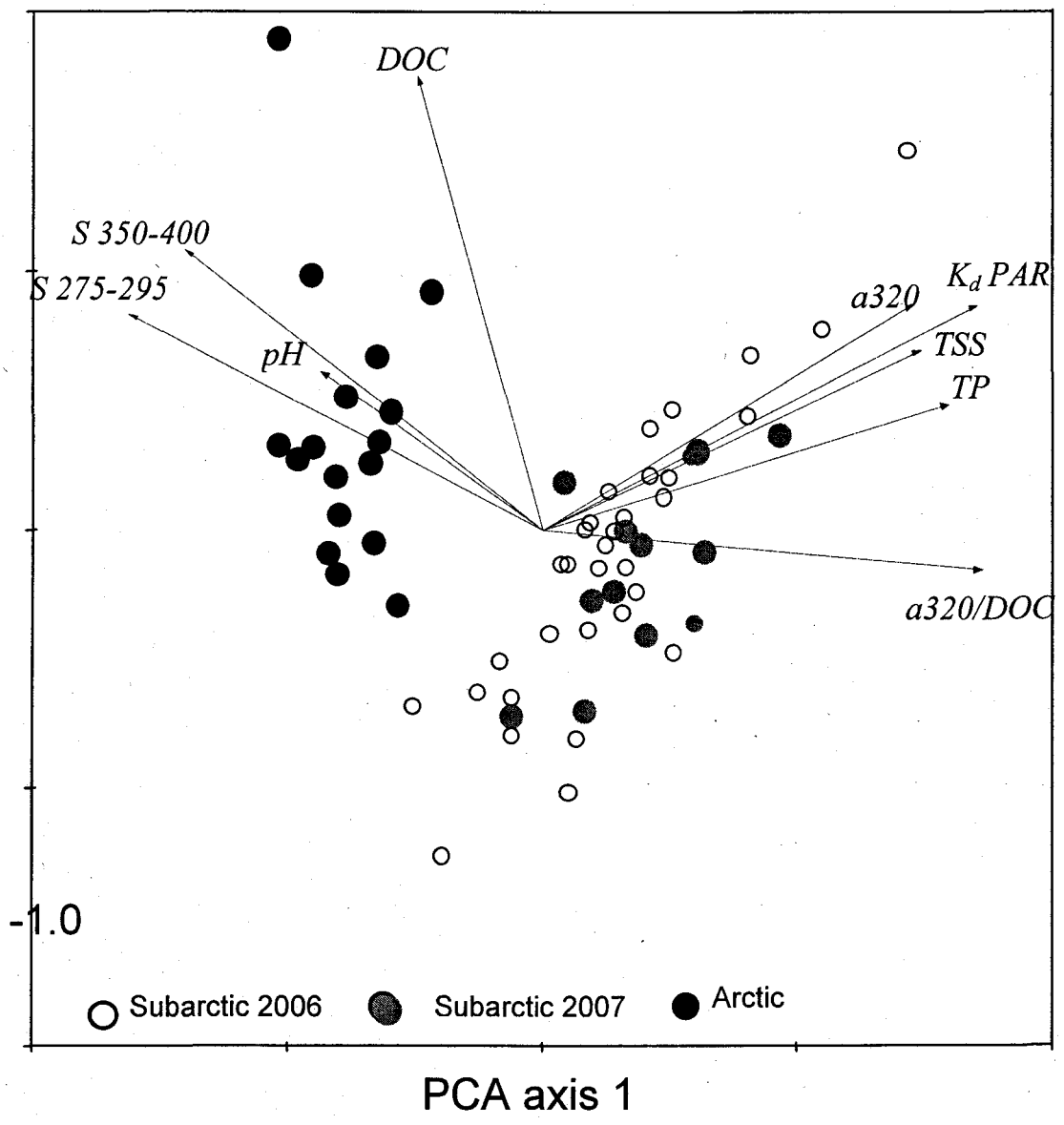

Figure 10 PCA of the physical and chemical variables for arctic and subarctic ponds (years 2006 and 2007). Pond names were removed for clarity purpose

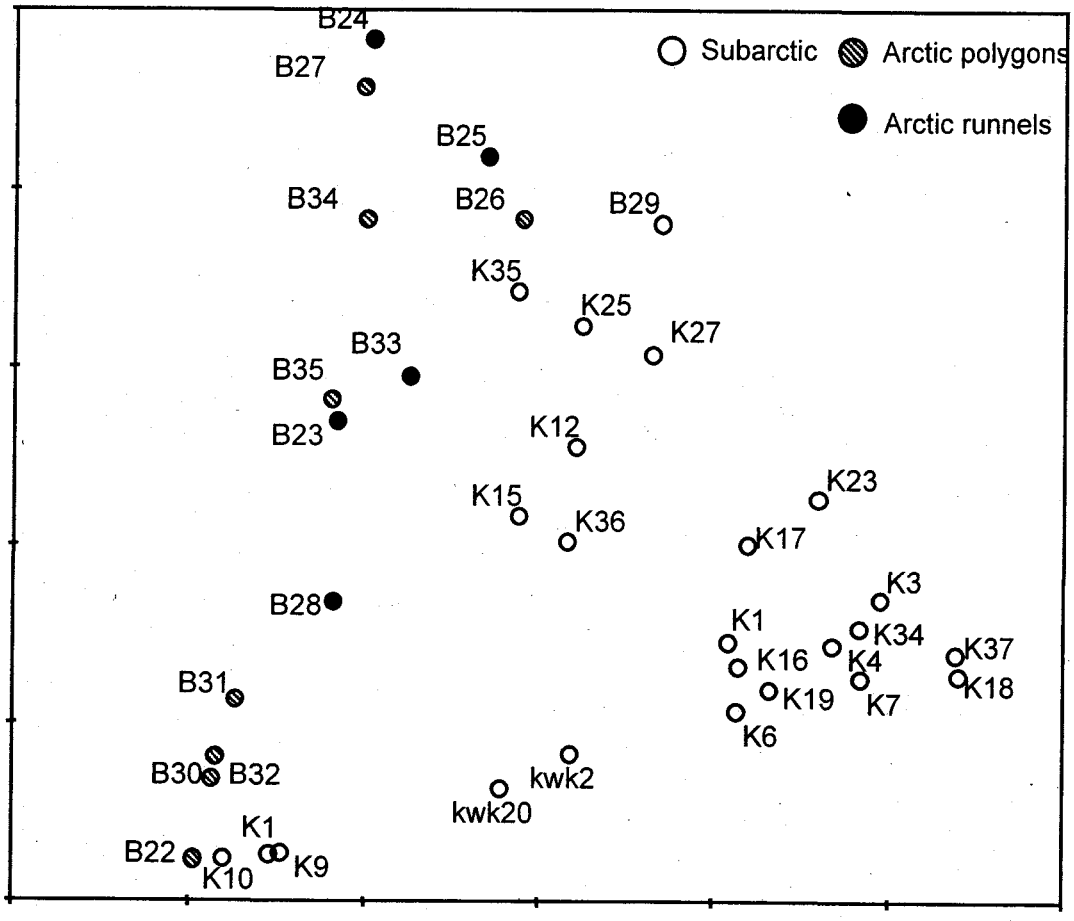

Figure 11 CA of the plankton cell abundance (autotrophs only) for arctic and subarctic ponds. K = subarctic ponds; B = arctic ponds

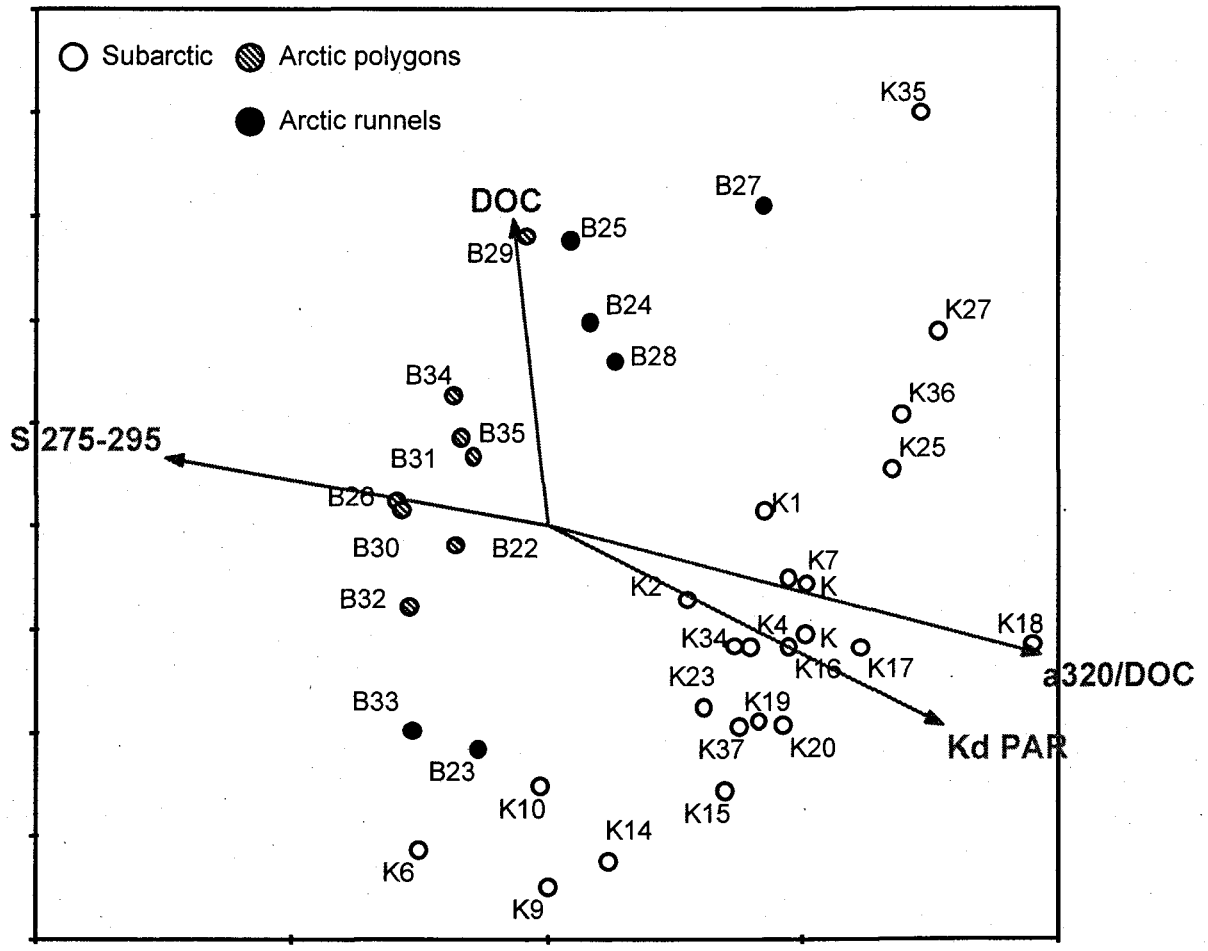

Figure 12 CCA of the plankton abundance (autotrophs only) relative to physicochemical characteristics for arctic and subarctic ponds. K = subarctic ponds; B = arctic ponds

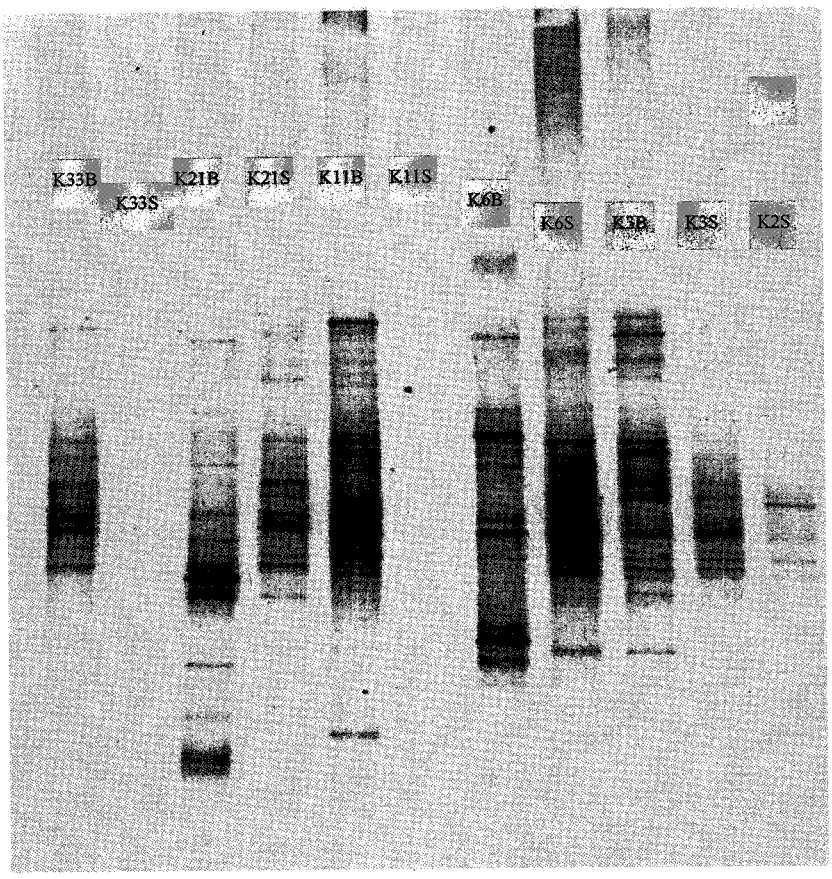

Figure 13 DGGE fingerprints (gel1) of 18rRNA eukaryotic PCR product from subarctic thaw ponds in July 2007. K = Subarctic ponds; B = Bottom sample; S = Surface sample.

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REMERCIEMENTS

Il me faudra certainement beaucoup plus qu'une page pour remercier adéquatement tous ceux qui m'ont soutenu de près ou de loin tout au cours de ma maîtrise! Tout d'abord, c'est à ma chère directrice de maîtrise Isabelle Laurion que je dois le plus! Isabelle, merci pour ton support, tes idées, tes projets qui me font toujours avancer, ta présence et ta gentillesse. Ce projet m'a permis de vivre des expériences vraiment fabuleuses qui sortent de l'ordinaire et je ne l'oublierai jamais. Je tiens aussi à remercier vivement ma co-directrice Connie Lovejoy qui m'a fait découvrir tout un pan de la science qui m'était plutôt inconnu, la biologie moléculaire. « Docteure » Leira Retamal, ton humour, ta débrouillardise hors du commun, ta vivacité d'esprit sont autant de traits que j'admire chez toi et qui m'ont grandement aidé sur le terrain et lors de ma rédaction. Un gros merci aussi à Julie Breton qui m'a patiemment expliqué comment le labo fonctionnait à mon arrivée à l'INRS et qui est toujours présente pour me donner un bon coup de pouce que ce soit matériel ou moral! Merci à Isabelle Lavoie qui m'a sauvée avec des statistiques! Je dois énormément à toute l'équipe du labo Lovejoy, j'ai atterri dans le monde de la biologie moléculaire et grâce à vous, j'ai appris beaucoup! Un gros merci donc à Anne-Dorothée qui m'a tellement aidé, Ramòn Terrado qui m'a prouvé que les DGGE peuvent vraiment fonctionner, Marianne Potvin qui sait décidément de quoi elle parle, à Karen Scarcella pour ses protocoles, à Pierre Galand pour les premières PCR, à Estelle pour ta présence et à Mary Taller pour ta bonne humeur. Côté labo Laurion, tous mes hommages à Catherine Vallières, la pro du microscope, Annabelle Warren, une amie qui m'a fait découvrir un tas de musique, Nicolas Rolland, « patenteux » par excellence et Sébastien Bourget. Un gros merci à tout le monde à Kuujjuarapik en 2006! Quel groupe super on faisait, je m'ennuie encore! Je ne peux pas nommer tout le monde mais je n'en oublie aucun dans ma tête je vous le jure. Spécialement, Marie-Ève Bédard qui s'oriente tellement mieux que moi sur les sites! Un gros merci à l'équipe thermokarst Kuujj 2007 : Alexandra Rouillard, qui m'a tellement soutenue et calmé dans les moments de panique, Warwick Vincent qui en plus de toutes ses qualités peut réparer une van dorn avec un débouche toilette ☺! Reinhart Pienitz, Pierre Francus, Shohei Watanabe pour ses analyses, Tommy Harding, Fred Bouchard avec qui je me suis bien amusée, merci pour ta guitare! Merci à tous les autres également. Tous les honneurs à l'équipe Bylot 2007, on a bien rit, bien mangé et nous avons été très bien accueillies

sur ces terres si éloignées mais plus grandes que nature, je n'oublierai jamais cette expérience nordique extraordinaire. Marie-Josée Martineau, ta compétence m'ont été d'une utilité inestimable. Le travail des techniciens de l'INRS, Michelle Bordeleau et Sébastien Duval mérite également d'être souligné, merci! Je tiens également à rendre hommage à tous mes collègues des Sciences de l'eau de l'INRS qui réussissent à me changer les idées et avec qui je me suis beaucoup amusée : (mention spéciale à Clem pour avoir été dans mon dos pour ces presque 3 ans! Bonne chance pour tout!) toutes les maîtrises pro, Gabrielle, Loïc, Nicolas, Imen, Patrick, Véronique, Guillaume, Isabelle, Brigitte et Jean-Nicolas. Merci à tous les autres INRSiens et ceux de l'asso également, un endroit rempli de personnes exceptionnelles! Équipe radio : Simon, Frédérique, Rémy et Nicolas pour m'avoir fait connaître un média puissant! Un clin d'œil spécial à Jimmy Poulin qui a du m'endurer comme voisine de bureau, j'espère ne pas avoir trop ralentit ta rédaction ☺! Merci aux Sciences de la Terre (hé oui!) : Geneviève, Aurélie, Clarisse, Francis et tous les autres! Encore un ptit clin d'œil aux Sciences de l'eau 2007 avec qui on s'est bien amusé (je pense à Audrey, Guillaume G et Guillaume B, Vincent, Joanie et tous les autres!). Un gros merci à Laurie Tremblay, ma chère colocataire qui a le don de me remonter le moral. Finalement, je tiens à souligner le support de mes chers amis biologistes: Éric N, Alexandra D, François D, Krystell H, Nicolas F, Annabelle G, Amélie G et compagnie! Grosse colle à mes deux acolytes : Chantal F et Marie-Hélène L. Finalement, merci à mes parents, à mon frère et à ma chère (feu) marraine pour leur support.

ANNEXE

Variability in greenhouse gas emissions from permafrost thaw ponds

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Running title: Greenhouse gas emissions from thaw ponds

Acknowledgements

We thank M.-È. Bédard, F. Bouchard, J. Breton, T. Harding, M.-J. Martineau, N. Rolland, A. Rouillard, M. Rousseau, D. Sarrazin, C. Vallières, and S. Watanabe for their essential support in the field and laboratory, G. Gauthier for support at the Bylot Island field station of the Centre d'études nordiques, M. Allard, Y. Prairie, J.-L. Fréchette, and V. St. Louis for their helpful comments and advice, two anonymous reviewers for their insightful suggestions, L. Marcoux for drafting the map, and the Fonds québécois de la recherche sur la nature et les technologies, Natural Sciences and Engineering Research Council of Canada, ArcticNet, Polar Continental Shelf Project (contribution 02409), Indian and Northern Affairs Canada, Parks Canada, International Polar Year, Centre d'études nordiques, and U.S. National Science Foundation grants DEB-0640953 and ARC-0714085 for logistic and financial support.

Abstract

Arctic climate change is leading to accelerated melting of permafrost and the mobilization of soil organic carbon pools that have accumulated over thousands of years. Photochemical and microbial transformation will liberate a fraction of this carbon to the atmosphere in the form of CO_2 and CH_4. We quantified these fluxes in a series of permafrost thaw ponds in the Canadian Subarctic and Arctic and further investigated how optical properties of the carbon pool, the type of microbial assemblages, and light and mixing regimes influenced the rate of gas release. Most ponds were supersaturated in CO_2 and all of them in CH_4. Gas fluxes as estimated from dissolved gas concentrations using a wind-based model varied from -20.5 to 114.4 mmol CO_2 m^{-2} d^{-1}, with negative fluxes recorded in Arctic ponds colonized by benthic microbial mats, and from 0.03 to 5.62 mmol CH_4 m^{-2} d^{-1}. From a time-series set of measurements in a subarctic pond over 8 days, calculated gas fluxes were on average 40% higher when using a newly derived equation for the gas transfer coefficient developed from eddy covariance measurements. The daily variation in gas fluxes was highly dependent on mixed layer dynamics. At the seasonal timescale, persistent thermal stratification and gas build-up at depth indicated that autumnal overturn is a critically important period for greenhouse gas emissions from subarctic ponds. These results underscore the increasingly important contribution of permafrost thaw ponds to greenhouse gas emissions, and the need to account for local and regional variability in their limnological properties for global estimates.

Introduction

The warming of subarctic and arctic regions is accompanied by permafrost melting and erosion and the production of numerous small basins that fill with water. These thaw (or thermokarst) lakes and ponds persist from days to hundreds of years depending on local geomorphology, climate and hydrology (Åkerman and Malmström 1986; Pienitz et al. 2008). They are usually surrounded by peaty soils and vegetation, and are therefore often rich in organic matter that will be partly mineralised and transferred to the atmosphere in the form of CO_2 and CH_4, two potent greenhouse gases (GHG). Thaw ponds need to be taken into account in global atmospheric carbon budgets and climate warming prediction models since they are significant sources of methane (Walter et al. 2006). These aquatic systems may contribute a positive feedback to climate warming (Schuur et al. 2008) and be partly responsible for the increase in global atmospheric CH_4 during the last deglaciation (Walter et al. 2007).

In general, small lakes and ponds represent a net source of CO_2 to the atmosphere (Sobek et al. 2005) and they are now recognized as important contributors to regional and global climate (Cole et al. 2007). Specific studies on thaw pond carbon cycling are scarce but they all report a large evasion and/or a supersaturation of GHG in these systems (Hamilton et al. 1994 in Hudson Bay region; Ström and Christensen 2007 in Scandinavia; Blodau et al. 2008 in Siberia). Several studies have considered how changes in the surface area covered by thermokarst systems could substantially alter the effect of high-latitude ecosystems on the atmosphere (Chapin et al. 2000). During a thaw-freeze cycle associated with thermokarst lake migration in Siberia, ~30% of yedoma carbon was apparently decomposed by microbes, converted to methane and released to the atmosphere (Zimov et al. 1997). Recently, Walter et al. (2006) attributed a 58% increase in CH_4 emission in northern Siberia to the expansion of thaw lakes between 1974 and 2000.

The rate at which GHG are liberated to the atmosphere partly depends on the physical structure of the water column which influences the light, temperature and oxygen content, thus the microbial assemblages and metabolic pathways. For example, Kankaala et al. (2006) showed in a boreal lake that 80% of CH_4 diffused from the sediment was consumed by methanotrophs and 20% was released to the atmosphere, proportions that were highly dependent on water column mixing. GHG efflux will also depend on the availability of dissolved compounds for microbial degradation and their reactivity to photolysis, and on the activity of primary producers that will determine the metabolic balance of these shallow water ecosystems. Thus, several factors controlled by climate (temperature, water column stability, allochthonous inputs) have the potential to affect the contribution of small polar lakes to the atmospheric carbon budget (Vincent 2009).

Large differences in carbon evasion rates have been observed in different permafrost regions, especially for CH_4 evasion, however little is known about the causes of this variability and how climate change may influence GHG production in the future. For terrestrial and wetland systems in permafrost regions, a complex set of variables has been suggested to control CH_4 emissions, including soil temperature, near-surface turbulence and atmospheric pressure, ground temperature and thaw depth, water table position, substrate availability to methanogens, and primary production (Sachs et al. 2008). Thaw lakes and ponds are a major, yet overlooked class of aquatic ecosystems in polar regions (Pienitz et al. 2008).

Permafrost thaw ponds in northeastern Canada present a broad range of limnological

properties that potentially influence their greenhouse gas production (Breton et al. in press). The objectives of the present study were to measure gas exchange and to examine its controlling factors in thaw ponds of the Canadian Subarctic and Arctic. These two regions differ in that permafrost is continuous in the Arctic but discontinuous in the Subarctic, which leads to limnological differences in their aquatic ecosystems. We examined spatial variability by sampling 30 subarctic ponds and 22 arctic ponds, and temporal variability via repeated measurements in 12 ponds over two summers. Additionally, we conducted an intensive week long study at one site with continuous measurements of gas concentrations in the surface water and meteorological variables in the overlying atmosphere. We used the latter data set to compare three gas transfer models for estimating GHG exchange.

Methods

Study sites– Field sampling was from 21 to 27 July 2006 and from 28 June to 24 July 2007 at two contrasting sites (Fig. 1). Thirty of the study ponds were located in Nunavik (12 of these ponds were resampled in 2007) in the subarctic discontinuous permafrost region at 55°16'N, 77°46'W, near the village of Whapmagoostui-Kuujjuarapik (named KWK ponds hereafter). Another 22 ponds were sampled in 2007 in Sirmilik National Park, Bylot Island, Nunavut, in the arctic continuous permafrost region at 73°09'N, 79°58'W near the village of Pond Inlet (named BYL ponds hereafter) (Table 1). The subarctic thaw ponds (Fig. 2a,b) are surrounded by dense shrubs (*Betula glandulosa, Salix* sp., *Alnus* sp., *Myrica gale*) and sparse trees (*Picea mariana, Picea glauca, Larix laricina*), with some areas colonized by *Sphagnum* spp. mosses. These thermokarst ponds are formed in depressions (1 to 3 m deep) left after the ice has melted below mineral mounds.

In the continuous permafrost area, the ponds are formed on low-center polygons and in runnels over melting ice-wedges (or runnel ponds) at the surface of permafrost terrain (Fig. 2c,d). These ponds are a natural phenomenon associated with the active layer dynamics of organic soils, but are likely increasing in importance with the accelerated warming and melting of permafrost (Schuur et al. 2008). At this site, there was a ca. 2.5 m thick peaty silt unit in the center of polygons, consisting of fibrous peat mixed with wind-blown sediment that originated from the nearby glaciofluvial outwash plain (Fig. 2e; *see* Fortier and Allard 2004 for geomorphological details). The active layer in this region is about 40 cm deep in the peaty silts. Four larger water bodies were sampled for comparison: BYL36 is a large pond about 5.5 m deep that was considered in the same class as the other thaw ponds (Fig. 2d); BYL 37 is a kettle lake located next to the camp; and BYL39 and BYL40 are two oligotrophic lakes with a rock floor and located in a nearby valley in the proximity of alpine glaciers.

Physicochemistry– Temperature, dissolved oxygen and pH were recorded with a 600R multiparametric probe (Yellow Spring Instrument). The oxygen probe was calibrated at the beginning of each sampling day in water-saturated air with a correction for barometric pressure. The temperatures at the surface (0.15 m) and bottom (2.0 m; max. depth ~2.2 m) of pond KWK16 were measured continuously from July 2007 through July 2008 with readings recorded every half hour (HOBOware™ U12 thermistors, Onset). Water samples were filtered through pre-rinsed cellulose acetate filters (0.2 μm pore size; Advantec Micro Filtration Systems) to measure the optical properties of dissolved organic matter (DOM). Dissolved organic carbon (DOC) concentrations were measured using a Shimadzu TOC-5000A carbon analyzer calibrated with potassium biphthalate.

To determine the chromophoric fraction of DOM (CDOM), absorbance scans were performed from 250 to 800 nm on a spectrophotometer (Cary 100, Varian) at a speed of 240 nm min^{-1} and a slit width of 2 nm. Total phosphorus (TP) was measured by spectrophotometry as in Stainton et al. (1977) on unfiltered water samples fixed with H_2SO_4 (0.15% final concentration) and digested with potassium persulfate. The total suspended solids (TSS) were collected onto pre-combusted and pre-weighed glass fiber filters (0.7 μm nominal pore size; Advantec MFS) that were dried for two hours at 60°C.

Meteorology– Meteorological variables were measured by nearby automated climate stations. At the arctic site, the climate station was up to 10 km from the sampled ponds (in three cases), but mostly < 500 m. At the subarctic site, the station was installed beside pond KWK2, and up to 370 m from the furthest sampled pond at this site. Incident short wave radiation (300-1100 nm, W m^{-2}), air temperature, relative humidity and wind speed (m s^{-1}) were measured at 2 m above ground and recorded every 30 min (WeatherHawk 511).

Bacteria and chlorophyll a– Water samples for bacterial abundance were fixed with a filtered solution of paraformaldehyde (1% final concentration) and glutaraldehyde (0.1% final concentration) after adding a protease inhibitor (phenylmethanesulphonylfluoride (PMSF) at a final concentration of 1 μmol L^{-1}, Gundersen et al. 1996) and were kept frozen until analysis (at -20°C in the field and -80°C once back in the laboratory). The bacteria were stained with 4',6-diamidino-2-phenylindole (DAPI, 5 μg L^{-1} final concentration) and counted using epifluorescence microscopy (Zeiss Axiovert). Water samples were additionally collected onto glass fiber filters for the determination of chlorophyll *a* (Chl *a*) concentrations in surface waters. Filters were kept frozen at -80°C until pigments were extracted into 95% MeOH. Chl *a* was determined by high pressure liquid chromatography (HPLC) using the method of Zapata et al. (2000), as adapted by Bonilla et al. (2005).

CO_2, CH_4, and O_2 concentrations– Dissolved CO_2 and CH_4 ($Gas_{(aq)}$) were determined by the equilibration of 2 liters of pond water into 20 mL of ambient air for 3 minutes, with the headspace sampled in duplicated vials (red stopper Vacutainer®) previously flushed with helium and vacuumed (Hesslein et al. 1990). Gas samples were taken within 5 min after collecting the pond water and the ponds were sampled between 09:00 h and 18:00 h. Gas samples were kept at 4°C until analysed by gas chromatography (Varian 3800 with a COMBI PAL Head Space injection system and a CP-Poraplot Q 25m × 0.53mm column and flame ionization detector). The dissolved gases were calculated according to Henry's Law:

$$Gas_{(aq)} = K_H \times pGas \quad (1)$$

where K_H is the Henry's constant adjusted for ambient water temperature and pGas (pCO_2 or pCH_4) is the partial pressure of the gas in the headspace. Although the CO_2 equilibrium in pond water is linked to pH, the method used (equilibrium of a headspace a hundred times smaller than the water volume) was unlikely to change the pH sufficiently to affect dissolved CO_2 estimations. For CH_4, even though the effect was minor (< 1%), gas movement during the equilibration was corrected for.

Concentrations of CO_2, CH_4, and O_2 were followed in surface water (~20 cm depth) of one pond (KWK2; max depth of 2.6 m) during 8 days, from 28 June to 06 July 2007, using a continuous gas monitoring system. The monitor

was first developed by the Canadian Department of Fisheries and Oceans and modeled after the design of Carignan (1998), and built by Environnement Illimité. Three types of sensors were used to measure CO_2 (infrared gas analyser LI820, LI-COR), CH_4 (measurements in humid air; metal oxide sensor PN-SM-GMT, Panterra, Neodym Technologies), and O_2 (Qubit Systems) on a gas stream that was equilibrated with the source water. Details are given in Bastien et al. (2008). Briefly, water was pumped from the source via a reversible peristaltic pump through a bundle of porous polypropylene tubes (contactor, 1.0 × 5.5 Liqui-Cell Minimodule polysulfone, Membrana) that act as a water-air exchanger. Solenoid valves controlled the gas flow from the contactor to the air as required. Water and air temperatures were also recorded. Control of all electrical devices, collection and storage of data was performed by an electronic data logger (CR10X, Campbell Scientific). The entering water was filtered using layers of Nitex of 250, 100, 53, and 10 μm. Measurements were taken automatically every 3 h (the monitoring operating cycle took 22 mins: one cycle of 20 mins in water with 2 measurements at 10 and 20 mins, and one cycle of 2 mins in air with one measurement). Duplicate measures taken by the system had on average a standard deviation of 1.8 μmol L^{-1}. Variations in water flow rate through the contactor were caused by Nitex clogging at the end of ~2 days (from 600 down to 150 mL min^{-1}), but in most cases the filters were changed daily to reduce this effect.

Estimation of fluxes with floating chamber and gas partial pressure– In 2007, CO_2 flux was directly estimated using a floating chamber (area = 0.179 m^2; volume = 0.0175 m^3) connected to an infrared CO_2 analyser (EGM-4, PP-Systems) in a closed path, and the equation:

$$\text{Flux} = (S \times MW \times V_{ch}) / (V_m \times A_{ch}) \times F \quad (2)$$

where S is the slope from the graph of gas concentration in the chamber vs. time (measurements taken for 25 to 60 min depending on flux rate), MW is the gas molecular weight, V_{ch} is the volume of chamber, V_m is the molar volume of gas at ambient temperature, A_{ch} is area of chamber and F is a unit conversion factor to obtain a daily flux value in mg gas m^{-2} d^{-1}.

CO_2 and CH_4 fluxes were also estimated for all the ponds studied in 2007 using dissolved gas concentrations, wind speed and a wind-based equation for the gas transfer coefficient k:

$$\text{Flux} = k (C_{sur}-C_{eq}) \quad (3)$$

where k is the gas transfer coefficient (cm h^{-1}), C_{sur} is the gas concentration in surface water (μmol L^{-1}) and C_{eq} is the gas concentration in equilibrium with the atmosphere (for the time series, direct measurements of gas concentration in the air were used instead of global atmospheric values as for all the other ponds). The gas transfer coefficient for a given gas was calculated as:

$$k = k_{600} (Sc/600)^{-0.5} \quad (4)$$

$$k_{600} = 2.07 + 0.215 \times U_{10}^{1.7} \quad (5)$$

where the value k_{600} is the gas exchange coefficient given by Cole and Caraco (1998) that is dependent on wind speed at 10 m height (U_{10}), Sc is the Schmidt number of that gas calculated from empirical third-order polynomial fits to water temperature and corrected for Sc at 20°C (600). Wind speed was averaged over the preceding hour.

We computed gas fluxes using the 3 hourly data from the continuous gas monitoring system

using three different models for gas exchange. We used two wind based models for the gas transfer coefficient, that of Cole and Caraco (1998) described above and one we derived using estimates of k_{600} based on eddy covariance measurements in an oligotrophic subarctic lake (Jonsson et al. 2008). Using non-negative values of k the regression is:

$$k_{600} = 0.81 + 1.087\ U_{10} + 0.085\ U_{10}^2 \quad (6)$$

We also used the small eddy version of the surface renewal model (MacIntyre et al. 1995) which takes into account physical processes in addition to wind that cause turbulence at the air-water interface (Banerjee and MacIntyre 2004). For this method, we calculated surface energy fluxes from the meteorological data and surface water temperatures measured with the continuous gas monitoring system following MacIntyre et al. (2002). We then computed the turbulence in the upper mixed layer following Imberger (1985) and MacIntyre et al. (1995). As we only had surface water temperatures, we ran the computation for mixed layer depths of 0.1, 0.3, and 1 m, as profile data from KWK2 and the nearby ponds indicated mixed layers that were typically 0.1-0.3 cm in depth during the day. Near uniform profiles of O_2 and CO_2 to 1 m indicated that nocturnal mixing might penetrate as deep as 1 m. We assumed net long wave radiation was -50 W m^{-2}, a value typical for somewhat cloudy days in the northern temperate zone and the Arctic (MacIntyre et al. in press). Values of the gas transfer coefficient for all models were averaged over three hours and then used to calculate fluxes.

Results

Pond limnological characteristics– Overall, the thaw ponds in subarctic and arctic regions were biologically productive ecosystems, with an average bacterial abundance of 11.2×10^6 cells mL^{-1} (measured in 2007 only), Chl *a* concentrations of 4.9 μg L^{-1} and total phosphorus concentration of 51 μg L^{-1} (Table 1 shows the 2007 data series; *see* Table 2 for comparison of ranges between both years). The pH varied from 5.8 to 9.7, with the arctic ponds showing the highest pH values. The subarctic ponds presented striking patterns of colors (Fig. 2a) generated by differing combinations of suspended particles (mostly clays and silts; Total Suspended Solids (TSS) averaged 20.0 mg L^{-1}), dissolved organic matter content and CDOM absorption properties (DOC averaged 8.3 mg L^{-1}; absorption coefficient at 320 nm a_{320} averaged 35.0 m^{-1}). The arctic ponds had lower TSS (average of 4.6 mg L^{-1}), higher DOC (11.6 mg L^{-1}) but lower CDOM (a_{320} average of 19.9 m^{-1}), and less productive pelagic waters (average of 2.8 μg Chl *a* L^{-1} and 27 μg TP L^{-1}) compared to the subarctic ponds. However, thick microbial mats were observed in ponds formed on low-center polygons, with a consortium of taxa dominated by oscillatorian cyanobacteria (Vézina and Vincent 1997). The mats were actively photosynthesizing under the continuous light exposure of the polar summer, and most likely the cause of higher pH values and low nutrients in the water column.

Different conditions were observed at the arctic sites in the two years, likely generated by variations in hydrology and climate. The snow pack was relatively thin at the end of winter in 2007 (21 cm on 01 June compared to a long-term average of 31 cm; G. Gauthier, Centre for Northern Studies, unpubl. data); temperatures were exceptionally warm and relative humidity low in 2007 (10 mm of rain in July). Therefore, several of the ponds sampled in 2006 had completely dried up in 2007, and in some cases retaining their orange benthic microbial mats that became exposed to the air (Fig. 2f).

Dissolved gases in surface waters– All subarctic ponds were supersaturated in both CO_2

and CH_4. Similarly, all arctic ponds were supersaturated in CH_4, however they were mostly undersaturated in CO_2 (Table 1). Global values of atmospheric partial pressures (IPCC 2007; 38.4 Pa of CO_2 and 0.18 Pa of CH_4) were used to determine the gas saturation (when converted to aqueous concentrations at equilibrium using ambient water temperatures, these values corresponded on average to 19.5 μmol L^{-1} of CO_2 and 0.0034 μmol L^{-1} of CH_4). At the subarctic site, differences in dissolved gases (departure from saturation is used to account for temperature differences among ponds and years) were observed between 2006 and 2007 (using the 12 ponds that were sampled in both years), with lower CO_2 (paired t-test, t = 2.051, df = 11, p = 0.065) and substantially lower CH_4 concentrations in 2007 (t = 4.932, df = 11, $p < 0.001$; Table 2). Departure from CO_2 saturation was inversely correlated with departure from O_2 saturation (Fig. 3; r = -0.733, $p < 0.0001$, n = 59) and positively correlated with DOM chromophoric properties such as a_{320} ($r = 0.619$, $p < 0.0001$, n = 63) or DOC-specific a_{320} (r = 0.584, $p <$ 0.0001), but not with DOC (r = 0.107, p = 0.402). The correlation with DOC improved when subarctic data were tested alone (r = 0.431, $p = 0.004$).

Profiles of temperature and gas concentrations– Despite their shallowness (maximum measured depth varied from 0.8 to 3.3 m), most of the subarctic ponds were thermally stratified at the times of sampling (Fig. 4). KWK36 was stratified to the surface (no mixed layer), while the other had mixed layer depths of ~0.1 to 1 m. The stratification results from the high attenuation of light in these systems due to high concentrations of DOC (average 8.3 mg L^{-1}) and suspended solids (average 20 mg L^{-1}). The Brünt Väisälä frequency (N) was calculated as an index of water column stability $N = (g/\rho_{max} \times \Delta\rho/\Delta z)^{1/2}$, where g is the acceleration due to gravity (9.8 m s^{-2}), ρ_{max} is the maximum density of the considered water column and $\Delta\rho/\Delta z$ is the vertical density gradient. N varied from 0.04 to 0.10 s^{-1} in the subarctic ponds (average 0.06 s^{-1}; n = 13; maximal depth of 3.3 m) and from 0.01 to 0.09 s^{-1} in those arctic ponds that were deep enough to obtain a profile (average 0.04 s^{-1}; n = 6; maximum depth of 1 m). The arctic lakes (maximum depths of BYL37, BYL39, and BYL40 were 3.2, 4.3, and 6.7 m, respectively) had a stability index lower than 0.023 s^{-1}.

Most subarctic ponds also had a hypoxic hypolimnion, with on average 23% of surface oxygen at the bottom of the hypolimnion (1.4% to 95%; n = 13). In most cases (11 out of 12 ponds profiled), dissolved CO_2 and CH_4 increased in the bottom waters, with on average 10 times more CO_2 (up to 24×) and 744 times more CH_4 (up to 2802×) at the bottom of stratified ponds, 10-20 cm above sediments. Surface CO_2 concentrations were not correlated with bottom values, but CO_2 and CH_4 concentrations above the sediments were significantly correlated ($r = 0.886$, $p < 0.0001$). The rapid increases in CO_2 and CH_4 and decrease in O_2 at ~1 m depth in these ponds indicates that this is a typical depth of nocturnal mixing and that mixing events to slightly deeper depths will entrain significant quantities of GHG to the surface. Gas profiles were not performed in the arctic ponds because of their shallowness.

Time series temperatures in pond KWK16– The year long monitoring of surface and bottom water temperatures of subarctic pond KWK16 revealed persistent stratification despite its shallow depth (Fig. 5). The temperature difference between surface and bottom waters was larger than 1°C for 87% of the year, with summer stratification occurring about 34% of the year (increasing to 42% with a stability criterion of 0.5°C difference between surface and bottom temperatures). Temperature inversions occurred on 28 October and 17 May. Winter water temperatures ranged between 2°C and 4°C and indicated that the pond did not

freeze. The ice was likely thin and covered by a thick layer of snow (total snow fall in 2007 at the village of Whapmagoostui-Kuujjuarapik was 2.4 m, but the snow likely accumulates on ponds due to the local topography) since the temperature at 0.15 m depth always remained above 0.08°C (winter average of 0.7°C). There were two principal periods of mixing: one long episode beginning in September until the end of October (but during which diurnal stratification was still occurring; Fig. 5b) and a short episode in May (Fig. 5c). Isolated mixing events of the whole water column were also observed during the summer (defined here as 01 June to 03 September), but on only two occasions in July, while the temperature difference between surface and bottom waters still remained above 0.2°C. However, the analysis of heat fluxes in one pond (KWK2, less turbid) revealed regular mixing events in the upper water column (*see* below).

Meteorological and gas measurements time series from pond KWK2– As in the other subarctic ponds, shallow mixed layers were observed in pond KWK2. During the study period, air temperatures were lower than surface water temperatures except on the two sunniest days, 02-03 July (Fig. 6). Relative humidity, typically above 80%, decreased on sunny days. Effective heat flux, which indicates whether the uppermost mixing layer will gain or lose heat, was well above 0°C on sunny days, indicating that the upper water column would have stratified on those days (Fig. 7). Cold, cloudy, windy conditions prevailed during the last two days of the study. Evaporation caused the greatest heat loss and increased during windy periods (Fig. 7a). The effective heat flux was always negative at night as well as throughout most of the cloudy days (Fig. 7b) and would cause mixed layer deepening at those times.

Oxygen concentrations were in near equilibrium with the atmosphere except on the afternoons of 02-03 July (Fig. 7c). The increased concentrations likely indicated increased photosynthesis due to higher insolation (significant correlation obtained between the departure to O_2 saturation and incident irradiance, $r = 0.393$, $p = 0.0017$), and by reduced exchanges with the atmosphere due to suppressed mixing in that day. From 04-06 July, concentrations were below saturation. CO_2 concentrations always exceeded saturation indicating the pond was net heterotrophic (dissolved CO_2 varied from 26.7 to 81.9 μmol L^{-1}, with an average of 51.1 μmol L^{-1}). Methane concentrations began to increase substantially relative to saturation on 02 July. Fluctuations in concentrations (or departure from saturation) in these three gases tended to coincide (positive correlation between CO_2 and CH_4 and negative correlation between CO_2 and O_2; $r > \pm 0.639$, $p < 0.0001$). The largest increases in CO_2 and largest decreases in O_2 occurred at the end of the day on 03 July. CH_4 concentrations also increased along with CO_2. These changes co-occurred with the effective heat flux decreasing below zero which signifies mixed layer deepening. Similar patterns were observed the preceding day (02 July). The lower O_2 concentrations on 04 and 05 July were likely due to lower photosynthesis (lower irradiance on cloudy days), but the concomitant sustained higher concentrations of CO_2 and CH_4 also indicate a deepening of the mixed layer and the associated entrainment of dissolved gases from deeper waters. Profile data taken on 07 July indicated that gas concentrations were uniform to at least 0.75 m which supports the idea that mixing occurred during the cold front at the end of the study period.

The gas transfer coefficients k computed using the data in Jonsson et al. (2008) were on average 40% higher than those computed following Cole and Caraco (1998), and up to 60% higher at the highest wind speed (Fig. 8). Higher k values based on the eddy covariance

data are not surprising since eddy covariance provides fluxes on the time scale of the meteorological forcing (e.g., minutes to hours). Since GHG fluxes increase non-linearly with wind speed, k estimates based on fluxes computed over time scales of days will be underestimates. Estimates of k with the surface renewal model depended upon the assumed mixed layer depth, with higher estimates for shallower mixed layers as the flux of energy which induces mixing is then trapped in a smaller volume of water. Estimates of k using surface renewal were similar to those from Cole and Caraco (1998) assuming the upper mixed layer was 1 m deep and similar to those obtained from eddy covariance data assuming a mixed layer of 0.1 m. As anticipated, the surface renewal model often gave higher values at night as it captured the effects of turbulence from heat loss. Because k estimated from surface renewal was bounded by the two other approaches and because we did not have time series measurements of mixed layer depth, we do not present fluxes computed with this method. The highest GHG fluxes occurred on 30 June and on 05 and 06 July (Fig. 9) in response to the higher wind speed on those days (Fig. 6). Sustained high fluxes at the end of the study were additionally caused by the increased gas concentrations in the upper water column due to the increased vertical mixing induced by cooling.

Gas fluxes in pond data series– Gas fluxes varied widely in thaw ponds data series (by 242% for CO_2 and by 195% for CH_4). The lowest CO_2 fluxes were obtained in arctic polygon ponds (down to -20.5 mmol m^{-2} d^{-1}) and the highest CO_2 and CH_4 fluxes were obtained in arctic runnel ponds (up to 114.4 and 5.6 mmol m^{-2} d^{-1} of CO_2 and CH_4 respectively). The method used to measure CH_4 concentration in water (2L of pond water in equilibrium with 20 mL headspace) for estimation of fluxes with the wind-based model most likely excluded CH_4 bubbles (especially those from sporadic bubbling on thaw lake edges as described in Walter et al. 2006). Therefore, these values must be considered diffusive fluxes (although the influence of small bubbles cannot be excluded; Semiletov et al. 1996) that provide a lower bound to total CH_4 flux. Also, wind speed varied from 0.2 to 8.4 m s^{-1} in July, generating variability in gas fluxes possibly of the same order of magnitude as spatial variations. For example, this range in wind speeds would generate CH_4 fluxes from 0.4 to 1.8 mmol m^{-2} d^{-1} in pond BYL31. Therefore, the values presented here, obtained from one discrete measurement during the day, should be considered first approximations.

The CO_2 fluxes obtained directly with the floating chamber in 2007 (Table 3) were correlated with those from the same ponds estimated from dissolved gas concentration following Cole and Caraco (1998; for paired fluxes, $r = 0.842$, $p < 0.0001$, $n = 27$). However, fluxes obtained with the floating chamber were generally lower than with the wind-based model (mean of 4 compared to 12 mmol m^{-2} d^{-1}, respectively), although those measurements were not always taken exactly at the same time as the dissolved gases.

Discussion

The two groups of thaw pond systems in this study showed striking differences in their morphological, physicochemical and biological properties, and also contrasted in their greenhouse gas characteristics. Even at the local scale, there was large variability between nearby waters. We measured negative CO_2 fluxes (net transfer from atmosphere to water) in arctic ponds colonized by benthic microbial mats, while the largest positive fluxes occurred in the adjacent humic-rich runnel systems. Gas exchange also varied greatly over all measured timescales: diel, weekly, and seasonal.

Supersaturated thaw ponds– Like most lakes and ponds of the temperate and boreal regions, the Canadian subarctic thaw ponds sampled in the present study were all supersaturated in CO_2, with concentrations varying from 20 to 105 μmol L^{-1}, corresponding to a partial pressure range of 44 to 230 Pa (n = 43, data from 2006 and 2007 combined). These values fall in the range obtained further to the south in a large survey of boreal lakes showing surface water CO_2 partial pressure between ~40 and 250 Pa (n = 2838; Sobek et al. 2005). Subarctic thaw ponds were also supersaturated in CH_4 (concentration varying from 0.04 to 1.34 μmol L^{-1}, or 0.1 to 3.1 Pa), with large increases observed in the isolated hypolimnion (*see* below). The automated measurements in pond KWK2 over 8 days (Fig. 7) give a sense of the short-term dynamics in CO_2 (ranging from 27 to 82 μmol L^{-1}) and CH_4 (from 0.02 to 0.56 μmol L^{-1}) that can occur in surface waters of a thaw pond in summer. Dissolved CH_4 varied to a larger extent than CO_2 in this time series (coefficients of variation of 69% and 19%, respectively), consistent with the results of Walter et al. (2006).

It is instructive to compare the results from two years sampled at different dates (end of July in 2006 and 3 weeks earlier in 2007; Table 2). Earlier in summer 2007, the surface water temperature was colder, dissolved O_2 concentration was higher and both CO_2 and CH_4 were lower. While these data could be indicative of reduced heterotrophic activity, they could also reflect more efficient exchanges with the atmosphere (negative effective heat flux) and/or lower accumulations of gases in deeper waters. Nevertheless, they indicate the high spatial and temporal resolution of meteorological data, water column structure and gas concentrations that are required to fully define GHG dynamics.

The arctic waters differed in their GHG characteristics. These ponds were often undersaturated in CO_2, principally when benthic cyanobacteria had developed active photosynthetic mats (in low center polygon ponds; although in 2008, several runnel ponds were colonized by *Sphagnum* spp. that likely also contributed to the CO_2 sink). However, the arctic ponds generally had higher CH_4 contents than the surface waters of subarctic ponds (Table 2). Other studies found the opposite trend, with larger CH_4 concentrations in sites located at lower latitudes (71.5°N compared to 68.5°N in Semiletov et al. 1996 and Nakano et al. 2000). The ponds developing on the periphery of polygons were in most cases supersaturated in both gases (averaged dissolved CO_2 and CH_4 were 48.8 and 2.2 μmol L^{-1} in runnel ponds, compared to 3.8 and 0.4 μmol L^{-1} in low center polygon ponds, respectively) and the concentration differed significantly between the two pond types (Mann-Whitney rank sum test, $p < 0.008$). The runnel ponds had high DOM and especially high chromophoric DOM (on average a_{320} = 30 m^{-1} and DOC = 13 mg L^{-1}), whereas polygon ponds apparently had photobleached DOM (a_{320}:DOC 1.6 times lower than in runnel ponds, with on average a_{320} = 15 m^{-1} and DOC = 10 mg L^{-1}). Therefore, photolysis of DOM into CO_2 was possibly acting with greater importance in the shallow arctic ponds exposed to longer daylight as compared to the turbid subarctic ponds. In the kettle lake and especially the two oligotrophic lakes, dissolved CO_2 (on average 17 μmol L^{-1}) and CH_4 (on average 0.03 μmol L^{-1}) were close to atmospheric equilibrium. These lakes had the lowest bacterial abundance (especially the oligotrophic lakes) and DOM concentration. Therefore, reduced inputs of allochthonous organic carbon and nutrients in these lakes likely dampened their role as gas conduits to the atmosphere.

Several factors are known to influence dissolved CO_2 in freshwaters, including DOM concentrations, availability to microbes and reactivity to sunlight (Granéli et al. 1996; Obernosterer and Benner 2004), vertical structure of the water column, benthic-pelagic

coupling and type of microbial assemblages (Huttunen et al. 2006; Kankaala et al. 2006), uptake by primary producers in productive ecosystems (del Giorgio et al. 1999) and inputs by groundwater and stream flow (Striegl and Michmerhuizen 1998). Groundwater and stream flow inputs were found to be minor in temperate and dystrophic bog lakes (Hanson et al. 2003) and are likely reduced in thaw ponds: their closed morphology and the presence of permafrost limit water circulation. This is particularly the case for the subarctic thermokarst ponds studied which are located in impermeable clay-silt beds (although small streamlets through breaches between adjacent ponds were sometimes observed). Therefore, the CO_2 supersaturation observed in surface waters of thaw ponds is thought to originate mainly from three sources: benthic respiration, pelagic respiration and DOM photolysis. Jonsson et al. (2001) demonstrated that 40% of dissolved CO_2 originated from benthic respiration, 50% from pelagic respiration and 10% from DOM photolysis in a deep humic lake in Sweden. A much larger contribution of benthic respiration is likely in shallow ponds (Kortelainen et al. 2006). In the turbid subarctic thaw ponds where light is rapidly attenuated (reduced production of CO_2 by DOM photolysis), surface water CO_2 was most likely derived from the respiration in littoral sediments located above the metalimnion (Bastviken et al. 2008) and, following vertical mixing at night and during cold fronts, from respiration deeper in the water column and from the sediments.

Stratified thaw ponds– A major consequence of the high turbidity and humic content of subarctic thaw ponds, combined with the low evaporation, high solar radiation, and warm air temperatures typical near ice-off, was the rapid establishment of steep thermal stratification in spring (spring mixing persisted only about 5 days in KWK16 in 2008; Fig. 5). The deeper water masses were rapidly isolated from the atmosphere, with anoxia leading to anaerobic metabolism. A stable thermocline in a 2 or 3 meter-deep water body with a stability index (N) of 0.04 to 0.1 s^{-1} was unexpected at these latitudes, but explained by the strong attenuation of shortwave solar radiation in these water bodies.

As a result of the stratification, both CO_2 and CH_4 increased at depth to attain values up to 836 μmol L^{-1} of CO_2 and 127 μmol L^{-1} of CH_4 (at 2 m depth in the anoxic water of pond KWK21). Gas build up in bottom waters is likely a consequence of the physical isolation of this water mass, while increasing concentrations towards sediments vary proportionally with the sediment area:water volume ratio of each water layer. Such high dissolved gas concentrations have also been reported in thaw lakes and ponds of the Kolyma River Lowland region in Siberia (Semiletov et al. 1996). Using the sediment-water CH_4 flux obtained by Huttunen et al. (2006) in a small boreal lake (27 mg m^{-2} d^{-1}), the time required to reach the hypolimnetic CH_4 concentration measured in pond KWK23 (1.97 mg CH_4 L^{-1} averaged from the 2 depths where data were recorded; Fig. 4) was estimated to 110 days (estimated hypolimnion volume = 427 m^3 and sediment area = 285 m^2). This pond was likely stratified only for about 40 days prior to sampling (based on the stratification regime established in the similar pond KWK16). However, if these ponds do not entirely mix in spring, concentrations may remain higher thus allowing for greater accumulation over time and supporting the flux calculations above. The increased specific conductivity in the bottom waters of some ponds also suggests incomplete mixing. Differences in the nature of the available organic matter and microbial communities (e.g., the balance between methanogens and methanotrophs) will also affect gas accumulation rates.

In subarctic pond KWK2, the combination of surface meteorology and time series measurements of gas concentrations allowed insights into the combined effects of biological

processes and drivers of vertical mixing on gas fluxes. Concentrations of CO_2 and CH_4 increased in the upper water column in response to cooling at the surface, which likely drove the convective entrainment of these gases from deeper in the water column. Cold fronts are most likely to cause increases in fluxes, which doubled in the present study during such events. The time series temperature data from KWK16 (Fig. 5) indicate cooling events occurred 4-6 times per month in the summer and usually persist for several days. Summer mixing events will be likely in shallower ponds, especially the least turbid and humic ones. These high resolution data point to methods to improve the accuracy of predicting changes in fluxes with climate warming. Gas ventilation would occur periodically in cool summers but could potentially be delayed until autumn in warm summers as the water column is more stable. Water column dynamics just prior to ice-on would determine whether these gases are vented to the atmosphere or sequestered until next spring, with increased opportunity for ongoing transformations (e.g., consumption of CH_4 by methanotrophs). The remarkable and persistent stability of the subarctic thaw ponds indicates that considerable concentrations of GHG may be vented during fall overturn. This autumnal venting from subarctic stratified ponds could be linked to the late-autumn shoulder consistently observed in the seasonal cycles of atmospheric methane at high latitudes (Dlugokencky et al. 1994). Thus, sampling efforts should also be made at this time of the year, as well as during the spring liberation of gases accumulated under the ice (Michmerhuizen et al. 1996). The accentuation of thermal stratification (faster in spring, more stable in summer) expected as a consequence of global warming (Jankowski et al. 2006) will also affect seasonal variations in GHG evasion.

Heterotrophic thaw ponds– The subarctic thaw ponds were relatively rich in organic matter and in phosphorus, but their low light environment would favour heterotrophy over phototrophy. The ponds indeed harboured abundant bacteria (Table 1) relative to concentrations found in temperate lakes (e.g., 1.7 to 5.9 $\times 10^6$ mL^{-1}, $n = 14$; del Giorgio et al. 1997). Therefore, net heterotrophy and the observed large CO_2 evasion were to be expected in these ponds. On the other hand, arctic ponds were often net autotrophic (mostly polygon ponds; Fig. 3). The arctic ponds that were net heterotrophic (runnel ponds) presented signs of recent peat erosion, higher DOC contents and higher aromaticity (a_{320}:DOC). Climate warming can lead to increased peat erosion in this type of landscape with the formation of abundant runnel ponds, resulting in the liberation of soil organic carbon and its respiration to the atmosphere (the highest C fluxes were measured in runnel ponds). In contrast, the polygon ponds colonized by benthic cyanobacterial mats are a carbon sink and are vulnerable to climate warming, with their water draining either through fissures that form across the polygons or during periods of reduced precipitation:evaporation ratios (Fig. 2f). It will be essential to monitor multiple successional stages in these ecosystems as climate warms to fully estimate their role in GHG production and global feedback effects.

Greenhouse gas fluxes– In the ponds where chamber flux measurements were taken several times during the day (one subarctic and 5 arctic ponds), variations were high (coefficients of variation of 37% to 210%), with values always higher when taken earlier in the day (not shown). This suggests that nocturnal mixing brought more gas to the air-water interface (Crill et al. 1988). Therefore, the time of sampling is a source of variability that needs to be considered. Our estimates of fluxes are limited by several factors. First, wind was estimated from a relatively distant climate station (but within 500 m in most cases), and local topographic variations, although slight, may have caused errors in our estimates of flux rates. The difference observed between fluxes obtained

with a floating chamber and with the conservative wind-based model of Cole and Caraco (1998; Table 3; on average 2.5 times lower when using the chamber) is likely the result of several factors. The wind was measured at 10 m above ground, while the model was developed for lakes of longer fetch length where shore topography is not affecting the boundary layer. At the present study sites, pond area was sometimes only a few square meters, and the local topography (at arctic site, Fig. 2e) and thick surrounding vegetation (at subarctic site, Fig. 2b) may have led to lower shear stresses than would have occurred in the larger water bodies where most of the empirical studies have been conducted to develop equations for the gas transfer coefficient (Kwan and Taylor 1994). Consequently, gas exchange may be lower than computed. Surface films of surfactants (Banerjee and MacIntyre 2004), abundant in those small, humic and microbially active thaw ponds, could also act to reduce gas exchanges through the interface, a factor that is not considered in wind-based models. Finally, chamber deployment has been shown to enhance gas transfer through disturbance of the surface boundary layer (Matthews et al. 2003), and cannot be completely excluded in the present study.

The highest CO_2 flux measured was in the Arctic for a humic pond (BYL38; DOC = 20.8 mg L^{-1}) that had apparently been formed by erosion of soils on the side of a moraine deposit (arrow in Fig. 2d). The flux measured with the floating chamber reached 909 mg C m^{-2} d^{-1} (at noon; the value given in Table 3 for BYL38 is an average of three separate measurements). This result suggests that newly formed runnel ponds may release large amounts of carbon at the beginning of the erosional cycle, followed by reduced evasions as labile carbon is used up.

To compare the CO_2 fluxes obtained in the present study with literature values, we extrapolated our instantaneous flux values to provide a first estimate of annual rates. Annual CO_2 fluxes ranged from 39 to 273 g C m^{-2} in the subarctic ponds (average of 122 g C m^{-2} yr^{-1}, n = 12). These values have a high level of uncertainty given the diel and seasonal variations, however they are likely to be underestimates since they were calculated using surface water concentrations of CO_2 and they neglect evasion during fall cooling as well as changes under the ice. Also, as demonstrated with the application of three models to compute gas transfer coefficients, the wind based model (Cole and Caraco 1998), which was used to calculate fluxes in the pond data series, is the most conservative. Given these caveats, the CO_2 fluxes estimated for subarctic ponds in the present study fall within the range reported by Kortelainen et al. (2006) for small boreal lakes (average of 102 g C m^{-2} yr^{-1} for lakes smaller than 0.1 km^2). They are higher than those estimated from a large series of boreal lakes in Sweden (0.63 to 5 g C m^{-2} yr^{-1}; Algesten et al. 2003) or from arctic lakes and rivers in Alaska (average of 24 g C m^{-2} y^{-1}; Kling et al. 1992), but much lower than those values reported for wetland ponds in the Hudson Bay Lowland (1350-4015 g C m^{-2} yr^{-1}, also derived from daily fluxes extrapolated to the full year; Hamilton et al. 1994). This simple comparison demonstrates the need for improved regional coverage of GHG flux measurements. Further studies are also needed to verify the 40% increase in fluxes obtained when using gas transfer coefficients based on eddy covariance data (Fig. 9), and, as noted above, to address the role of surfactants and the changes in the characteristics of the atmospheric boundary layer over small water bodies and the predicted gas transfer coefficients.

The area covered by thaw ponds in northeastern Canada is unknown at this stage, but it was estimated to cover 8-12% of the landscape in Hudson Bay lowlands (Hamilton et al. 1994), and 15-40% in the arctic coastal plain of northern Alaska (Hinkel et al. 2005).

Considering that Canada has 4 million km^2 occupied by permafrost and assuming that 5% of this area is occupied by thaw ponds involving soil erosion (thermokarst), a global carbon evasion from Canadian thaw ponds can be estimated. Using the average surface flux values from subarctic and arctic ponds (2007 data series, excluding the polygon ponds with cyanobacterial mats), we obtain annual diffusive flux from Canadian thaw ponds of 95 Tg CO_2 and 1.0 Tg CH_4. Current estimates of CO_2 evasion from lakes range from 257 to 550 Tg CO_2 yr^{-1} (Cole et al. 2007), an estimate that does not include thaw ponds as studied here. In general, methane losses by ebullition exceed diffusive fluxes. For example, Walter at al. (2006) estimated that molecular diffusion of methane was 5% of total emissions from Siberian thermokarst lakes. The present study CH_4 fluxes were indeed lower than what was measured in other thermokarst lakes and ponds (Walter et al. 2006; Wickland et al. 2006; Blodau et al. 2008) or permafrost-influenced aquatic systems (Nakano et al. 2000; Ström and Christensen 2007) but close to estimates based on the eddy covariance method in polygonal tundra (similar to the present study arctic site; Sachs et al. 2008). However, ebullition in Canadian thaw ponds is possibly lower than ebullition from thick yedoma organic sediments beneath Siberian thaw lakes (Walter et al. 2008). Therefore, even though this estimation of annual methane flux from Canadian thaw ponds is possibly underestimated, it is comparable to the global annual evasion of 3.8 Tg CH_4 calculated for Northern Siberian thaw lakes and significant in comparison to the 6-40 Tg CH_4 emitted annually by northern wetlands (Walter et al. 2006). Moreover, in these annual estimations, we assumed a constant evasion of gases at summer rates (surface water fluxes were only including the contribution by benthic respiration from the littoral zone comprised in the mixing layer) and did not consider the accumulation of gases in deeper strata of the water column during the summer (subsidized by anoxic respiration below the mixing layer). Using the gas profile obtained in one stratified subarctic thaw pond (KWK23, Fig. 4), and a trapezoidal integration of the gases trapped below the mixed layer (< 0.75 m), we estimate that the autumnal mixing period could release to the atmosphere more than two times the CH_4 evasion calculated for continuous loss over 365 days using surface water July rate. On the other hand, the shallower arctic ponds are frozen to the bottom for a large part of the year and therefore most likely not releasing greenhouse gases during this period.

Indicators of gas content– Efforts have been made to predict CO_2 from DOC in lake systems. Methods to estimate past DOC using a paleolimnological approach (Pienitz and Vincent 2000) or remote sensing (Kutser et al. 2005) would help us to scale up our predictions of CO_2 concentrations or evasion rates in time and space. However, contrasting results have been reported on the predictive strength of DOC (as bulk measure of DOM) for the estimation of CO_2 in lake waters, with for example significant correlations found in boreal lakes (Sobek et al. 2005) and non-significant correlations in small (Kortelainen et al. 2006) or large boreal lakes (Rantakari and Kortelainen 2005) and in northern temperate lakes (del Giorgio et al. 1997). In the present study, when both sites and years were combined, we did not obtain a significant correlation between CO_2 and DOC, but the relation became significant when the subarctic ponds were considered alone (although r remained low at 0.411, p = 0.007). The differing trends revealed by these studies may result from the difficulty to adequately describe the huge variability in microbial availability and photochemical reactivity of DOM in aquatic systems simply using DOC. It could also be linked to more complex interactions between DOM, food web structure and sedimentation carbon losses (Flanagan et al. 2006).

Other DOM descriptors, such as the absorbance and fluorescence properties, were

used more successfully than DOC to explain the differing rates of microbial and photochemical degradation (Stedmon and Markager 2005). We obtained a better relationship between dissolved CO_2 and the chromophoric portion of DOM (i.e., the absorption coefficient). Additionally, we observed a correlation between CO_2 and a_{320}:DOC. These results suggest that: 1) a significant part of the CO_2 originates from DOM photolysis since it is related to DOM chromophoricity (Opsahl and Benner 1998); 2) the chromophoric components of DOM (generally considered of a higher molecular weight) were used more successfully by the microbial community (as found in marine waters by Amon and Benner 1994); or 3) DOM exerts an indirect effect on CO_2 through its effect on temperature, light availability and water column structure, hence the chromophoric properties of DOM are prevalent in these relationships. Overall, these empirical relationships have a relatively low predictive power, and the models developed for one ecosystem type may be poorly applicable to other systems. For example, CO_2 concentrations estimated from a model developed on 176 boreal lakes by Algesten et al. (2003, using TOC equal to DOC) were correlated with CO_2 concentrations measured in the present study for subarctic ponds (r = 0.429 and p = 0.005), but the values differ on average by 28%.

Thawing of permafrost has been identified as one of the five most vulnerable carbon pools that can have drastic consequences for atmospheric carbon through positive feedback mechanisms (Gruber et al. 2004). However, the size of this high latitude pool, the processes affecting it and the timescale of change involved are yet to be quantified (Schuur et al. 2008). Climate change not only has the potential to mobilize a large pool of stored carbon, it will also affect water temperature, with direct effects on microbial growth and respiration. Perhaps most importantly, climate change will affect the duration of ice cover and the precipitation regime, hence the light and stratification patterns and the hypolimnetic oxygen concentrations, which all have consequences on GHG evasion rates. With a total coverage estimated at about 4.6 million km^2 (Downing et al. 2006), the great abundance of small lakes and ponds combined with their high biogeochemical activities imply that they play a significant role in the global carbon budget and should be incorporated in climate change projections. This is especially the case for permafrost thaw ponds, which account for a vast surface area and lie on melting, carbon-rich soils. Though most lakes and ponds represent net sources of GHG to the atmosphere, processes such as methanotrophy and primary production at certain sites can reduce or even reverse the GHG fluxes. Changes in stratification dynamics will further influence the likelihood of sequestration vs. gas evasion. These limnological sources of variability in gas exchange will require close attention to fully assess GHG efflux and extent of climate feedback in the rapidly warming polar regions.

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Table 1. Limnological properties of the subarctic and arctic thaw ponds and lakes sampled in July 2007, including pH, total suspended solids (TSS), dissolved organic carbon (DOC), absorption coefficient by chromophoric dissolved organic matter at 320 nm (a_{320}), total phosphorus (TP), chlorophyll *a* (Chl *a*), bacterial abundance, and departure from gas saturation ($depCO_2$ and $depCH_4$) obtained from the difference between surface water concentration and the concentration in equilibrium with the atmosphere. RUN = pond formed in runnels over melting ice-wedges; POL = pond on low-center polygons; KL = kettle lake; OL = oligotrophic lake; na = not available.

Pond name	type	pH	TSS ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	a_{320} (m^{-1})	TP ($\mu g\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	Bacteria ($\times 10^6$ cell L^{-1})	$depCO_2$[1] ($\mu mol\ L^{-1}$)	$depCH_4$[1]
Whapmagoostui-Kuujjuarapik, Nunavik										
KWK1	brown	7.36	11.7	7.6	39.46	48.2	10.3	6.79	10.9	0.04
KWK2	black	8.46	5.1	5.2	30.99	37.6	2.4	9.86	23.2	0.04
KWK3	beige	6.97	42.0	8.7	41.85	52.8	6.4	12.79	47.6	0.29
KWK 6	green	6.89	6.9	3.1	9.91	33.6	2.1	12.93	15.4	0.04
KWK 7	brown	7.20	11.3	9.5	45.39	59.7	1.4	15.13	39.3	0.06
KWK 11	black	6.77	9.5	9.4	38.29	95.6	23.8	15.23	10.3	0.05
KWK 16	beige	6.53	24.3*	7.5	35.7*	na	5.2*	na	22.8*	0.44*
KWK 21	beige	6.87	24.0	7.7	40.97	86.0	4.0	16.32	20.2	0.04
KWK 23	beige	7.18	16.8	6.6	33.80	69.5	5.2	15.83	24.1	0.05
KWK 33	brown	6.20	25.1	9.8	56.14	108.9	7.2	16.49	56.1	0.13
KWK 35	brown	7.25	10.6	10.5	42.35	41.2	8.1	9.53	25.8	0.05
KWK 36	black	6.52	8.0	8.1	40.38	36.9	2.1	10.34	45.8	0.11
KWK 38	brown	7.33	5.5	6.0	19.50	54.2	1.9	10.52	38.8	0.43
Bylot Island, Nunavut										
BYL 1	POL	9.6	3.3	10.4	13.48	26.6	0.8	13.84	-17.7	0.39
BYL 22	POL	9.49	4.4	8.9	15.32	34.2	7.9	17.78	-17.1	0.17
BYL 23	RUN	8.87	2.6	18.4	40.09	41.8	1.0	5.68	6.4	1.86
BYL 24	RUN	8.99	6.2	11.0	24.21	43.0	3.8	6.70	-18.0	0.74
BYL 25	RUN	8.08	4.0	12.4	23.76	16.7	0.5	10.93	36.9	0.63

BYL 26	POL	9.59	2.8	10.8	13.17	18.0	1.0	7.91	-17.1	0.21
BYL 27	RUN	8.54	4.5	14.4	45.99	28.6	1.5	14.68	78.1	5.17
BYL 28	RUN	7.88	4.4	12.4	30.60	30.2	0.8	13.72	81.9	2.71
BYL 29	POL	9.65	1.7	12.7	20.38	28.0	0.6	10.25	-16.5	0.19
BYL 30	POL	9.46	5.7	10.7	13.40	26.5	7.4	10.25	-17.1	0.45
BYL 31	POL	9.20	9.0	14.2	22.56	28.3	3.6	9.34	-16.5	0.94
BYL 32	POL	9.61	5.0	10.4	15.56	26.2	7.3	17.73	-14.4	1.24
BYL 33	RUN	9.38	1.5	8.2	14.86	14.3	1.0	4.67	-14.9	1.87
BYL 34	POL	9.53	13.7	11.8	15.66	35.1	5.0	12.71	-16.5	0.35
BYL 35	POL	9.40	3.3	8.7	14.36	35.4	1.9	9.95	-17.1	0.70
BYL 36	POL	8.52	1.1	4.3	5.92	21.9	1.2	8.14	-10.8	0.05
BYL 38	RUN	na	na	20.8	na	na	na	na		
BYL 41	POL	7.19	na	8.9	13.20	20.8	2.9	8.38	-18.7	0.16
BYL 42	POL	8.11	na	11.3	15.88	4.2	1.5	2.23	-14.5	0.12
BYL 37[2]	KL	8.13	1.8	5.2	18.43	16.5	1.8	7.37	-7.5	0.02
BYL 39[2]	OL	7.06	1.4	1.5	4.70	3.2	0.9	1.61	-2.7	0.02
BYL 40[2]	OL	7.18	na	1.5	3.79	3.6	0.6	1.20	0.6	0.04

*Values not available in 2007 but results from 2006 are given as an indication. [1]Average gas concentrations in equilibrium with the atmosphere for the 2007 data series are as follows (if concentrations are preferred to departure from saturation): 19.5 μmol L^{-1} CO_2 and 0.0034 μmol L^{-1} CH_4. [2]Three lakes are shown for comparison.

Table 2. Comparison of 12 subarctic ponds sampled both in 2006 and 2007 and of 18 arctic ponds (sampled in 2007). Surface water temperature at sampling (T), dissolved organic carbon (DOC), DOC-specific absorption coefficient at 320 nm (a_{320}:DOC), and departure from gas saturation ($depO_2$, $depCO_2$, and $depCH_4$) obtained from the difference between surface water concentration and the concentration in equilibrium with the atmosphere.

	KWK in 2006	KWK in 2007	BYL in 2007
	Min-Max (average ± standard deviation)		
Sampling period	21– 27 July	28 June – 04 July	15 – 23 July
T (°C)	16.4 – 22.7 (19.7 ± 1.7)	9.5 – 19.5 (13.4 ± 3.4)	7.0 – 12.0 (10.4 ± 1.6)
DOC (mg C L^{-1})	3.8 – 12.9 (8.8 ± 2.7)	3.1 – 10.5 (7.7 ± 2.1)	4.3 – 18.4 (11.1 ± 3.0)
a_{320}:DOC (L mg C^{-1} m^{-1})	2.2 – 4.8 (3.9 ± 0.7)	3.2 – 6.0 (4.7 ± 0.9)	1.2 – 3.2 (1.7 ± 0.5)
$depO_2$ (μmol L^{-1})	-60 – 17 (-12 ± 28)	-32 – 47 (-3 ± 21)	-87 – 59 (17 ± 43)
$depCO_2$ (μmol L^{-1})	4 – 79 (42 ± 20)	10 – 56 (30 ± 15)	-19 – 82 (-1 ± 32)
$depCH_4$ (μmol L^{-1})	0.23 – 1.34 (0.48 ± 0.33)	0.04 – 0.43 (0.11 ± 0.12)	0.05 – 5.2 (1.0 ± 1.3)

Table 3. Gas fluxes measured with the floating chamber (CO_2) compared to estimations from dissolved gas concentration and wind speed following Cole and Caraco (1998). na = not available.

Pond name	CO_2 flux chamber	CO_2 flux wind speed	CH_4 flux wind speed	O_2 flux wind speed
	(mmol m^{-2} d^{-1})			
Whapmagoostui-Kuujjuarapik, Nunavik				
KWK1	2.3	8.9	0.04*	-13.6
KWK2	10.6	19.3	0.04*	-22.1
KWK3	na	30.1	0.18	-4.7
KWK 6	5.2	18.3	0.05*	-13.0
KWK 7	24.3	36.7	0.05	-5.4
KWK 11	4.3	13.0	0.06	21.7
KWK 16	10.3	na	na	na
KWK 21	3.5	13.5	0.03*	-3.1
KWK 23	23.2	35.0	0.07*	-49.4
KWK 33	11.1	62.2	0.14	-9.2
KWK 35	4.8	16.2	0.03	4.9
KWK 36	na	39.2	0.09	6.6
KWK 38	14.0	40.8	0.45	52.6
Bylot Island, Nunavut				
BYL 1	-14.6	-14.3*	0.32	-0.1
BYL 22	-10.8	-19.9*	0.20	20.0
BYL 23	-3.1	8.0	2.33	75.3
BYL 24	-2.3	-20.5*	0.84	16.0
BYL 25	16.3	20.3	0.35	-1.2
BYL 26	-14.9	-9.8*	0.12	31.4
BYL 27	23.9	85.4	5.62	-82.3

BYL 28	28.2	114.4	3.77	-127.2
BYL 29	-18.2	-11.9*	0.14	40.0
BYL 30	-17.1	-13.0*	0.34	29.5
BYL 31[1]	-9.7	-11.8*	0.67	36.4
BYL 32[1]	-11.1	-10.1	0.87	27.7
BYL 33[1]	-7.0	-10.6	1.32	38.4
BYL 34	-13.8	-8.0*	0.17	30.1
BYL 35	-8.3	-12.6*	0.52	40.8
BYL 36	-3.2	-6.6	0.03	-2.3
BYL 38	60.0	na	na	na
BYL 41	na	-8.9	0.08	-10.3
BYL 42	31.5	-6.6	0.05	5.9
BYL 37[2]	-1.4	-5.3	0.01	-1.3
BYL 39[2]	3.5	-1.5	0.01	-3.3
BYL 40[2]	5.9	0.4	0.03	-11.6

*Dissolved CO_2 or CH_4 in surface waters were close to the detection limit. This detection limit was used to calculate the gas flux, thus, those values are only approximate. [1]These ponds were localised in another valley on Bylot Island, at ca 10 km north of the climate station, therefore wind-based estimations in gas flux are given only as an indication. [2]Three lakes are shown for comparison.

Figure legends

Figure 1. Location of the two sampling sites as indicated by the stars.

Figure 2. Thaw pond study sites. (a) subarctic thaw ponds (~20-27 m diameter), (b) dense shrubs around a turbid subarctic pond, (c) thaw ponds on low-center polygons (~10-20 m diameter) and in runnels (~1-4 m wide) over melting ice-wedges in the arctic site, (d) general view of the arctic site on the side of a moraine (arrow) with a larger pond similar to BYL36 (white color is floating ice), (e) soil erosion beside an arctic thaw pond (~1 m wide), (f) orange benthic mat exposed to the air after a long period without rain in 2007, and peat layer erosion along the bank of the proglacial river.

Figure 3. Correlation between O_2 and CO_2 (departure from gas saturation, $depO_2$, and $depCO_2$) in subarctic thaw ponds (squares), arctic thaw ponds (polygon ponds: polygons; runnel ponds: triangles), and arctic lakes (circles).

Figure 4. Profiles of temperature and dissolved concentrations of O_2, CO_2, and CH_4 in four subarctic ponds (CH_4 were multiplied by 10 to fit CO_2 scale).

Figure 5. Temperature at the surface (0.15 m) and bottom (2.0 m) of pond KWK16 (maximal depth ~2.2 m) (a) followed over one complete year from 06 July 2007 to 11 July 2008, (b) showing diurnal stratification during the autumnal mixing period, and (c) during spring mixing.

Figure 6. (a) Wind speed at 10 m above ground, (b) air (thin line) and water (thick line) temperatures, (c) relative humidity, and (d) incident short wave radiation (300-1100 nm) at pond KWK2 during the 8 day period of gas measurements in surface water.

Figure 7. (a) Sensible heat (thick line), latent heat (thin line), and longwave (dashed line) fluxes, (b) surface heat flux (sum of sensible, latent and net long wave, thick line) and effective heat flux (sum of net shortwave and surface heat flux, thin line), and (c) difference between concentration of O_2 (thin), CO_2 (thick), and CH_4 (dashed) in surface water and the gas concentration in equilibrium with the atmosphere (depGas) of pond KWK2. Difference for CH_4 has been multiplied by 100. Effective heat flux was computed assuming that the mixing layer was 0.3 m deep.

Figure 8. Gas transfer coefficients calculated following Cole and Caraco (1998; thick line), Jonsson et al. (2008; thin line), and the surface renewal model (MacIntyre et al. 1995; dashed line) with surface renewal calculations assuming a mixed layer depth of (a) 0.1 m, (b) 0.3 m, and (c) 1 m.

Figure 9. Greenhouse gas fluxes computed using the two wind based models for the gas transfer coefficient: Cole and Caraco (1998; thick line) and Jonsson et al. (2008; thin line). (a) CO_2, (b) CH_4.

Fig. 1

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Fig. 2

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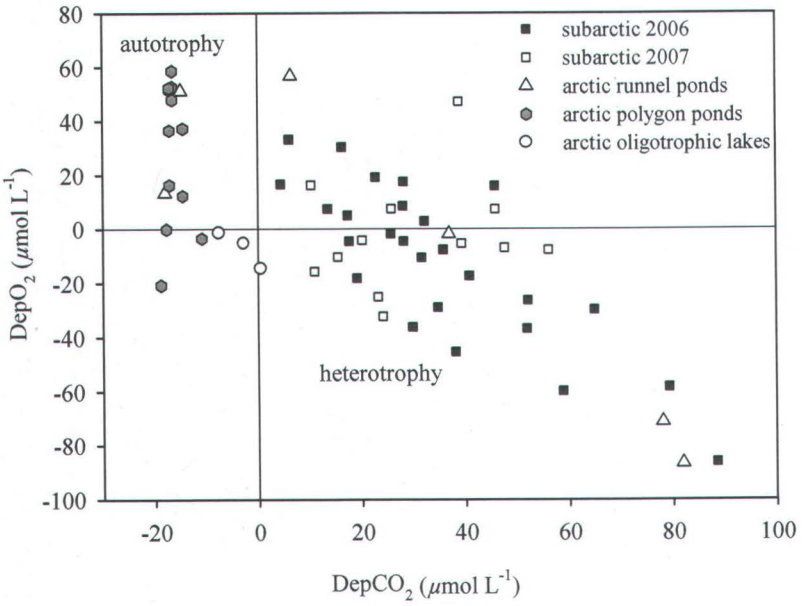

Fig. 3

Laurion et al. 2009

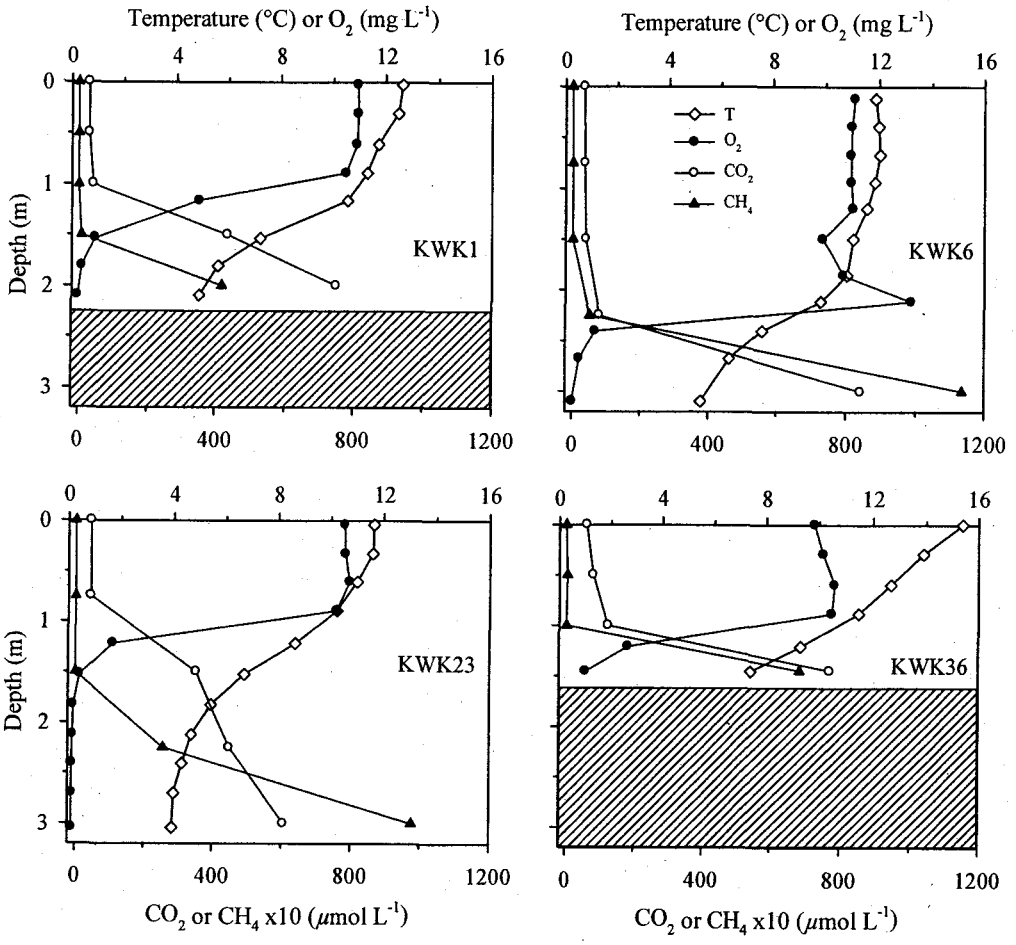

Fig. 4

Laurion et al. 2009

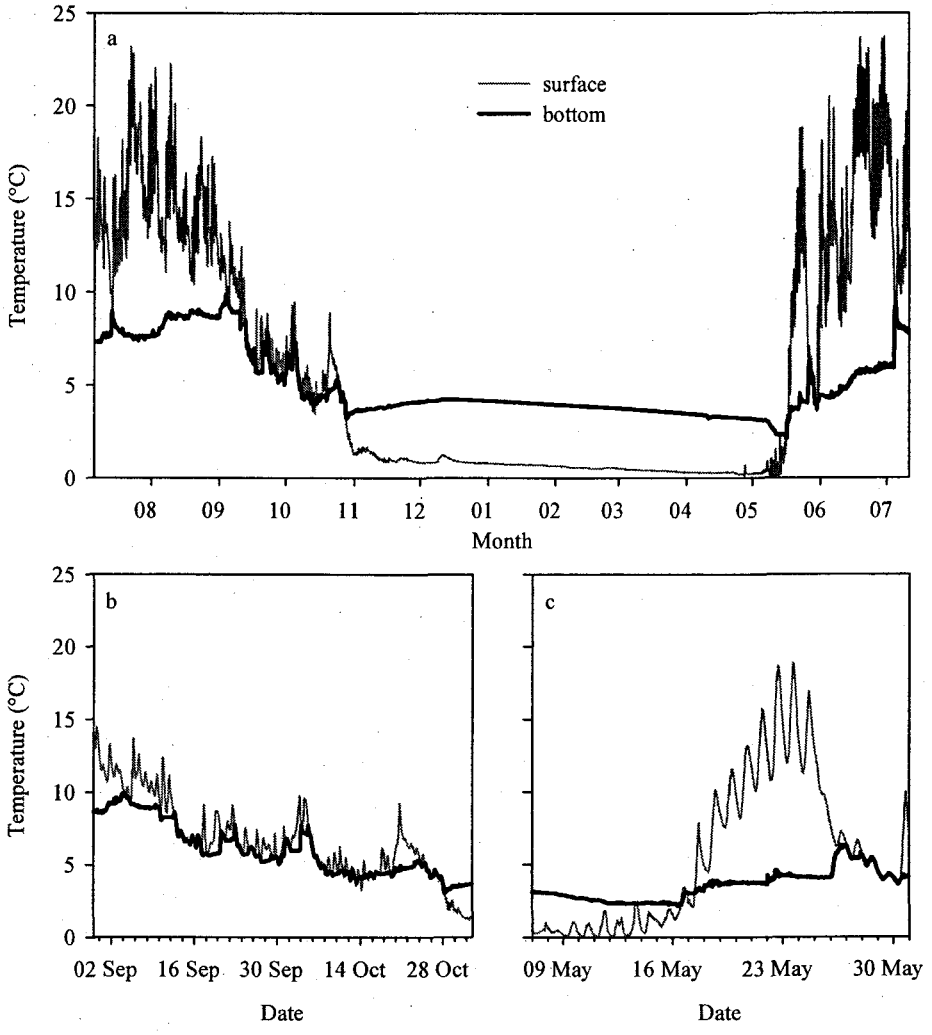

Fig. 5

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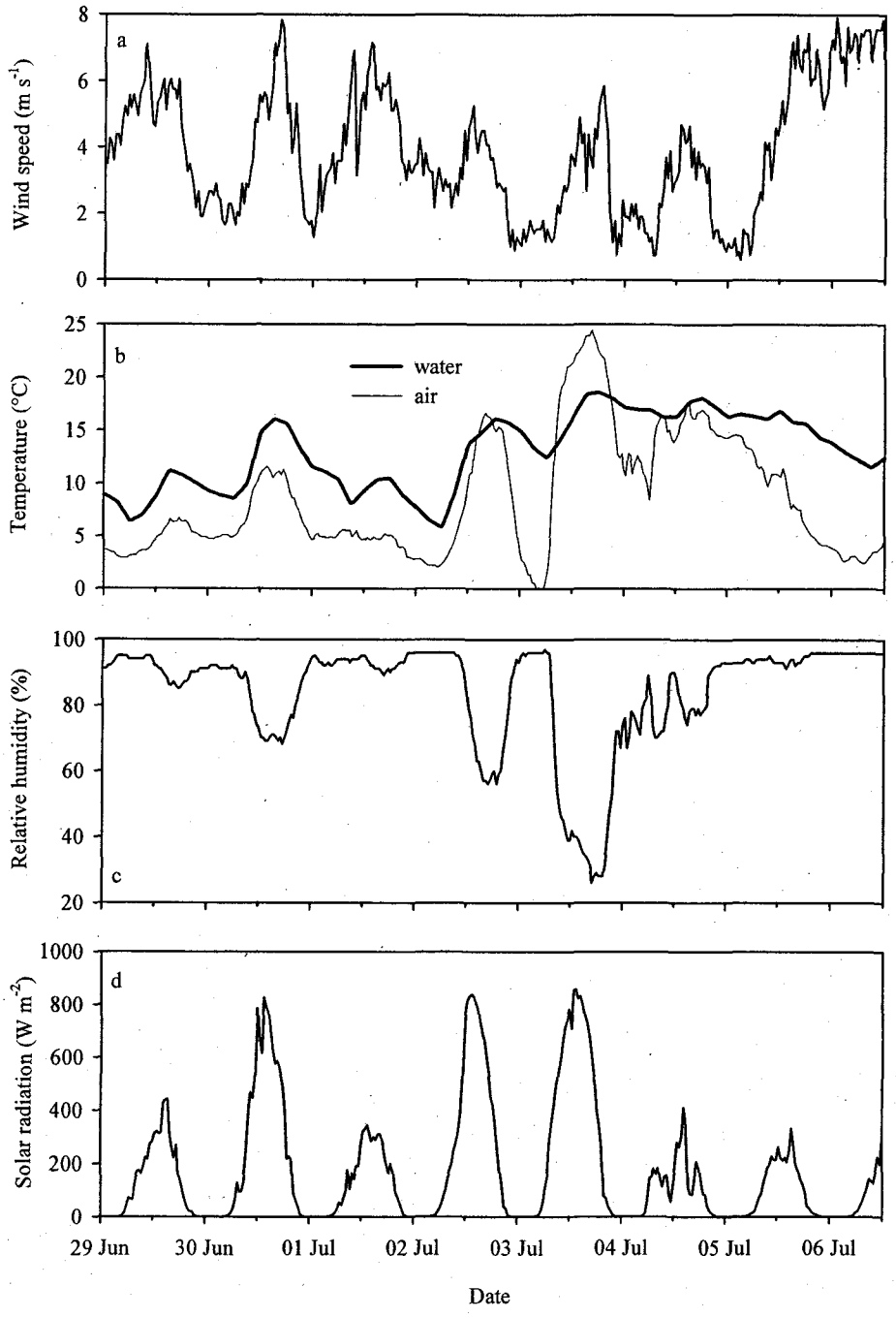

Fig. 6

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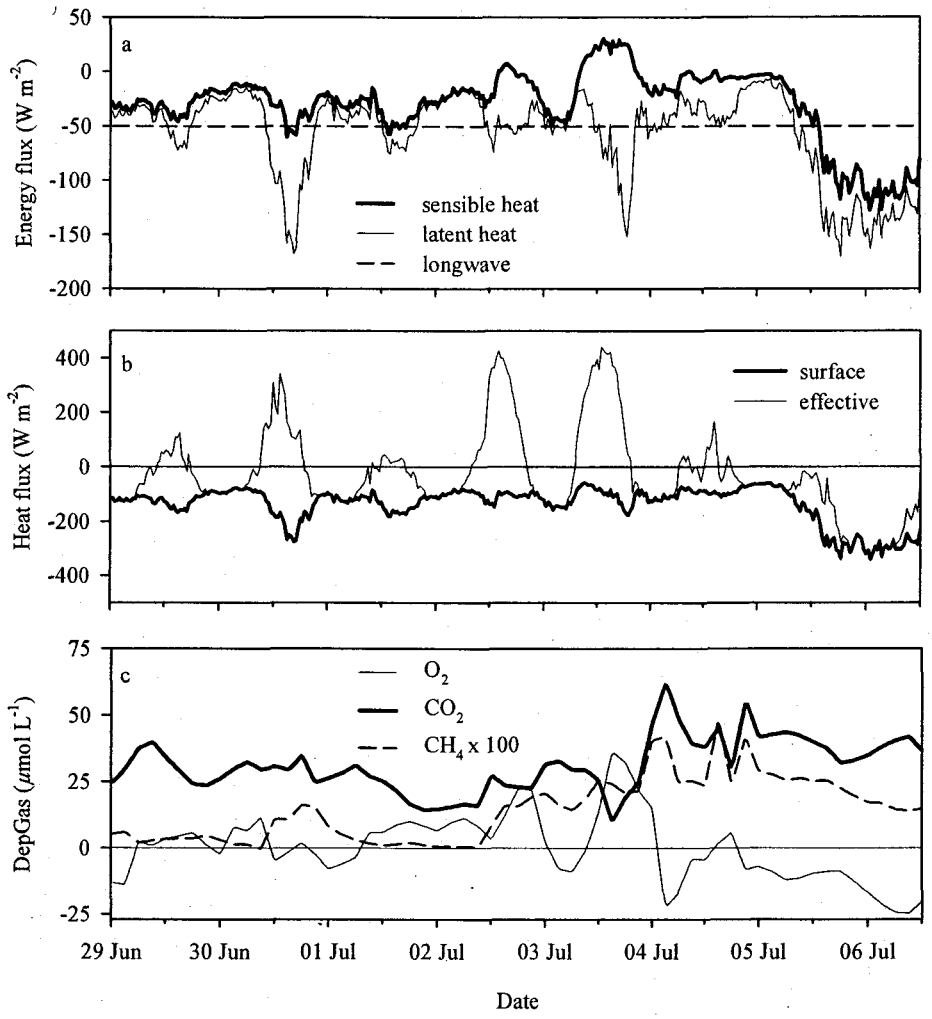

Fig. 7

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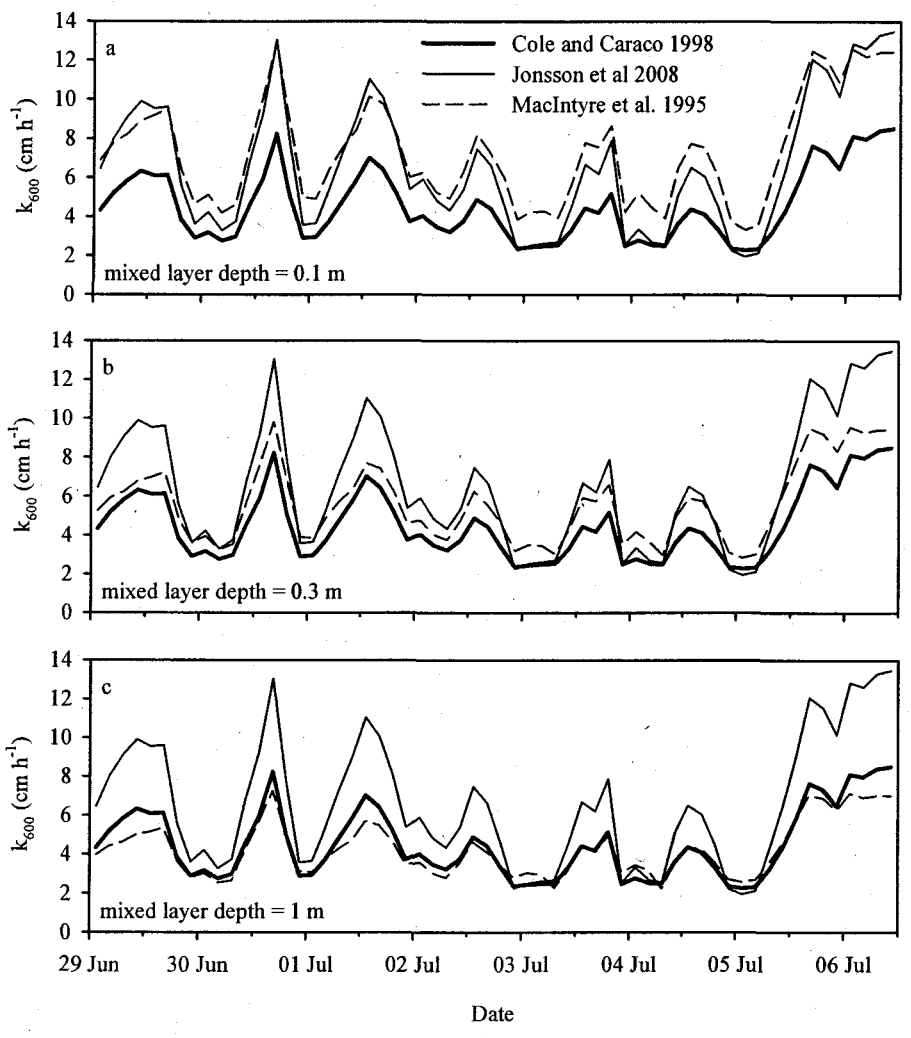

Fig. 8

Laurion et al. 2009

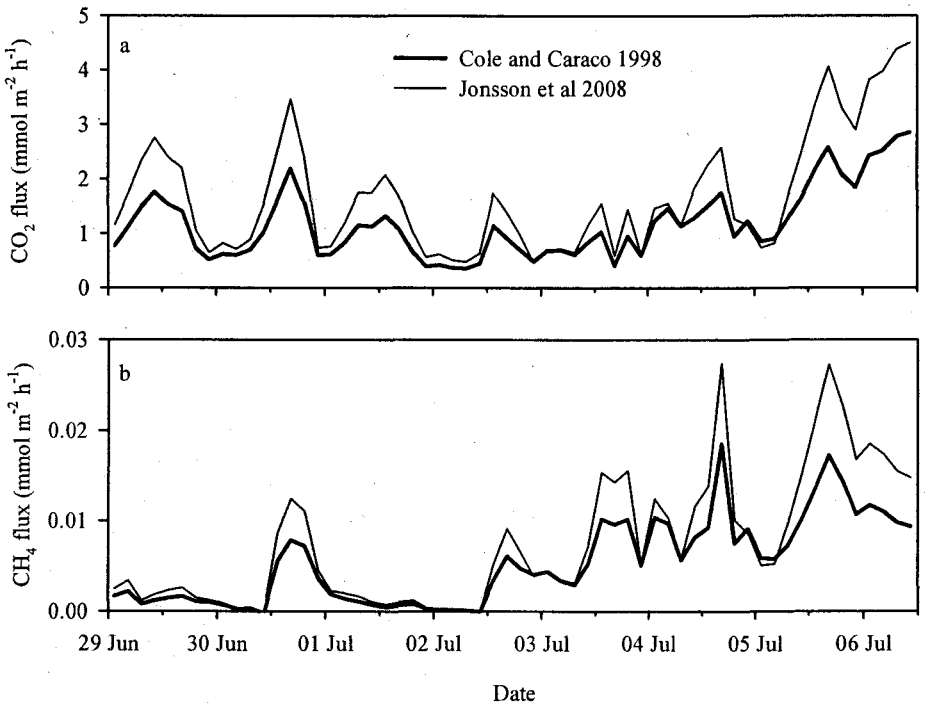

Fig. 9

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