UNIVERSITÉ DU QUÉBEC À RIMOUSKI

VIABILITÉ DU PHYTOPLANCTON ET DES ALGUES DE GLACE DANS LA MER DE BEAUFORT (ARCTIQUE CANADIEN)

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PAR

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A Carol, la meva família i amics

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RÉSUMÉ

Cette thèse examine pour la première fois l'influence de diverses variables environnementales, dont l'éclairement, sur la photo-physiologie et la viabilité cellulaire des communautés algales de la mer de Beaufort lors de la transition entre le printemps et l'été. Nous avons d'abord comparé deux techniques permettant de déterminer la viabilité cellulaire (pourcentage de cellules vivantes (% LC) et de cellules mortes) : la méthode de digestion cellulaire (CDA, sans colorant) et le kit BacLight^{TM} (une méthode de coloration). Au début de cette thèse, aucune étude n'avait encore été publiée sur la possibilité d'appliquer ces méthodes aux algues polaires. Pour la diatomée pennale *Nitzschia frigida,* une espèce associée à la glace de mer, les deux méthodes ont donné des résultats similaires. La méthode CDA a donc été choisie pour comparer le % LC retrouvé sous des sites aux couverts de neige variables (mince et moyen) et ainsi étudier l'influence de l'éclairement sur la viabilité des communautés naturelles d'algues de glace. En général, les plus faibles % LC étaient trouvés sous les minces couverts de neige (ca. 30%). L'influence de l'éclairement sur la viabilité des algues cellulaires a aussi été étudiée en exposant des cultures discontinues d'une diatomée centrale, *Attheya septentrionalis,* à différentes intensités lumineuses en laboratoire. Après quatre jours d'exposition à des intensités lumineuses fortes pour cette algue de glace (170-180 µmol photons m^{-2} s^{-1}), le % LC a diminué jusqu'à 10%. La méthode CDA apparaît donc comme une technique valide pour déterminer la viabilité cellulaire des algues polaires et son utilisation a permis de fournir de précieuses informations quant à l'influence de l'éclairement sur les communautés algales arctiques.

Les changements de la composition taxonomique et de l'état photo-physiologique des communautés algales associées à la couche inférieure de la glace dans la mer de Beaufort ont été examinés au printemps. En utilisant l'information fournie par les marqueurs pigmentaires chémotaxonomiques, nous avons démontré que les diatomées de Type 2 (type pigmentaire probablement associé à des diatomées pennales) dominent le bloom d'algues de glace au printemps et sont ensuite remplacées par les chlorophytes, les prasinophytes et les dinoflagellés à la fin du bloom. Les conditions de couverture de neige affectent significativement la composition de la communauté algale seulement au commencement du bloom. À ce moment, les sites avec un épais couvert de neige et un faible éclairement étaient caractérisés par la présence de diatomées de Type 1 (diatomées centrales) et de chlorophytes, lesquels n'étaient pas présents sous les couverts de neige plus minces. Un éclairement plus élevé sous les conditions de mince couverture neigeuse a favorisé la présence de diatomées de Type 2. Nous avons aussi observé des différences significatives dans les pigments photoprotecteurs entre les trois différents couverts de neige au

commencement du bloom, la photoprotection étant accrue sous les faibles couverts de neige. Cette réponse, et le fait que la variable environnementale la mieux corrélée avec le ratio de pigments photoprotecteurs/pigments photosynthétiques soit l'éclairement moyen à la base de la glace pendant les 3 jours précédents l'échantillonnage, suggèrent que la neige reste en place suffisamment longtemps pour permettre à la photo-acclimatation de prendre place. Les conditions de faible couvert de neige ont toutefois favorisé la croissance des algues de glace au commencement du bloom puisque la biomasse de chlorophylle *a* (chl *a*) y était plus grande. À cet accroissement de biomasse algale était associée une augmentation des concentrations de pigments de dégradation de la chl *a*, peut-être en lien avec la présence d'espèces présentant de fortes concentrations internes de chlorophyllide *a* (possiblement les diatomées pennales). Cette étude souligne l'importance du rôle de la lumière dans le contrôle des communautés d'algues de glace de l'Arctique pendant le printemps, avant la fin du bloom. L'extrapolation de nos résultats en fonction du réchauffement climatique actuel de l'Arctique suggère que le bloom printanier d'algues de glace pourrait bénéficier d'une réduction du couvert de neige (influence bénéfique de la lumière au commencement du bloom), mais pourrait être de plus courte durée en raison d'une saison de glace raccourcie et possiblement d'une mortalité accrue associée à un éclairement plus important.

La viabilité cellulaire du phytoplancton a été examinée dans les eaux de surface de la mer de Beaufort pendant la transition printemps/été. Le pourcentage de cellules vivantes était très variable. Cette variabilité s'expliquait partiellement par les différences dans la composition des communautés et était influencée par la température de l'eau et par l'éclairement, avec des tendances distinctes pour les stations couvertes de glace et les stations d'eau libre. La performance photosynthétique et le % LC diminuaient avec une augmentation de l'éclairement, suggérant un effet négatif des fortes intensités lumineuses sur la condition physiologique des cellules. Pour les stations couvertes de glace, les plus faibles % LC étaient associés aux températures de fonte de glace, alors qu'ils s'associaient plutôt aux fortes intensités lumineuses dans les stations d'eau libre. Un pigment que nous avons appelé « simili »-pyrophéophorbide *a* et qui devra être mieux identifié avec des techniques appropriées (telles que le LC-MS) a montré un potentiel comme marqueur pigmentaire pour la perte de viabilité cellulaire dans l'environnement arctique. Cette étude souligne la complexité des réponses des communautés algales naturelles lorsqu'elles doivent faire face à des changements environnementaux. Il semble que les diatomées se portaient mieux que d'autres groupes pendant la période de fonte de glace entre le printemps et l'été, montrant qu'elles semblent occuper des niches écologiques spécifiques dans lesquelles elles sont favorisées. Cette thèse a identifié l'éclairement, la température et l'stratification de la colonne d'eau comme des facteurs environnementaux importants qui influencent la condition physiologique et la viabilité des algues de l'Arctique.

Mots clés : Arctique, algues de glace, phytoplancton, mort cellulaire, indice d'activité photosynthétique, pigments, CHEMTAX, photoprotection

ABSTRACT

This thesis examines for the first time the influence of irradiance and other environmental factors on the photo-physiology and cell viability of algal communities in the Canadian Beaufort Sea during the spring-summer transition. We tested the applicability of two techniques to determine cell viability (as percentages of living (% LC) and dead cells): the cell digestion assay (CDA, non-staining) and the BacLight^{TM} Kit (a staining method). At the beginning of this thesis, no study had been published on the applicability of these methods on polar algae. The two methods gave similar results when applied to the sea ice pennate diatom *Nitzschia frigida*. The influence of irradiance on the viability of natural ice algal communities was investigated by comparing sites with various snow cover depths and measuring % LC with the CDA. In general, lower % LC values were found under the lowest snow cover depths (ca. 30%). The influence of irradiance on algal cell viability was also examined by exposing batch cultures of the centric diatom *Attheya septentrionalis* to various irradiances in the laboratory. After 4 days of exposure to irradiances considered high for this ice algal species (170-180 µmol photons m^{-2} s^{-1}), % LC decreased down to 10%. The CDA method is a valid technique to determine viability of polar algae and it provided valuable data on the influence of light on Arctic algal communities.

Changes in the taxonomic composition and the photo-physiological state of the bottom ice algal community of the Beaufort Sea were studied during the spring. Using information on chemotaxonomic marker pigments, we showed that diatoms Type 2 (pigment type likely associated with pennate diatoms) dominate the sea ice algal bloom during spring, followed by seasonal replacement with chlorophytes, prasinophytes and dinoflagellates during the post-bloom period. Snow cover conditions affected community composition only during the early bloom, when the low irradiance, high snow cover sites were characterized by the presence of diatoms Type 1 (centric diatoms) and chlorophytes which were not detected in the other snow cover sites. Higher irradiances under low snow cover conditions favoured the presence of diatoms Type 2. We also observed significant differences in photoprotective pigments among the three snow cover conditions in early bloom, with enhanced photoprotection under low snow cover. This response, and the fact that the ratio of photoprotective to photosynthetic pigments was best correlated with the average bottom ice irradiance over the preceding three days, suggests that snow remained in place long enough for photoacclimation to take place. These conditions favoured the growth of sea ice algae, since the chlorophyll *a* (chl *a*) biomass was greater under low snow cover conditions in the early bloom period. This was also associated with an increase in chl *a* degradation pigments possibly related to the presence of species with particularly abundant chlorophyllide *a* (possibly pennate diatoms). This study highlights the important role of light in controlling the algal communities in Arctic sea ice during spring, before the demise of the ice algal bloom. Extrapolation of our results to continued climate warming in

the Arctic suggests that the ice algae spring bloom could benefit from a reduction of the snow cover (beneficial influence of light early on) but may be cut short by the shorter ice season and possibly by the increased mortality of sea ice algae associated with increased irradiance.

Phytoplankton cell viability was examined in the surface waters of the Beaufort Sea during the spring-summer transition. The percentage of living cells varied widely, this variation being explained partly by differences in the community composition and was influenced by water temperature and irradiance with distinct trends for ice-covered and open-water stations. Photosynthetic performance and the relative abundance of living cells decreased with increasing irradiance, suggesting a detrimental effect of high irradiances on the physiological condition of cells. The lower % LC values were related to melting temperatures at ice-covered stations and to the highest irradiances at open-water stations. A pigment that we called pyropheophorbide *a*-"like" and that will need to be fully identified with appropriate techniques (such as LC-MS), shows potential as a marker pigment for the loss of cell viability in this environment. This study highlights the complexity of the responses of natural communities coping with changes of the environmental variables. However diatoms seemed to thrive better than other groups during the spring-summer melting conditions, showing that they seem to occupy specific ecological niches in which they are favored. This thesis identified irradiance, temperature and stratification as important environmental factors influencing the physiological conditions and viability of Arctic algae.

Keywords : Ice algae, phytoplankton, cell death, photosynthetic activity index, pigments, CHEMTAX, photoprotection.

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INTRODUCTION GÉNÉRALE

Les producteurs primaires sont à la base du réseau alimentaire pélagique marin. La dynamique des populations algales dépend de la balance entre le gain et la perte de cellules. Au cours du dernier siècle, les études portant sur les cycles biogéochimiques aquatiques n'ont pris géneralement en compte que le broutage par le zooplancton, l'advection horizontale et le flux vertical vers l'océan profond comme facteurs de perte cellulaire et ont supposé qu'en leur absence, les cellules phytoplanctoniques se multiplieraient indéfiniment (e.g. Walsh 1983). Cependant, des études conduites en lacs ont montré que la mort cellulaire et la décomposition pouvaient conduire à des pertes significatives de biomasse phytoplanctonique lorsque les conditions environnementales différaient de la zone de tolérance des cellules (Jassby & Goldman 1974). La perte de phytoplancton par la mort cellulaire en eaux douces a reçu une attention particulière dans la littérature des années 1970 et 1980, lorsqu'on s'est aperçu que l'abondance cellulaire ne pouvait être expliquée simplement par un équilibre entre les taux de croissance phytoplanctonique et les taux de broutage et de flux vertical (Kalff & Knoechel 1978, Reynolds et al. 1982). Rapidement des tentatives de quantification de la mort des cellules phytoplanctoniques dans le milieu marin ont été réalisées en identifiant les frustules de diatomées vides et de cellules brisées par microscopie inversée (Kalff & Knoechel 1978, Brussaard & Riegman 1998). Bien que la mort cellulaire soit un processus fondamental dans la dynamique des populations, ses causes et son importance dans l'écologie des populations phytoplanctoniques et dans les cycles biogéochimiques ne sont toujours pas bien comprises et sa quantification est quasi-inexistante (Agustí et al. 1998, Berges & Falkowski 1998, Kirchman 1999). Les informations disponibles sur le sujet sont relativement rares et en constante évolution, essentiellement parce que le développement et l'utilisation des techniques pour mesurer la viabilité cellulaire sont récentes, mais aussi parce que la mort cellulaire et la viabilité ne

sont pas facilement définissables à l'aide d'un seul paramètre physiologique ou morphologique (Kirchman 1999, Bidle & Falkowski 2004, Franklin et al. 2006).

Le développement de la microscopie électronique en transmission (TEM) a permis l'observation de particules semblables au virus (VLPs) dans les algues eucaryotes et a permis, pour la première fois, d'isoler des virus infectant des microalgues (Mayer & Taylor 1979, Van Etten et al. 1983, Brussaard 2004). L'infection d'une cellule par un virus, ou tout autre organisme pathogène, est à présent considérée comme un facteur important causant la mort cellulaire des microalgues dans l'environnent marin (Imai et al. 1993, Veldhuis & Brussaard 2006). En effet, une infection virale peut décimer une floraison de phytoplancton en quelques jours (Bratbak et al. 1993, Brussaard et al. 1995, Baudoux et al. 2006, Llewelyn et al. 2008).

Dans les études récentes de mort cellulaire algale, les paradigmes qui ont été développés pour les métazoaires quant à la mortalité cellulaire ont été transposés au domaine de la phycologie (voir Franklin et al. 2006). La découverte récente de la mort cellulaire par autocatalyse, aussi bien pour les procaryotes que pour le phytoplancton est un autre mécanisme, indépendant des attaques virales ou parasitiques, qui explique les taux élevés de lyse parfois retrouvés (Berges & Falkowski 1998, Bidle & Falkowski 2004, Franklin et al. 2006).

Les cellules phytoplanctoniques vivent dans un environnement complexe et fortement variable. Une étude récente sur la mortalité du phytoplancton suggère un certain nombre de facteurs de stress naturel pouvant provoquer la mort du phytoplancton. Parmi ces facteurs, le manque de lumière (Berges & Falkowski 1998, Segovia et al. 2003, Segovia & Berges 2005), la forte intensité lumineuse (Agustí 2004, Agustí & Llabrés 2007), le rayonnement ultraviolet (UV, 280-400nm) (Labres & Agustí 2006, Llabrés & Agustí 2010), la température (Lasternas & Agustí 2010) et la limitation en éléments nutritifs (Brussaard et al. 1997, Berges & Falkowski 1998, Alonso-Laita & Agustí 2006) peuvent influencer la mort phytoplanctonique. D'autres facteurs de stress comme l'accumulation de dérivés réactifs de l'oxygène ont aussi été identifiés comme pouvant déclencher des

réactions de mort cellulaire similaires à l'apoptose retrouvée chez les organismes multicellulaires (Berman-Frank et al. 2004, Bidle & Falkowski 2004). Le développement récent de nouvelles méthodes a permis l'étude de la mort et de la lyse cellulaire dans l'environnement naturel. Par exemple, des mesures d'activité d'estérase dissoute (DEA) (Van Boekel et al. 1992, Brussaard et al. 1995, Agustí et al. 1998, Riegman et al. 2002) ont identifié la lyse phytoplanctonique comme un processus important de perte dans les communautés naturelles. La lyse phytoplanctonique est d'ailleurs reconnue comme le facteur responsable de la fin des floraisons phytoplanctoniques dans la mer du Nord (Van Boekel et al. 1992, Brussaard et al. 1995). Des niveaux de lyse phytoplanctonique équivalent à 50% du taux de croissance brute du phytoplancton ont été mesurés dans les eaux de surface de la mer Méditerranée (Agustí et al. 1998) et sont responsables de 10 à 50% de la perte totale des cellules de pico-eucaryotes dans les eaux oligotrophes subtropicales de l'Atlantique du Nord-Est (Baudoux et al. 2007).

De nouvelles techniques, qui distinguent les cellules saines (en vie) des cellules mortes, ont été développées afin de déterminer le statut des cellules de phytoplancton dans les communautés naturelles (Veldhuis et al. 2001, Agustí & Sanchez 2002). La plupart de ces techniques sont basées sur un point critique de la progression vers la mort cellulaire : la perte de la capacité à maintenir l'homéostasie de la cellule qui se traduit par une augmentation importante de la perméabilité de la membrane cellulaire. Cet événement irréversible caractérise le processus de mort des cellules en nécrose ou en autocatalyse-apoptose (Wyllie et al. 1980, Ellis et al. 1991, Darzynkiewicz et al. 1994). Les cellules soumises à ce processus augmentent l'activité de leur protéase et perdent finalement leur contenu cellulaire (Ning et al. 2002).

Les tests permettant de déterminer l'état des cellules (vivantes ou mortes) sont habituellement basés sur la perméabilité cellulaire de la membrane et sur l'incapacité des colorants ou des enzymes à pénétrer les cellules dont la membrane plasmique est intacte. Par exemple, ces tests sont (1) le colorant d'ADN spécifique « mortal stain SYTOX Green » (Veldhuis et al. 1997, 2001, Vardi et al. 1999, Franklin & Berges 2004,

Timmermans et al. 2007), (2) le colorant « vital Fluoresceine diacetate (FDA) » (Jochem 2000, Agustí & Sanchez 2002, Franklin & Berges 2004, Garvey et al. 2007, Llabrés & Agustí 2008) ou (3) le kit de viabilité Baclight™ utilisé pour déterminer la viabilité des diatomées et des cyanobactéries (Agustí et al. 2006, Llabrés & Agustí 2008). La méthode de digestion cellulaire (CDA) est, quant à elle, une méthode non-colorante développée dans un premier temps pour la biologie cellulaire comme un test de perméabilité cellulaire des membranes (Darzynkiewicz et al. 1994) et modifiée par la suite pour étudier les communautés naturelles de phytoplancton marin (Agustí & Sánchez 2002). Cette méthode utilise des enzymes digestives (ADNase I et Trypsine) qui pénètrent dans le cytoplasme des cellules dont les membranes cellulaires sont endommagées (c'est-à-dire nécrosée ou présentant une apoptose avancée). Une fois dans le cytoplasme, les enzymes digèrent entièrement la cellule. Ces enzymes n'ont aucun effet notable sur la viabilité, la morphologie ou la fonction de cellules vivantes (Darzynkiewicz et al. 1994, Agustí & Sánchez 2002). Le retrait sélectif des cellules mortes permet le comptage des cellules vivantes en utilisant les méthodes conventionnelles de microscopie inversée, d'épifluorescence, ou de cytométrie en flux (Agustí & Sánchez 2002). Jusqu'à maintenant, cette méthode a été appliquée pour étudier la mort cellulaire des communautés phytoplanctoniques de la mer Méditerranée (Agustí et al. 1998), de l'océan Atlantique (Agustí 2004), de l'océan Pacifique (Hayakawa et al. 2008), des environnements polaires (Llabrés & Agustí 2008) et pour les communautés d'eau douce (Agustí et al. 2006). Les résultats de ces études indiquent que la proportion de cellules mortes varie à l'intérieur des communautés phytoplanctoniques et entre les taxons composant ces communautés (Agustí & Sánchez 2002, Agustí 2004, Agustí et al. 2006).

L'importance écologique de la mort cellulaire est d'autant plus grande lorsque l'on s'intéresse à la boucle microbienne puisque la lyse phytoplanctonique peut contribuer à la production de matière organique dissoute (MOD) et de carbone organique dissous (COD) qui peut alors être utilisé par les bactéries (Figure 1). La lyse cellulaire a aussi des répercussions sur la quantité et la composition de la MOD libérée (Kirchman 1999). La lyse cellulaire du phytoplancton représente une perte de nourriture pour les herbivores et une

réduction de l'export par le flux vertical. Cependant, le carbone produit peut entrer dans la boucle microbienne et éventuellement soutenir la production recyclée (Agustí et al. 1998, Fuhrman 1999, Suttle 2005).

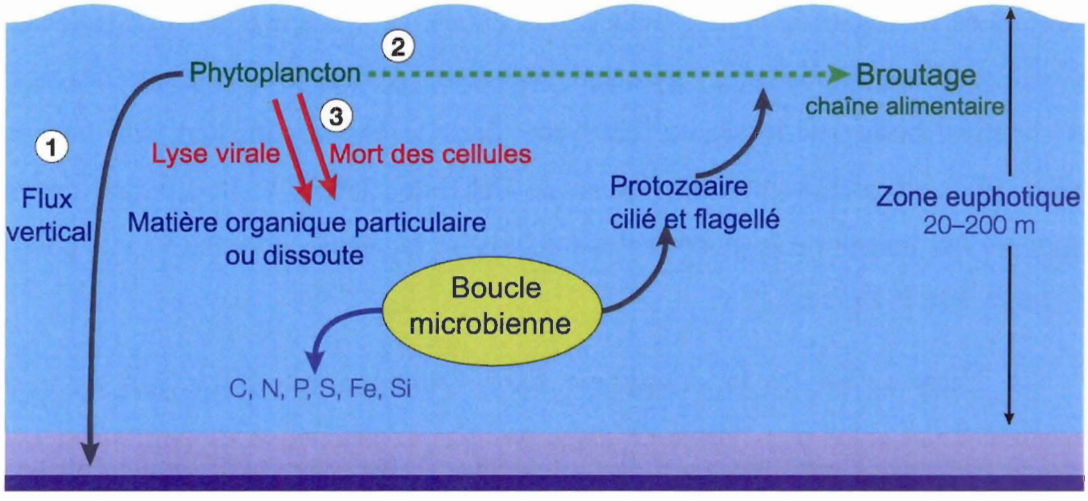

Fig. 1. Diagramme des trois principales voies que peuvent suivre les cellules de phytoplancton dans le milieu marin, illustrant les conséquences de la mort cellulaire du phytoplancton (autocatalyse ou lyse virale). Modifié de Biddle & Falkowski (2004)

L'océan Arctique et la mer de Beaufort

L'océan Arctique est un océan presque complètement fermé et entouré par les continents (Longhurst 1998, Jakobsson et al. 2004, Macdonald et al. 2004). L'océan Arctique reçoit à la fois les apports d'eau de l'océan Pacifique et de l'océan Atlantique par les détroits de Béring et de Fram (Michel et al. 2006). Le système des courants de surface dans l'Arctique est composé d'une gyre anticyclonique (qui tourne dans le sens des aiguilles d'une montre) dictée par le régime des vents dans le bassin canadien, soit la gyre de Beaufort (Pickard & Emery 1990, Tynan & DeMaster 1997), et du courant de dérive transpolaire qui transporte la glace et l'eau depuis les plateaux eurasiens vers le pôle nord puis vers le détroit de Fram.

L'état actuel de la cryosphère dans l'Arctique est défini par la présence permanente d'eau gelée sous forme de glace de mer, de glaciers et de permafrost (ACIA 2005, Carmack & Wassmann 2006). Le couvert de glace a une influence importante sur la réflectance, ou albédo, de l'océan, sur la formation des nuages, sur la chaleur et les transferts d'humidité avec l'atmosphère et les courants océaniques (ACIA 2005). La couverture saisonnière de glace de mer atteint son maximum annuel en mars et son minimum en septembre (Comiso 2003).

La mer de Beaufort est fortement influencée par l'apport en eau douce en provenance du continent, lequel est responsable de la forte stratification de la colonne d'eau (Macdonald et al. 2004) et d'un important apport de sédiments (Holmes et al. 2002). La portion canadienne de la mer de Beaufort est caractérisée par le plateau du Mackenzie et par le golfe d'Amundsen (Jakobsson et al. 2004). Le golfe d'Amundsen est situé à environ 150 km à l'est de la rivière Mackenzie et contient la troisième plus grande polynie arctique, soit la polynie du cap Bathurst, d'environ 25000 km^2 à son étendue maximale (Arrigo & Van Dijken 2004). La gyre de Beaufort, généralement influencée par des vents du nord-est, dirige la circulation superficielle océanique locale. À une profondeur de 50 m se trouve le courant sous-jacent de Beaufort, lequel tourne dans le sens des aiguilles d'une montre et s'écoule vers l'est (Aagaard 1984). Il contribue à l'apport en éléments nutritifs au large des

eaux côtières (Macdonald et al. 1987, Ingram et al. 2008). La colonne d'eau dans la portion canadienne de la mer de Beaufort est principalement formée de trois couches (Carmack et al. 2004). La surface ou la couche polaire mixte (CMP) s'étend de la surface jusqu'à une profondeur de 25 à 50 m et sa température, sa salinité et son contenu en éléments nutritifs sont fortement variables. De 50 à 200 m se trouve une couche formée par des eaux du Pacifique en provenance de la mer de Béring et au fond (> 200 m), il y a la couche d'eau Atlantique. La couche Pacifique a une salinité inférieure et est plus riche en éléments nutritifs que la couche Atlantique.

L'Arctique sous un scénario de changement global

Les dernières décennies ont été synonymes de changements environnementaux radicaux à l'échelle de la planète. L'influence de l'homme sur la biosphère depuis le début de l'industrialisation a contribué au réchauffement de la planète par l'émission et l'accumulation de dioxyde de carbone et d'autres gaz à effet de serre dans l'atmosphère. Ces gaz absorbent une partie du rayonnement infrarouge (Ledley et al. 1999, Millero 2006, Sarmiento & Gruber 2006) et altèrent ainsi le flux radiatif vers la troposphère (Boyd & Doney 2003), entraînant le réchauffement de surface de la planète. La température moyenne mondiale de l'air a ainsi augmenté de 0,7 °C au cours du dernier siècle (Hoegh-Guldberg et al. 2007) et une augmention de 6 °C aux hautes latitudes de l'hémisphère nord est prévue vers la fin du 21ème siècle (IPCC 2007).

Les émissions de chlorofluorocarbones (CFCs) représentent un autre impact de l'activité humaine et elles représentent un facteur majeur de la dégradation de la couche d'ozone stratosphérique (McKenzie et al. 2003). En conséquence du réchauffement global, une perte croissante de la couche d'ozone a été observée dans l'Arctique au printemps et au début de l'été (Rex et al. 2004). L'épuisement d'ozone stratosphérique conduit à une augmentation du rayonnement UVB (280-315 nm) qui peut facilement endommager les

molécules organiques des organismes vivants, notamment les composantes du système photosynthétique (Jordan 1996, Ryan et al. 2002).

L'océan Arctique est particulièrement sensible aux changements globaux (Semtner 1987, Morison et al. 2000, Chen et al. 2003, Carmack et al. 2006, Comiso et al. 2008), puisqu'en altérant le couvert de neige et de glace de mer, le réchauffement modifie l'albédo de l'océan, créant une rétroaction positive (Holland & Bitz 2003). Le couvert de glace de l'Arctique a en effet changé au cours des dix dernières années. Ces changements incluent la modification dans la durée du couvert de glace (congélation plus tardive et fonte devancée), l'augmentation des zones de fonte, le recul des glaciers, le dégel du permafrost et l'augmentation des températures superficielles (Maslanik et al. 1996, Polyakov et al. 1999, Rothrock et al. 1999, Comiso & Parkinson 2004, ACIA 2005, Carmack et al. 2006). En plus de la fonte de la glace, l'augmentation de la décharge des rivières arctiques (Peterson et al. 2002) pourrait diminuer la salinité et augmenter la stratification de l'océan Arctique (Peterson et al. 2002, 2006, Macdonald et al. 2004). La réduction de la durée et de l'étendue de la glace de mer induit des changements tant dans la circulation océanique que dans les caractéristiques des masses d'eau, modifiant ainsi la stratification et la turbidité de la colonne d'eau, de même que sa composition en éléments nutritifs. Ces changements affecteront à leur tour la production primaire et le transfert d'énergie vers les niveaux trophiques supérieurs (Michel et al. 2006), entraînant alors des répercussions pour l'écosystème entier (Wassmann 2006).

Importance d'étudier et de quantifier la viabilité cellulaire dans le changement Arctique

Les études sur la lyse des cellules algales sont peu nombreuses (e.g. Brussaard et al. 1995, 1996, Agustí et al. 1998, Veldhuis et al. 2001), cependant ils suggèrent d'importants taux de mortalité ou de lyse cellulaire chez le phytoplancton marin et des taux fortement variables dans le temps et dans l'espace (Agustí et al. 1998, Agustí & Duarte

2000, Brussaard 2004). Tel que mentionné précédemment, il a été démontré que la mort physiologique et la lyse subséquente du phytoplancton pouvait résulter d'un bon nombre de facteurs, y compris des pathogènes comme les bactéries, les champignons et les virus, ou encore l'exposition à des substances toxiques ou à des extrêmes environnementaux de lumière, de température et de concentration en nutriments. Ces facteurs de mort cellulaire sont communs dans l'océan, mais les liens entre les facteurs biotiques et abiotiques pour la mort cellulaire ne sont toujours pas bien compris (Franklin et al. 2006). De plus, des études de terrain ont montré une influence des conditions d'éclairement et du rayonnement UV sur la viabilité du phytoplancton en mer Méditerranée (e.g. Sommaruga et al. 2005), dans l'océan Atlantique (e.g. Agustí 2004, Llabrés & Agustí 2006) et dans l'océan Antarctique (Llabrés & Agustí 2010). Des travaux récents en Antarctique suggèrent aussi que la viabilité cellulaire est fortement déterminée par le niveau de photoprotection développé par le phytoplancton (Van de Poll et al. 2005, 2006, 2011). La température de l'eau et la disponibilité des nutriments ont aussi été identifiés comme étant des facteurs potentiels pouvant influencer la viabilité des cellules dans les communautés naturelles (Alonso-Laita & Agustí 2006, Lasternas & Agustí 2010).

Les océans polaires représentent des environnements extrêmes avec des faibles températures, la présence saisonnière d'un couvert de glace et des variations saisonnières extrêmes dans les régimes de lumière contrôlant la croissance du phytoplancton (Smith & Sakshaug 1990, Harrison & Cota 1991). Ces conditions difficiles et variables peuvent affecter la viabilité des algues de glace et des cellules phytoplanctoniques, mais peu d'études se sont attardées sur le sujet (Llabrés & Agustí 2008, Lasternas & Agustí 2010, Echeveste et al. 2011).

Dans l'océan Arctique, la majeure partie de la production biologique se produit dans la glace de mer et dans la portion supérieure de la colonne d'eau (Jakobsson et al. 2004) et donc, les producteurs primaires sont exposés à ces conditions fortement variables. L'étude de la proportion de cellules mortes et vivantes dans l'océan Arctique peut donner des informations sur la dynamique des populations algales, notamment sur la réponse

physiologique de ces communautés face au changement rapide de leur environnement. De plus, cette étude pourrait permettre de mieux situer le rôle de la mort cellulaire parmi les processus qui entraînent la perte de cellules, lesquels sont des éléments clés dans la structure des communautés arctiques (Lasternas & Agustí 2010).

La glace de mer

La glace de mer est un habitat majeur pour les communautés algales associées à la glace, celles-ci étant généralement dominées par les diatomées pennales (Horner & Schrader 1982). La glace de mer est caractérisée par de forts gradients de température, de salinité et de lumière. Ces propriétés sont fortement variables et surtout dictées par la température ambiante et le couvert de neige (Thomas & Dieckmann 2002). Les conditions lumineuses sous la glace varient en fonction de l'épaisseur de glace, puisqu'elle atténue rapidement la lumière et ce, particulièrement en présence de neige. Une couche de neige de 10 cm d'épaisseur peut réduire l'intensité lumineuse de 80 à 99% (Sakshaug & Skjoldal 1989). Les microalgues sciaphiles vivant à la base de la glace de mer sont potentiellement sensibles à la photo-inhibition (Neale et al. 1994, Prézelin et al. 1998, Belzile et al. 2000). Cette dernière se produit lorsque le complexe photosynthétique absorbe la lumière au-delà de la capacité de photosynthèse de la cellule. Par conséquent, une intensité lumineuse relativement faible pourrait suffire à photo-inhiber les cellules sciaphiles présentes dans la couche inférieure de la glace de mer (Neale 1987, Falkowski 1994).

Les changements climatiques dans l'Arctique pourraient modifier l'exposition lumineuse des algues de glace en influençant la couverture de neige et de glace, via l'augmentation de la température de l'air et des précipitations. Le moment de la débâcle et la vitesse de fonte de la glace déterminent en partie l'intensité du stress physiologique des algues de glace (Moline et al. 2008). En effet, le degré d'exposition des communautés algales au rayonnement UV est dépendant de la présence et de l'épaisseur du couvert de glace et de neige (Belzile et al. 2000). De plus, la fonte de la glace entraîne des

changements rapides dans la salinité ambiante, créant ainsi un stress osmotique et photosynthétique pour les producteurs primaires (Ralph et al. 2007).

Une étude récente a montré que les communautés de protistes retrouvées dans la couche inférieure de la glace de mer variaient en fonction de l'épaisseur du couvert de neige, suggérant une réponse différentielle de ces communautés face aux changements dans le régime de lumière lors de la transition hiver/printemps (Różańska et al. 2009). Pendant le printemps la plupart de la variabilité spatiale de l'éclairement à la base de la glace est influencé par l'épaisseur du couvert de neige et la formation d'étangs de glace (Mundy et al. 2005, Perovich et al. 1998). Néanmoins, peu de choses sont connues quant à l'influence de la couverture de neige, et de son atténuation de la lumière, sur la photoprotection et la viabilité des algues de glace dans l'Arctique (Juhl & Krembs 2010, Mundy et al. 2011).

La colonne d'eau

Dans l'océan Arctique, la saumure produite lors de la formation de la glace en hiver a tendance à déstabiliser la colonne d'eau lui permettant alors de se mélanger. À l'inverse, à l'été, la fonte de la glace, dont la salinité se rapproche de celle de l'eau douce, augmente la stratification (Jakobsson et al. 2004). Cela peut alors se traduire par une diminution des remontées d'éléments nutritifs depuis l'océan profond. Les cellules peuvent aussi subir une rapide augmentation de leur exposition à la lumière suivant la disparition soudaine de la glace de mer, notamment lors de la débâcle printanière (Kashino et al. 2002, Griffith et al. 2009). La stratification de la partie supérieure de la colonne d'eau, amplifiée par le réchauffement de l'Arctique, pourrait maintenir les cellules près de la surface pour une plus longue période.

Agustí (2004) a suggéré que la forte lumière et le manque d'éléments nutritifs pouvaient être les causes principales de la mort cellulaire au centre de l'océan Atlantique. Dans un autre environnement stratifié, la mer Méditerranée, la lyse du phytoplancton se révélait être un facteur de perte cellulaire important dans les eaux de surface (Agustí et al.

1998). La lyse des cellules pourrait donc contribuer de manière importante à la perte de phytoplancton dans les systèmes oligotrophes (Baudoux et al. 2007). La disponibilité des nutriments peut modifier l'efficacité photosynthétique du phytoplancton marin; la limitation en phosphore peut par exemple causer une diminution de la capacité photosynthétique des cellules (Lippemeier et al. 2003, Shelly et al. 2005). La photo-inhibition est plus prononcée dans les régions pauvres en nutriments de l'océan, où les cellules peuvent manquer d'éléments nutritifs pour réparer les centres de réaction photosynthétiques endommagés par la lumière (Herzig & Falkowski 1989). De plus, l'efficacité quantique du photosystème II peut se voir grandement réduit par le stress nutritif (Falkowski 1994). Des stress environnementaux comme les faibles températures peuvent aussi rendre l'appareil photosynthétique plus sensible à la photo-inhibition (Falk et al. 1990). Les résultats de Falk et al. (1990) soutiennent d'ailleurs l'hypothèse selon laquelle les cellules acclimatées à des températures basses sont plus dépendantes de processus de réparation efficaces pour éviter la photo-inhibition que les cellules acclimatées à des températures élevées. Gallegos et al. (1983) et Gallegos & Platt (1985) ont suggéré que l'intensité de la photo-inhibition dans l'Arctique pouvait être liée au degré de stratification de la colonne d'eau (Harrison & Platt 1986). À ce sujet, des études récentes dans l'Antarctique ont montré que les cellules exposées à du mélange vertical pouvaient augmenter leur concentration interne de pigments photoprotecteurs de type xanthophylle, alors que les concentrations de ces pigments ne changeaient pas, ou diminuaient même, chez les cellules exposés à un environnement statique (Griffith et al. 2009). Les cellules près de la surface sont exposées non seulement à la lumière visible, mais aussi au rayonnement UV, les courtes longueurs d'onde étant particulièrement nuisibles (UV-B). L'inhibition de la photosynthèse par la radiation UV a été observée dans plusieurs régions des océans incluant les zones polaires (e.g. Llabrés & Agustí 2006, 2010). La découverte que les populations de picophytoplancton (d'une taille inférieure à 2 μm) sont fortement sensibles à la radiation UV soutient l'hypothèse selon laquelle le rayonnement solaire pourrait être un facteur de plus à considérer dans les causes possibles de la mort des cellules de phytoplancton dans l'océan (Agustí & Llabrés 2007). Cette dernière étude suggère de plus que les communautés de pico-eucaryotes et les

populations de *Synechococcus* possédant une meilleure capacité de réparation, ou un système photoprotecteur plus efficace contre l'exposition aux radiations UV, auraient une viabilité plus élevée par rapport aux populations de *Prochlorococcus*. L'acclimatation du phytoplancton à la radiation UV inclut donc souvent une augmentation des pigments photoprotecteurs (Stambler 2003).

En résumé, peu de choses sont actuellement connues sur la viabilité cellulaire et sur l'impact quantitatif de la lyse cellulaire, notamment sur leur rôle clé dans la structure des communautés d'algues de glace et de phytoplancton de l'océan l'Arctique. Il devient impératif d'étudier les facteurs environnementaux qui peuvent influencer la viabilité cellulaire des algues de glace et du phytoplancton dans une région en plein changement. Avec tous les scénarios climatiques prévoyant un réchauffement considérable de l'Arctique au cours des 100 prochaines années (ACIA 2005, IPCC 2007), la disparition éventuelle du couvert de glace de mer à l'été n'est plus à mettre en doute, mais la perte potentielle des communautés d'algues de glace et leur remplacement par des communautés pélagiques demandent toujours à être étudiés. Dans un tel scénario, le flux vertical de carbone organique et le couplage entre la zone photique et le benthos pourraient être altérés (Walsh et al. 1989). Bien qu'ils soient des outils puissants dans la compréhension des facteurs contrôlant la dynamique des producteurs primaires et de leur réponse face aux changements environnementaux, les modèles biogéochimiques dans l'Arctique sont rares (Lavoie et al. 2005, 2009, Sibert et al. 2011). De plus, les quelques modèles existants utilisent les taux de mortalité du phytoplancton ou des algues de glace en provenance de la littérature et les considèrent souvent comme étant constants. Sinon, ils utilisent seulement les taux de broutage ou de flux verticaux pour évaluer les taux de perte de cellules. Le présent projet présente donc pour la première fois une étude sur le phytoplancton et sur la viabilité d'algues de glace dans l'océan Arctique côtier.

L'objectif général de cette étude était de déterminer la viabilité cellulaire des algues de glace et des communautés de phytoplancton dans l'océan Arctique côtier lors

de la transition printemps-été. Cette recherche a été réalisée dans le cadre de l'étude sur le chenal de séparation circumpolaire (CFL), une composante du programme de l'Année polaire internationale (IPY).

Les hypothèses centrales du projet CFL étaient :

1) La variabilité climatique affecte le timing et l'étendue du chenal de séparation circumpolaire par l'intermédiaire de contrôles océaniques et atmosphériques prévisibles. Avec le réchauffement climatique, ces changements physiques résulteront en une augmentation de la productivité de l'écosystème et une intensification du cycle du carbone;

2) La variabilité climatique affecte l'écosystème de la glace côtière qui est adjacente au chenal en contrôlant le timing des précipitations de neige et la formation/débâcle de la glace de mer. Cette variabilité climatique influencera également la contribution de la production sympagique par rapport à la production pélagique. Avec le réchauffement climatique, les processus pélagiques seront favorisés.

Dans le projet CFL, les deux principales questions concernant la productivité biologique étaient les suivantes :

1) Est-ce que la composition et la structure des communautés phytoplanctoniques sont différentes entre les eaux couvertes de glace et les eaux libres?
2) Est-ce que la production biologique de ces régions est limitée par la lumière et/ou les éléments nutritifs?

Au cours du projet CFL, j'ai examiné l'importance de la mortalité des algues unicellulaires dans la glace de mer et les eaux de surface au printemps et à l'été. Cette thèse comprend une introduction générale, trois chapitres sous forme d'articles scientifiques et une conclusion générale.

Dans le premier chapitre, j'ai examiné la méthodologie nécessaire à la quantification des cellules vivantes et mortes dans l'océan Arctique côtier. Au commencement de ce projet, aucune méthode n'avait été publiée concernant la quantification de la viabilité cellulaire des algues dans les eaux polaires. J'ai déterminé la viabilité cellulaire de l'espèce arctique *Nitzschia frigida*, une diatomée dominante dans la glace de mer. J'ai utilisé la méthode de digestion cellulaire (CDA) modifiée pour des eaux polaires (Llabrés & Agustí 2008 - *non publié au début de ce travail*) et la méthode de colorant BacLight™ Kit. J'ai alors comparé les résultats obtenus avec les deux méthodes. J'ai aussi étudié l'influence de la lumière sur la diatomée centrale *Attheya septentrionalis* dans des cultures discontinues soumises à différentes conditions de lumière en m'attardant à la viabilité (avec la méthode CDA) des cellules et à leur condition physiologique. J'ai également appliqué la méthode CDA sur les communautés naturelles d'algues de glace de la mer de Beaufort en comparant les résultats obtenus pour différentes épaisseurs de neige. Les résultats de cette partie du travail ont indiqué que la lumière avait une forte influence sur la viabilité cellulaire, et c'est pourquoi le chapitre suivant a été consacré à un examen approfondi de l'effet de la lumière sur les algues de glace.

Le deuxième chapitre porte donc sur l'influence de l'épaisseur du couvert de neige sur les algues de glace dans l'Arctique côtier au cours du printemps et utilise les marqueurs pigmentaires pour mesurer cette influence. Les objectifs spécifiques de ce chapitre étaient de 1) déterminer si la composition taxonomique des algues (à partir de leurs pigments) varie au cours du bloom printanier et ce, sous différentes conditions de neige, (2) examiner la réponse photoprotectrice des communautés algales sous différentes conditions de neige et (3) déterminer si les cellules exposées à une forte intensité lumineuse présentaient une mauvaise condition physiologique.

Le troisième chapitre traite de la viabilité cellulaire des communautés de phytoplancton dans les eaux couvertes de glace et dans les eaux libres pendant le printemps et l'été. Les objectifs étaient de (1) déterminer la variabilité de la viabilité des

cellules phytoplanctoniques dans la mer de Beaufort pendant la période de fonte de la glace, (2) identifier les facteurs environnementaux contribuant à la perte de la viabilité et enfin (3) déterminer s'il existait une relation entre les pigments de dégradation, la faible viabilité des cellules et les faibles performances photosynthétiques.

CHAPITRE 1

DÉTERMINATION DE LA MORT CELLULAIRE DU PHYTOPLANCTON ARCTIQUE : TEST DE DIGESTION CELLULAIRE, INFLUENCE DE L'INTENSITÉ LUMINEUSE SUR DES CULTURES ET SUR DES ALGUES DE GLACE SOUS DIFFÉRENTS COUVERTS DE NEIGE

Une version abrégée de ce chapitre a été présentée à la conférence ASLO Aquatic Sciences Meeting à Nice, France, à l'hiver 2009.

1.1 RÉSUMÉ

Dans les écosystèmes marins, la mort cellulaire est une variable importante dans la compréhension de la dynamique des producteurs primaires, cependant, très peu d'informations sont disponibles sur la mort cellulaire du phytoplancton polaire. C'est pourquoi, nous avons voulu déterminer pour la première fois, la mort cellulaire des algues de glace dans l'Arctique durant l'étude sur le chenal de séparation circumpolaire (CFL) au printemps 2008 dans la mer de Beaufort (Canada). Les pourcentages de cellules vivantes (% LC) et de cellules mortes ont été estimés en utilisant deux méthodes : par des essais de digestion cellulaire enzymatique (CDA, sans coloration) et par une méthode de coloration (BacLight^{TM} Kit). Nous avons obtenu des résultats similaires pour ces deux méthodes. L'influence de la lumière sur la viabilité des cellules a aussi été examinée en comparant les sites en fonction de l'épaisseur du couvert de neige et en mesurant le % LC avec la méthode CDA. Les pourcentages les plus faibles de cellules vivantes (env. 30%) ont été observés sous les couverts de neige les plus minces (plus forte intensité lumineuse). Des résultats similaires ont été trouvés après l'exposition d'une culture de la diatomée polaire centrale *Attheya septentrionalis* (CCMP2083) sous différentes intensités lumineuses en laboratoire. Les pourcentages de viabilité cellulaire ont diminué à 10% après 4 jours d'une exposition des cellules à une intensité lumineuse relativement forte pour ces algues (170-180 µmol photons m^{-2} s^{-1}). Cette étude a aussi permis de valider la méthode de viabilité cellulaire utilisée durant cette thèse et a fourni un aperçu de l'application possible de la méthode CDA et l'influence de la lumière dans les communautés d'algues de glace dans l'Arctique.

Mots-clé : Arctique, algues de glace, mort cellulaire, méthodes de viabilité, pigments

1.2. DETERMINING PHYTOPLANKTON CELL DEATH IN THE ARCTIC: A TEST OF THE CELL DIGESTION ASSAY AND THE INFLUENCE OF IRRADIANCE IN CULTURES AND IN ICE ALGAE UNDER VARIOUS SNOW COVERS

ABSTRACT

Cell death is a significant factor to understand the dynamics of primary producers in marine ecosystems, however little information is available on phytoplankton cell death in general and in polar ecosystems in particular. Here, we determined cell death of ice algae for the first time in the Arctic, during the Circumpolar Flaw Lead system study (CFL) during spring 2008 in the Canadian Beaufort Sea. Percentages of living (% LC) and dead cells were estimated using two methods: an enzymatic cell digestion assay (CDA, non-staining) and a staining method (BacLight^{TM} Kit). We found that these two methods gave similar results. The influence of irradiance on cell viability was also examined by comparing sites with various snow cover thicknesses and measuring % LC with the CDA. The lowest percentages of living cells (ca. 30%) were observed in sites under low snow cover (higher irradiances). Similar results were found after exposing cultures of the polar centric diatom *Attheya septentrionalis* (CCMP2083) to a range of irradiances in the laboratory, with the percent of living cells decreasing down to 10% after the cells were exposed for 4 days to relatively high irradiances for these algae (170-180 µmol photons m^{-2} s^{-1}). This study served as a validation for the cell viability method used during this thesis and it provides insights on the application of the CDA and the influence of light in the ice algae communities in the Arctic.

Keywords : Arctic, ice algae, cell death, viability methods, pigments

INTRODUCTION

Interest in phytoplankton cell viability in the marine environment is increasing with the realization of the importance of lysis rates in the oceans (Kirchman 1999). A variety of methods are available to assess viability (including staining and non staining methods). However, the applicability of methods for cell viability has rarely been tested for polar phytoplankton. This was particularly the case for the enzymatic cell digestion assay, since the first test of its application in cold conditions was published after the beginning of this work (Llabrés & Agustí 2008). Thus, there was a need to test cell viability methods under cold conditions before a more widespread use in the Arctic – this constitutes the first part of this thesis.

The snow and ice cover in the Arctic have been changing with the currently warming climate. In particular, a decline in snow depth (Warren et al. 1999, Derksen & Brown 2012), ice thickness (Kwok & Rothrock 2009) and extent (Comiso et al. 2008) have been observed. A decrease in sea ice will directly impact the ice algal community through loss of habitat; however climate change in the Arctic is also modifying the bottom ice light environment through its influence on snow and ice cover. We focused on snow depth, as its control on the transmission of photosynthetically active radiation (PAR) is a factor of magnitude greater than ice thickness (Mundy et al. 2005). The low temperature of their environment may also enhance the sensitivity of ice algae to light (Huner et al. 1998). Hence, conditions exist in sea ice for algal cell viability to be affected by the rapidly changing ice conditions. Photodamage has been previously observed, leading to the disappearance of some species of ice algal communities (Mangoni et al. 2009). As shade-acclimated sea ice algae could be harmed by increasing irradiance, we examined cell death along with the algal pigment signature which can provide information on deteriorating physiological condition (degradation pigments, e.g. Spooner et al. 1994).

To our knowledge, this study represents the first estimate of the proportion of dead and live cells in the ice algal community. These estimates are important to understand the

dynamics of ice algae in their response to the changing Arctic environment, especially considering that loss terms (here, cell death) are seldom measured.

The objectives of the present chapter were to (1) test the methods used to determine cell viability in polar natural samples, more specifically to demonstrate whether the enzymatic cell digestion assay (CDA) gave results similar to the BacLight™ staining method for the Arctic pennate diatom *Nitzschia frigida*, (2) examine the cell viability response of the ice algae community to various snow cover depths, and (3) test the influence of light on cell viability under controlled conditions, using a culture of the Arctic centric diatom *Attheya septentrionalis* exposed to various irradiance conditions.

MATERIALS AND METHODS

Sampling

The field work was conducted aboard the Canadian Coast Guard Ship (CCGS) *Amundsen* as part of the International Polar Year Circumpolar Flaw Lead system study (IPY-CFL; Barber et al. 2010). The sampling station (D43) was located in the Canadian Beaufort Sea (approximately 70° 43'N - 123° 20'W; Fig. 1). The ice cover consisted of first-year sea ice ranging in thickness from 1.2 to 1.4 m. Sampling took place in the morning (8:00 to 12:00) every third day from 26 April to 5 May 2008. On each sampling day, three separate snow depth sites, consisting of a low (<5 cm), medium (5-15 cm) and high (>15 cm) snow depth, were chosen for sampling within close proximity (<30 m apart). At each site, downwelling irradiance (PAR, 400-700 nm) was measured at the surface and at the ice-water interface, using Li-Cor 2 π sensors (LI-190SA quantum and LI-192SA underwater quantum sensors). Sub-ice measurements were made using an under-ice arm as described in Mundy et al. (2007). Sea ice was sampled with a Mark II ice corer (9 cm internal diameter, Kovacs Enterprises). Four to five ice cores were extracted at each snow depth site and snow depth and ice thickness were measured at each core location. For each site, the bottom 3 cm of each ice core was pooled in a single dark isothermal plastic

container and sub-samples from the pooled ice cores were split among several researchers. The ice cores were slowly melted overnight in filtered surface seawater (filtered through 0.2 μm polycarbonate membrane filters) to minimize osmotic stress on the ice algae (Bates & Cota 1986, Garrison & Buck 1986).

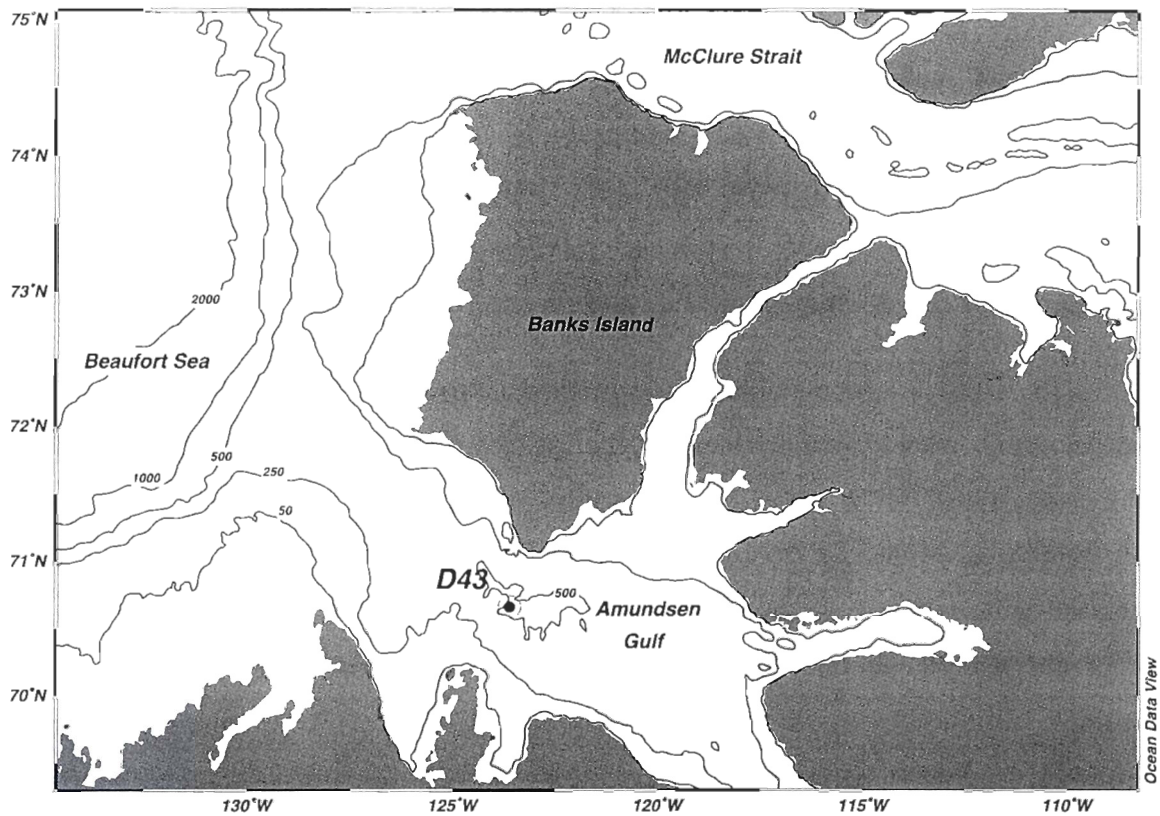

Fig. 1 Location of the sampling station in the Amundsen Gulf, Canadian Beaufort Sea, in April-May 2008. Water depth is in meter

Cell viability

The two methods used in this study for determining cell death are based on cell membrane permeability tests, which have been developed to discriminate between living and dead cells (Wyllie et al. 1980, Ellis et al. 1991, Darzynkiewicz et al. 1994, Agustí & Sánchez 2002). These methods include the cell digestion assay (CDA) and the BacLight™ kit (Molecular Probes® Fluorescent Dyes and Probes, Invitrogen).

The CDA is a non-staining vital method (Darzkynkiewicz et al. 1994, Agustí & Sánchez 2002), which was modified for the low temperature encountered in polar waters (Llabrés & Agustí 2008). The CDA was applied to duplicate 3 mL seawater samples. For each sample, a volume of 200 µL of DNAse I solution (400 µg mL^{-1} in HBSS Hanks' Balanced Salts solution) was added per mL of sample, followed by an incubation at 25°C for 15 min. After incubation, 200 µL of Trypsin solution (2% in HBSS) were added per mL of sample, followed by incubation for an additional 30 min at 25°C. Immediately following the second incubation period, ice algal cells from the cell digestion assay were counted by transmitted light microscopy, focusing on the most abundant taxa. Duplicate 3 mL blank samples without the addition of digestion enzymes were under the same conditions. The blanks were fixed with glutaraldehyde (1% final concentration) and were counted following the same procedure as that for the CDA samples. The CDA and blank sample counts provided a concentration estimate of living and total algal cells, respectively, and dead cells were calculated as the difference between total and living cells.

The accuracy of the CDA to quantify living and dead cells was tested by comparing the results with the CDA with those obtained with an independent vital stain, the BacLight™ kit (Molecular Probes® Fluorescent Dyes and Probes, Invitrogen), a vital stain that works well with diatoms (a major component of the ice algae community; Różańska et al. 2009). With this method, diatoms show a clear and bright staining (Llabrés & Agustí 2008). For the BacLight™ method, duplicate 1 mL samples were stained with 0.2 µL of BacLight™ kit component B: SYTO 9 dye (1.67 mM) and Propidium Iodide (1.83 mM) both in DMSO, and incubated for 10-15 min in the dark following the same procedure as in

Agustí et al. (2006) and Llabrés & Agustí (2008). Quantitative analysis was conducted on board the ship with an Olympus epifluorescence microscope fitted with a blue filter (excitation 470 nm, emission 520-560 nm). A total of 200 cells were counted, distinguishing green cells (living cells), red cells (dead cells) and not well-stained cells. The not well-stained cells always represented < 5% of the total abundance. The percentage of dead cells was calculated as the ratio of red cells to total cell abundance.

Pigments

The identity and concentration of algal degradation pigments were determined by reverse-phase high performance liquid chromatography (HPLC). Samples for pigments were filtered onto 25 mm Whatman GF/F filters (maximum volume of 500 mL) and the filters were then stored on board the ship in liquid nitrogen. At the end of the field mission, filters were sent to Rimouski in a dry-shipper and thereafter kept in a –80°C freezer until analysis. Algal pigments were extracted from the filter in 95% methanol, sonicated (Sonicator Ultrasonic Processor XL 2010) for a few seconds and centrifuged for 5 min at 3700 g. Pigment extracts were then filtered through a 0.22 µm polytetrafluoroethylene syringe filter and poured in an autosampler vial which was gently sparged with argon before closing the lid to limit oxidation. A 50 µL extract was injected in a reverse-phase Waters Symmetry C8 column (150 x 4.6 mm, 3.5 µm). Gradient elution was controlled by a Thermo Separation (TSP) P4000 pump with solvents as indicated in the HPLC method developed by Zapata et al. (2000). Pigments were detected with a TSP UV 6000 LP diode-array absorbance detector (400 to 700 nm) and a TSP FL3000 fluorescence detector to confirm the presence of chlorophyll-related compounds. Calibration was done with external pigment standards obtained commercially from DHI Lab Products (Hørsholm, Denmark) and extinction coefficients were taken from Jeffrey (1997). Limits of detection and quantification were estimated as in Bidigare et al. (2005) and pigments with concentrations less than the limit of quantification were not reported. Marker pigments were identified through comparison with the retention time and spectral properties of pigment standards.

Algal culture experiment under controlled light conditions

To verify the influence of light on cell viability, an experiment was set up at the Institut des sciences de la mer's (ISMER) laboratory. Cultures of *A. septentrionalis* CCMP2083 (provided by Dr. C. Lovejoy), a diatom species present in the water column and also in the sea-ice in the Canadian Arctic (Poulin et al. 2011), were grown in f/2 enriched seawater media (Guillard 1975, Andersen et al. 2005) in the laboratory, under an irradiance of 70 µmol photons m^{-2} s^{-1}, a salinity of 32 psu and a temperature of 4°C. These cultures were then exposed to three irradiance treatments for a period of 12 days. The treatments consisted in (1) a continuous photoperiod of 70 µmol photons m^{-2} s^{-1}, (2) progressively increased irradiances every two days from 90 to 180 µmol photons m^{-2} s^{-1}, and (3) continuous high irradiances of 170-180 µmol photons m^{-2} s^{-1}. Light was supplied by fluorescent cool white lamps (Osram). Sampling was done simultaneously in the cultures exposed to the three treatments measuring systematically an index of phototosynthetic activity (fluorescence F_v/F_m) and the percentage of living cells (% LC). Minimum and maximum fluorescence (F_0, F_m) were measured after 30 min of darkness using a Turner Designs fluorometer (model AU-10). F_m was measured after adding DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea, $3x10^{-3}$M), an inhibitor that blocks electron transport at the electron acceptor Q in photosystem II (PSII), causing an increase in chlorophyll fluorescence (Roy & Legendre 1979, Jochem 2000). The index of photosynthetic performance F_v/F_m was calculated as $(F_m-F_0)/F_m$. A decrease in the maximum quantum yield of PSII photochemistry, measured as dark-adapted F_v/F_m, is an indicator of photoinhibition or down-regulation of PSII (Critchley 2000). The percentage of living cells was calculated using the CDA (as detailed previously for the field work).

RESULTS AND DISCUSSION

Comparison of the two cell death methods

The two methods, CDA and BacLight™, were compared on three occasions while onboard the CCGS *Amundsen* during the IPY-CFL system study. An experiment was performed to test the effect of external stressors, including prolonged darkness and high temperature compared to the natural environment, to ascertain the applicability of our cell death determination methods. To do so, cells collected at the bottom of the sea ice under the medium snow cover on 29 April were incubated in the dark at room temperature (15-20°C) for 10 days. Cell death was determined with the CDA method at the beginning and at the end of the experiment. The percentage of living cells of *N. frigida* (the dominant species present) decreased from 86.3 ± 4.9% (mean ± SE) at the beginning of the experiment, to 14.2 ± 1.4% at the end of the 10-day period on 9 May. Mann-Whitney *U*-tests revealed that there were no significant differences in the percentage of living cells of *N. frigida* between the CDA and the BacLight™ methods when sampled on 2 May ($p = 0.35$), 5 May ($p = 0.35$) and 9 May ($p = 0.06$), from the dark and room temperature test (Table 1). These results confirmed the applicability of the CDA assay for the species *N. frigida* (Table 1).

Bottom ice irradiance conditions

The three snow depth categories showed a clear difference in sub-ice irradiance (Table 2). Transmitted irradiance was greater by a factor of about 4 and 2 under low and medium snow depths in comparison to the high snow depth cover, respectively (Table 2) and sub-ice irradiance was significantly higher under the low snow cover compared to the other two conditions (ANOVA, $p < 0.05$). Sub-ice irradiance increased from 29 April to the end of the sampling period (Table 2).

Table 1 Percent living cells of *Nitzschia frigida* (mean ± SE) estimated using the enzymatic cell digestion assay (CDA) and the BacLight™ Viability Kit. Cell viability was determined on bottom ice samples collected under medium snow cover on 2 and 5 May and at the end of the stress experiment on 9 May. Number of observations: n = 4 for CDA; n = 2 for the BacLight™ Kit

	Living cells (%)		
Method	2 May	5 May	9 May
CDA	86.3 ± 4.9	22.7 ± 7.1	14.2 ± 1.4
BacLight™ Kit	91.5 ± 0.8	15.7 ± 1.3	10.0 ± 3.0

Table 2 Physical characteristics of the sea ice environment under low, medium and high snow cover in the Amundsen Gulf from 26 April to 5 May 2008 (calculation of bottom ice irradiance as in chapter II).

Date	Snow cover	Snow depth (cm)	Ice thickness (cm)	Bottom ice irradiance (μmol photons m^{-2} s^{-1})
26 Apr	Low	4.4	131	20.8
	Medium	9.3	122	8.5
	High	25.2	127	4.1
29 Apr	Low	4.5	133	18.7
	Medium	12.5	128	8.8
2 May	Low	5.5	136	24.8
	Medium	14.1	131	10.3
	High	24.7	124	6.9
5 May	Low	4.0	144	44.3
	High	17.6	136	16.6

Cell viability under various snow covers

When comparing the two snow cover conditions for each day, a significantly lower percentage of living cells was observed under the low snow cover for large centric diatoms (>25 μm) on 29 April ($p < 0.05$) and 2 May ($p < 0.01$) and for *N. frigida* on 2 May (Student's *t*-test, $p < 0.05$). In contrast, a significantly lower percentage of small diatoms (5-25 μm) were observed under the medium snow cover on 29 April (Student's *t*-test, $p < 0.05$). In fact, the percentage of living cells was below 50% for all diatom taxa under the low snow cover on 2 May, except for small cells (< 5 μm), which remained at approximately 70% (Fig. 2b). The percentage of living cells for these small cells also ranged between 60 and 70% under the medium snow cover, indicating that irradiance had little effect on these cells.

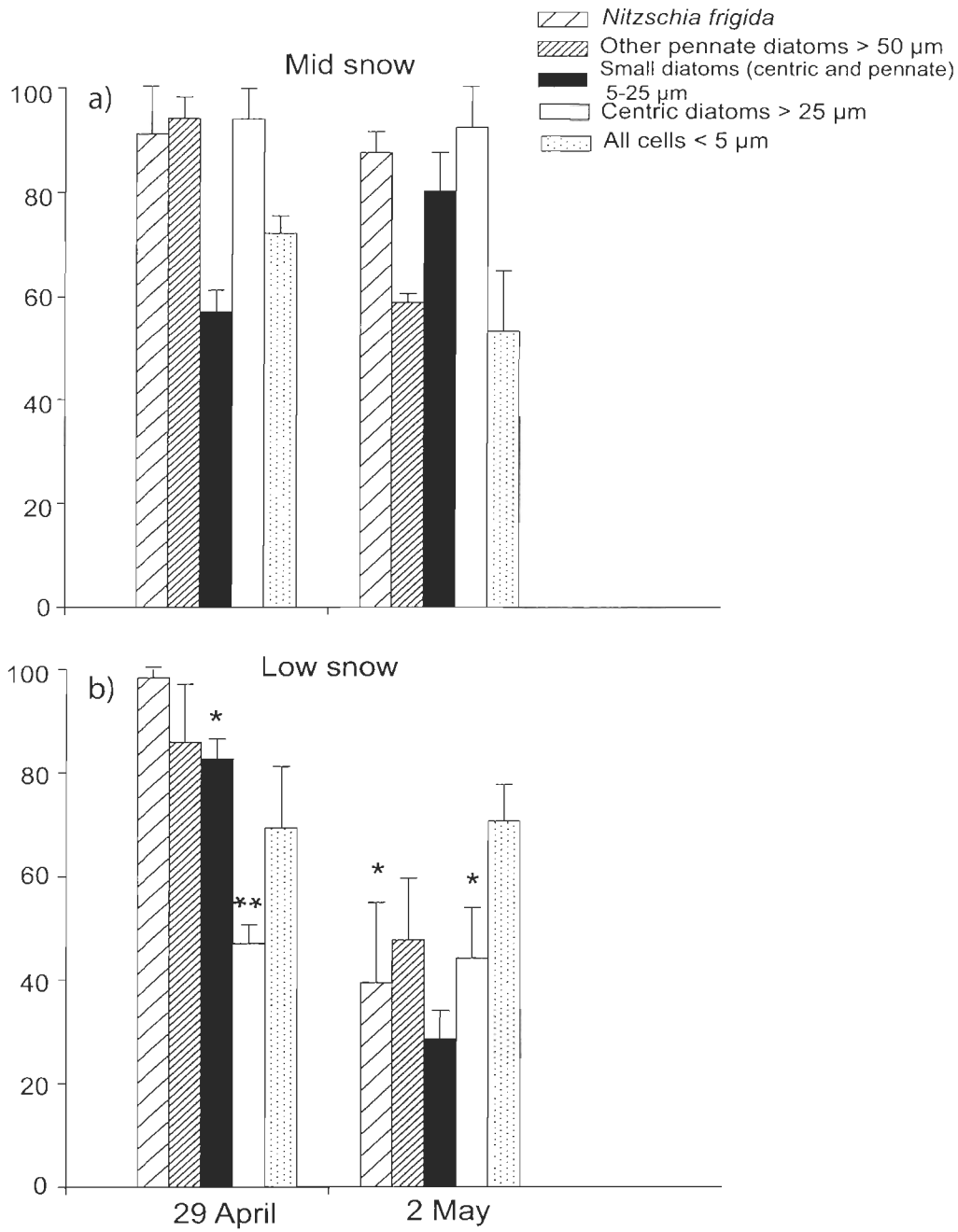

Fig. 2 Percentage of living cells (% LC, by the CDA method) for various bottom ice algal groups collected under (a) medium (MS), and (b) low (LS) snow cover on 29 April and 2 May 2008. Means and SE are shown. Significant differences between the two snow cover depths (Student's *t*-test, * $p < 0.05$, ** $p < 0.01$)

Chlorophyll biomass and degradation pigments

Bottom ice chlorophyll *a* (chl *a*) concentrations ranged from 4 to 12.6 mg m^{-2} during the study (Fig. 3a). The three-fold changes in chl *a* concentration were not obviously related to sub-ice irradiance (no significant Pearson's linear correlation (r) between these two variables: $r = 0.13$, $p = 0.73$). There was no clear trend in chl *a* concentration among the three snow cover conditions.

The chlorophyll degradation product, chlorophyllide *a* (chlide *a*), is often observed in senescent diatoms (Jeffrey & Hallegraeff 1987, Spooner et al. 1994, Llewellyn et al. 2008). Here, the ratio of chlide *a* to chl *a* showed an increase mostly from 2 to 5 May under low and high snow covers (Fig. 3b), associated with nearly a doubling of bottom ice irradiance (Table 2). Indeed, a significant relationship was observed between the chlide *a*:chl *a* ratio and bottom ice irradiance ($r = 0.77$, $p < 0.05$) although there was no significant difference among the three snow cover conditions (ANOVA, $p = 0.64$). Another chlorophyll degradation product, the major allomer of chl *a* (peak preceding chl *a* in the chromatograms, likely 13^2-hydroxy-chlorophyll *a*: Spooner et al. 1994), showed similar trends. The ratio of this allomer to chl *a* also increased significantly with bottom ice irradiance ($r = 0.76$, $p < 0.05$) and showed higher values under the low snow cover (ANOVA, $p < 0.05$; Fig. 3c). This pigment forms under oxic degradation of algae, including diatoms (Spooner et al. 1994, Louda et al. 1998, Szymczak-Żyła et al. 2008). The chl *a* specific ratios of these two degradation pigments showed an increase with cell death that was not statistically significant ($p=0.13$) but there were few data.

Pheophorbide *a*, a pigment frequently associated with grazing (Welschmeyer & Lorenzen 1985) or cellular senescence (Spooner et al. 1994), was also present at concentrations reaching nearly 50% of the chl *a* values (data not shown) The pigment reported here was observed in all samples and it matched the retention time of the pheophorbide *a* pigment standard. Other pheophorbide *a* derivatives were occasionally present but these were not considered here because their concentration was much lower. Pheophorbide *a* showed similar trends as for chl *a* and its concentration relative to chl *a*

showed no particular features with respect to time or snow cover (data not shown) and was not significantly correlated with chlide *a* ($r = 0.64$, $p = 0.09$), suggesting an association with grazing rather than an indication of cellular senescence.

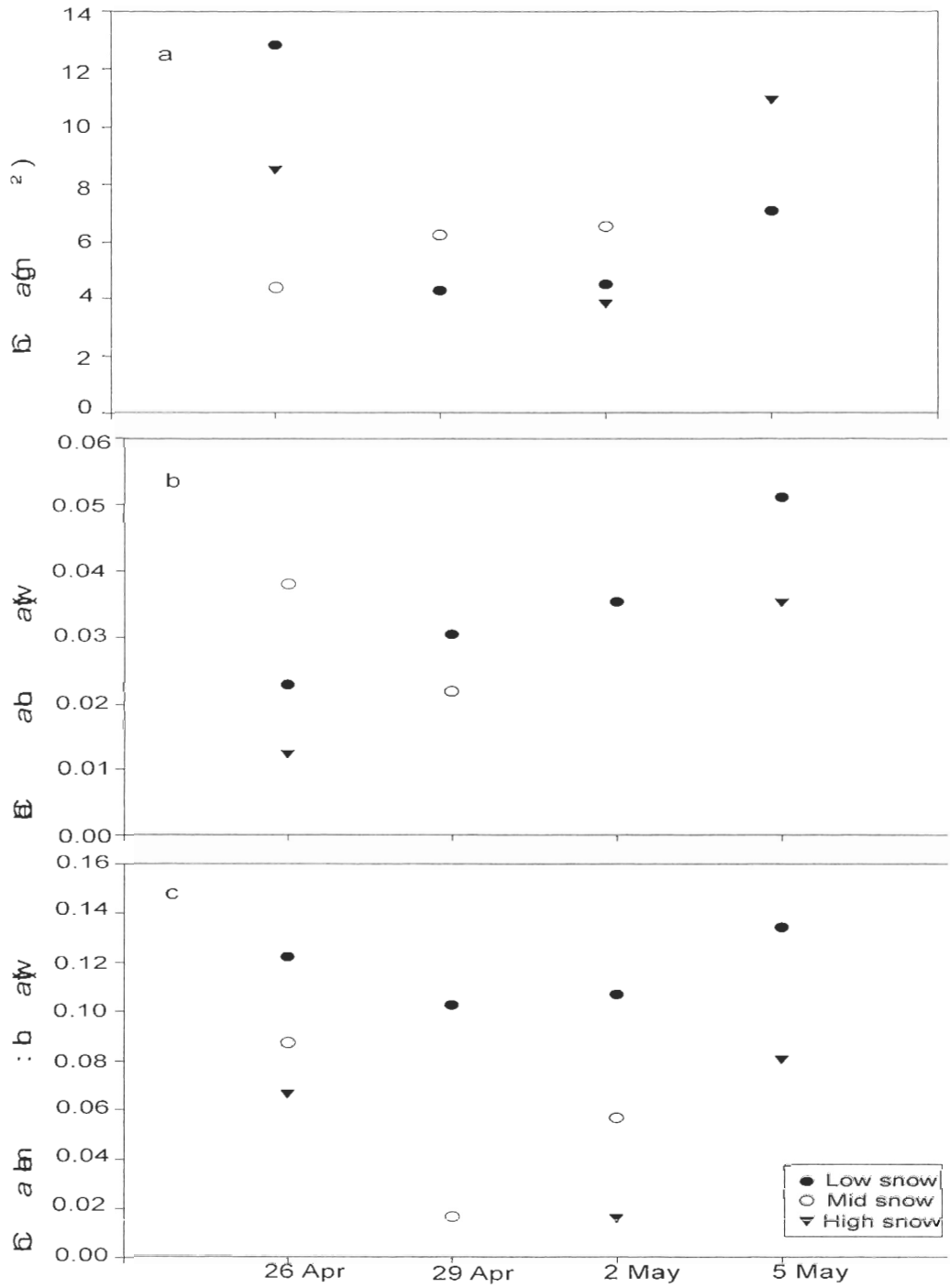

Fig. 3 Temporal changes in (a) the chlorophyll *a* concentration, the ratios of (b) chlorophyllide *a* to chlorophyll *a* (childe *a*:chl *a*) and (c) chlorophyll *a* allomer to chlorophyll *a* (chl *a* allomer:chl *a*) under low, mid and high snow cover depths

Culture experiment

The % LC of the culture *A. septentrionalis* ranged from 81.6 to 86.2% for the control treatment (constant irradiance ~70 µmol photons m^{-2} s^{-1}) throughout the experiment (Fig. 4a, b). The F_v/F_m values remained between 0.52-0.75. After exposure to the treatments of gradually increasing and of constant high irradiances, both % LC and F_v/F_m decreased over time (Fig. 4b, c). The lowest values for % LC were obtained after the eight-day exposure to the gradually increasing irradiance (mean ± SE, 13.4 ± 0.9%), and on the last day of the experiment, for the culture exposed to the constant high irradiance treatment (13.2 ± 3.2%). For that last culture, there was a sharp decline of % LC on the fourth day (13.8 ± 1.2%; Fig. 4b), with no apparent recovery of the cells, as indicated by the low F_v/F_m values (< 0.2) (Fig. 4c).

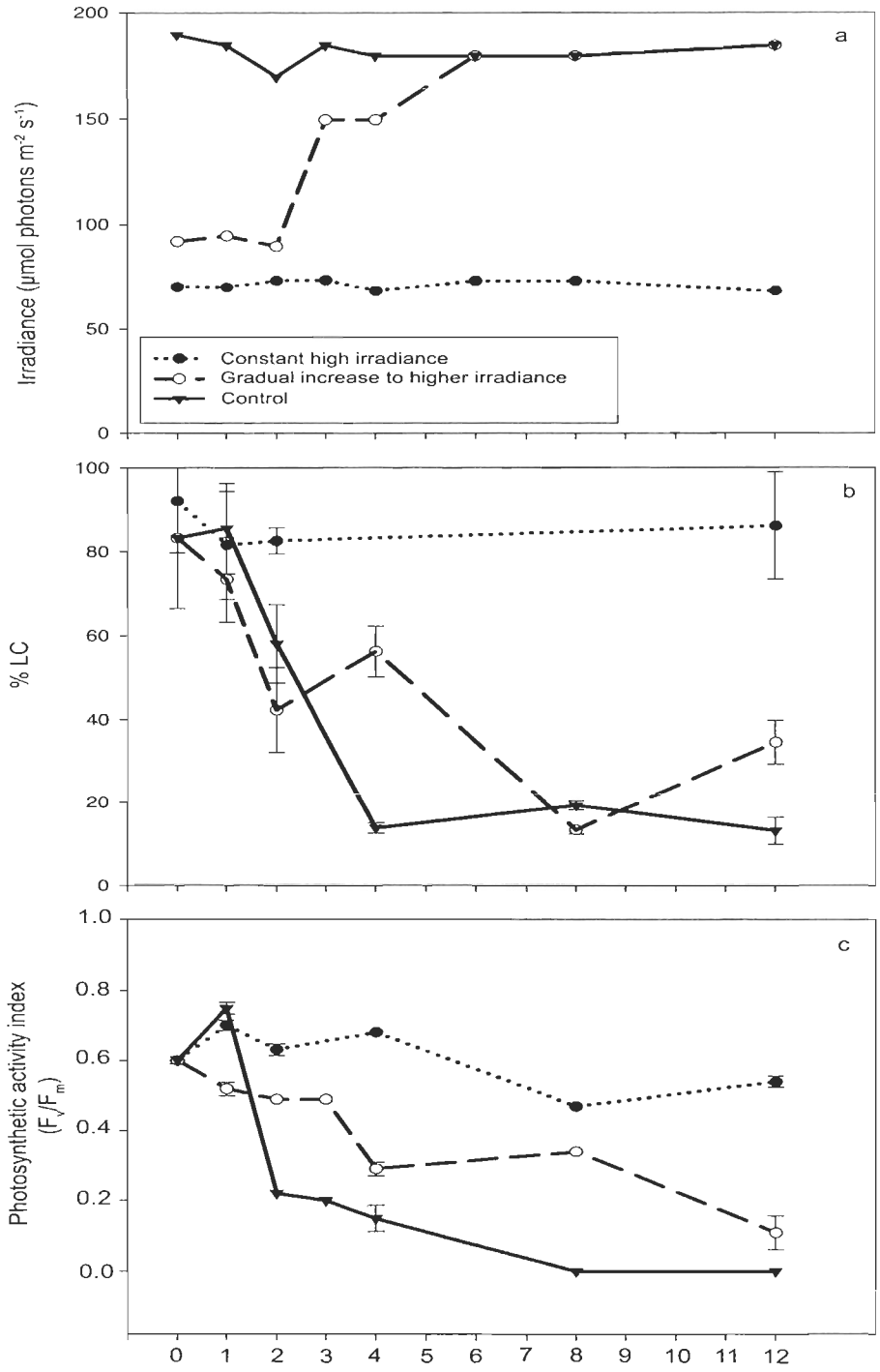

Fig. 4 Temporal changes during the 12 days culture experiment using *A. septentrionalis*: (a) irradiance (PAR), (b) percentage of living cells (%LC), measured with the CDA, and (c) photosynthetic activity index. In (b) and (c), mean ± SE

Cell viability

Validation of the methodology used to determine cell viability was performed by comparing the CDA (non-staining) and the BacLight™ (staining) methods. The BacLight™ method was chosen among others because it uses double staining (contains Syto 9 and Propidium Iodide), avoiding the potential ambiguity of other stains with low fluorescence signals (e.g. fluorescein diacetate, FDA) (Agustí & Sánchez 2002, Garvey et al. 2007, Llabrés & Agustí 2008). The low percentage of not well-stained cells (< 5% of the total abundance) obtained with the BacLight™ method indicated the efficacy of the stain. Similar to Llabrés & Agustí (2008) for the Arctic diatom *Nitzschia* sp., we found no differences between the two methods, CDA and BacLight™, for the determination of cell viability in the species *N. frigida*.

Ice algae can be extremely shade-adapted (Cota 1985, Thomas & Dieckmann 2002, Moline et al. 2008), and therefore, they can experience photoinhibition at relatively low irradiance (Neale 1987, Falkowski 1994). Inhibition of photosynthesis in ice algae has been reported at light levels above 20 μmol photons m^{-2} s^{-1} (Booth 1984, Palmisano et al. 1985, Kirst & Wiencke 1995). During the present study the measured bottom ice irradiance was above 20 μmol photons m^{-2} s^{-1} on two occasions and it reached 44.3 μmol photons m^{-2} s^{-1} on 5 May (low snow cover, Table 2), these conditions were potentially damaging for ice algae.

Indeed, the percentage of living cells observed particularly on 2 May (no data for cell death on 5 May) reached less than 40% for the dominant ice algal species, *N. frigida*, as well as for small-sized diatoms (Fig. 2). Similar values have been observed in phytoplankton cells from temperate oligotrophic waters (Agustí et al. 1998, 2001), with values as low as 15% living cells for *Synechococcus* in the South Atlantic subtropical gyre (Agustí 2004). The significant decrease in percent living cells between the 29 April and 2 May for the above two groups of ice algae was not matched with a large increase in bottom ice irradiance, since values for the low snow cover increased only from 19 to 25 μmol photons m^{-2} s^{-1} between these two days (Table 2), but these values are both in the

range of the threshold for photoinhibition mentioned above. A likely delay between photodamage and cell death combined with limitations of our dataset probably account for the absence of a significant relationship between living cell values and bottom ice irradiance, although living cell values were generally low when bottom ice irradiances were elevated. Nitrate concentrations in bottom sea ice ranged between 0.8 and 5.8 μM (2.9 to 4.2 μM in surface waters, B. Philippe, personal communication). These nitrate levels were low but not entirely depleted so it was not considered a likely cause of cell death, although this was not tested specifically.

We examined changes in ice algal biomass that could result from this cell viability loss through the analysis of pigments. The chl *a* biomass was quite variable among the four dates and three snow cover conditions, ranging from 4 to 12.6 mg m^{-2} (Fig. 3). These values were within the range previously observed for the study region (e.g., Różańska et al. 2009). The large variability reflects the patchy distribution of ice algae (Gosselin et al. 1986). Nevertheless, studies have shown that the variation in thickness of the snow and ice cover, which determines the irradiance at the bottom of the ice, is the major factor controlling the algal biomass distribution over the scale of less than 100 m (Gosselin et al. 1986, Rysgaard et al. 2001, Mundy et al. 2005). Our results show no significant relationship between chl *a* and bottom ice irradiance, most likely influenced by our limited dataset, but this could also be related to physical factors such as ice type (i.e. drifting polynya ice was sampled in our study), ice age and to bottom ice erosion associated with the polynya environment. These physical factors would influence the accumulation of bottom ice algal biomass and therefore, the relationship of chl *a* with bottom ice irradiance. Also, we do not observe a relationship between estimates of living cells and algal biomass (expressed as chl *a*), but rather lower viability (lower percentages of living cell estimates) when ice algal biomass was high. We note that if dead cells were to remain in their environment for some time and were still pigmented, one could expect a mismatch between these pigment variables and the percentage of living cells on the short-term, as observed here.

However, we expect signs of a deteriorating physiological condition when the percentage of living cells decreases. Two of the pigments observed in our study were indicative of such deterioration, chlide *a* and the major allomer of chl *a* (allomerization is an oxidative degradation of chl *a*). Pheophorbide *a* can also occur under these conditions (Spooner et al. 1994), but as it can also result from grazing activity, its interpretation is more difficult. As pheophorbide *a* showed no relationship with irradiance or cell viability and no correlation with chlide *a*, while it was strongly correlated with fucoxanthin, we assumed that its presence reflected the presence of grazers more than cellular senescence. Both chlide *a* and the chl *a* allomer (relative to chl *a*) showed a significant increase with irradiance, supporting our suggestion that increases in light levels were detrimental to the cells. However, only the ratio of chl *a* allomer to chl *a* was significantly higher under the low snow cover compared to the other two snow conditions, because chlide *a* showed more variability among snow covers (Fig. 4a). When plotted against percent living cells, these two pigment ratios demonstrated a negative relationship, but the trends were not significant, likely because of the small dataset. The delay between unhealthy cells turning into dead cells could also have contributed to a mismatch between these variables.

In summary, this study presents the first determination of cell viability for Arctic ice algae. Since then, the CDA has been successfully applied to other natural communities of polar environments (Agustí et al. 2009, Lasternas & Agustí 2010, Llabrés & Agustí 2010, Echeveste et al. 2011).

Under controlled laboratory conditions, cultures of *A. septentrionalis* exposed to a light stress showed a loss in viability, with the lowest percentages of living cells and photosynthetic activity by the end of the experiment. These conditions, and possibly others not examined in this study, appear to contribute to the loss of cell viability. These culture results support our interpretation that increasing irradiance may be a leading factor in affecting cell viability in the Arctic.

Acknowledgements This work is a contribution to the International Polar Year-Circumpolar Flaw Lead system study (IPY-CFL 2008), supported through grants from the Canadian IPY Federal Program office and the Natural Sciences and Engineering Research Council (NSERC) of Canada. EA received post-graduate scholarships from ISMER and Université du Québec à Rimouski and a stipend from Québec-Océan. NSERC discovery grants to SR and to MG also helped to support this work. We are grateful to Dr C. Lovejoy for providing the cultures of *A. septentrionalis*. We thank the officers and crew of the CCGS *Amundsen* for logistical support and B. Philippe, A. Sallon, M. Lionard, S. Gracia, C. Bourgault and J. Corriveau for assistance in the field or laboratory.

CHAPITRE 2

LE COUVERT DE NEIGE AFFECTE LA COMPOSITION DES PIGMENTS D'ALGUES DE GLACE DE L'OCÉAN ARCTIQUE CÔTIER PENDANT LE PRINTEMPS

Ce deuxième article, intitulé "*Snow cover affects ice algae pigment composition in the coastal Arctic Ocean during spring*" fut corédigé par moi-même, les professeurs Christopher-John Mundy, Suzanne Roy, Michel Gosselin, et Susana Agustí. En tant que premier auteur, ma contribution à ce travail fut l'essentiel de la recherche sur la composition pigmentaire chez les algues de glace et l'influence du couvert de neige, l'élaboration du plan d'échantillonnage, la réalisation des campagnes de terrain, les analyses en laboratoire, le traitement statistique des résultats et la rédaction de l'article. Les professeurs C. J. Mundy, S. Roy, et M. Gosselin ont fourni l'idée originale. Ils ont aidé à la définition de la problématique, à l'élaboration du plan d'échantillonnage ainsi qu'à la révision de l'article. Les professeurs M. Gosselin et C. J. Mundy ont contribué à l'échantillonnage sur le terrain. Différents éléments de cet article ont été présentés dans plusieurs conférences nationales et internationales : CFL-all Hands Meeting à Winnipeg en novembre 2009, l'Assemblée annuelle de Québec-Océan à Québec en novembre 2010 et la conférence ASLO Aquatic Sciences Meeting au Japon en juillet 2012. Ce chapitre a été publié dans le journal *Marine Ecology Progress Series* en janvier 2013.

2.1 RÉSUMÉ

Certains pigments et leurs produits de dégradation peuvent fournir une quantité considérable d'informations sur la composition taxonomique et l'état photo-physiologique des communautés algales. Cependant, jusqu'à maintenant, aucune étude ne s'est intéressée à la composition pigmentaire des algues de glace du Haut-Arctique canadien. Durant le bloom printanier de 2008 (mars à juin), nous nous sommes donc penchés sur cette lacune et avons examiné l'assemblage pigmentaire des algues localisées à la base de la glace dans la portion canadienne de la mer de Beaufort et ce, sous différents couverts de neige. Du développement du bloom jusqu'à l'atteinte de la biomasse maximum, les diatomées pennales (de type pigmentaire 2, contenant de la chlorophylle [chl] c_2 et c_3) dominaient la biomasse. Les diatomées contenant de la chl c_1 (de type pigmentaire 1) et les chlorophytes n'ont été retrouvées que sous les épais couverts de neige. Une communauté plus diversifiée était présente pendant le déclin du bloom, lorsque seuls les sites avec une mince couverture de neige étaient toujours présents, dû à la fonte. Cette communauté avait une proportion relativement plus élevée de chlorophytes, prasinophytes et de dinoflagellés en raison du déclin des diatomées, qui étaient représentées par un nombre grandissant de frustules vides (observations au microscope) et qui présentaient plusieurs signes de détérioration physiologique (augmentation de la chlorophyllide *a* et de l'allomère de la chl *a*). Le ratio entre les pigments caroténoïdes photoprotecteurs et ceux photosynthétiques était généralement plus élevé sous les minces couverts de neige et augmentait au fil de la saison avec l'accroissement de l'éclairement à la base de la glace. Les sites avec de minces couverts neigeux se distinguaient également par une plus grande abondance de diatomées de Type 2 et de pigments photoprotecteurs et par une plus forte biomasse de chl *a* au début du bloom. De plus, ces sites montraient davantage de pigments de dégradation de la chl *a* qui pourraient cependant être dû à la présence de diatomées pennales riches en chlorophyllide puisque leur biomasse croissante au début du bloom suggère que les cellules étaient en bon état physiologique à ce moment. Cette étude souligne l'influence

considérable de la lumière et témoigne de la plasticité des algues de glace de l'Arctique dans leur acclimatation face à la lumière.

Mots-clé : Arctique, algues de glace, pigments, CHEMTAX, photoprotection

2.2 Snow cover affects ice algae pigment composition in the coastal Arctic Ocean during spring

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ABSTRACT

Specific pigments produced by algae and their degradation products can provide considerable information on the taxonomic composition and photo-physiological state of algal communities. However, no previous study has looked at ice algal pigment composition in the high Arctic. We examined the bottom ice algal pigment composition in the Canadian Beaufort Sea under various snow cover conditions during the spring bloom (March to June 2008). During the early and peak bloom periods, pennate diatoms (Pigment Type 2, containing chlorophyll [chl] c_2 and c_3) dominated the chl *a* biomass. Diatoms containing chl c_1 (Pigment Type 1) and chlorophytes were only present under high snow cover. A more diverse community was observed during the post-bloom when only low snow sites remained due to snow melt, with higher relative contributions of chlorophytes, prasinophytes and dinoflagellates, associated with the loss of diatoms, along with an increased abundance of large empty diatoms (from microscopy) and with signs of a deteriorating physiological condition (increases in chlorophyllide *a* and the allomer of chl *a*). The ratio of photoprotective to photosynthetic carotenoid pigments was generally higher at low snow cover sites, increasing seasonally with the bottom ice irradiance. Low snow cover sites differed also by having more Type 2 diatoms, increased photoprotection and greater chl *a* biomass during the early bloom. In addition, these sites showed increases in chl *a* degradation pigments that may be due to the presence of chlorophyllide-rich pennate diatoms, since the increasing biomass suggests healthy physiological conditions at that time. This study highlights the important influence of light and the light-acclimation plasticity in Arctic sea ice algae.

Keywords **:** Arctic, ice algae, pigments, CHEMTAX, photoprotection

INTRODUCTION

Sea ice is a major habitat for the microbial community and ice-associated microalgae, generally dominated by pennate diatoms (Poulin et al. 2011). Under-ice light conditions present special problems to cells living in this habitat because photosynthetically active radiation (PAR) is reduced. The observed decrease of the sea ice cover over the last 30 yr in the Arctic (NSIDC 2012) together with an earlier ice melt onset in spring and later freeze-up in autumn (Markus et al. 2009), will influence the ice ecosystem, however, the extent of this influence has not yet been determined. Potential impacts include perturbations to the bottom ice light environment, where the majority of ice algal biomass can be observed in the Canadian Arctic (Smith et al. 1990). The bottom ice light regime in the Arctic is strongly affected not only by the ice but also by the snow cover (Mundy et al. 2005). While ice thickness decreases as spring progresses, most of the spatial variability in bottom ice irradiance is due to snow depth and melt pond coverage (Perovich et al. 1998). With melting of the snow and sea ice cover, ice algae are exposed to increases in irradiance, including ultraviolet radiation which has been shown to penetrate sea ice (Belzile et al. 2000). If the amount of light absorbed exceeds the capacity for utilization by photochemistry, photodamage can occur, especially at low temperatures that could enhance the sensitivity of cells (Huner et al. 1998). A recent study suggested that photodamage of cells during high irradiance exposure controls phytoplankton growth in the Southern Ocean rather than the low levels of light or inadequate iron supply (Alderkamp et al. 2010). However, examination of the photoprotective mechanisms employed by bottom sea ice algae from Antarctica indicated that these algae possess a high level of plasticity in their light acclimation capabilities, notably due to an active xanthophyll cycle associated with non-photochemical quenching of chlorophyll fluorescence which reduces the excitation pressure on photosystem II (Petrou et al. 2011). The degree of plasticity in light acclimation has been suggested to explain the changes in algal community in the Antarctic (Arrigo et al. 2010, Petrou & Ralph 2011) and the Arctic (Ban et al. 2006).

Since timing and rate of loss of the ice and snow cover determine the degree of photo-physiological stress in ice algae, it is important to know how changes in the light regime might affect the sea ice community. In the present work, we focus on the influence of the snow cover on the community composition and pigment-related photoprotective response of ice algae during the sea ice diatom bloom and secondarily examine the changes that take place as this bloom progresses. The main objectives were to (1) determine if ice algae taxonomic composition was different under the various snow cover conditions and periods of the bloom, (2) investigate the photoprotective response of ice algae communities under three contrasting snow cover conditions (low, mid and high snow depths), and (3) determine if cells exposed to higher irradiances (low snow cover depth) showed a detrimental physiological condition. In order to study changes in the taxonomic composition of the major algal groups we used chemo-taxonomy (CHEMTAX factorization, Mackey et al. 1996) on high performance liquid chromatography (HPLC) pigment signatures with confirmation from microscopy. CHEMTAX factorization has been used successfully in polar waters (Ishikawa et al. 2002, Wright et al. 2010), but no study has yet been done for the Arctic sea ice.

MATERIALS AND METHODS

Study site and sampling

This study was conducted in the Canadian Beaufort Sea (Fig. 1) as part of the International Polar Year–Circumpolar Flaw Lead system study (IPY–CFL; Barber et al. 2010), onboard the Canadian Coast Guard Ship (CCGS) *Amundsen.* Sampling was carried out on 27 separate days from 18 March to 22 June 2008 on first-year drifting sea ice ("D" stations) of the Amundsen Gulf and on the first-year landfast ice ("F" stations) of Franklin and Darnley bays, Prince of Wales Strait and M'Clure Strait's entrance (northwestern Banks Island) (Fig. 1).

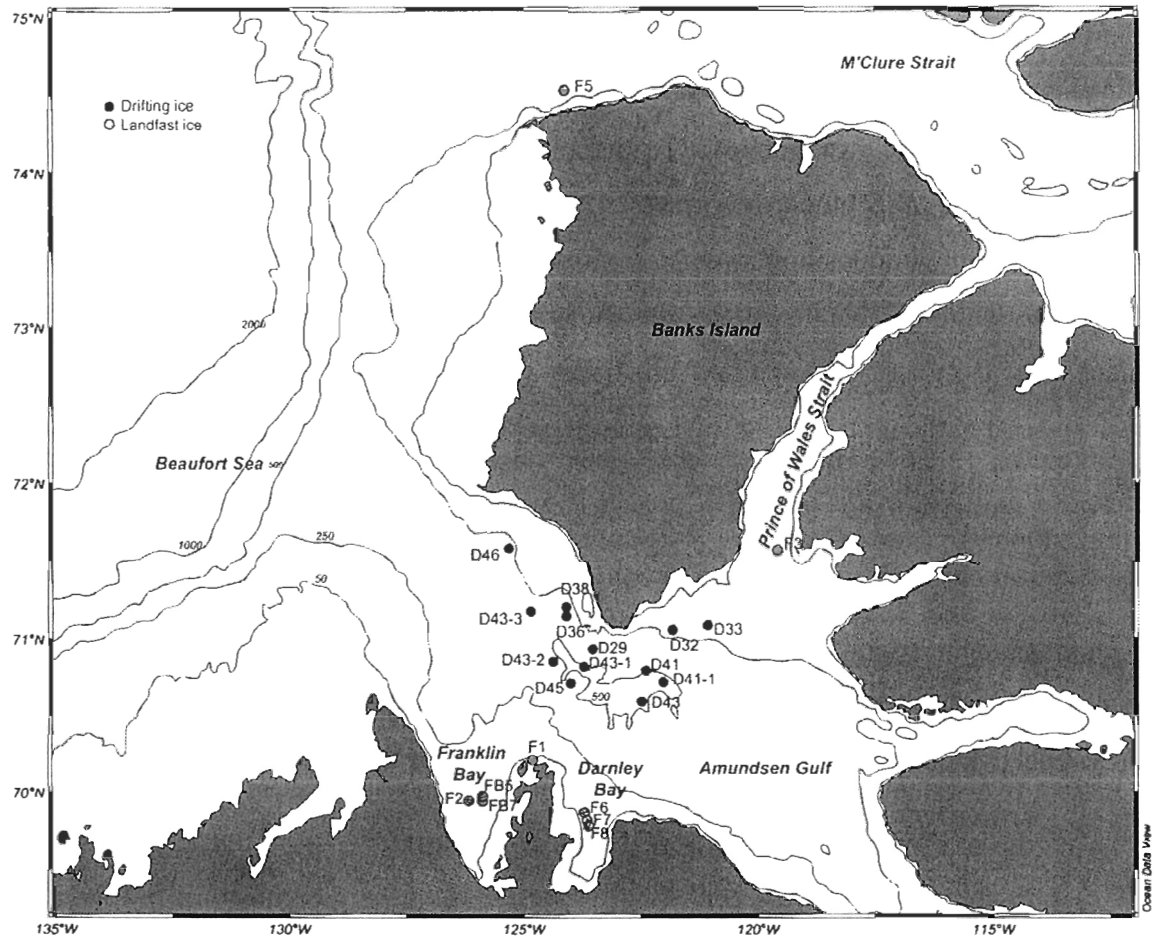

Fig. 1. Locations of sampling stations in the Canadian Beaufort Sea during the International Polar Year-Circumpolar Flaw Lead system study, March to June 2008. Water depth is in meters

Downwelling PAR (400-700 nm) incident irradiance was continuously monitored (PARLite™ quantum sensor, Kipp & Zonen) on a tower at the bow of the ship commencing on 1 April. PAR values were integrated to obtain daily incident PAR. PAR downwelling incident irradiance was also measured at every sampling site (between 9:00 and 11:00) at the surface (E_0) and at the ice-water interface (E_s), using Li-Cor 2 π sensors (LI-190SA quantum and LI-192SA underwater quantum sensors) between 26 April and 2 June. Sub-ice measurements were taken using an under-ice arm as described in Mundy et al. (2007). Irradiance reaching the top of the bottom 3 cm algal layer (bottom ice irradiance, E_b in μmol photons m^{-2} s^{-1}) was estimated from that at the ice-water interface, using Beers law (details provided in Supplements 1 & 2). The percent transmission of incident irradiance reaching the top of the bottom ice algal layer was defined as $\%E_b = (E_b/E_0) \times 100$. The $\%E_b$ was multiplied by the daily incident irradiance, then averaged over 3 days leading up to the sample collection date in order to determine the mean irradiance received by bottom ice algae.

Routine ice sampling was performed at low (<5 cm), medium (8-15 cm), or high (>15 cm) snow depth sites until 20 May. Afterwards, only low snow cover sites remained to be sampled due to snow melting. Sampling sites for the different snow cover conditions were within close proximity of each other at the various stations (<30 m apart). Bottom ice cores (3 cm) were collected with a Mark II ice corer (9 cm internal diameter, Kovacs Enterprises). Snow depth and ice thickness were measured at each core location. Bottom ice cores used for bulk ice salinity and nutrient measurements were extracted and melted slowly in the dark in sterile bags (Nasco Whirl–Pak®). For biological measurements, four to five replicate ice cores were extracted and pooled in a single dark isothermal container. Melting was done overnight in a variable volume (1.2 – 3.5 l) of 0.2 μm filtered surface seawater to minimize osmotic stress on the ice algae (Garrison & Buck 1986).

Salinity and nutrients

A WTW 330i conductivity meter was used to determine salinity of the bottom ice samples. Nitrate plus nitrite ($NO_3 + NO_2$), nitrite (NO_2), silicic acid [$Si(OH)_4$)] and phosphate (PO_4) were determined on board the ship with a Bran-Luebbe III nutrient autoanalyzer (adapted from Grasshoff et al. 1999). Prior to nutrient analysis, samples were filtered through pre-combusted Whatman GF/F filters. Nutrient concentrations were corrected for the influence of salinity using a simple linear correction (Grasshoff et al. 1999).

Microscopic analysis

Samples for microscopic analysis were preserved with acidic Lugol's solution and stored in the dark at 4 °C until analysis. Cells > 2 μm were identified using an inverted light microscope (WILD Heerbrugg). A minimum of 400 cells were enumerated over at least three transects. Detailed taxonomic analysis will be presented elsewhere (Mundy et al. 2011, Philippe 2013), but the abundance of the major algal groups is shown below.

Pigment analysis using HPLC

The identity and concentration of algal pigments were determined by reverse-phase HPLC. Samples were filtered onto 25 mm Whatman GF/F filters (maximum volume of 500 ml and maximum time of filtration of 20 min). The samples were immediately placed in liquid nitrogen over at least a 24 h period, and then transferred to a -80 °C freezer on board the ship. Samples were sent every 6 wk by plane to Rimouski in a liquid nitrogen dry-shipper and thereafter kept in a –80 °C freezer until analysis. Algal pigments were extracted in 95% methanol, sonicated (Sonicator Ultrasonic Processor XL 2010) for 15 s on ice and centrifuged for 5 min at 3700 x *g*. Extracts were filtered through a 0.22 μm polytetrafluoroethylene syringe filter and poured in an autosampler vial which was gently sparged with argon to limit oxidation. A volume of 50 μl was injected in a Waters Symmetry C8 column (150 x 4.6 mm, 3.5 μm). Gradient elution was controlled by a Thermo Separation (TSP) P4000 pump with solvents as indicated in Zapata et al. (2000).

Pigments were detected with a TSP UV 6000 LP diode-array absorbance detector (400 to 700 nm) and a TSP FL3000 fluorescence detector to confirm the presence of chlorophyll-related compounds. Calibration was done with external pigment standards obtained from DHI Water & Environment (Hørsholm, Denmark) and extinction coefficients were taken from Jeffrey (1997). Limits of detection and quantification were estimated as in Bidigare et al. (2005), and pigments with concentrations less than the limit of detection were not reported. Marker pigments were identified through comparison with the retention time and spectral properties of pigment standards. A pigment-based index of diversity was calculated using the formula $(1/n)\sum n_i(\log n_i - \log n)$, where n is the total concentration of all marker pigments (chlorophyll *a* [chl *a*]) excluded) and n_i is the concentration of each specific marker pigment (Shannon 1948, see Roy et al. 2006).

CHEMTAX analysis

The contribution of major algal groups to total chl *a* was determined using the CHEMTAX software (Mackey et al. 1996, version 1.95, Wright et al. 2009) with confirmation from the microscopic analysis. This new version of the software sets up multiple (60) initial pigment ratio matrices and directs the program to use each of them in turn to obtain more stable final values ("successive runs" strategy, as recommended by Latasa 2007). Since CHEMTAX should be applied to data sets with stable pigment ratios (Mackey et al. 1996), samples were separated into 5 data sets with similar environmental (snow depth) and biological (algal bloom phase) conditions. For the early bloom period, we split the samples into 3 groups: (1) low (n=10 samples), (2) mid (n=7), and (3) high (n=9) snow cover depths. Initial pigment ratios for these three groups differed according to irradiance (e.g., low light pigment ratios for high snow cover and higher light pigment ratios for low snow cover conditions, with pigment ratios taken from the polar literature given below or from Higgins et al. 2011). The fourth data set was the peak bloom period (n=14 samples), with similar pigment ratios under all snow covers, and the last was the post-bloom period (n=11 samples). CHEMTAX was used separately on each data set. The initial pigment ratio matrices were based on previous CHEMTAX analyses from polar

water studies (Mackey et al. 1996, Ishikawa et al. 2002, Rodríguez et al. 2002, Suzuki et al. 2002, Wright et al. 2010, Higgins et al. 2011, Kozlowski et al. 2011).

Statistical analyses

Kruskal-Wallis test, Mann-Whitney *U*-test and Wilcoxon's signed-rank test were performed to seek differences among snow cover depths or sampling periods. Descriptive statistics, Spearman's rank order correlations (r_s) and Pearson's linear regressions were obtained using SigmaStat 3.5 and SigmaPlot 10.0 (Systat Software).

RESULTS

Algal biomass

As observed in previous studies from the Canadian Arctic, ice algal biomass increased from March until early May and decreased in June (Fig. 2). The early bloom period lasted from 18 March to 19 April, with chl *a* concentrations ranging from <0.01 to 14 mg m^{-2}, followed by the peak bloom period from 26 April to 16 May (10 to 100 mg chl *a* m^{-2}). The post-bloom period took place in the second half of May and in June, with much lower concentrations of chl *a*, reaching a minimum of 0.02 mg m^{-2} at station F8. Maximum chl *a* concentrations found in this study were up to 3 times higher than what has been observed in the past (30-31 mg m^{-2}; Różańska et al. 2009). These high values have been attributed to enhanced under-ice nutrient availability due to wind-driven upwelling events (Tremblay et al. 2011). During the early bloom period, chl *a* concentrations were significantly higher for the low snow (mean ± SD: 4.7 ± 4.9 mg m^{-2}) than for the mid (1.2 ± 2.2 mg m^{-2}) or high (1.7 ± 2.8 mg m^{-2}) snow cover conditions (Kruskal-Wallis test, $p < 0.05$). However, no significant difference in chl *a* between snow cover conditions was observed during the peak bloom period (Kruskal-Wallis test, $p > 0.05$).

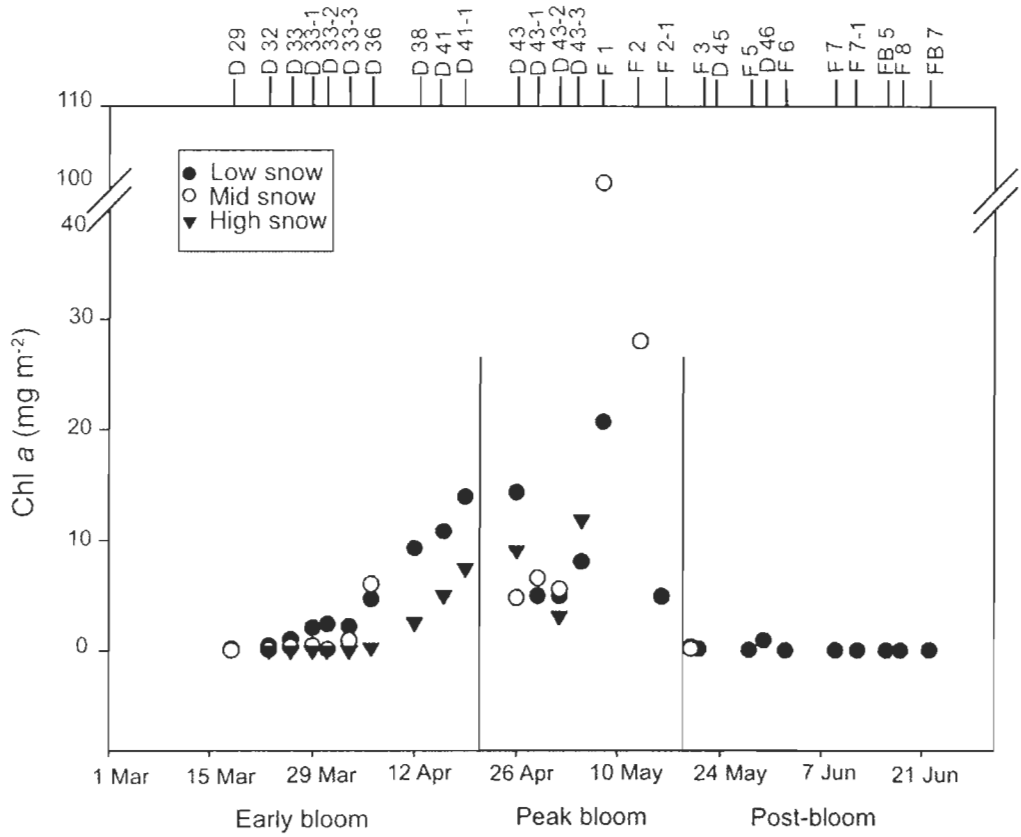

Fig. 2. Temporal change in chlorophyll *a* (chl *a*) concentration during the 3 bloom periods under low, mid and high snow cover conditions. Sampling stations shown in Fig. 1 are indicated at the top of this figure

Light and chemical variables

Incident daily PAR increased seasonally, from 20.6 mol photons $m^{-2} d^{-1}$ on 1 April, to 66.9 mol photons $m^{-2} d^{-1}$ on 18 June (Fig. 3a). Bottom ice irradiance (E_b) differed among the snow cover conditions during the early bloom (Fig. 3b), with significantly higher values for low snow (E_b and $\%E_b$ [mean ± SD] = 25.6 ± 6.2 μmol photons $m^{-2} s^{-1}$, 11.2 ± 3.6%, respectively) than for mid (9.2 ± 3.3 μmol photons $m^{-2} s^{-1}$, 4.5 ± 1.6%) or high snow cover conditions (3.7 ± 4.1 μmol photons $m^{-2} s^{-1}$, 1.0 ± 1.1%) during the early bloom (Kruskal-Wallis test, $p < 0.05$). During the peak bloom, after the removal of an outlier (station F2, assumed to be a measurement error), E_b showed less variability between snow cover conditions. However it was still significantly higher under low snow (43.4 ± 32.6 μmol photons $m^{-2} s^{-1}$, 4.0 ± 3.1%) than under mid (13.2 ± 5.5 μmol photons $m^{-2} s^{-1}$, 1.2 ± 0.4%) or high snow cover conditions (7.0± 2.0 μmol photons $m^{-2} s^{-1}$, 0.7 ± 0.3%, Wilcoxon's signed-rank tests, $p < 0.05$). During the post-bloom, E_b increased to a maximum value of 153.1 μmol photons $m^{-2} s^{-1}$ (12.3%) observed at station F6. Unfortunately, sub-ice irradiances (E_s) used to estimate E_b were not measured after 2 June and high and mid snow conditions had all but disappeared by that time, due to snow melting.

The average sea ice thickness was about 1.3 m (Fig. 3c) and it did not differ significantly among the bloom periods, although it decreased during the post-bloom, reaching a minimum on 30 May (0.8 m, station D46). No significant difference in sea ice thickness was observed between the different snow cover conditions during the early bloom and peak bloom periods (Kruskal-Wallis tests, $p > 0.05$).

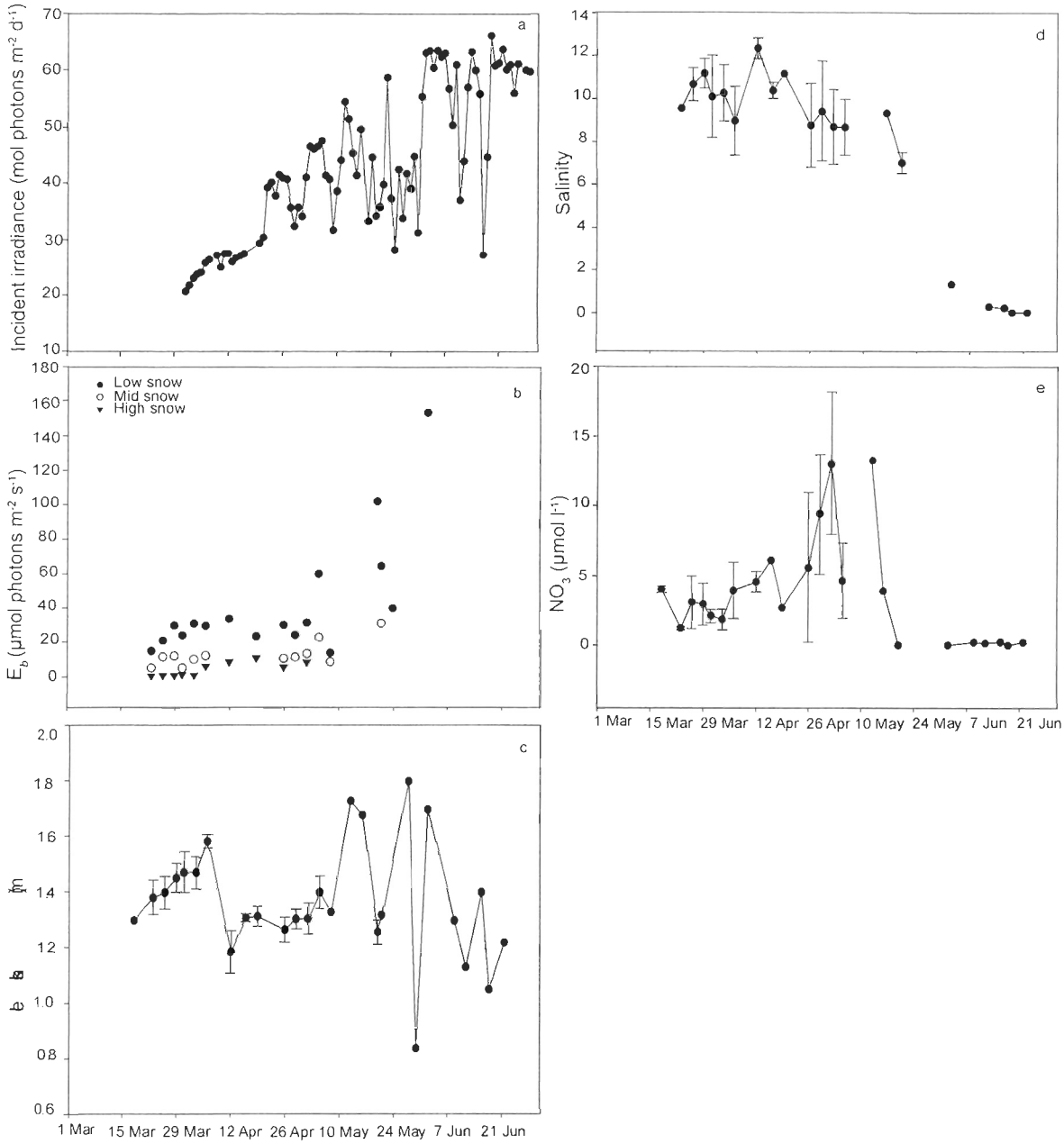

Fig. 3. Temporal changes in (a) daily incident irradiance, (b) bottom ice irradiance (E_b) under the various snow cover conditions, (c) ice thickness, (d) bottom ice bulk salinity, and (e) bottom ice nitrate (NO_3) concentration from March to June 2008. In panels c, d and e, mean values (± SD) are shown

Bottom bulk ice salinity (Fig. 3d) averaged (± SD) 10.4 ± 1.3 for the early bloom and 9.0 ± 1.5 for the peak bloom, remaining relatively constant during these two periods, but decreased to a value of 3.0 ± 3.2 during post-bloom (Kruskal-Wallis test, $p < 0.05$). There were no significant differences in salinity among the snow cover conditions for the early bloom and peak bloom periods.

Over the entire study, the concentrations of NO_3 ranged from <0.05 to 18.9 μmol l^{-1} (Fig. 3e), $Si(OH)_4$ from <0.2 to 22.5 μmol l^{-1}, and PO_4 from <0.02 to 4.2 μmol l^{-1}. There were no significant differences among snow cover conditions, except for PO_4 during the early bloom, which showed greater concentrations under low snow cover (data not shown, Kruskal-Wallis test, $p < 0.05$). NO_3 averaged (± SD) 3.1 ± 1.6 μmol l^{-1} for the early bloom, 8.4 ± 5.3 μmol l^{-1} during the peak bloom (increase due to local upwelling: Tremblay et al. 2011) and 0.1 ± 0.1 μmol l^{-1} during the post-bloom period. $Si(OH)_4$, PO_4, and NO_3 concentrations were all significantly lower during post-bloom (Mann-Whitney *U*-test, $p < 0.01$). The NO_3:PO_4 and NO_3:$Si(OH)_4$ molar ratios decreased throughout the study and were lower than the critical values of 16 for NO_3:PO_4 (Redfield et al. 1963) and of 1.1 for NO_3:$Si(OH)_4$ (Brzezinski 1985), suggesting that nitrogen was the limiting nutrient for algal growth.

Pigment composition and distribution

The pigments determined with HPLC and used in the initial matrix for the CHEMTAX analyses, included the chlorophylls *a*, *b*, c_1, c_2, c_3, Mg-2,4-divinyl pheoporphyrin a_5 monomethyl ester (MgDVP) and the carotenoids peridinin, fucoxanthin, neoxanthin, violaxanthin, alloxanthin, lutein and zeaxanthin. Pigments representing < 0.01 mg m^{-2} or having sporadic presence were not considered.

The relative concentration of the major marker pigments is presented in Fig. 4. Fucoxanthin dominates, with 61 and 65% of the sum of accessory pigments during the

early and peak bloom periods, respectively. The temporal distribution of fucoxanthin displayed the same trend as chl *a*, with which it was strongly correlated ($r_s = 0.99$, $p < 0.0001$, $n = 27$). This suggests a high contribution of diatoms to the total algal biomass, which was confirmed by microscopy and the CHEMTAX analyses (see following subsection). Chl c_2 was the pigment with the next highest relative concentration (12 to 16%; Fig. 4). During post-bloom, the decrease in relative concentrations of fucoxanthin and chl c_2 was accompanied by an increase in other accessory pigments such as chl *b* (present in chlorophytes and prasinophytes) and peridinin (present in some dinoflagellates). We did not detect any pigments specific to haptophytes (such as 19'-hexanoyloxyfucoxanthin). Microscopic observations confirmed that this group was scarcely present. Therefore, we attributed the presence of fucoxanthin to diatoms throughout our study.

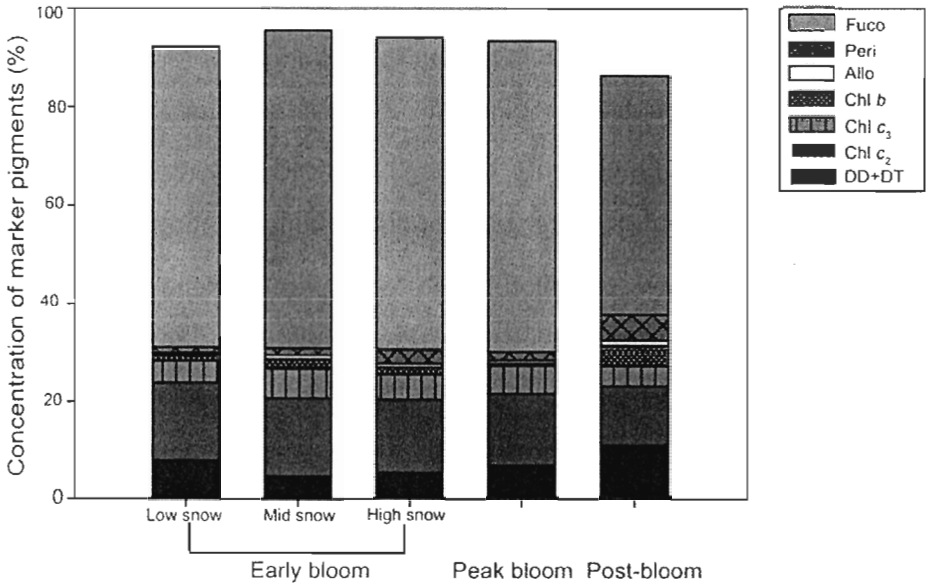

Fig. 4. Mean relative concentration of the main marker pigments to total accessory pigments (wt:wt) under different snow cover conditions during the early bloom and under all snow covers during the peak bloom and the post-bloom period. The coefficients of variation for individual pigments ranged between 2 and 36%. DD+DT: sum of diadinoxanthin and diatoxanthin . See Table 1 for pigment abbreviations

Relative contribution of taxonomic groups (CHEMTAX and microscopy)

An example of the initial marker pigment to chl *a* ratios (wt:wt) for the various algal groups considered and of the final ratios after optimization by CHEMTAX is presented in Tables 1 & 2 (see Supplement 1 for remaining ratios). These ratios were used to calculate the relative contribution of each sea ice algal group to total chl *a* (Figs. 5a,b,c & 6a,b). Some of these groups (e.g., diatoms, dinoflagellates) possess several pigment-based algal types; the most recent description of these can be found in Jeffrey et al. (2011). In general the final pigment ratios differed little from the starting literature values and were within the ranges of these values with only a few exceptions. During the early bloom, for the mid snow cover conditions, the ratio of alloxanthin to chl *a* for cryptophytes decreased from 0.253 (initial ratio) to 0.061 (final CHEMTAX ratio), this value being somewhat lower than the minimum value found in the literature (0.104, Higgins et al. 2011). For the same period and snow cover conditions, the chl *b* attributed to prasinophytes Type 2 showed an increase in its ratio to chl *a* from 0.786 to 1.116. This last value is only slightly higher than that reported by Rodríguez et al. (2002) for the prasinophyte *Pyramimonas* spp. (0.977). During the peak bloom period, the ratio of fucoxanthin to chl *a* for diatoms Type 2 increased from 1.1 to 1.5, close to the maximum value found in Suzuki et al. (2002) (1.4) or in Higgins et al. (2011) (1.3).

Most of the CHEMTAX-identified diatoms belonged to pigment Type 2 (Higgins et al. 2011), which contains fucoxanthin and chl c_2 and c_3. These were likely associated with pennate diatoms as observed microscopically. Cryptophytes (with alloxanthin as diagnostic pigment) were present according to CHEMTAX and also observed microscopically (*Rhodomonas baltica*). Peridinin-containing dinoflagellates (labeled Type 1 in Higgins et al. 2011) likely included the genus *Amphidinium*, also observed during the present study. The prasinophytes present (designated Type 2 in the present study and Type 2A in Higgins et al. 2011) lacked prasinoxanthin, and may have included species from the genus *Pyramimonas*, which were observed by microscopy in the present study, as in Różańska et al. (2009).

Table 1. Example of initial pigment to chlorophyll (chl) *a* ratios for each algal group encountered in the sea ice as used for CHEMTAX calculations and final ratios after optimization by CHEMTAX. Matrices are shown here for the low snow cover conditions during early bloom. β,β-carotene was not included in the CHEMTAX analyses since it is ambiguous as a biomarker (present in several algal groups). MgDVP: Mg-2,4-divinyl pheoporphyrin a_5 monomethyl ester; Peri: peridinin; Fuco: fucoxanthin; Neo: neoxanthin; Viola: violaxanthin; Allo: alloxanthin; Prasino2: prasinophytes Type 2; Dino1: dinoflagellates Type 1; Crypto: cryptophytes; Chloro: chlorophytes; Diatom2: diatoms Type 2

	Chl c_3	Chl c_2	Chl c_1	MgDVP	Peri	Fuco	Neo	Viola	Allo	Chl *b*	Chl *a*
Initial ratio matrix- early bloom low snow cover											
Prasino2	0	0		0.023	0	0	0.056	0.055	0	0.786	1
Dino1	0	0.162		0.004	0.675	0	0	0	0	0	1
Crypto	0	0.204		0	0	0	0	0	0.253	0	1
Diatom2	0.066	0.299		0	0	0.755	0	0	0	0	1
Output ratio matrix- early bloom low snow cover											
Prasino2	0	0		0.025	0	0	0.048	0.067	0	0.532	1
Dino1	0	0.138		0.004	0.467	0	0	0	0	0	1
Crypto	0	0.173		0	0	0	0	0	0.129	0	1
Diatom2	0.032	0.109		0	0	0.504	0	0	0	0	1

Table 2. Example of initial pigment to chlorophyll (chl) *a* ratios for each algal group encountered in the sea ice as used for CHEMTAX calculations and final ratios after optimization by CHEMTAX. Matrices are shown here for all snow covers during the post-bloom. As in Table 1, β,β-carotene was not included. Zea: zeaxanthin; Lut: lutein; Chloro: chlorophytes; see Table 1 for other abbreviations

	Chl c_3	Chl c_2	Chl c_1	MgDVP	Peri	Fuco	Neo	Viola	Allo	Zea	Lut	Chl *b*	Chl *a*
Initial ratio matrix- post-bloom													
Prasino2	0	0		0.023	0	0	0.120	0.160	0	0	0.009	0.945	1
Dino1	0	0.300		0.008	1.063	0	0	0	0	0	0	0	1
Crypto	0	0.200		0	0	0	0	0	0.229	0	0	0	1
Chloro	0	0		0	0	0	0.020	0.040	0	0.009	0.203	0.263	1
Diatom2	0.066	0.299		0	0	0.755	0	0	0	0	0	0	1
Output ratio matrix- post-bloom													
Prasino2	0	0		0.026	0	0	0.125	0.200	0	0	0.009	0.810	1
Dino1	0	0.350		0.010	1.352	0	0	0	0	0	0	0	1
Crypto	0	0.183		0	0	0	0	0	0.223	0	0	0	1
Chloro	0	0		0	0	0	0.018	0.050	0	0.010	0.176	0.093	1
Diatom2	0.068	0.116		0	0	0.682	0	0	0	0	0	0	1

Relative contributions of the various algal groups showed that diatoms were already dominant in early bloom under all snow cover conditions, ranging from 40 to 90% for both CHEMTAX (Fig. 5a,b,c) and microscopy (Fig. 5d). Cryptophytes were the second major group (2 to 37%), followed by generally smaller contributions from prasinophytes (2 to 29%) and dinoflagellates (1 to 12%), according to CHEMTAX. For microscopy, groups other than diatoms represented from 1 to 32% of the total number of cells (Fig. 5d).

Relative contributions of the various algal groups resulting from CHEMTAX showed some differences among the 3 snow cover conditions (Fig. 5a,b). For the group of stations D32 to D36 (studied under the 3 snow cover conditions), we observed a higher percentage of prasinophytes (5 ± 12%) and cryptophytes (19 ± 17%) under high snow conditions than for low (prasinophytes: 3 ± 2%, cryptophytes: 8 ± 10%) or mid snow cover conditions (prasinophytes: 3 ± 1%, cryptophytes: 13 ± 7%), respectively. However these differences were not significant (Kruskal-Wallis test, $p > 0.05$). Only the percentage of diatoms Type 2 was significantly lower for high snow cover (54 ± 2%) than for low (83 ± 14%) or mid (78 ± 3%) snow cover conditions (Kruskal-Wallis test, $p < 0.05$). The communities present under the high snow cover were also distinguished by the absence of dinoflagellates, the presence of chl *b*-containing chlorophytes, matching with *Dunaliella* spp. (observed by microscopy) and the presence of a second type of diatoms (Type 1; Higgins et al. 2011), characterized by the presence of chl c_1. This second type was likely associated with the observed presence of the centric diatom genera *Chaetoceros* and *Thalassiosira*, which are more typical of the phytoplankton community in the Arctic (Poulin et al. 2011).

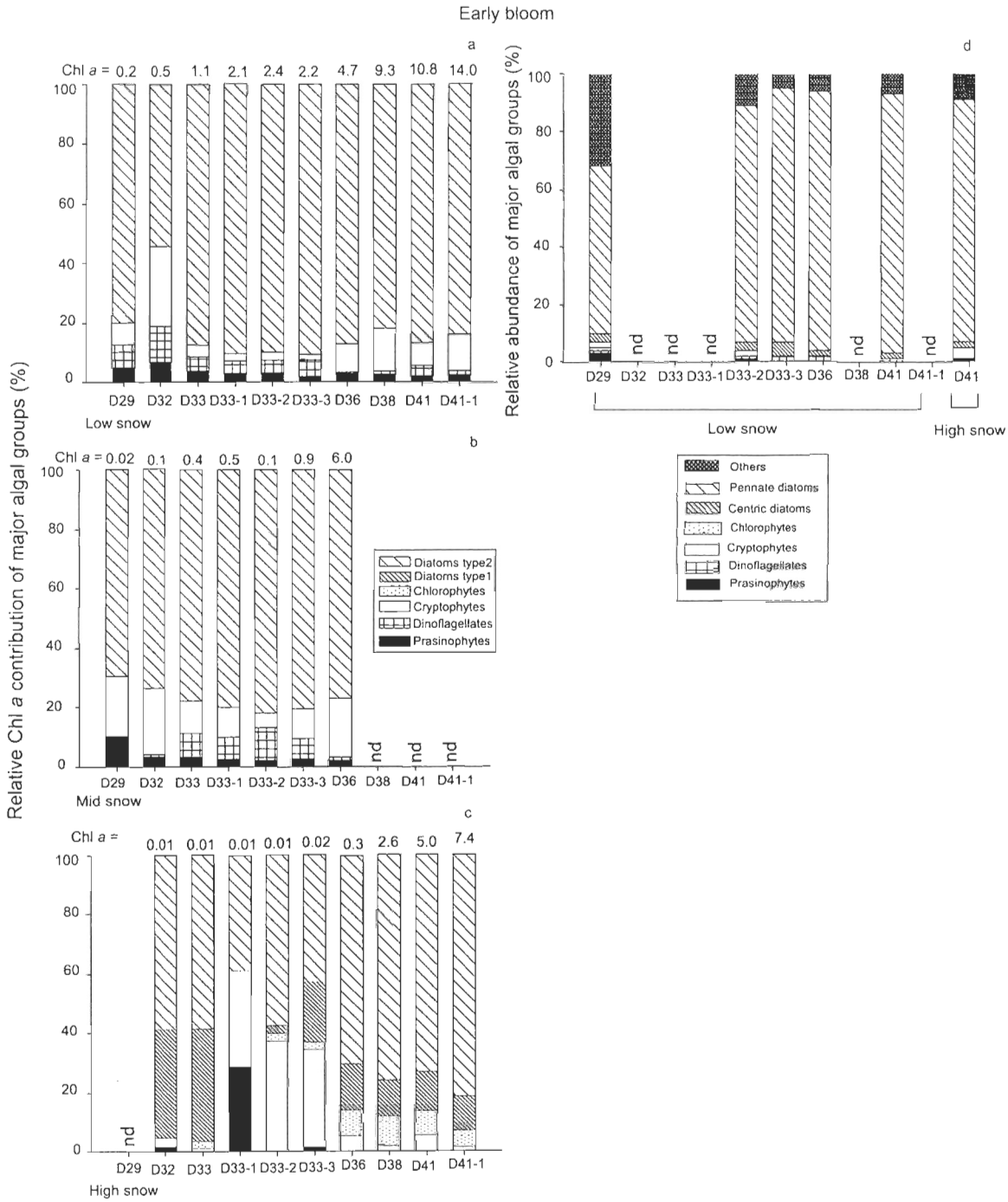

Fig. 5. (a-c) Contribution of major algal groups to total chlorophyll *a* concentrations (CHEMTAX analysis) during early bloom (18 March to 19 April) under (a) low, (b) mid and (c) high snow cover conditions. Chlorophyll *a* concentration (mg m^{-2}) for each station is shown at the top of the bars. (d) Relative abundance (cell counts) of major algal groups to the total community from the microscopy analysis, under low and high snow

cover conditions. The group called 'Others' includes chrysophytes, dictyochophytes, euglenophytes, prymnesiophytes and unidentified flagellates. nd: no data

During the peak bloom period, the CHEMTAX results indicated that diatoms Types 1 and 2 were still present, but diatoms Type 1 represented < 4% of total chl *a* (Fig. 6a). Diatoms Type 2 continued their domination (87 to 100%) followed by cryptophytes (1 to 8%), with minor contributions from dinoflagellates (1 to 4%) and prasinophytes (<2%). Microscopy confirmed that the bloom was mostly composed of pennate diatoms (Fig. 6c), with species such as *Nitzschia frigida*, *Fossula arctica*, *Navicula arctica*, *Nitzschia promare*, *Navicula* sp., and *Entomoneis* sp., along with a few centric diatoms such as *Attheya septentrionalis*. There were no differences among snow cover conditions during this period.

Community composition was more variable during the post-bloom period, along with a strong decline of the algal biomass. Microscopy results showed a growing importance of groups other than diatoms for this period (average [± SD] for these other groups = 32 ± 19%), mostly due to unidentified flagellates. The relative contribution of diatoms (CHEMTAX) was also significantly lower than in the two previous periods (Kruskal-Wallis test, $p < 0.001$). Dinoflagellates ranged from <1% to 37% and cryptophytes from <1% to 35%, with trends generally opposite to those of dinoflagellates. The maximum contribution of chlorophytes was found at station F7-1 (61%), but this group was absent from stations F6 and F7. Prasinophytes were also present, with a maximum of 23% at station F6 (Fig. 6b).

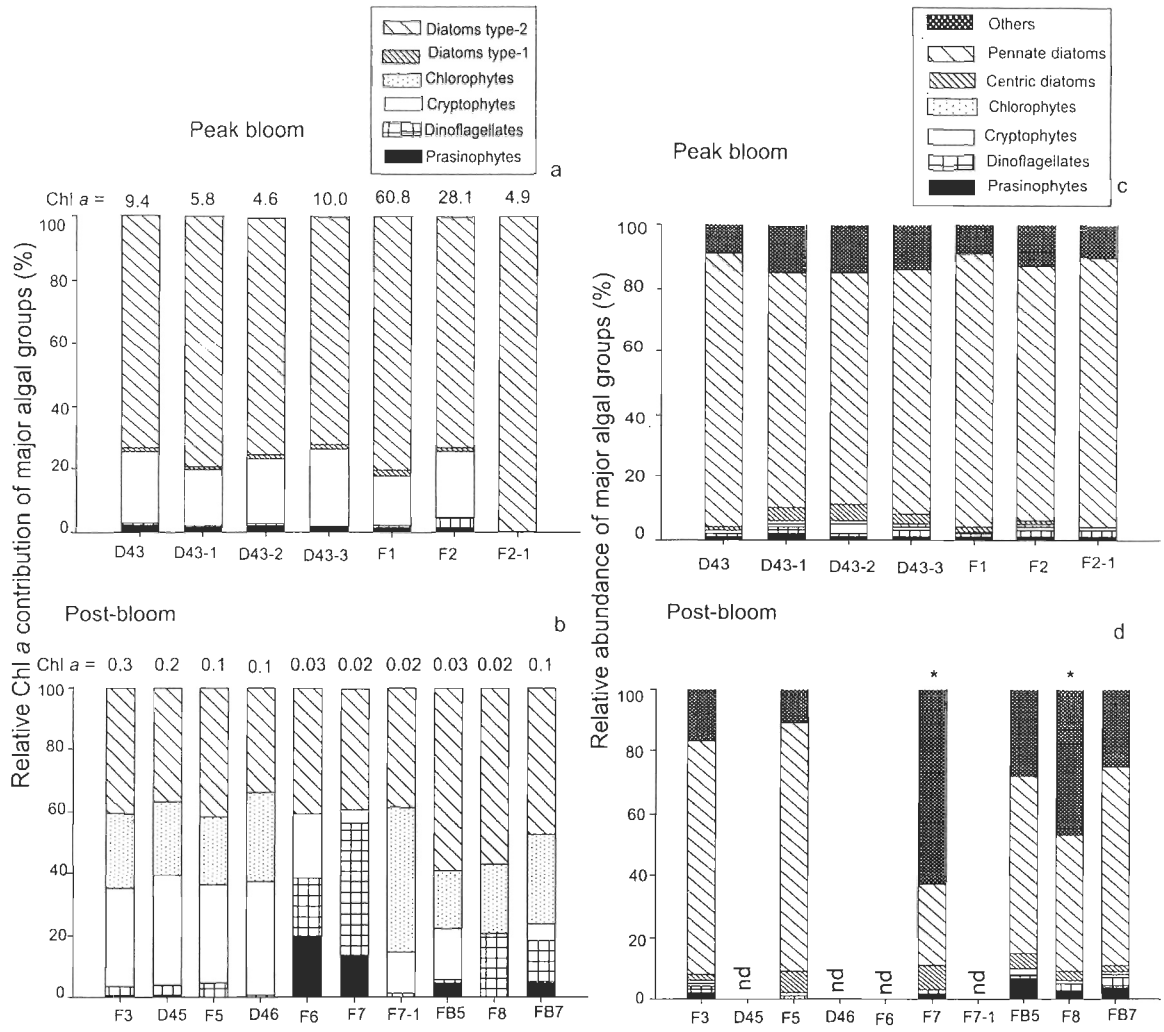

Fig. 6. Same as Fig. 5, but for the peak bloom (a,c) and post-bloom periods (b,d). (a,b) pigment-based contribution of major algal groups to total chlorophyll (chl) *a* concentrations (CHEMTAX analysis). (c,d) relative abundance of major algal groups (microscopy). Chl *a* concentration (mg m^{-2}) for each station is shown at the top of the bars. For stations D43, D43-1, D43-2, D43-3, F1 and F3, chl *a* values are averages over the different snow cover conditions. For microscopy, the group called 'Others' includes chrysophytes, dictyochophytes, euglenophytes, prymnesiophytes and unidentified flagellates. In (d), asterisks above two of the bars indicate that microscopically unidentified flagellates (2-20 μm) represented a large proportion of the total community at those stations; nd: no data

The pigment-based diversity index of the community was lowest at the peak of the bloom and it was highest during the post-bloom period (Table 3). During the early bloom and peak bloom, the communities under the low, mid and high snow cover conditions showed generally similar diversity.

Table 3. Mean (SD in parentheses) pigment-based index of diversity under different snow cover conditions during the early bloom (18 March to 19 April), peak bloom (26 April to 16 May) and post-bloom periods (20 May to 22 June); n: number of observations; n.d.: no data

Stage	Snow cover		
	Low	Mid	High
Early bloom	0.24 (0.09) n = 10	0.20 (0.03) n = 7	0.29 (0.08) n = 9
Peak bloom	0.15 (0.02) n = 6	0.15 (0.02) n = 5	0.18 (0.03) n = 3
Post-bloom	0.40 (0.12) n = 10	0.57 n = 1	n.d.

Photosynthetic (PSC) and photoprotective (PPC) carotenoids

The highest concentrations of chl *a*, PSC (sum of fucoxanthin, peridinin, neoxanthin and alloxanthin) and PPC (sum of diadinoxanthin, diatoxanthin, violaxanthin, zeaxanthin, lutein and β,β-carotene) were observed during the peak bloom period, under mid snow cover conditions at stations F1 near Cape Parry (54.0 mg PSC m^{-2} and 6.4 mg PPC m^{-2}) and F2 in Franklin Bay (13.0 mg PSC m^{-2} and 2.0 mg PPC m^{-2}). All other stations had much lower concentrations. High PPC concentrations were mostly due to the presence of diadinoxanthin (DD), β,β-carotene and diatoxanthin (DT), with an average contribution to total PPC of 57, 23, and 18%, respectively.

The PPC:PSC ratio increased seasonally (Fig. 7a) as downwelling incident irradiance increased (Figs. 3a). Irradiance clearly influenced this response, with a PPC:PSC ratio varying from 0.13 to 0.45 (wt:wt) at stations with daily incident irradiance <50 mol photons m^{-2} and from 0.97 to 3.50 for the stations exposed to higher daily incident irradiance (during post-bloom).

The PPC:PSC ratio was also significantly higher under low snow (mean ± SD: 0.19 ± 0.03, Mann-Whitney *U*-test, $p < 0.05$) than under mid or high snow cover conditions during the early bloom. This was also the case during the peak bloom period (low snow cover condition: 0.20 ± 0.02, Mann-Whitney *U*-test, $p < 0.01$), indicating a response to the (also significantly) higher transmitted irradiance under low snow cover conditions during these 2 periods (Fig. 3b).

Similar trends were observed with the main diatom xanthophyll cycle pigments (DD+DT). The (DD+DT):chl *a* ratio increased over time (Fig. 7b), paralleling the seasonal increase in daily incident irradiance ($r_s = 0.70$, $p < 0.001$, Fig. 3a). Values were significantly higher (Mann-Whitney *U*-test, $p < 0.001$) for stations with daily incident irradiance >50 mol photons m^{-2}, with a maximum (DD+DT):chl *a* value of 0.74 for station F8. As for PPC:PSC, there was no difference in (DD+DT):chl *a* between the mid or high snow cover conditions, but this ratio was significantly higher under low snow (Mann-Whitney *U*-tests, $p < 0.01$, after removal of two outliers at stations D33-1 and D33-2, which had concentrations of chl *a* and DT ≤ 0.01 mg m^{-2}). The increase in irradiance between high and mid snow cover conditions was likely insufficient to elicit a response in terms of photoprotective pigments. The level of deepoxidation or relative fraction of DT, i.e., DT:(DD+DT), showed relatively constant values ranging from 0.16 to 0.26 with no significant differences across snow cover conditions (Kruskal-Wallis test, $p = 0.19$) or bloom periods (Kruskal-Wallis test, $p = 0.10$).

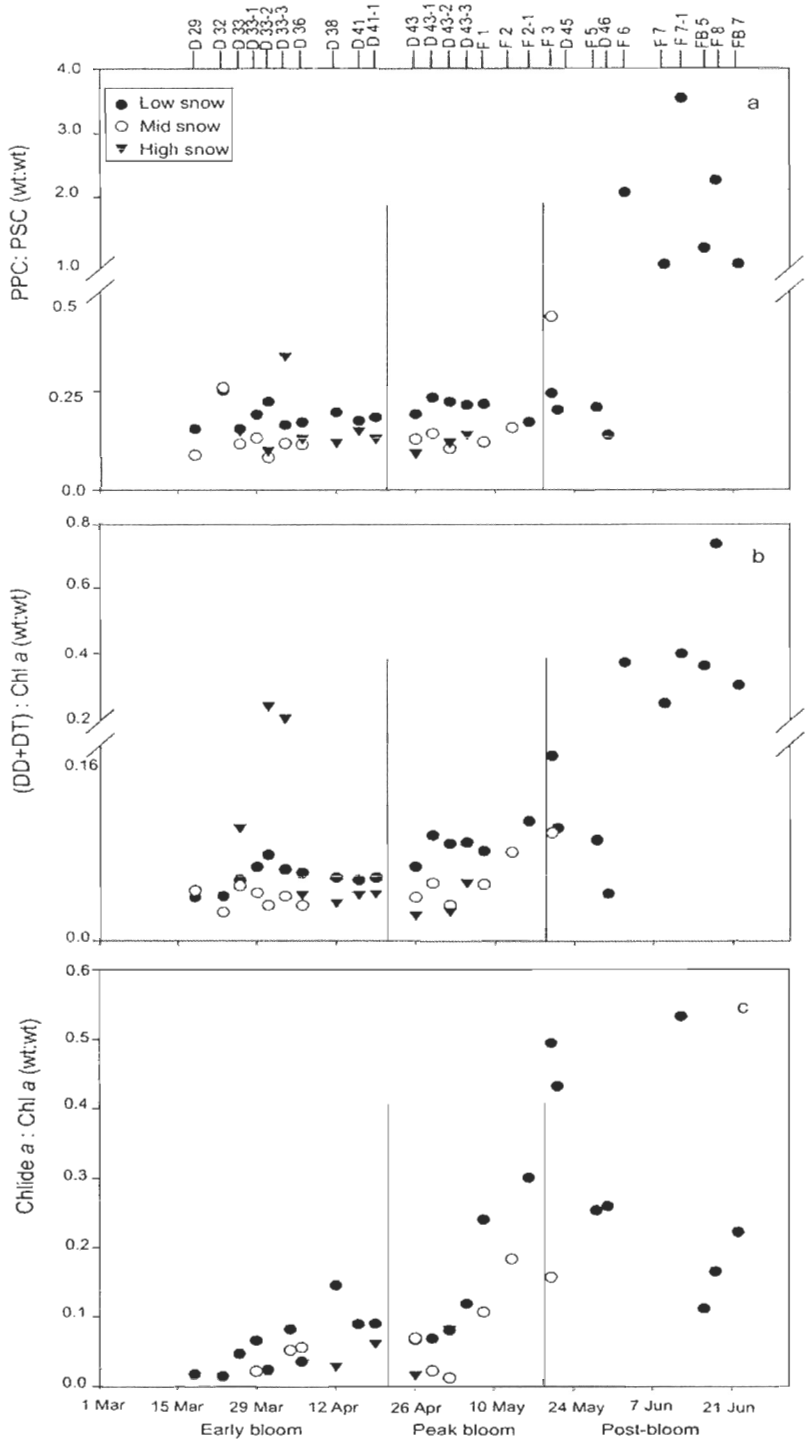

Fig. 7. Temporal changes in the ratios of (a) photoprotective (PPC) to photosynthetic carotenoids (PSC), (b) sum of diadinoxanthin and diatoxanthin (DD+DT) to chlorophyll *a* (chl *a*), and (c) chlorophyllide *a* (chlide *a*) to chl *a* under low, mid and high snow cover conditions. Sampling stations are indicated at the top of the upper panel

Degradation pigments

We identified 6 chlorophyll degradation products: chl *a* allomer and epimer, chlorophyllide *a*, monovinyl-chlorophyllide *a*, pheophorbide *a* and pheophytin *a*. Chl *a* allomers and epimers were at their minimum concentrations during the early bloom and peak bloom periods and they increased during the post-bloom. The sum of chl *a* allomer and epimer generally made up <15% of chl *a*, except for stations D32, D33, D45, F3, and F5 which had greater values (maximum of 38% for station D32). The ratio of chl *a* allomer to chl *a* was significantly higher during the post-bloom (mean ± SD: 0.15 ± 0.06) compared to the early bloom (0.07 ± 0.03; Kruskal-Wallis test, $p < 0.05$). Chlorophyllide *a* (chlide *a*), which is frequently observed in senescent cells (Llewellyn et al. 2008), followed a similar trend to the allomers when normalized to chl *a*, with significantly higher values during the post-bloom period (Fig. 7c, Kruskal-Wallis test, $p < 0.05$).

Differences were also noted according to snow cover conditions. During the early bloom and the peak bloom periods, concentrations of chlide *a* were generally higher under low snow cover conditions. For low snow cover conditions, the chlide *a*:chl *a* ratio was correlated with daily incident irradiance ($r_s = 0.50$, $p < 0.05$). The chl *a* allomer:chl *a* ratio also increased significantly with daily incident irradiance for low snow cover sites throughout all three bloom periods ($r_s = 0.72$, $p < 0.01$).

The increase of the chlide *a*:chl *a* ratio was accompanied by a greater percentage of large empty diatom cells during the post-bloom (Table 4; significant correlation between these variables: $r_s = 0.98$, $p < 0.05$). Although there was no significant relationship between these two variables during the early or peak bloom periods, both increased over time.

Table 4. Mean (SD in parentheses) abundance of empty diatom cells relative to total number of cells and ratio of chlorophyllide *a* (chlide *a*) to chlorophyll *a* (chl *a*) during the early bloom (18 March to 19 April), peak bloom (26 April to 16 May) and post-bloom periods (20 May to 22 June); n: number of observations

Stage	**Empty diatoms (%)**	**Chlide *a*:Chl *a* (wt:wt)**
Early bloom	11.5 (5.2)	0.07 (0.05)
	n = 6	n = 5
Peak bloom	16.2 (9.2)	0.20 (0.10)
	n = 10	n = 10
Post-bloom	36.2 (21.4)	0.33 (0.19)
	n = 5	n = 5

Pheophytin *a* was only present at 3 stations during the bloom period (maximum concentration: 4.1 mg m^{-2}). Pheophorbide *a* was more frequently observed, with a maximum pheophorbide *a*:chl *a* ratio of 0.97 (wt:wt), found at station D45 during the post-bloom. There were no significant differences for the pheophorbide *a*:chl *a* ratio throughout the different study periods or between different snow cover conditions. Although pheophorbide *a* and chlide *a* have been reported in association with senescent cells, there was no significant correlation between these pigments in the present study.

DISCUSSION

The present study showed that, aside from changes in algal composition that took place during the progression of the sea ice algal bloom during spring in the Arctic, sites that differed in terms of snow cover conditions also showed differences in terms of community composition as well as photoprotection, which highlights the important role of light in this environment.

Community composition

Diatoms dominated the sea ice algal bloom in both pigment and microscopic results, in agreement with past studies in the Arctic with pennate diatoms contributing on average 68% of the abundance of sea ice eukaryote taxa in the Canadian Arctic (Poulin et al. 2011). The largest changes in the community composition occurred during the post-bloom, with increases in chlorophytes, prasinophytes, and dinoflagellates and a decrease in diatoms Type 2. However, this decrease was markedly observed only in a subset of stations (F6, F7, FB5, F8 and FB7). Since most of these stations were sampled near the end of the sampling (in June), they likely represent a later stage of the post-bloom.

The species succession described above is similar to that from other studies describing the Arctic bottom ice community. In general the community composition is characterized by the presence early in the season of flagellates along with a few solitary diatom species. As the season progresses the species composition changes to colony-forming pennate diatoms (such as *Nitzschia frigida*, *Navicula pelagica* and *Fragilariopsis*

cylindrus) which dominate the community during the spring bloom period (Różańska et al. 2009). By the end of the bloom, in summer, diatoms are replaced with flagellates (Różańska et al. 2009, Mundy et al. 2011). In broad terms, this species succession is similar to the general pattern seen for pelagic environments (Margalef 1978).

Differences in community composition were observed for the various snow cover conditions, particularly in early bloom. These are thought to reflect different stages in the succession of species, influenced by the amount of light that reaches the bottom of the ice layer. The low irradiance high snow cover conditions likely reflect an early stage of that succession, bearing some similarity with the low light conditions of early spring in that environment. Sites with these characteristics showed the presence of small flagellates such as prasinophytes and cryptophytes along with chlorophytes and one type of diatoms (Type 1) which gradually disappeared later on, whereas high-irradiance, low-snow cover sites were characterized by a significantly greater proportion of diatoms Type 2 (pennate diatoms). These observations match those from the study by Różańska et al. (2009) who showed a greater abundance of small flagellates under high snow conditions in the early bloom period (earlier in fact than the start of sampling during the present study), and more diatoms under low snow cover conditions. With the seasonal increase in irradiance in the present study (or a decrease in the snow cover during the early bloom and peak bloom periods), the community composition changed to pennate diatoms, followed by a gradual increase of dinoflagellates.

Photoprotective response

Sea ice algae cannot avoid exposure to excess irradiance by vertical displacement, as can, in a limited way, phytoplankton in the water column. Cells that perform in such an environment must be able to acclimate to the changes in irradiance (Petrou et al. 2011). Sudden changes can have major negative effects, as observed when the entire snow layer above the ice is removed experimentally, resulting in a decrease in algal biomass and growth rate (Juhl & Krembs 2010). In the present study, we examined *in situ* acclimation to the changing irradiances associated with different snow cover conditions. Our results show that, although generally dominant in sea ice, diatoms were favoured under low-

snow, higher relative irradiance conditions. This is not surprising as this algal group is recognized for its photoprotective performance, enabling cells to support wide variations in irradiance (Lavaud et al. 2004), including sea ice algae in Antarctica (Petrou et al. 2011). Our results show that the pool size of the xanthophyll-cycle pigments ([DD+DT]:chl *a*) or of all photoprotective pigments (PPC:chl *a* and PPC:PSC) increased significantly with increasing irradiance and was significantly higher under low snow cover. This response was observed with bottom ice irradiances as low as 26 μmol photons m^{-2} s^{-1} (early bloom, low snow conditions - compare with values less than 10 μmol photons m^{-2} s^{-1} for mid or high snow cover conditions at that time). There was little difference in photoprotective pigments between high and mid snow cover conditions, suggesting that the increase in irradiance observed under the mid snow cover conditions relative to high snow was not strong enough to elicit a photoprotective response in terms of pigments (high snow and mid snow cover bottom ice irradiances were respectively 4 and 9 μmol photons m^{-2} s^{-1} in early bloom, and 7 and 13 μmol photons m^{-2} s^{-1} during peak bloom). Maximum values of (DD+DT):chl *a* were in the same range as for other polar regions (Kropuenske et al. 2009, Petrou et al. 2011), reaching a value of 0.40 (wt:wt) when irradiances were high. Values > 0.40 have been suggested to occur during nutrient limitation for the Antarctic diatom, *Chaetoceros brevis* (van de Poll et al. 2005, van de Poll & Buma 2009). The highest values of (DD+DT):chl *a* observed here were during post-bloom, and were also likely associated with nutrient limitation since nitrate concentrations were near zero during this period.

The fact that significantly greater concentrations of photoprotective pigments were found under low snow cover conditions further suggests that there was enough time for photoacclimation to take place and that it varied on small spatial scales since the different snow cover sites were within close proximity at each station. This implies that snow cover conditions remained relatively stable for several days. Juhl & Krembs (2010) estimated that the minimum acclimation time required by ice algae was of the order of 3 to 6 days, and, indeed, the best relationship that we observed between the PPC:PSC ratio and the daily bottom ice irradiance was when irradiance was averaged over the preceding 3 days (Fig. 8).

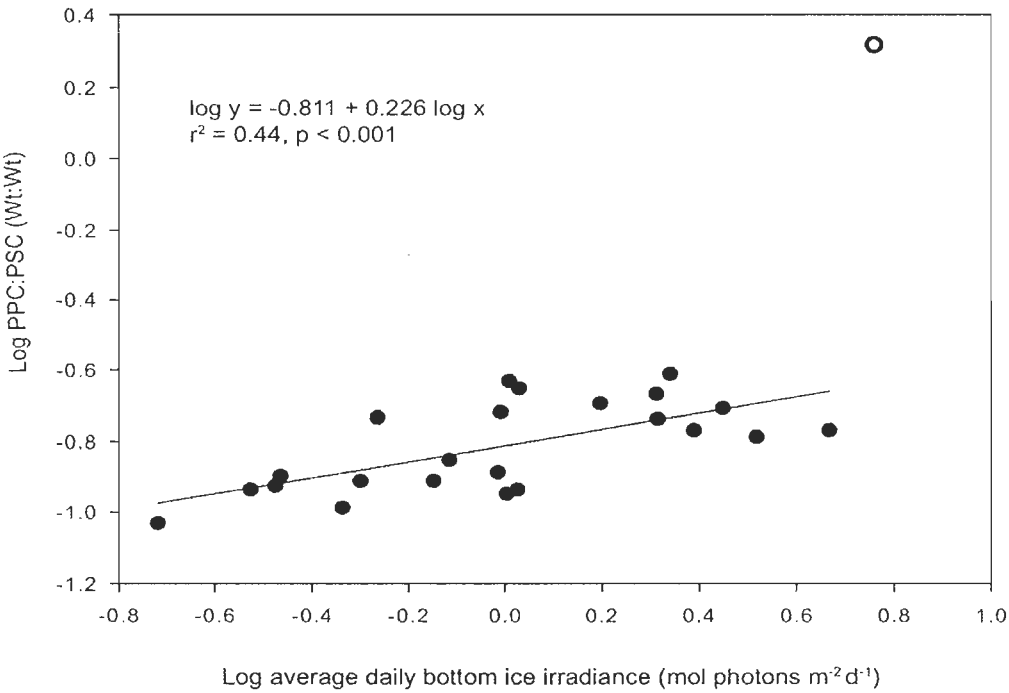

Fig. 8. Relationship between the ratio of photoprotective (PPC) to photosynthetic carotenoids (PSC) and average daily bottom ice irradiance (averaged over the preceding 3 days). One data point (white circle, station F6) was omitted to compute the regression. Note the log scale

The xanthophyll cycle is characterized by a fast (a few minutes) and a slow (acclimation) component (van de Poll & Buma 2009). Changes in the pool size (as above) belong to the latter. The fast component refers to the deepoxidation of diadinoxanthin into diatoxanthin. However, the usual sampling method by which sea ice is slowly melted in filtered seawater in darkness (>15 h), gives ample time for the back transformation of DT to DD. Indeed, there were no significant differences among snow covers for the deepoxidation index, DT:(DD+DT). The fact that this ratio remained at relatively high values (0.16 to 0.26) even though cells stayed in the dark for several hours during ice melting, suggests that chlororespiration took place, inducing a proton gradient across the thylakoid membrane which favours the accumulation of DT (Jakob et al. 1999).

Physiological status of the cells

The physiological status of the cells (based on degradation products of chl *a*) differed according to the snow cover conditions during the early bloom and peak bloom periods. It also showed clear deterioration during the post-bloom period in late May to June. During the early bloom and peak bloom periods, pigments generally indicative of a deteriorating physiological condition (such as chlide *a* or the allomer of chl *a*) showed an increase under low snow cover conditions (higher relative irradiances) compared to the other snow covers. However, this was accompanied by increases in chl *a* biomass and in photoprotective pigments, suggesting rapid algal multiplication and adequate photoprotection rather than poor physiological health. Excess irradiance can harm cells but small increases can be beneficial, as observed by Juhl & Krembs (2010) when only thin layers of snow were removed from the ice surface. Nutrient concentrations were low during early bloom (e.g. 2.0 to 3.7 μmol l^{-1} for nitrate) but not depleted, as found later during post-bloom. Hence, the presence of these chl *a* degradation pigments was perhaps related to the death and replacement of photosensitive species with phototolerant ones that were better photoprotected and growing faster (pennate diatoms). Other studies also observed the presence of chlide *a* during algal blooms, and various explanations have been proposed (e.g. cell autolysis; Llewellyn et al. 2008). Alternatively, changes in community composition under low snow cover could have favoured species showing high concentrations of chlide *a*, as seen for certain pennate diatoms (Quijano-Scheggia et

al. 2008). In such a case, the presence of this pigment would be associated with a change in species rather than with poor physiological health.

During the post-bloom period, there were several indications of cell senescence as the diatom bloom declined, including the significant relationship of the chlide *a*:chl *a* ratio with the abundance of empty diatoms (Llewellyn et al. 2008). Algal senescence was likely related to nutrient depletion during post-bloom and possibly associated with high irradiances. The group of late-sampled stations mentioned above (F6, F7, FB5, F8, and FB7) showed fewer signs of senescence accompanied by strong photoprotection, suggesting that a transition had occurred to a community better acclimated to the low nutrient, high irradiance conditions.

The two pheopigments observed in our study (pheophytin *a* and pheophorbide *a*) showed no relationship to the algal senescence markers; therefore we suggest that they were associated more with grazing than with a deteriorated physiological condition (Szymczak-Żyła et al. 2008). Copepod grazers were present in this environment (Wold et al. 2011) and Marquardt et al. (2011) reported the highest abundance of sympagic meiofauna in the bottom 3 cm of the ice during April and May of the same year. There were no significant differences in the concentration of pheophorbide *a* relative to chl *a* between snow cover conditions or between the different periods of the bloom, suggesting that grazing was not affected greatly by irradiance or bloom progression.

CONCLUSIONS

Using information on chemotaxonomic marker pigments in ice algae from the Beaufort Sea, we show that diatoms (pigment Type 2, pennates from microscopic observations) dominate the sea ice algal bloom during spring, followed by seasonal replacement with chlorophytes, prasinophytes and dinoflagellates during the post-bloom period. Snow cover conditions affected community composition only during the early bloom, when the low irradiance high snow cover sites were characterized by the presence of diatoms Type 1 and chlorophytes which were not detected in the other snow cover

sites. Higher relative irradiances under low snow cover conditions favoured the presence of diatoms Type 2 (pennate diatoms). We also observed significant differences in photoprotective pigments among the three snow cover conditions in early bloom, with enhanced photoprotection under low snow cover. This response, and the fact that the ratio of photoprotective to photosynthetic pigments was best correlated with the average bottom ice irradiance over the preceding 3 days, suggests that snow remained in place long enough for photoacclimation to take place. These conditions favoured the growth of sea ice algae, since the chl *a* biomass was greater under low snow cover conditions in the early bloom period. This was also associated with an increase in chl *a* degradation pigments possibly related to the presence of species with particularly abundant chlide *a* (possibly pennate diatoms). This study highlights the important role of light in controlling the algal communities in Arctic sea ice during spring, before the demise of the ice algal bloom. Extrapolation of our results to continued climate warming in the Arctic suggests that the ice algae spring bloom could benefit from a reduction of the snow cover (beneficial influence of light early on) but may be cut short by the shorter ice season.

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Supplementary material

Supplement 1. Initial (before CHEMTAX analysis) and final (optimized after analysis) pigment to chlorophyll *a* ratios for each algal group encountered in the sea ice. Matrices are shown here for the various snow cover conditions during early bloom, peak bloom and post-bloom periods (see main chapter for details). Empty pigment columns indicate that these pigments were absent or that CHEMTAX results were improved by removing some pigments from the analysis. See Table 1 & 2 for pigment abbreviations; RMS: root mean square error.

	Chl c_3	Chl c_2	Chl c_1	MgDVP	Peri	Fuco	Neo	Viola	Allo	Chl *b*	Chl *a*
Initial ratio matrix early bloom low and mid snow cover											
Prasino2	0	0		0.023	0	0	0.056	0.055	0	0.786	1
Dino1	0	0.162		0.004	0.675	0	0	0	0	0	1
Crypto	0	0.204		0	0	0	0	0	0.253	0	1
Diatom2	0.066	0.299		0	0	0.755	0	0	0	0	1
Output ratio matrix early bloom low snow cover RMS=0.022											
Prasino2	0	0		0.025	0	0	0.048	0.067	0	0.532	1
Dino1	0	0.138		0.004	0.467	0	0	0	0	0	1
Crypto	0	0.173		0	0	0	0	0	0.129	0	1
Diatom2	0.032	0.109		0	0	0.504	0	0	0	0	1
Output ratio matrix early bloom mid snow cover RMS=0.033											
Prasino2	0	0		0.027	0	0	0.054	0.055	0	1.116	1
Dino1	0	0.130		0.004	0.592	0	0	0	0	0	1

Crypto	0	0.257	0	0	0	0	0	0.061	0	1
Diatom2	0.073	0.119	0	0	0.646	0	0	0	0	1

	Chl c_3	Chl c_2	Chl c_1	MgDVP	Peri	Fuco	Neo	Viola	Allo	Chl *b*	Chl *a*
Initial ratio matrix early bloom high snow cover											
Prasino2	0		0	0.023		0			0	0.729	1
Crypto	0		0	0		0			0.253	0	1
Chloro	0		0	0		0			0	0.330	1
Diatom2	0.066		0	0		1.100			0	0	1
Diatom1	0		0.110	0		0.722			0	0	1
Output ratio matrix early bloom high snow cover RMS=0.153											
Prasino2	0		0	0.023		0			0	1.02	1
Crypto	0		0	0		0			0.405	0	1
Chloro	0		0	0		0			0	0.306	1
Diatom2	0.067		0	0		1.172			0	0	1
Diatom1	0		0.060	0		0.435			0	0	1

	Chl c_3	Chl c_2	Chl c_1	MgDVP	Peri	Fuco	Neo	Viola	Allo	Chl *b*	Chl *a*
Initial ratio matrix peak bloom											
Prasino2	0	0	0	0.023	0	0			0	0.786	1
Dino 1	0	0.282	0	0.004	0.714	0			0	0	1
Crypto	0	0.170	0	0	0	0			0.278	0	1
Diatom2	0.066	0.299	0	0	0	1.100			0	0	1

Diatom1	0	0.189	0.070	0	0	0.700			0	0	1	
Output ratio matrix peak bloom RMS=0.197												
Prasino2	0	0	0	0.022	0	0			0	0.762	1	
Dino1	0	0.235	0	0.004	0.662	0			0	0	1	
Crypto	0	0.166	0	0	0	0			0.218	0	1	
Diatom2	0.093	0.394	0	0	0	1.523			0	0	1	
Diatom1	0	0.098	0.047	0	0	0.451			0	0	1	

	Chl c_3	Chl c_2	Chl c_1	MgDVP	Peri	Fuco	Neo	Viola	Allo	Zea	Lut	Chl *b*	Chl *a*
Initial ratio matrix post-bloom													
Prasino2	0	0		0.023	0	0	0.120	0.160	0	0	0.009	0.945	1
Dino1	0	0.300		0.008	1.063	0	0	0	0	0	0	0	1
Crypto	0	0.200		0	0	0	0	0	0.229	0	0	0	1
Chloro	0	0		0	0	0	0.020	0.040	0	0.009	0.203	0.263	1
Diatom2	0.066	0.299		0	0	0.755	0	0	0	0	0	0	1
Output ratio matrix post-bloom RMS=0.071													
Prasino2	0	0		0.026	0	0	0.125	0.200	0	0	0.009	0.810	1
Dino1	0	0.350		0.010	1.352	0	0	0	0	0	0	0	1
Crypto	0	0.183		0	0	0	0	0	0.223	0	0	0	1
Chloro	0	0		0	0	0	0.018	0.050	0	0.010	0.176	0.093	1
Diatom2	0.068	0.116		0	0	0.682	0	0	0	0	0	0	1

Supplement 2. Calculation of the bottom ice irradiance

Since sub ice irradiance (E_s in µmol photons m^{-2} s^{-1}) depends not only on the ice and snow but also on the algal biomass present in sea ice, we estimated irradiance reaching the top of the bottom 3 cm algal layer (bottom ice irradiance, E_b in µmol photons m^{-2} s^{-1}) using Beers law:

$$E_b = \frac{E_s}{\exp(-K_d z)} \quad [1]$$

where K_d is the diffuse attenuation coefficient of PAR (m^{-1}) and z is the thickness of the bottom ice algal layer (0.03 m). The diffuse attenuation coefficient of the bottom ice algal layer was calculated using the equation derived in Kirk (1994 and references therein), which has been suggested appropriate for sea ice (Maffione 1998):

$$K_d = \frac{1}{\mu_d}\left[\left(a_{ice} + a_{ph}\right)^2 + 0.238\left(a_{ice} + a_{ph}\right)b_{tot}\right]^{0.5} \quad [2]$$

where μ_d is the average cosine of the downwelling hemisphere of the light field (dimensionless), a_{ice} and a_{ph} are the pure sea ice and algal absorption coefficients (m^{-1}) for the bottom ice algal layer, respectively, and b_{tot} represents the total volume scattering coefficient for the bottom ice (m^{-1}). μ_d was set to 0.7 based on observations and modeling of Ehn et al. (2008b). Pure ice spectral absorption from Warren (1984) was similarly integrated over PAR to obtain a_{ice} = 0.071 m^{-1}. A specific absorption coefficient of 0.006 m^2 mg^{-1} chlorophyll *a* (average of values from SooHoo et al. (1987) and Arrigo et al. (1991)) was used to determine a_{ph} from chlorophyll *a* concentration. Finally, b_{tot} was set to 165 m^{-1}, from previous observations in the same region (Ehn et al. 2008a).

CHAPITRE 3

VIABILITÉ DES CELLULES PHYTOPLANCTONIQUES DANS LES EAUX DE SURFACE DE LA MER DE BEAUFORT (CANADA) PENDANT LA PÉRIODE DE TRANSITION PRINTEMPS-ÉTÉ

Ce troisième article, intitulé "*Phytoplankton cell viability in surface waters of the Canadian Beaufort Sea during the spring-summer transition*" fut corédigé par moi-même, les professeurs Suzanne Roy, Michel Gosselin, et Susana Agustí. La version présentée ici sera soumise au journal *Marine Ecology Progress Series* au cours des prochains mois. En tant que premier auteur, ma contribution à ce travail fut l'essentiel de la recherche sur la détermination de la viabilité cellulaire du phytoplancton, l'élaboration du plan d'échantillonnage, la réalisation des campagnes de terrain, les analyses en laboratoire, le traitement statistique des résultats et la rédaction de l'article. Les professeurs S. Roy, M. Gosselin et S. Agustí ont fourni l'idée originale. Ils ont aidé à la définition de la problématique, à l'élaboration du plan d'échantillonnage ainsi qu'à la révision de l'article.

3.1 RÉSUMÉ

Nous avons examiné la viabilité des cellules phytoplanctoniques (déterminée par la méthode de digestion cellulaire) dans les eaux de surface de la mer de Beaufort (Canada) pendant la période de transition printemps-été et nous avons essayé de la relier aux facteurs environnementaux (éléments nutritifs, lumière) et aux changements dans les communautés phytoplanctoniques (identifiés grâce à la signature pigmentaire). Deux catégories de stations ont été visitées en mai et juin 2008 : les stations ayant un couvert de glace (IC) et exposées à une faible lumière et les stations d'eau ouverte (OW) exposées à une lumière beaucoup plus élevée. Nous avons observé une grande variabilité de la proportion de cellules vivantes par rapport aux cellules totales (% LC). Aucune tendance évidente n'a été trouvée entre la concentration de nitrates (l'élément nutritif probablement limitant dans cet environnement) et le % LC. Cependant, nos résultats suggèrent que la température de l'eau, la lumière et la stratification de la colonne d'eau ont influencé la proportion de cellules vivantes, avec des changements au niveau de la composition de la communauté algale, particulièrement pour les stations IC.

L'augmentation de la lumière a amélioré la viabilité cellulaire sous la glace, mais pour les stations OW, la grande augmentation saisonnière de lumière a été associée à de faibles viabilités cellulaires, avec un déclin de la performance photosynthétique (F_v/F_m), particulièrement aux intensités lumineuses supérieures à 600 µmol photons $m^{-2}s^{-1}$. Pour l'ensemble des stations, il y avait une corrélation significative entre le % LC et la performance photosynthétique (F_v/F_m) pendant toute la période de l'étude. La température de l'eau avait une influence différente pour les deux catégories de stations : pour les stations IC, le % LC a diminué aux températures supérieures à 0°C, tandis que pour des stations OW, il a augmenté, suggérant que la fonte de la glace permettrait le transfert vers l'eau des cellules de glace en mauvais état dans un cas, et que le réchauffement saisonnier favoriserait les communautés présentes dans les eaux ouvertes.

La photoprotection a été examinée en utilisant le ratio entre les pigments caroténoïdes photoprotecteurs et les pigments caroténoïdes photosynthétiques. Ce ratio a augmenté du début du mois de mai jusqu'au début du mois de juillet et il était plus élevé pour les stations OW, caractérisées par des températures, une lumière et une stratification plus élevées. Deux réponses photoprotectrices différentes ont été trouvées dans nos résultats : l'une a été associée à une communauté principalement composée de diatomées (en moyenne 34% du total de la chl *a*) et des valeurs de PPC :PSC < 0.5, tandis que l'autre a été trouvée dans une communauté dominée principalement par les prasinophytes (moyenne de 21%) et dans une moindre mesure par les diatomées (moyenne de 14%) et avec des valeurs de PPC :PSC > 0.5. Un pigment de dégradation de la chlorophylle *a* provisoirement identifié comme «simili»-pyrophéophorbide *a*, a montré une relation négative significative entre sa concentration normalisée par unité de chl *a* et le % LC ansi que F_v/F_m. Ce pigment était positivement corrélé à l'index de stratification, suggérant que les conditions de fonte avaient une influence sur sa répartition et qu'il pourrait donc être utilisé comme un marqueur de la faible viabilité cellulaire des algues de glace relâchées dans les eaux de surface.

Mots-clé : Arctique, viabilité cellulaire, index d'activité photosynthétique, pigments de dégradation

3.2 Phytoplankton cell viability in surface waters of the Canadian Beaufort Sea during the spring-summer transition

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ABSTRACT

We examined phytoplankton cell viability (determined using the cell digestion assay) in surface waters of the Canadian Beaufort Sea during the spring-summer transition, with an aim to understand how the changes in phytoplankton communities (determined using pigment signatures) were influenced by environmental factors (nutrients, irradiance). Two categories of stations were visited in May and June 2008: ice-covered (IC) stations, exposed to low irradiances, and open-water (OW) stations, exposed to higher irradiances. We observed a large variation in living cells relative to total cells (% LC). No obvious trend was found between nitrate concentration (the nutrient likely limiting in this environment) and % LC. Instead, our results suggest that water temperature, irradiance and water column stratification influenced the distribution of living cells, along with changes in the community composition, especially at IC stations.

Increasing the *in situ* irradiance improved cell viability at ice-covered and open-water stations. However, *in situ* irradiances greater than 600 μmol photons m^{-2} s^{-1} were associated with low cell viabililty, along with a decline in photosynthetic performance (F_v/F_m). There was a significant positive correlation between % LC and F_v/F_m throughout the study period when considering all stations. Water temperature had a different influence for the two sets of stations: for IC stations, % LC declined at temperatures above 0°C, whereas for OW stations, it increased, suggesting that ice melting resulted in the release in surface water of unhealthy cells from the bottom ice in one case, and that seasonal warming favored the communities present in open waters.

Photoprotection was examined using the ratio of photoprotective to photosynthetic carotenoid pigments. It increased from the beginning of May up to the beginning of July and was highest at OW stations, characterized by higher temperatures, irradiances and stronger stratification. Two different photoprotective responses were found during this study: one was associated with a community dominated by diatoms (mean: 34% of the total chl *a*) and PPC:PSC values < 0.5, whereas the other was found in a community with a higher contribution of prasinophytes (mean 21%) and lower contribution of diatoms

(mean 14%) as well as PPC:PSC values > 0.5. A chlorophyll degradation pigment tentatively identified as pyropheophorbide *a*-"like" showed a significant negative relationship between its concentration (relative to chlorophyll *a*) and the % LC and F_v/F_m and was positively correlated with the stratification index, suggesting that the melting conditions influenced its distribution and that it may be useful as a marker for low cell viability of ice algae being released in surface waters.

Keywords: Arctic, cell viability, photosynthetic activity index, degradation pigments

INTRODUCTION

Seasonal variations in solar radiation and sea-ice cover have a large influence on primary production in the Arctic (Gosselin et al. 1990, Rysgaard et al. 1999, Mundy et al. 2011). Pelagic production is maximal in the summer months between sea-ice melt in spring and freeze-up in fall (Horner & Schrader 1982, Wang et al. 2005, Tremblay et al. 2012). With the change in climate currently underway, some researchers predict that the annual phytoplankton production in the Arctic Ocean will be enhanced due to the decrease of the minimum summer ice extent that could result in a longer phytoplankton growing season (Arrigo et al. 2008, Zhang et al. 2010). However during summer, the freshwater inputs from rivers and ice melting (Macdonald et al. 2004, Peterson et al. 2006) can lead to strong stratification of the water column, especially if this is enhanced by the increase of river inflows (Peterson et al. 2002), which could lead to a depletion of nutrients in the surface layer (Tremblay & Gagnon 2009) and to the exposure of phytoplankton to higher irradiances. The accelerated changes in the Arctic caused by sea-ice cover decline (Stroeve et al. 2007, Comiso et al. 2008, Overland & Wang 2013) and the rapidity of the melting will influence the new conditions to which cells are exposed.

Recent studies have focused on the responses of the algal community to some of these changes (Li et al. 2009, Tremblay et al. 2012), however there is still much uncertainty about how the algal community will adjust with these ongoing changes. Knowledge about cell viability under these changing conditions could help us understand their influence on the physiology of phytoplankton cells and the dynamics of phytoplankton populations, but it is not frequently determined in field programs and there are few data from the Arctic (Llabrés & Agustí 2008, Lasternas & Agustí 2010, Echeveste et al. 2011). Field studies in regions other than polar environments have highlighted a high proportion of dead cells, especially in surface waters (van Boekel et al. 1992, Hayakawa et al. 2008) and in oligotrophic environments (Agustí 2004, Alonso-Laita et al. 2005, Alonso-Laita & Agustí 2006). Some studies looked for marker compounds that could provide information on cell viability, such as chlorophyll *a* (chl *a*) degradation products that are produced during the processes of senescence and death of

the cells (Spooner et al. 1994, Szymczak-Żyła et al. 2008). These degradation pigments could be useful as markers for the loss of cell viability (Franklin et al. 2012).

The overall objective of this study was to investigate phytoplankton cell viability in the Canadian Beaufort Sea during the spring-summer transition. The specific objectives were to (1) examine the variability in phytoplankton cell viability, specifically over the ice melt period in spring, (2) to identify factors contributing to the loss of viability, if any, such as nutrient depletion or high irradiance, and (3) examine signs of a decline in physiological condition of the algae, such as low photosystem II (PSII) efficiency or an increase in degradation pigments.

MATERIALS AND METHODS

Study site and sampling

This study was conducted in the Canadian Beaufort Sea (Fig. 1), as part of the International Polar Year–Circumpolar Flaw Lead system study (IPY–CFL; Barber et al. 2010), onboard the Canadian Coast Guard Ship (CCGS) *Amundsen*. Sampling was carried out on 73 occasions from 1 May to 13 July 2008. Water samples were collected in ice-covered (IC) and open-water (OW) stations (Fig. 1). First-year drifting sea ice stations (noted "D") were located in the Amundsen Gulf whereas first-year landfast ice stations (noted "F") were situated in Franklin and Darnley bays and at the M'Clure Strait's entrance (northwestern Banks Island) (Fig. 1). After 24 June, Franklin Bay's stations were free of ice.

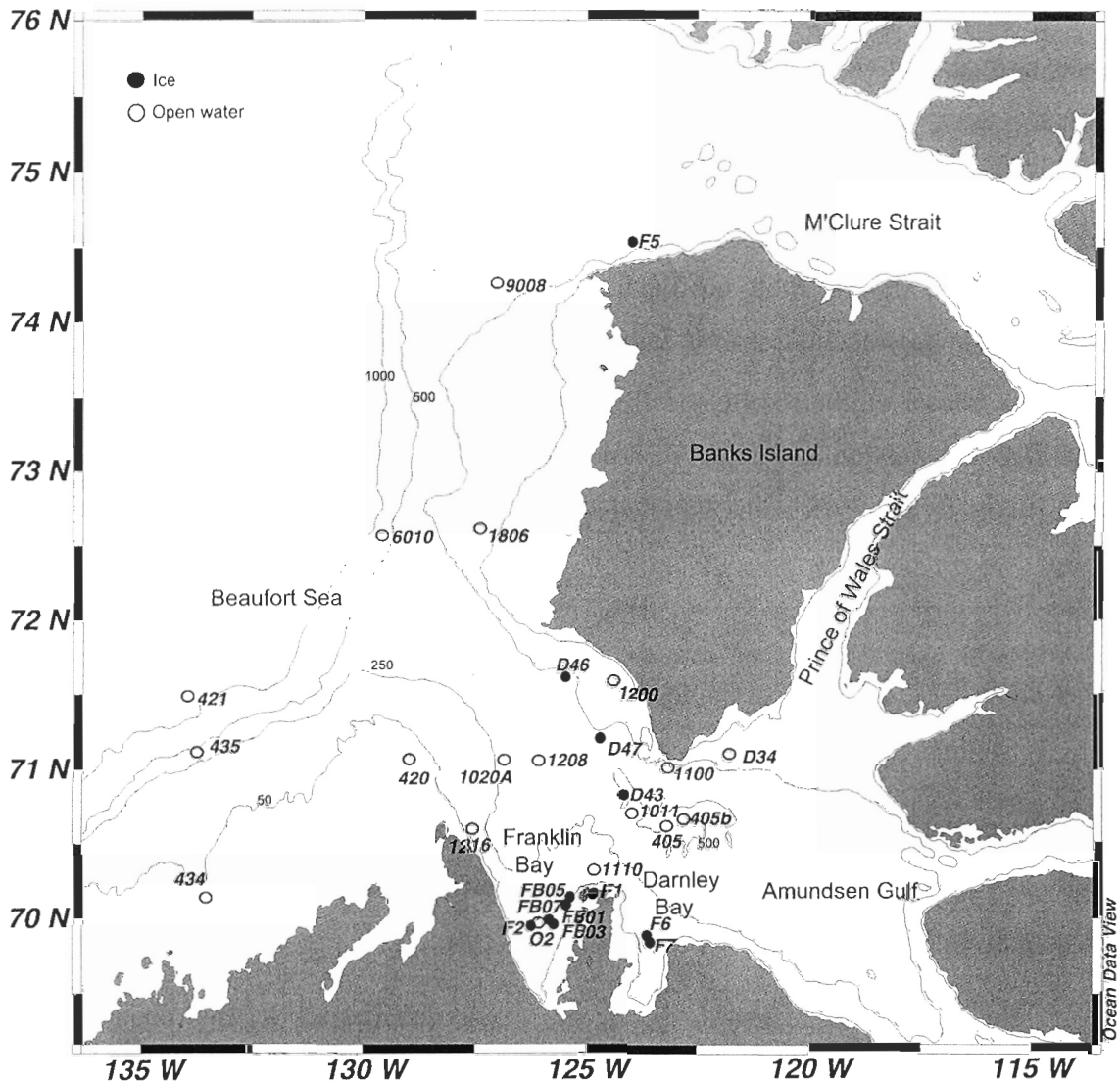

Fig. 1. Location of the sampling stations in the Canadian Beaufort Sea during the IPY-CFL system study, May-July 2008. Water depth is in meters

At each station, vertical profiles of water temperature, salinity, photosynthetically active radiation (PAR, 400 to 700 nm) and *in vivo* fluorescence were measured using a SBE-911*plus* CTD probe (Sea-Bird Electronics) equipped with a QCP-2300 PAR sensor (Biospherical Instruments) and an *in situ* fluorometer (Seapoint Sensor). The probe and sensors were mounted on a rosette equipped with 12-24 l Niskin-type bottles (Ocean Test Equipment). A vertical PAR profile was also performed with a PNF-300 radiometer (Biospherical Instruments) at OW stations. The vertical profiles of irradiance from the irradiance sensor on the rosette and from the PNF-300 radiometer were used to estimate the diffuse attenuation coefficient of downwelling PAR (K_d, m^{-1}) at IC and OW stations, respectively. *In situ* PAR at the sampled depth was calculated using Beer's law as:

$$E_z = E_0 e^{-K_d z}$$

where E_0 is the PAR value at the ice-water interface for IC stations and at the sea surface for OW stations (µmol photons m^{-2} s^{-1}) and Z is the sampled depth (m).

Downwelling incident PAR irradiance was measured at 1 min intervals with a PARLite™ quantum sensor (Kipp & Zonen) installed on a tower at the bow of the ship. These data were used to calculate daily incident PAR.

The surface mixed-layer depth (Z_m) was determined as the depth for the shallowest extreme curvature of density and temperature profiles (de Boyer Montégut et al. 2004, Table 1). An index of the vertical stratification of the water column ($\Delta\sigma_t$) was estimated as the difference in the sigma-*t* (σ_t) between 80 and 10 m from 1 May to 24 June and between 80 and 5 m after 24 June. At stations where bottom depth was < 80 m, the deepest depth was used for the calculation. The mean irradiance in the surface mixed layer (E_{zm}, µmol photons m^{-2} s^{-1}) was calculated as in Riley (1957):

$$E_{zm} = \frac{E_0(1 - e^{-K_d Z})}{K_d Z}$$

where Z is the depth of Z_m (m).

Water samples were generally collected within the surface mixed layer (Z_m), which ranged from 4 to 44 m (Table 1). At IC stations, samples were collected in the upper 5 m of the water column through a hole in the sea ice made ~500 m from the ship, using a Kemmerer water sampler. Samples collected at depths > 5 m were taken via the ship's moonpool with the CTD-rosette system. At OW stations, samples from the upper 5 m of the water column were collected with an electric submersible pump (12 V standard engineered plastic pump, model Cyclone, Proactive Environmental Products) whereas samples at depths > 5 m were taken with the moonpool's CTD-rosette system. After 25 June, samples were collected with the CTD-rosette system deployed from the ship's upper deck.

Nutrients

Nitrate plus nitrite (NO_3+NO_2), nitrite (NO_2), phosphate (PO_4), and silicic acid [$Si(OH)_4$] concentrations were measured immediately after sampling using an onboard Bran-Luebbe 3 Autoanalyzer (adapted from Grasshoff et al. 1999).

Cell viability

The proportion of living cells in the algal community (% LC) was determined by applying a cell membrane permeability test, the cell digestion assay (CDA) (Agustí & Sánchez 2002), recently extended for quantification of dead phytoplankton cells in cold waters (Llabrés & Agustí 2008, 2010). The CDA consists in exposing the phytoplankton communities to an enzymatic cocktail (DNAse and Trypsin) that enters the cytoplasm and digests cells with compromised membranes, i.e. dead or dying cells, which are thus removed from the sample. The cells remaining in the samples after the CDA are considered as living cells. For quantification of nano- and microphytoplankton viability, samples were concentrated from an initial volume of 0.3-2 l to 50 to 70 ml, using a device similar to the Millipore cell concentrator chamber used in previous studies (Agustí & Sánchez 2002, Alonso-Laita & Agustí 2006, Lasternas & Agustí 2010). The CDA was applied to duplicate 10 ml aliquots of the cell concentrate by adding 2 ml of DNAse I solution (400 $\mu g\ ml^{-1}$ in Hanks' Balanced Salt Solution [HBSS]), followed by 15 min incubation at 25°C in a water bath (Haake). After this time, 2 ml of Trypsin solution (1%

in HBSS) was added, followed by 30 min at 25°C. Additional duplicate 10 ml aliquots from the concentrate without enzymes were incubated to obtain the blanks. After incubation, samples with enzyme additions were then placed in ice to stop the enzymatic cell digestion process and then both blanks and samples containing enzymes were filtered onto polycarbonate 0.4 μm pore diameter black filters, washed several times with filtered seawater, fixed with glutaraldehyde (1% final concentration) and stored frozen at –80°C until microscopic counts. Phytoplankton cells were counted at magnifications of 400 and 1000× using an epifluorescence microscope (Zeiss Axiovert©) fitted with a blue excitation red fluorescence filter. Cells were grouped by size into three groups: >20 μm, 5-20 μm and <5 μm.

The cell counts in the samples after the CDA assay represent the abundance of living cells. The cell abundance in the blank samples represents the total cell abundance, i.e. both living and dead cells (Agustí & Sánchez 2002). The % LC was calculated as the ratio of the concentration of cells obtained after applying the CDA to the concentration of cells in the blanks.

Taxonomic analysis

At a few stations, samples were taken for taxonomic analysis, preserved with acidic Lugol's solution (0.4% final concentration) and stored in the dark at 4 °C until analysis. Cells > 2 μm were identified by an experimented phytoplankton taxonomist (S. Lessard), using an inverted light microscope (WILD Heerbrugg). A minimum of 400 cells were enumerated over at least three transects of the chambre (Lund et al. 1958).

Index of photosynthetic activity (F_v/F_m)

The photosynthetic performance of algae from the surface layer (0-15 m) was assessed by examining the changes in chlorophyll fluorescence with the electron transport inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) that blocks electron transport at the electron acceptor Q in PSII, causing an increase in chlorophyll fluorescence (Roy & Legendre 1979, Jochem 2000). Minimum and maximum fluorescence (F_0, F_m) were measured after 30 min of dark adaptation using a Turner Designs 10-AU fluorometer. F_m

was measured after adding DCMU (3×10^{-3} M). F_v/F_m was calculated as $(F_m-F_0)/F_m$, providing an index of photosynthetic performance. A decrease in the maximum quantum yield of PSII photochemistry, measured as dark-adapted F_v/F_m, is an indicator of photoinhibition or down-regulation of PSII (Critchley 2000).

Pigment analysis

The identity and concentrations of algal pigments were determined by reverse-phase high performance liquid chromatography (HPLC). Under dim light, water samples (0.25-2 l) were filtered onto 25 mm Whatman GF/F filters (maximum time of filtration of 20 min). The samples were immediately placed in liquid nitrogen for at least 24 h, and then transferred to a -80°C freezer on board the ship. Samples were sent every six weeks by plane to Rimouski in a nitrogen dry-shipper and thereafter kept in a -80°C freezer until analysis. Algal pigments were extracted in 95% methanol, sonicated (Sonicator Ultrasonic Processor XL 2010) for 15 seconds on ice and centrifuged for 5 min at 3700 g. Extracts were filtered through a 0.22 μm polytetrafluoroethylene syringe filter and poured into an autosampler vial which was gently sparged with argon to limit oxidation. A volume of 50 μl was injected in a Waters Symmetry C_8 column (150 x 4.6 mm, 3.5 μm). Gradient elution was controlled by a Thermo Separation (TSP) P4000 pump with solvents as indicated in Zapata et al. (2000). Pigments were detected with a TSP UV6000 LP diode-array absorbance detector (400 to 700 nm) and a TSP FL3000 fluorescence detector to confirm the presence of chlorophyll-related compounds. Calibration was done with external pigment standards obtained from DHI Lab Products (Hørsholm, Denmark) and extinction coefficients were taken from Jeffrey (1997). Limits of detection and quantification were estimated as in Bidigare et al. (2005) and pigments with concentrations less than the limit of detection are not reported in the present study. Marker pigments were identified through comparison with the retention time and spectral properties of pigment standards (Egeland et al. 2011).

Statistical analyses

Spearman's rank order correlations (r_s) were used to determine if there were any significant correlations among physical, chemical and biological variables. Mann-Whitney *U*-test was performed to seek differences among ice-covered and open-water stations. Descriptive statistics, Spearman's rank order correlations (r_s) and Pearson's linear regressions were obtained using SigmaStat 3.5 and SigmaPlot 10.0 (Systat Software, Inc).

RESULTS

Physico-chemical variables

Irradiance in the surface layer was influenced by the ice cover: it differed significantly between the two sets of stations, ice-covered and open-water. The percent sea ice cover ranged from 20 to 95% in May, decreasing to 10% in June and reaching a minimum in July (<5%) (Forest et al. 2011). We present results separately for both sets of stations.

The seasonal increase in incident daily PAR ranged from 23.6 on 17 May to 66.1 mol photons m^{-2} d^{-1} on 20 June (Fig. 2a).

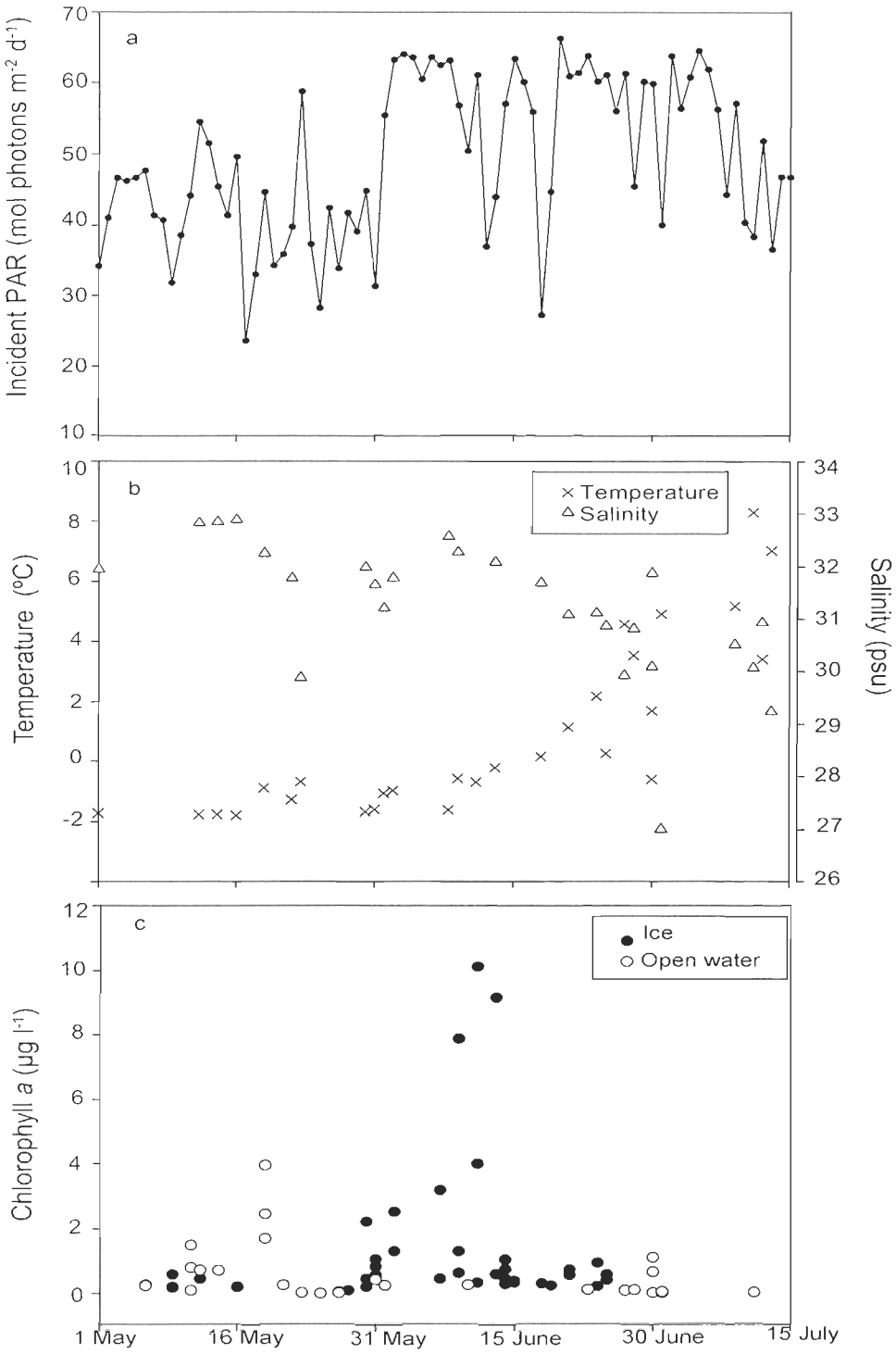

Fig. 2. Temporal changes in (a) daily incident irradiance (b) surface layer water temperature and salinity, and (c) surface layer chlorophyll *a* (chl *a*) concentration at ice-covered and open-water stations from May to July 2008

The PAR at the sampling depth (E_z) and the mean irradiance in the surface mixed layer (E_{zm}) were significantly higher (Mann-Whitney *U*-test, $p < 0.001$) for OW (mean ± SE: 418.7 ± 82.6 and 364.6 ± 62.1 μmol photons m^{-2} s^{-1}, respectively) than for IC stations (7.4 ± 2.0 and 10.9 ± 2.7 μmol photons m^{-2} s^{-1}, respectively) (Table 1).

Water temperatures for the surface layer during the study period were significantly higher (Mann-Whitney *U*-test, $p < 0.05$) in OW (mean ± SE: 2.57 ± 0.94°C) compared to IC stations (– 0.63 ± 0.33°C). Temperatures increased throughout the sampling period and reached maximum values at the end of the study on 11 July (8°C, station 1100) and 13 July (7°C, station D34) (Figs. 1 & 2b). Salinity values ranged from 27 to 33 with mean (± SE) values of 30.56 ± 0.42 at OW and 31.91 ± 0.18 at IC stations. Lower salinity was measured at stations 421 and D34 at the end of the study (Fig. 2b). The stratification index ($\Delta\sigma_t$) ranged from 0.60 to 4.90 kg m^{-3} for OW (mean ± SE: 1.67 ± 0.25 kg m^{-3}) and from 0.36 to 1.78 for IC (mean ± SE: 0.88 ± 0.07 kg m^{-3}). Significant differences were found for salinity and the stratification index between the OW and IC stations, with salinity being significantly higher at IC stations (Mann-Whitney *U*-test, $p < 0.01$), and the stratification index significantly higher at OW stations (Mann-Whitney *U*-test, $p < 0.01$) and increasing throughout the season (see Table 1). The surface mixed layer (Z_m) averaged (± SE) 14.58 ± 1.82 m throughout the study period and did not differ significantly between the OW and IC stations (Mann-Whitney *U*-test, $p > 0.05$) (Table 1).

Table 1. Physical and chemical characteristics of ice-covered and open-water stations in the Beaufort Sea during spring and summer 2008; Z_m, surface mixed-layer depth; $\Delta\sigma_t$, stratification index; PAR; E_0, downwelling incident PAR; E_{zm}, mean irradiance in the surface mixed layer; E_z, PAR at the sample depth; NO_3, nitrate; $[Si(OH)]_4$, silicic acid; PO_4, phosphate. nd: not determined

Station	Date	Bottom depth (m)	Z_m (m)	$\Delta\sigma_t$ (kg m^{-3})	E_0 (µmol m^{-2} s^{-1})	E_{zm} (µmol $m^{-2}s^{-1}$)	Sample depth (m)	E_z (µmol $m^{-2}s^{-1}$)	NO_3 (µmol l^{-1})	$Si(OH)_4$ (µmol l^{-1})	PO_4 (µmol l^{-1})
Ice-covered station											
D43	1 May	457	57	0.56	148	1	10	2	3.50	8.93	1.00
F1	10 May	53	15	0.36	650	2	0	3	nd	nd	nd
F1							2		nd	nd	nd
F1							5		nd	nd	nd
F2	14 May	192	21	0.63	1023	4	5	6	nd	nd	nd
F2	16 May	183	31	0.57	874	2	0	7	9.54	20.85	1.56
F2							5	5	9.49	20.91	1.55
F2							10	3	9.71	21.54	1.61
F5	28May	368	14	nd	742	28	0	45	<0.05	2.51	0.77
D46	30 May	289	32	0.53	618	3	0	6	2.04	6.72	0.97
D46							5	4	3.02	8.58	1.04
D46							10	3	3.79	10.35	1.15
D47	31 May	273	16	0.47	475	2	0	3	nd	nd	nd
D47							5	3	nd	nd	nd
D47							10	2	1.36	6.31	0.94
F6	2 June	71	12	0.87	1213	7	5	7	0.18	5.37	0.91
F6							12	2	1.88	6.93	1.02
F7	8 June	78	13	0.82	1270	7	0	11	nd	7.55	0.83
F7							5	7	nd	5.79	0.77
F7							12	4	nd	18.14	1.17

F7	9 June	78	11	0.85	175	1	0	2	nd	nd	nd
F7							5	1	nd	nd	nd
F7							10	1	nd	nd	nd
F7	11 June	80	nd	0.51	216	nd	0	11	0.49	3.26	nd
F7							5	7	0.45	nd	nd
F7							12	4	5.94	17.61	nd
F7	13 June	79	13	0.91	855	3	5	4	nd	nd	nd
F7							12	1	nd	nd	nd
FB01	14 June	98	11	1.25	678	2	5	1	1.13	8.66	0.87
FB03	15 June	105	12	1.17	1096	8	5	8	1.25	7.30	0.80
FB05	15 June	107	10	1.46	1624	3	0	4	0.91	9.92	0.79
FB05							5	3	0.40	6.60	0.82
F7	18 June	80	13	1.07	205	69	12	28	nd	3.54	0.65
F7	19 June	125	13	1.44	590	42	2	72	1.66	3.80	0.64
FB07	21 June	112	14	1.61	152	11	12	7	0.62	4.14	0.76
F7	24 June	88	6	1.63	1552	29	0	55	nd	nd	nd
F7							6	13	0.59	2.19	0.54
FB07	25 June	103	5	1.78	459	46	0	81	nd	nd	nd
FB07							6	17	0.96	5.91	0.71

Table 1. Continued

Station	Date	Bottom depth (m)	Z_m (m)	$\Delta\sigma_t$ (Kg m^{-3})	E_0 (µmol m^{-2} s^{-1})	E_{zm} (µmol m^{-2} s^{-1})	Sample depth (m)	E_z (µmol m^{-2} s^{-1})	NO_3 (µmol l^{-1})	$Si(OH)_4$ (µmol l^{-1})	PO_4 (µmol l^{-1})
Open-water station											
1020 A	6-May	276	42	0.6	1226	252	0	1226	4.83	9.78	1.17
1020 A							5	690	4.83	9.78	1.17
O2	12-May	205	21	0.67	178	67	0	178	nd	nd	nd
O2							2	142	nd	nd	nd
O2							5	100	9.45	20.47	1.56
O2							11	50	9.59	21.2	1.35
405b	19-May	537	17	0.68	1508	495	0	1508	nd	nd	<0.02
405b							5	648	nd	nd	1.11
405b							11	235	0.95	nd	0.92
1011	22-May	459	13	0.62	476	280	0	476	2.66	7.99	nd
1011							8	232	2.67	8.04	nd
1806	23-May	137	12	2.25	809	515	0	809	nd	nd	nd
1806							10	356	<0.05	2.63	0.75
9008	27-May	346	20	1.27	945	380	0	945	nd	nd	nd
9008							5	543	<0.05	3.03	0.8
405b	10-Jun	562	15	2.03	1544	739	7	695	nd	nd	nd
1216	23-Jun	207	9	3.1	1363	1363	0	1363	nd	nd	nd
1200	27-Jun	199	8	2.53	1250	852	0	1250	nd	nd	nd
1200							7	608	0.4	4.53	0.73
1208	28-Jun	400	6	1.81	181	135	7	88	<0.05	nd	0.77
434	30-Jun	40	4	2.13	270	205	5	132	nd	2.48	0.15
434							13	42	nd	1.91	0.36
421	1-Jul	1142	8	4.90	223	166	9	110	<0.05	3.71	0.31
420	9-Jul	43	6	1.88	316	238	7	158	<0.05	4.77	0.35
1100	11-Jul	265	6	3.15	1044	800	7	545	0.66	4.78	nd
1110	12-Jul	96	4	2.15	35	27	7	14	<0.05	3.27	<0.02
D34	13-Jul	182	4	4.27	1384	1176	10	599	<0.05	4.28	0.70

Nutrient concentrations varied over the entire study: for NO_3, values ranged from <0.05 to 9.6 μmol l^{-1} (mean ± SE: OW = 2.2 ± 0.9 μmol l^{-1}, IC = 2.7 ± 0.6 μmol l^{-1}), for $[Si(OH)_4]$, from 1.9 to 21.5 μmol l^{-1} (OW = 6.8 ± 1.6 μmol l^{-1}, IC = 9.2 ± 1.2 μmol l^{-1}) and for PO_4, from <0.02 to 1.60 μmol l^{-1} (OW = 0.73 ± 0.12 μmol l^{-1}, IC = 0.96 ± 0.06 μmol l^{-1}). There were no significant differences between OW and IC stations (Mann-Whitney *U*-test, $p = 0.13$ for NO_3; $p = 0.07$ for PO_4 and $Si(OH)_4$). The NO_3:PO_4 and NO_3:$[Si(OH)_4]$ molar ratios decreased over the study and were lower than the critical values of 16 for NO_3:PO_4 (Redfield et al. 1963) and of 1.1 for NO_3: $[Si(OH)_4]$ (Brzezinski 1985), suggesting that nitrogen was the potentially limiting nutrient for algal growth.

Algal biomass

The chl *a* concentration was significantly lower (Mann-Whitney *U*-test, $p < 0.05$) at OW (0.67 ± 0.22 μg l^{-1}) than at IC stations (1.50 ± 0.41 μg l^{-1}) (Fig. 2c). The algal biomass reached a maximum for OW stations on 19 May (3.95 μg l^{-1}, station 405b, 0 m) and for IC stations on 11 June (10.13 μg l^{-1}, station F7, 5 m), the peak of the observed bloom. Wind-driven upwelling events enhanced under-ice nutrient availability (Mundy et al. 2009, Tremblay et al. 2011) causing the high chl *a* concentrations encountered during this study at IC stations in Darnley Bay (Fig. 2c). After this period, chl *a* concentrations decreased to 0.01 μg l^{-1} at OW stations. The low chl *a* values encountered at the end of the study co-occurred with the lowest nutrient concentrations, suggesting nutrient-depleted post-bloom conditions. There was no significant relationship between chl *a* concentration and any of the nutrients determined at IC stations whereas at OW stations, chl *a* was positively correlated with NO_3 ($r_s = 0.76$, $p < 0.01$) and $[Si(OH)_4]$ ($r_s = 0.68$, $p < 0.05$).

The chl *a* concentration did not show any significant relationship with water temperature, salinity and daily incident irradiance at IC and OW stations, but it was negatively correlated with the stratification index for OW stations ($r_s = -0.68$, $p < 0.05$).

Pigment markers for taxonomic groups

The major marker pigments detected were used to assess the composition of phytoplankton groups (Higgins et al. 2011) along with the microscopic examination (few data) and past observations from this polar region (Lovejoy et al. 2007, Poulin et al. 2011). These marker pigments are listed in Table 2; they include fucoxanthin for diatoms (fucoxanthin is also present in a few other groups but diatoms were clearly dominant so we used this pigment as a marker for diatoms here), prasinoxanthin for prasinoxanthin-containing prasinophytes, peridinin for peridinin-containing dinoflagellates, alloxanthin for cryptophytes, and 19'-hexanoyloxyfucoxanthin (hex-fuco) and 19'-butanoyloxyfucoxanthin (but-fuco) for haptophytes. Chl *b*, micromonal and zeaxanthin were also present and strongly correlated with prasinoxanthin ($r_s = 0.74$, 0.86 and 0.92, respectively), suggesting that they belonged to prasinophytes. Complete analysis of the pigment data set is outside of the scope of this work, but the relative importance of these algal groups can be obtained by dividing the mean concentration of these marker pigments for the two groups of stations (IC and OW) by the mean field-derived marker pigment:chl *a* ratio for the respective algal groups (from Higgins et al. 2011, using low-light ratios for IC stations). Results show that overall, for both sets of stations, diatoms and prasinophytes dominate (diatoms: 30% of total chl *a* for IC stations, 40% for OW stations; prasinophytes: 20% for IC stations, 35% for OW stations), followed by cryptophytes and dinoflagellates (roughly 10% for both sets of stations) and by haptophytes (roughly 5%). Microscopic observations confirmed the presence of diatoms, ranging from 20 to 90% (relative abundance contribution to the total phytoplankton community for the samples analyzed), followed by dinoflagellates (2-12%), prasinophytes (4-8%) and cryptophytes (< 3%), and finally unidentified flagellates ranged from 3 to 40%.

Table 2. Main marker pigments, photoprotective pigments and degradation pigments measured at ice-covered (IC) and open-water (OW) stations. chlorophyll *b*, Chl *b*; peridinin, Peri; fucoxanthin, Fuco; alloxanthin, Allo; prasinoxanthin, Prasino; 19'-hexanoyloxyfucoxanthin, Hex-fuco; 19'-butanoyloxyfucoxanthin, But-fuco; micromonal, micral; zeaxanthin, Zea; chlorophyll *a*, Chl *a*; photoprotective carotenoids, PPC; photosynthetic carotenoids, PSC, see text for the detailed pigments for each group; DD+DT, sum of diadinoxanthin and diatoxanthin. Mean values ± SE are shown; n= number of samples; p values for significant differences between IC and OW stations; ns: no significant difference

	IC stations		**OW stations**		
Concentration and ratios	mean ± SE	n	mean ± SE	n	p
Marker pigments					
Chl *b* (µg l^{-1})	0.17±0.02	39	0.05±0.01	28	<0.01
Peri (µg l^{-1})	0.04±0.004	29	0.03±0.004	15	ns
Fuco (µg l^{-1})	0.48±0.16	40	0.18±0.06	30	<0.01
Allo (µg l^{-1})	0.01±0.001	25	0.01±0.000	13	ns
Prasino (µg l^{-1})	0.01±0.001	25	0.002±0.02	13	<0.01
Hex-fuco (µg l^{-1})	0.02±0.002	21	0.02±0.01	18	ns
But-fuco (µg l^{-1})	0.01±0.002	28	0.01±0.002	11	ns
Micral (µg l^{-1})	0.02±0.002	20	0.01±0.000	7	<0.01
Zea (µg l^{-1})	0.02±0.001	33	0.01±0.000	23	< 0.05
Chl *b*:chl *a* (wt:wt)	0.24±0.03	29	0.25±0.04	30	ns
Peri:chl *a* (wt:wt)	0.07±0.01	29	0.08±0.02	16	ns
Fuco:chl *a* (wt:wt)	0.26±0.02	30	0.35±0.07	32	ns
Allo:chl *a* (wt:wt)	0.02±0.002	25	0.03±0.01	13	ns
Prasino:chl *a* (wt:wt)	0.05±0.03	37	0.09±0.02	25	ns
Hex-fuco:chl *a* (wt:wt)	0.03±0.003	22	0.05±0.01	17	ns
But-fuco:chl *a* (wt:wt)	0.02±0.002	28	0.03±0.003	11	ns
Micral:chl *a* (wt:wt)	0.03±0.004	20	0.03±0.004	8	ns
Zea:chl *a* (wt:wt)	0.03±0.003	27	0.14±0.06	25	< 0.05
Chl *a* and photoprotective pigments					
Chl *a* (µg l^{-1})	1.50±0.41	36	0.67±1.02	20	<0.05
PPC (µg l^{-1})	0.12±0.02	36	0.07±0.02	18	<0.05
PSC (µg l^{-1})	0.62±0.18	36	0.25±0.09	20	<0.01
PPC:PSC (wt:wt)	0.36±0.03	36	0.53±0.14	18	ns
PPC:chl *a* (wt:wt)	0.14±0.06	30	0.24±0.05	18	<0.05
(DD+DT):chl *a* (wt:wt)	0.07±0.01	36	0.15±0.03	18	<0.01

Degradation pigments					
Chl *a* allomer ($\mu g\ l^{-1}$)	0.22±0.09	27	0.10±0.03	10	ns
Chlorophyllide *a* ($\mu g\ l^{-1}$)	0.22±0.07	23	0.20±0.08	11	ns
Pheophorbide *a* ($\mu g\ l^{-1}$)	0.41±0.12	37	0.18±0.28	19	<0.05
Pheophytin *a* ($\mu g\ l^{-1}$)	0.25±0.07	12	0.14±0.06	4	ns
Pyropheophorbide *a*-"like" ($\mu g\ l^{-1}$)	0.65±0.08	19	0.30±0.03	5	<0.05
Sum all degradation pigments ($\mu g\ l^{-1}$)	1.1±0.30	37	0.43±0.14	19	ns
Chl *a* allomer:chl *a* (wt:wt)	0.08±0.05	27	0.13±0.03	10	ns
Chlorophyllide *a*:chl *a* (wt:wt)	0.09±0.05	23	0.12±0.03	11	ns
Pheophorbide *a*:chl *a* (wt:wt)	0.26±0.09	36	0.33±0.07	19	ns
Pheophytin *a*:chl *a* (wt:wt)	0.07±0.03	11	0.18±0.09	4	ns
Pyropheophorbide *a*-"like":chl *a* (wt:wt)	0.86±0.66	19	1.90±1.4	5	ns
Sum all degradation pigments:chl *a* (wt:wt)	0.88±0.63	36	1.70±0.38	19	ns

Temporal patterns for these various groups are shown in Fig. 3. Diatoms (expressed as fucoxanthin concentrations and the ratio fucoxanthin:chl *a*, Fig. 3a, d) were present throughout the study period at both IC and OW stations, representing nearly a third of the total chl *a*. For IC stations, maximum concentrations were observed on 9-13 June (as for chl *a*), while for OW stations, concentrations were lower with a first maximum around 19 May and a second smaller maximum in late June. The fucoxanthin:chl *a* ratio was around 0.3, slightly lower for IC stations particularly after the bloom in early June. Prasinophytes (Fig. 3b, e) represented less than 30% of total chl *a* and showed different trends for the two groups of stations: for IC stations there was an increase over time, both in terms of concentration of prasinoxanthin and of the prasinoxanthin:chl *a* ratio, whereas for OW stations, maximum concentrations of prasinoxanthin were seen around 10 June (station 405b) and at the end of June (station 434). Dinoflagellates (Fig. 3c, f) were also present throughout the study, at roughly 10% of the total chl *a*, with a pattern that resembled fucoxanthin.

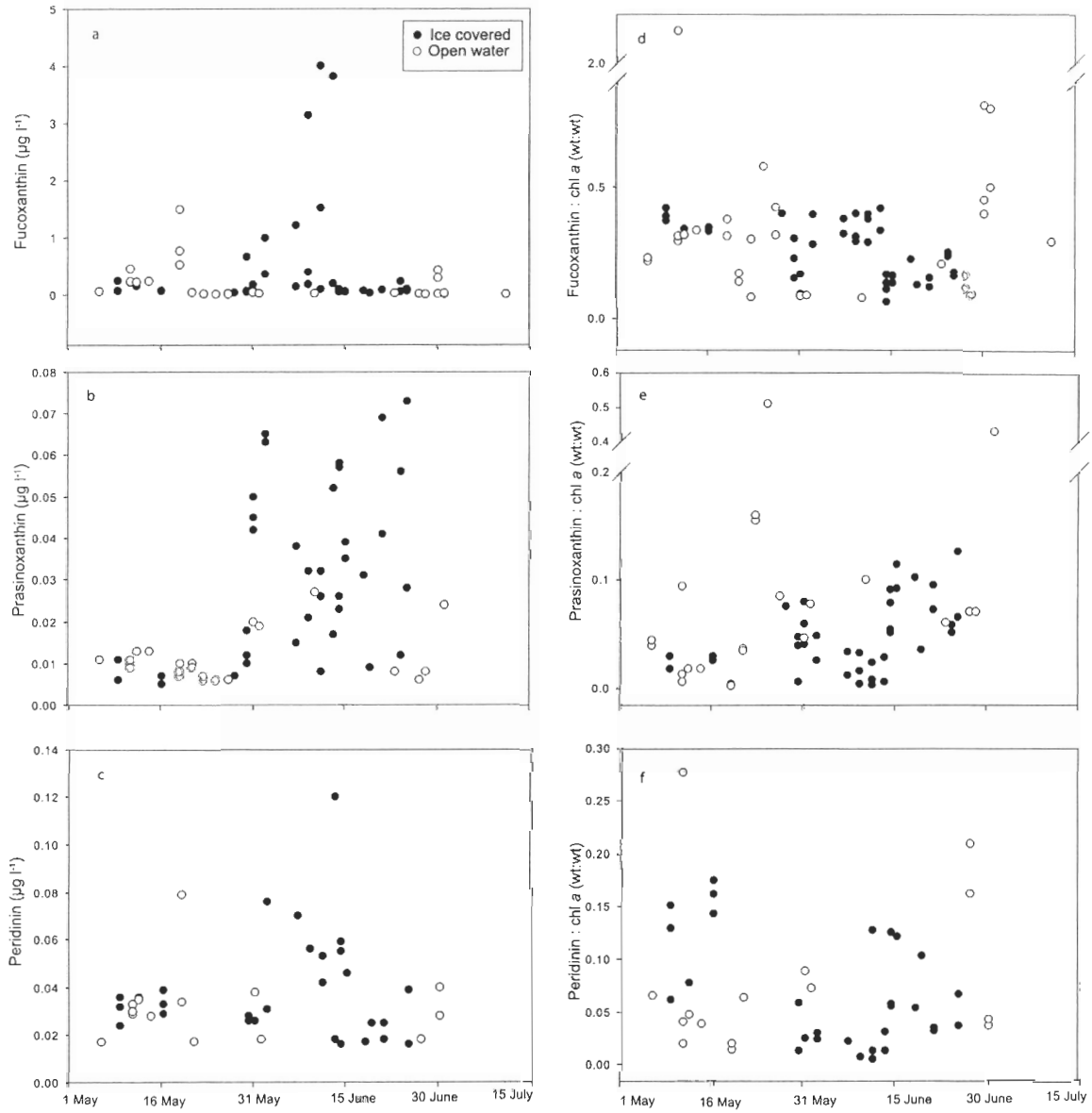

Fig. 3. Temporal changes in the concentrations of (a) fucoxanthin, (b) praxinoxanthin and (c) peridinin and in the ratios of (d) fucoxanthin, (e) prasinoxanthin and (f) peridinin to chlorophyll *a* at ice covered and open water stations from May to July 2008

Photoprotective pigments

The ratio of photoprotective carotenoids (PPC; diadinoxanthin: DD, diatoxanthin: DT, violaxanthin, zeaxanthin, lutein and β,β-carotene) to photosynthetic carotenoids (PSC; fucoxanthin, peridinin, neoxanthin, alloxanthin, prasinoxanthin, hex-fuco, but-fuco) increased seasonally (Fig. 4a). This increase was greater for OW stations (PPC:PSC, 0.16 – 2.52, wt:wt) than for IC stations (0.09 – 0.77). A similar trend was seen for the PPC:total chl *a* ratio (not shown), which ranged from about 0.04 to 0.26 at IC stations and 0.07 to 0.60 at OW stations. The increase of the PPC concentration was mostly due to diadinoxanthin (33% of total PPC), β,β-carotene (20%) and violaxanthin (14%). The PPC:PSC, or other similar ratios indicating the relative importance of photoprotective pigments such as PPC:chl *a* and (DD+DT):chl *a* ratios, were significantly higher at OW stations (see Table 2), suggesting that the most abundant taxonomic group, diatoms (which contain fucoxanthin, DD and DT) increased photoprotection through the xanthophyll cycle in open water stations in response to the higher irradiances of OW stations.

For irradiances (E_z) > 600 μmol photons m^{-2} s^{-1} at these stations, the results indicated two different photoprotective responses (Fig. 4b) with a group of samples with PPC:PSC values < 0.5 and another group showing values > 0.5. The composition of the first group was dominated by diatoms (34%) with low contributions of prasinophytes (4%) and other groups (< 4%); whereas the group of stations showing a higher photoprotective response was composed of 21% prasinophytes, 15% dinoflagellates, 14% diatoms and < 5% for other groups. Hence, under conditions of high irradiance in OW stations, samples dominated by diatoms showed signs of low photoprotection.

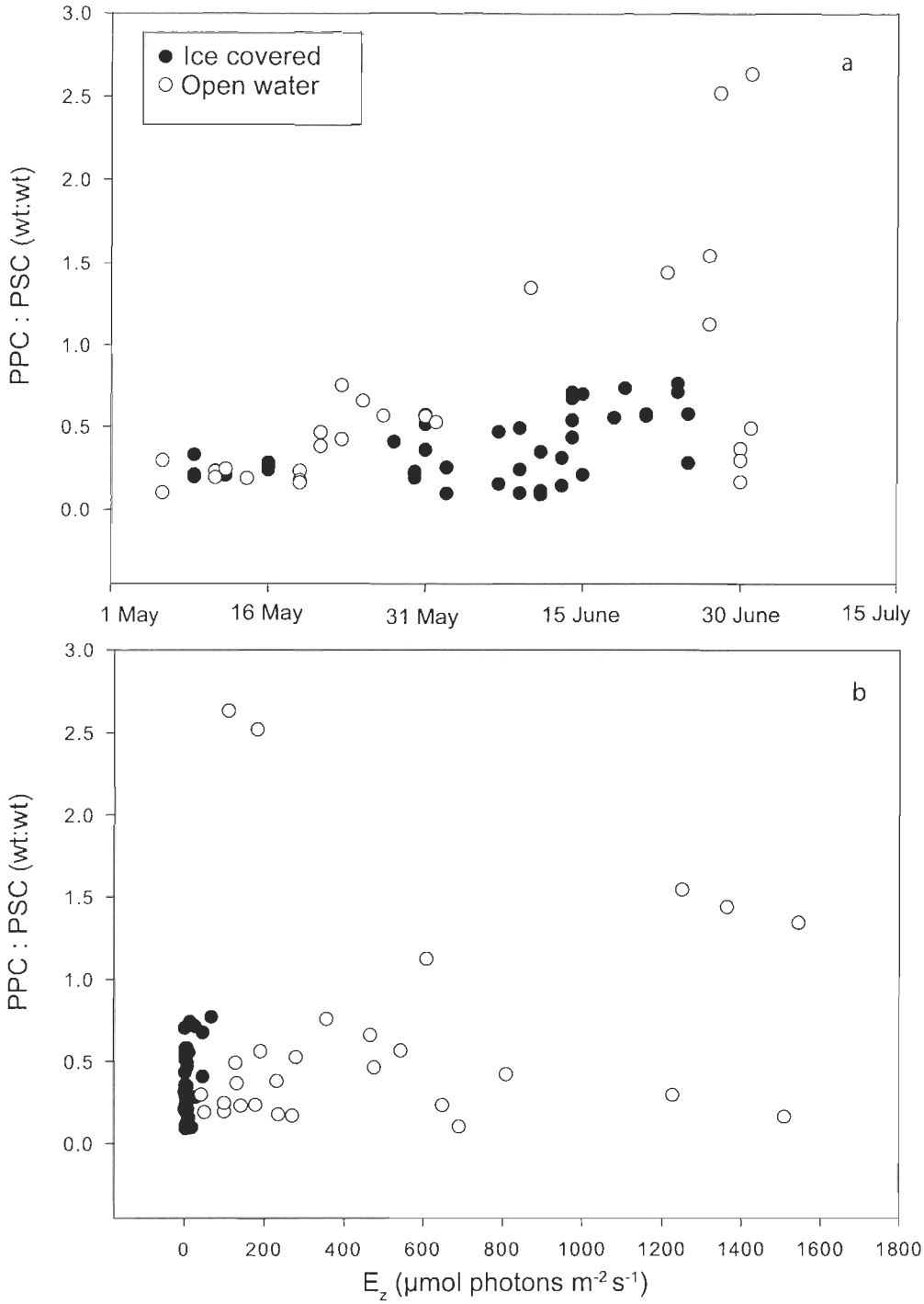

Fig. 4. (a) Temporal change in the ratio of photoprotective (PPC) to photosynthetic carotenoids (PSC) and (b) relationship between PPC:PSC ratio and PAR at sampling depth (E_z) at ice-covered and open-water stations

Living cells and physicochemical variables

For all stations (OW and IC), the total percentage of living cells (% LC) was most strongly correlated with the abundance of the 5-20 μm cells ($r_s = 0.90$, $p < 0.001$), followed by cells > 20 μm ($r_s = 0.78$, $p < 0.001$) and < 5 μm ($r_s = 0.60$, $p < 0.001$). This suggests that mortality was higher in the smallest size category.

The % LC of IC and OW stations varied widely with environmental variables throughout the study (Fig. 5). The % LC did not show a significant relationship with nitrate concentration (Fig. 5a, d). We note however that the lowest % LC values (less than 20%) encountered at low nitrate concentrations (less than 0.2 μmol l^{-1}) are characterized by a greater abundance of prasinophytes relative to diatoms.

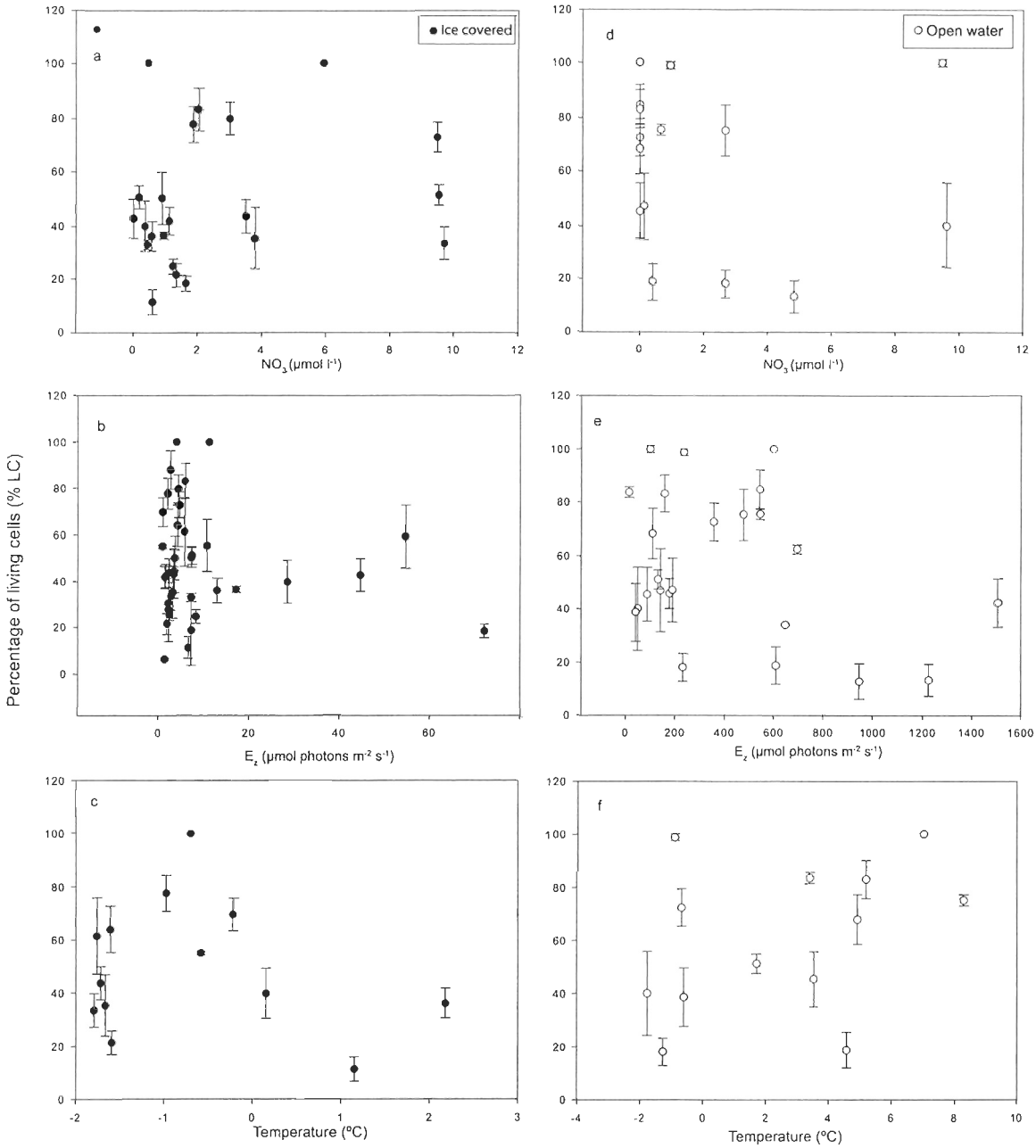

Fig. 5. Relationships between (a,d) percentage of living cells (% LC) and nitrate (NO_3) concentration, (b,e) % LC and PAR at sampling depth (E_z) and (c,f) % LC and water temperature at (a-c) ice-covered and (d-f) open-water stations. For % LC, mean values and SEs are presented

The relationship between % LC and *in situ* irradiance (E_z) was slightly different between the IC and OW stations. For IC stations, % LC varied widely with E_z and did not show a significant relationship. We examined whether there were changes in algal community that could explain the high variability of the % LC for irradiances <15 μmol photons m^{-2} s^{-1} by examining at the pigment composition of samples having % LC > 75% and samples with % LC < 25% (n = 6). The stations with > 75% LC had, on average, 39% diatoms, 5% prasinophytes and minor contributions from a few other groups to the total chl *a*. Stations with < 25% LC presented a higher contribution of prasinophytes (21%), followed by diatoms (18%) and cryptophytes (8%). The % LC tended to increase for E_z values between 15 and 60 μmol photons m^{-2} s^{-1} and decrease at higher irradiances (station F7, 24 June) (Fig. 5b). Community composition of these samples showed that prasinophytes dominated (33%) followed by diatoms (22%) and other groups (<5%). At OW stations, the % LC maximum values (86.68 ± 7.16%) were found at irradiances between 400 and 600 μmol photons m^{-2} s^{-1} (Fig. 5e) with a trend to increase from 10 to 600 μmol photons m^{-2} s^{-1}, however this relationship was not statistically significant ($p > 0.05$). There was a clear decrease in % LC with higher irradiances: lowest % LC values (30.52 ± 8.02%) were found at irradiances > 600 μmol photons m^{-2} s^{-1} (Fig. 6). Phytoplankton community at OW stations with values > 50% LC (n = 5) and E_z less than 200 μmol photons m^{-2} s^{-1} was composed of 40% diatoms, 6% prasinophytes and other groups contributing < 6%. For stations with < 50% LC and E_z less than 200 μmol photons m^{-2} s^{-1} (n = 5), the algal community was composed of 26% diatoms, 26% prasinophytes and 23% cryptophytes. Finally, samples with low % LC and high irradiances (greater than 600 μmol photons m^{-2} s^{-1}) were characterized by a dominance of diatoms (39% diatoms, 8% cryptophytes and 7% prasinophytes). Hence in open water stations, high percent living cells were seen only at relatively low irradiances (less than 500 μmol photons m^{-2} s^{-1}) and were dominated by diatoms, but a large proportion of these cells died when irradiances increased above this threshold.

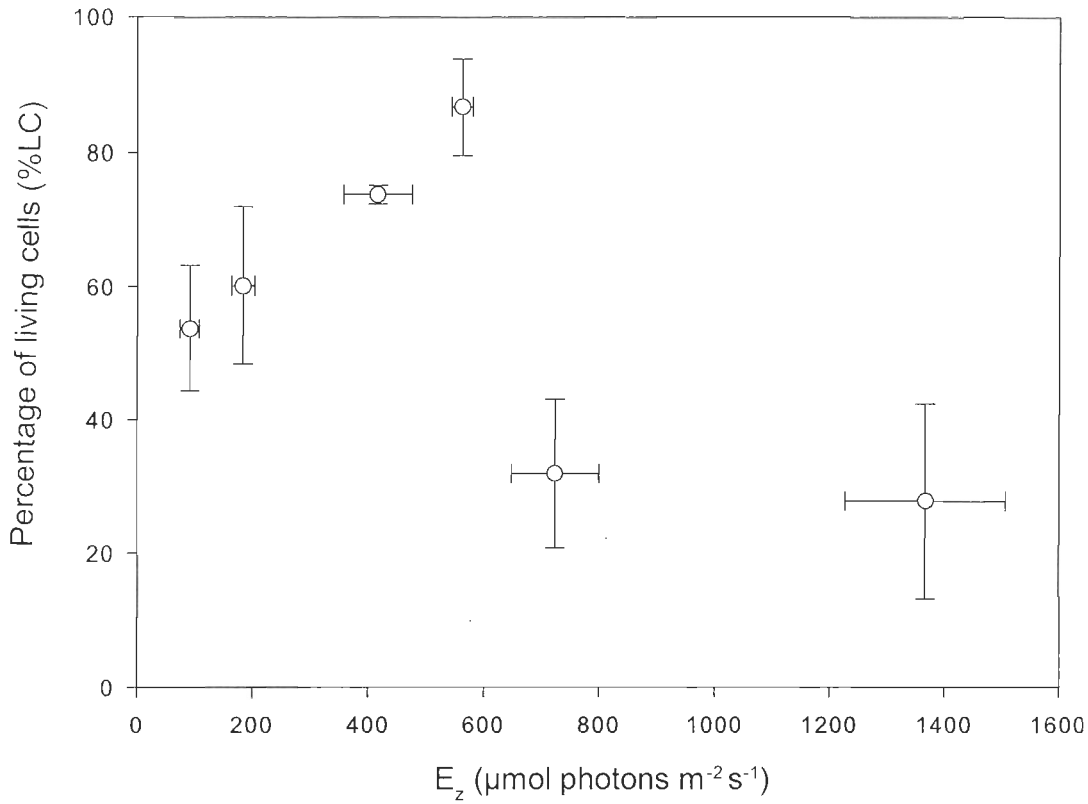

Fig. 6. Relationship between percentage of living cells (% LC) and PAR at sampling depth (E_z) at open-water stations. The data were binned for 5 irradiance intervals (i.e. < 100, n = 6; 100 - < 250, n = 6; 250 - < 500, n = 2; 500 - < 600, n = 3; 600 - < 1000, n = 4 and ≥ 1000, n = 2). Mean values and SEs for % LC and mean values and ranges for E_z are shown

The relationship between % LC and water temperature differed between IC and OW stations, with a clear decrease of % LC at temperatures above -1°C for IC stations (Fig. 5c), whereas % LC increased above 0°C for OW stations (Fig. 5f). At temperatures less than 0°C, % LC varied between 20 and 100% for both sets of stations. The community composition showed a dominance of prasinophytes at IC stations with temperatures > 0°C (n = 3; 33% prasinophytes, 19% diatoms and 10% cryptophytes). The OW stations with % LC increasing with temperatures were characterized by a dominance of diatoms (45% diatoms and 34% of prasinophytes with < 5% contribution from other groups).

Living cells and photosynthetic performance

During the period of study, the total percentage of living cells (% LC) was significantly correlated with F_v/F_m for all stations and depths ($r_s = 0.34$, $p < 0.05$, $n = 53$, Fig. 7a). This correlation was improved when considering only IC stations ($r_s = 0.36$, $p < 0.05$); indeed when only OW stations were considered, there was no statistically significant relationship ($p > 0.05$) between F_v/F_m and % LC values. The relationship between F_v/F_m and mean irradiance in the surface mixed layer (E_{zm}) differed between the two sets of stations. Our results showed no significant relationship between these variables at IC stations. However at OW stations we observed a significant negative relationship between the F_v/F_m values and E_{zm} (Fig. 7b) when an outlier, station 1110 (799 µmol photons m^{-2} $s^{-}1$, $F_v/F_m = 0.8$, 75% LC), was omitted, suggesting that high irradiance had a negative influence on the photosynthetic performance of cells for the OW stations. No other statistically significant relationships were found between F_v/F_m and other environmental variables.

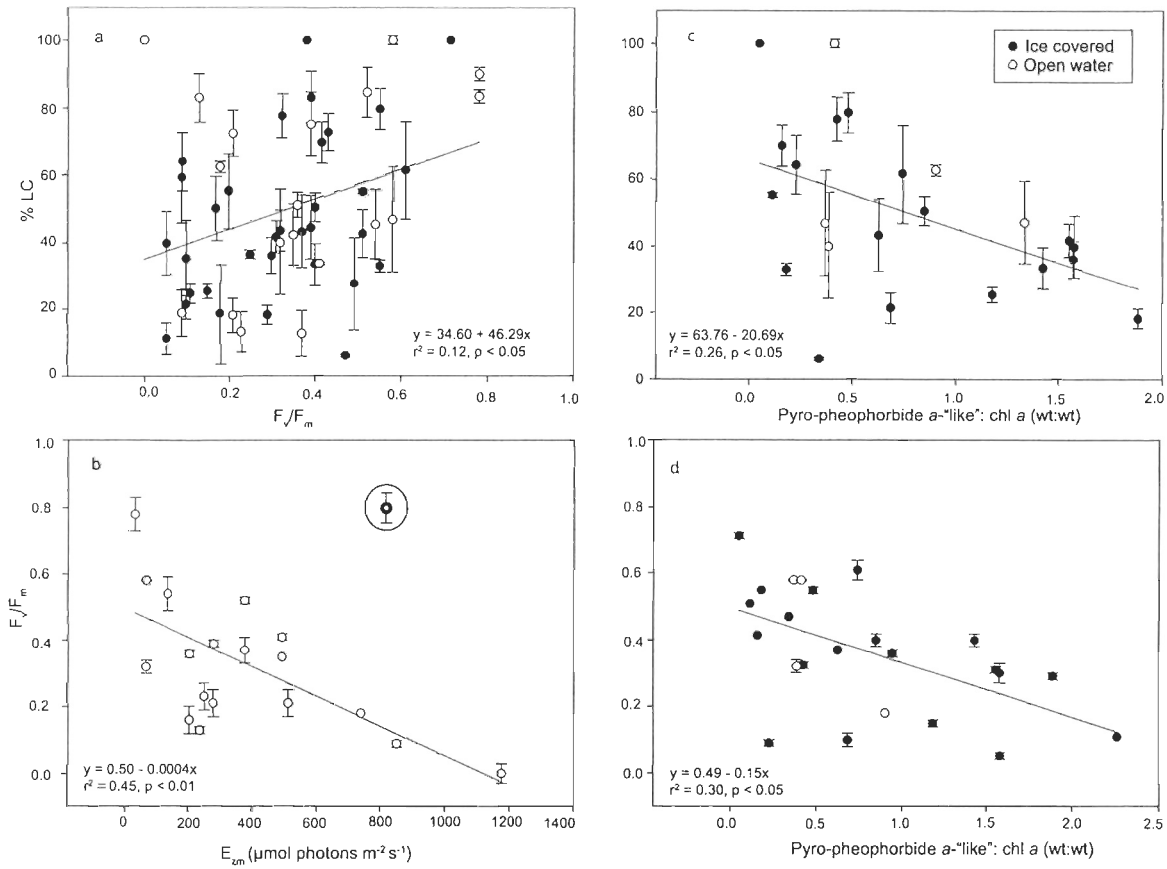

Fig. 7. Relationships between (a) percentage of total living cells (% LC) and photosynthetic performance (F_v/F_m), (b) F_v/F_m and mean PAR in the surface mixed layer (E_{zm}), (c) % LC and pyro-pheophorbide *a*-"like":chl *a* ratio and (d) F_v/F_m and pyro-pheophorbide *a*-"like":chl *a* ratio at ice-covered and/or open-water stations. For % LC and F_v/F_m, mean values and SEs are presented. A model I regression line is shown for each graph. In (b), the circled value was not used for computing the regression

Degradation pigments

We identified six chlorophyll degradation products: chl *a* allomer and epimer, chlorophyllide *a*, pheophorbide *a*, pheophytin *a* and a pyropheophorbide *a*-"like" pigment (see Table 2 and Fig. 8 for the absorption spectrum of this pigment). The sum of chl *a* allomer and epimer generally made up less than 15% of chl *a*. The concentrations of pheophorbide *a* and pyropheophorbide *a*-like were significantly greater at IC than OW stations (Table 2), as was the concentration of chl *a*. When normalized to chl *a* (wt:wt), there were no differences in the concentration of any of degradation pigments detected between IC and OW stations.

The ratio of the pigment pyropheophorbide *a*-"like" to chl *a* (wt:wt) was significantly correlated with the stratification index ($\Delta\sigma_t$) at IC stations ($r_s = 0.49$, $p < 0.05$, $n = 20$). No other significant relationship was found between any of the degradation pigment (normalized to chl *a*) and other physico-chemical variables at IC stations ($p > 0.05$).

After the removal of one outlier (assumed to be an artifact due to the presence of fecal pellets), the pyropheophorbide *a*-"like" concentration (normalized to chl *a*) presented a significant negative relationship with the % LC and with F_v/F_m for IC and OW stations (Fig. 7c,d), suggesting that it could be used as a tracer of low cell viability conditions.

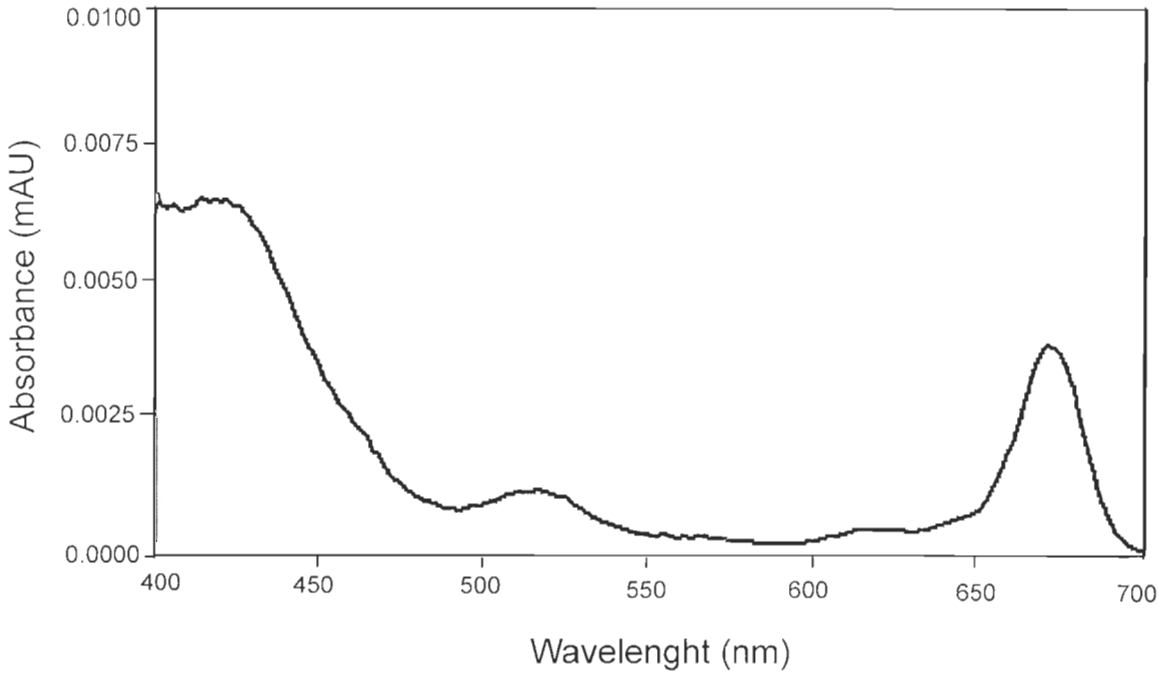

Fig. 8. Spectrum of the detected degradation pigment pyropheophorbide *a*-"like" in the HPLC solvent system of Zapata et al. (2000)

DISCUSSION

Water temperature and salinity values observed during this study were characteristic for the upper water layer in the area, the Polar mixed layer (PML), highly influenced by freezing-melting and river inputs ($S < 33$, $T \approx -1.5°C$, Carmack & Macdonald 2002). The spring-summer 2008 was characterized by an unusually low sea ice cover and warm sea surface temperatures (Forest et al. 2011). As the season progressed, the surface temperatures increased reaching the observed maximum values, coinciding with the complete retreat of the sea ice cover in the region by the end of the study. There was a lowering of the surface salinity due to the summer sea ice melting that increased stratification and reduced the depth of the surface mixed layer (Tremblay et al. 2012). The distribution of nutrients and phytoplankton biomass during the study period have been discussed elsewhere (Mundy et al. 2009, Brown et al. 2011, Tremblay et al. 2011); three consecutive wind-driven upwelling events enhanced the under-ice nutrient availability leading to the observed peaks in chl *a* concentrations at IC stations. Lower nutrient concentrations were observed at OW stations likely because of high stratification, especially later in the season, reducing nutrient replenishment. Vertical stratification and nutrient availability are thought to drive the structure and shape of phytoplankton communities of the surface waters in the Beaufort Sea (Hill et al. 2005). The Arctic community includes sea-ice-associated and pelagic organisms. The landfast ice is characterized by the high abundance of pennate diatoms (Poulin et al. 2011) and other groups during the ice melting period (e.g., prasinophytes, Mundy et al. 2011). The pelagic community includes diatoms, nano- and pico-prasinophytes (especially *M. pusilla*, Lovejoy et al. 2007), and other groups such as cryptophytes or prymnesiophytes (Lovejoy et al. 2006). Our results based on pigments are consistent with these observations.

We observed a large variability in the % LC at both IC and OW stations. This agrees with already reported estimates of cell lysis rates in natural phytoplankton communities that show a large variability in time and space (Agustí et al. 1998, Agustí & Duarte 2000, Brussaard 2004). Bacterial lysis has been studied in the Canadian Beaufort Sea in spring and summer (Payet & Suttle 2013). However, to our knowledge, no previous

study had looked at algal lysis in the western Arctic Ocean. Here we investigated the detailed proportion of living and dead algal cells in the phytoplankton communities using the CDA technique. We believe that using the appropriate precautions as was done here (Llabrés & Agustí 2008 and Chapter 1 of this thesis), it is possible to use the CDA as for other natural communities of the Arctic and Southern Ocean (Lasternas & Agustí 2010, Llabrés & Agustí 2010, Echeveste et al. 2011). Positive correlation with the photosynthetic performance index (F_v/F_m) supports the findings obtained with % LC, although both show a fair deal of variability.

The environmental variables examined (NO_3, irradiance and temperature) had a different influence on the viability of phytoplankton cells, according to the group of stations considered. The distribution of % LC did not show a clear pattern with the concentration of nutrients, as expected from past studies in other oceanic regions (Agustí et al. 1998, Alonso-Laita & Agustí 2006, Lasternas et al. 2010). Nitrate-depleted conditions promote the growth of picophytoplankton over large cells (Li et al. 2009, Ardyna et al. 2011, Balzano et al. 2012a), however in our results the lowest % LC were associated with a community with more prasinophytes relative to diatoms at OW stations. These samples corresponded to the end of May, beginning of June (when nitrate was depleted after consumption): this could be due to the increased presence of prasinophytes during this time and the underestimation of smaller cells in the microscopic analyses. However the % LC for the size group < 5 μm for these samples ranged from 40 to 81% LC; the algal group "prasinophytes" comprises different genera such as *Micromonas*, *Pyramimonas*, *Nephroselmis*, the way these different genera can thrive and survive to different nutrient concentrations and prevail over others might have influenced the observed results (e.g., *M. pusilla* grows over a wide range of nitrate concentrations, Lovejoy et al. 2007, Balzano et al. 2012b).

Irradiance influenced differently the % LC at IC and OW stations; cells at IC were clearly exposed to much lower irradiances than cells at OW stations. Under low irradiances (< 20 μmol photons m^{-2} s^{-1}), the highest % LC at IC stations matched with high percentages of diatoms, likely well acclimated to low irradiances, such as bottom pennate diatoms that

are released and enter the water column. These diatom cells might be photoinhibited at higher irradiances (25-50 μmol photons m^{-2} s^{-1}, McMinn et al. 2007, Mangoni et al. 2009) with potential loss of viability (van de Poll et al. 2005). At higher irradiances (> 20 μmol photons m^{-2} s^{-1}), % LC showed an increasing trend with irradiance at IC stations, along with a community formed of prasinophytes and diatoms likely able to cope with the increasing irradiances of the ice melting conditions (Petrou et al. 2011). These stations were located in landfast ice while the ice cover was retreating or completely retreated (as for stations F7 and FB07, 24-25 June) with the exception of station F5 (the 28 May, ~20% ice cover). For OW stations, the higher % LC values were associated with the diatom bloom in May. These communities had a threshold of optimum irradiance less than 600 μmol photons m^{-2} s^{-1}, showing a clear loss of viability at higher irradiances. This observation is consistent with photoinhibition at irradiances > 600 μmol photons m^{-2} s^{-1} observed in other polar studies (van de Poll et al. 2005, Petrou & Ralph 2011). The significant loss of photosynthetic performance (F_v/F_m) with the increasing irradiance (E_{zm}) confirmed the detrimental physiological condition of the cells. These high irradiance OW stations were also characterized by low photoprotection potential. There was nonetheless a detectable photoprotective response, with the highest PPC:PSC ratios corresponding to a community with a dominance of prasinophytes, matching with high PPC:PSC values in communities dominated by green algae in other oceanic regions (Allali et al. 1997, Roy et al. 2008). The lower values of PPC:PSC ratios in our results corresponded to a community with a higher percentage of diatoms relative to the other groups. These values were comparable to other polar regions (Kropueske et al. 2009, Petrou et al. 2011). Photoprotection capacity might be a key factor explaining cell viability and the fact that this capacity differs among diatoms (Dimier et al. 2007, Lavaud et al. 2007) might explain some of the variability in the % LC encountered in our results for irradiances lower than 600 μmol photons m^{-2} s^{-1}. Values of (DD+DT):chl *a* above 0.4 wt:wt have been suggested to occur during nutrient limitation in Antarctic diatoms (van de Poll et al. 2005, van de Poll & Buma 2009). However the thinning of the mixed layer, the increase of stratification and the marked seasonal increase of daily irradiances, probably exposed the cells to high

irradiances (including potentially damaging UV) for longer periods (day length 24 h), especially by the end of the season when irradiances and stratification were higher, likely causing the observed viability loss in diatoms. Unfortunately nutrient concentrations were not always determined at the stations with the measured higher irradiances, making it difficult to determine if there was a combined effect of nutrient limitation and excessive irradiance causing the observed loss of viability (van de Poll et al. 2005).

Within the ranges of temperatures observed during the present study there was a combination of meltwater (– 2 to 2°C) and pelagic (> 2°C) communities occupying these distinct ecological niches (see Petrou & Ralph 2011). These two communities responded differently to temperature. The ice-covered community showed a decrease of % LC above -1°C, with the lowest % LC stations being characterized by a dominance of prasinophytes. In contrast, the increase of temperatures at OW stations seemed to favor diatoms. Some polar algae have the capacity to adjust to higher temperatures (> 7°C, Michel et al. 1989) with no influence on photosynthetic performance (F_v/F_m~0.6, Petrou et al. 2012). In our results F_v/F_m did not show a good relationship with % LC at OW stations, however high F_v/F_m values are not always evidence of the absence of dead cells (Franklin et al. 2009) and these values can vary widely in natural communities from polar environments (McMinn & Hegseth 2004).

The degradation pigment pyropheophorbide *a*-"like" (relative to chl *a*) was the only pigment that showed a good relationship with both % LC and F_v/F_m. In contrast with some other studies (Llewellyn et al. 2008, Szymczak-Żyła et al. 2008), we did not find a good relationship of % LC (or F_v/F_m) with chlorophyllide *a* often considered a marker of cell senescence. In a recent study on cell senescence in the haptophyte *Emiliania huxleyi* and the diatom *Thalassiosira pseudonana*, Franklin et al. (2012) found species-specific senescence responses, which included increases in the pigment methoxychlorophyll *a* only in *T. pseudonana*. In the natural communities examined in the present study, the pigment pyropheophorbide *a*-"like" appears to be a good marker for senescence, more likely to be associated with the ice-covered community.

CONCLUSIONS

This study provides the first assessment of algal cell viability (as percentages of living and dead cells) in the surface waters of the Beaufort Sea. The % LC varied widely, this variation was related in part by differences in the community composition. % LC was influenced by temperature and irradiance showing distinct trends for IC and OW stations. Photosynthetic performance and living cells decreased with increasing irradiance, suggesting a detrimental effect of high irradiances on the physiological condition of phytoplankton. The lower % LC values were related to melting temperatures at IC stations and to the highest irradiances at OW stations. A pigment that we called pyropheophorbide *a*-"like" and that will need to be fully identified with appropriate techniques (such as LC-MS) shows potential as a marker pigment for the loss of cell viability in this environment. Based on the major marker pigments detected, diatoms seemed to thrive better than other groups during the spring-summer melting conditions, likely because they occupy specific ecological niches in which they are favored. This study highlights the complexity of the responses of natural communities submitted to different perturbation factors of the environment.

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CONCLUSION GÉNÉRALE

La dynamique des populations algales est basée sur le bilan entre les gains et les pertes de biomasse. Cependant, la mortalité du phytoplancton comme facteur de perte n'a reçu que très peu d'attention jusqu'à maintenant. Il y a peu de connaissances sur les facteurs qui peuvent influencer et/ou déclencher la mort cellulaire dans les communautés naturelles, particulièrement dans les régions polaires. Cette thèse fournit les premières informations sur la viabilité des communautés algales de la portion canadienne de la mer de Beaufort pendant le printemps et l'été. Les résultats montrent une proportion moyenne de cellules phytoplanctoniques vivantes pour la mer de Beaufort de 51.6 ± 3.3%, ce qui est comparable aux résultats obtenues par Lasternas (2012) dans les eaux de Svalbard au nord de la Norvège ainsi que pour la région Antarctique (Tableau 1).

Tableau 1. Pourcentages (moyenne ± SE) des cellules phytoplanctoniques vivantes déterminés dans la présente étude et dans d'autres régions océaniques (voir Lasternas 2012)

	Arctique	Arctique	Arctique	Antarctique	Atlantique	Mediterranée
	Mer de Beaufort	Mer de Beaufort	Svalbard			
	Eaux ouvertes	Eaux couvertes de glace				
	Cet étude	Cet étude	Lasternas 2012	Lasternas 2012	Lasternas 2012	Lasternas 2012
% LC	57.3 ± 5.8	48.0 ± 3.9	44.4 ± 6.8	58.8 ± 3.7	55.9 ± 3.2	61.9 ± 2.9

Dans le chapitre 1, j'ai testé la méthode de digestion cellulaire (CDA) afin de vérifier la viabilité des communautés d'algues de glace en Arctique. Dans ce chapitre, j'ai aussi examiné l'influence de l'intensité lumineuse sur la viabilité cellulaire en comparant des sites aux couverts de neige variables et en mesurant le pourcentage de cellules vivantes (% LC) avec la méthode CDA. Pendant la floraison printanière, la viabilité cellulaire algale était influencée par l'intensité lumineuse; les % LC les plus faibles étaient trouvés sous les couverts de neige minces (éclairements plus élevés, ~25 μmol photons m^{-2} s^{-1}). Ces résultats sont en accord avec des études précédentes rapportant de la photo-inhibition chez les algues de glace à des niveaux d'éclairement dépassant les 20 μmol photons m^{-2} s^{-1} (e.g. Booth 1984). Cependant, les petites cellules algales (< 5 μm) ne semblaient pas affectées par des intensités lumineuses plus élevées. Outre les faibles valeurs en % LC mesurées sous de minces couverts de neige, j'ai trouvé des indices de détérioration physiologique à partir des signatures de deux pigments de dégradation de la chlorophylle *a* (chl *a*), la chlorophyllide *a* et l'allomère de la chl *a*. La concentration de ces deux pigments (relativement à la chl *a*) montrait une augmentation significative suivant l'augmentation de l'éclairement retrouvé à l'interface glace-eau. Une relation positive (mais non significative) a aussi été montrée entre ce dernier paramètre et le pourcentage de cellules mortes. La disparité entre ces observations pourrait être due au nombre limité de données de l'étude et au fait que la mort cellulaire prend plus de temps à survenir que la dégradation de l'état physiologique, lequel est rapidement visible dans les données pigmentaires. J'ai aussi étudié l'influence de la lumière sur la viabilité cellulaire en utilisant des cultures discontinues de la diatomée centrale *Attheya septentrionalis*, une espèce présente aussi bien dans la glace de mer que dans la colonne d'eau en Arctique. Les cultures ont été soumises à différentes intensités lumineuses. Après 4 jours, les cellules exposées à une lumière constante de 170-180 μmol photons m^{-2} s^{-1} ont montré une performance photosynthétique ($F_v/F_m < 0.2$) et des % LC (< 20 %) inférieurs aux cellules exposées à 70 μmol photons m^{-2} s^{-1} (contrôle; F_v/F_m = 0.6, % LC = env. 80%). Ces résultats ont montré que l'intensité lumineuse est un facteur clé influençant la photo-physiologie et la viabilité des communautés des algues de glace au printemps.

Les résultats présentés dans cette thèse mettent en évidence l'influence des variables environnementales sur la physiologie et la viabilité cellulaire algale et aident à répondre aux deux principales questions concernant la productivité biologique dans le projet CFL. Tandis que le chapitre 2 aide à comprendre l'influence de la lumière pour la structure de la communauté algale associée à la glace, le chapitre 3 met en évidence l'influence de la lumière et des éléments nutritifs sur la biomasse et la composition phytoplanctonique dans la région d'étude. Dans ce dernier chapitre, mes résultats indiquent le rôle mineur des nutriments comme facteur de contrôle de la dynamique du phytoplancton dans cette région, ce qui a déjà été suggéré dans d'autres études (Estrada et al. 2009, Lasternas 2012).

Spécifiquement, dans le chapitre 2, j'ai examiné l'influence d'un couvert de neige d'épaisseur variable sur la composition pigmentaire des algues de glace pendant les trois périodes distinctes de la succession algale durant la floraison printanière, soit le commencement de la floraison, le moment où le bloom atteint son intensité maximale et la fin du bloom. J'ai utilisé des pigments spécifiques produits par les algues et leurs produits de dégradation pour déterminer la composition taxonomique et l'état photo-physiologique des communautés d'algues de glace. Au-delà des changements dans la composition de la communauté algale qui prennent place au fil du bloom, les conditions de couverture de neige vont aussi affecter la composition de la communauté algale à l'intérieur d'une même période. Ceci met donc en évidence le rôle important de la lumière dans cet environnement.

Pendant les deux premières périodes du bloom, les diatomées contenant de la chlorophylle c_2 (chl c_2) et de la chl c_3 (diatomées de type pigmentaire 2) dominaient la biomasse de la chl *a* tandis que les diatomées contenant de la chl c_1 (type pigmentaire 1) et les petits flagellés (par exemple chlorophytes et prasinophytes) étaient seulement présents aux sites avec un couvert de neige épais (faible éclairement). À la fin de la floraison, j'ai observé une plus grande diversité pigmentaire à la base de la glace avec une plus grande proportion de chlorophytes, prasinophytes et de dinoflagellés, au détriment des diatomées.

La réponse photoprotectrice (comme le ratio pigments photoprotecteurs/ pigments photosynthétiques) et le statut physiologique des cellules (basé sur les pigments

produits lors de la dégradation de la chl *a*) varient aussi entre les différentes épaisseurs de couvert de neige. Les réponses photoprotectrices les plus élevées et les signatures pigmentaires associées à des conditions physiologiques détériorées, telles que l'augmentation de la chlorophyllide *a* et de l'allomère de chl *a*, ont été trouvées aux sites présentant un couvert de neige mince pendant la période de l'étude. Comme l'augmentation de la chlorophyllide *a* et de l'allomère de chl *a,* relativement à la chl *a*, pour les sites au couvert de neige mince était accompagnée d'une augmentation de la biomasse et des pigments photoprotecteurs, nous avons suggéré que la plus grande disponibilité de la lumière au début et au milieu du bloom devait être avantageuse pour les cellules, comme suggéré par Juhl et Krembs (2010). La présence des produits de dégradation de la chl *a* pourrait alors être liée à la mort et au remplacement d'espèces photosensibles par des espèces plus tolérantes à la lumière dotées d'une forte croissance et d'un bon potentiel de photoprotection. De plus, le fait que le ratio pigments photoprotecteurs/pigments photosynthétiques présentait la meilleure corrélation avec l'intensité lumineuse moyenne au bas de la glace au cours des 3 journées précédentes, suggère que la photoacclimatation a eu lieu. Ces résultats mettent en évidence l'influence importante de la lumière et la capacité d'acclimatation des algues de glace de mer en Arctique.

Pendant la période « post-bloom », il y avait plusieurs indications de sénescence algale, notamment l'augmentation de l'abondance de grandes diatomées vides et l'augmentation des concentrations de la chlorophyllide *a* et de l'allomère de la chl *a*. La sénescence algale est probablement liée à l'épuisement des éléments nutritifs et aux fortes intensités lumineuses. L'extrapolation de ces résultats dans le contexte du réchauffement climatique en Arctique suggère que le bloom printanier d'algues de glace pourrait profiter d'une réduction du couvert de neige (influence avantageuse de la lumière au début du bloom), mais pourrait être de plus courte durée en raison d'une saison de glace raccourcie.

Dans le chapitre 3, j'ai étudié l'influence de certains facteurs-clé de l'environnement sur la viabilité du phytoplancton dans les eaux de surface pendant la période de transition du printemps à l'été. Pendant cette période, les cellules phytoplanctoniques sont exposées à

de plus fortes intensités lumineuses, en raison de l'augmentation saisonnière de l'éclairement incident, de la fonte du couvert de glace et des changements dans l'épaisseur de la couche de mélange de surface.

La viabilité des cellules phytoplanctoniques a montré une grande variabilité spatiale et temporelle. Cette variabilité s'expliquait partiellement par les différences dans la composition des communautés et était influencée par la température de l'eau et l'éclairement. Pour les stations couvertes de glace, les plus faibles % LC étaient associés aux températures de fonte de glace, alors qu'ils s'associaient plutôt aux fortes intensités lumineuses dans les stations d'eau libre. Les communautés en eau libre ont montré une perte nette de viabilité à des intensités lumineuses supérieures au seuil de 600 µmol photons m^{-2} s^{-1}. Ces découvertes sont en accord avec des études précédentes menées dans les environnements polaires qui montrent une forte photo-inhibition des algues soumises à des forts éclairements (van de Poll et al. 2005, Petrou et Ralph 2011).

Un pigment que nous avons appelé « simili »-pyrophéophorbide *a* et qui devra être mieux identifié avec des techniques appropriées (telles que le LC-MS) a montré un potentiel comme marqueur pigmentaire pour la perte de viabilité cellulaire dans la mer de Beaufort.

Cette étude souligne la complexité des réponses des communautés algales naturelles lorsqu'elles doivent faire face à des changements environnementaux. Il semble que les diatomées pélagiques se portaient mieux que d'autres groupes pendant la période de fonte de glace, au printemps-été, montrant qu'elles semblent occuper des niches écologiques spécifiques dans lesquelles elles sont favorisées.

Perspectives de recherche

La présente étude montre que des mesures de pigments algaux combinées avec la méthode CDA et des observations microscopiques peuvent fournir de précieuses informations sur l'écologie du phytoplancton arctique et en particulier sur les mécanismes impliqués dans les pertes de biomasse phytoplanctonique dans la colonne d'eau. Dans des travaux futurs, je propose d'utiliser la méthode CDA, combinée avec une analyse pigmentaire HPLC, afin de mesurer la viabilité cellulaire, comme dans Hayakawa et al. (2008). Cette approche a l'avantage d'être plus rapide que le dénombrement des cellules en microscopie. De plus, le couplage du CDA avec la cytométrie en flux pourrait améliorer les évaluations futures pendant les périodes où de petites cellules (<5 μm et 5-20 μm) représentent une fraction importante de la communauté algale.

Dans les chapitres 1 et 2, j'ai utilisé la méthode conventionnelle selon laquelle la glace de mer est lentement fondue à l'obscurité dans l'eau de mer filtrée (>15 h). Comme les diatomées sont des composantes importantes des communautés d'algues de glace et que ce groupe algal est reconnu pour sa performance photosynthétique et sa capacité photoprotectrice, il sera essentiel dans des travaux futurs d'étudier comment la méthode actuelle (i.e. fonte de l'eau de mer filtrée à l'obscurité) affecte la composante rapide du cycle des xanthophylles, soit la déépoxydation de la diadinoxanthine (DD) en diatoxanthine (DT). En effet, la procédure actuelle est suffisamment longue pour que la DT puisse être transformée en DD pendant la fonte de la glace à l'obscurité. De nouvelles études sont nécessaires pour déterminer s'il existe une influence de la durée de décongélation de l'échantillon de glace sur le cycle des xanthophylles.

Les résultats du chapitre 3 indiquent que les diatomées pélagiques semblaient mieux se porter que d'autres groupes algaux pendant la saison de fonte de la glace de mer. Ce résultat est en accord avec d'autres études conduites dans l'océan Antarctique côtier (van de Poll et al. 2011) qui suggèrent qu'une plus forte capacité photoprotectrice des diatomées par rapport à d'autres groupes (e.g. haptophytes) expliquerait leur dominance dans les eaux stratifiées en raison de la fonte.

L'identification des espèces de diatomées les plus importantes, notamment de celles présentes durant la période de fonte, avec des outils moléculaires et par la taxonomie, pourrait aider à déterminer s'il existe des différences interspécifiques dans la capacité photoprotectrice. Ceci pourrait aider à mieux comprendre les seuils au-delà desquels des réponses de photoacclimatation et des dommages causés par la lumière apparaissent, ainsi que leur influence sur la viabilité cellulaire.

Malgré que les algues de glace et le phytoplancton aient montré des réponses photoprotectrices à de fortes intensités lumineuses, elles ne mourraient pas nécessairement suite à cette exposition. Aucune relation significative entre le pourcentage de cellules vivantes et les pigments associés à la photoprotection n'a été trouvée au cours de cette étude. De nouvelles études sont nécessaires pour évaluer les différents mécanismes de photoprotection des espèces clés de l'Arctique et leurs relations avec la mort et la survie des algues.

Comme suggéré par mes résultats, le rayonnement UV pourrait être un facteur important expliquant la perte de viabilité observée dans les communautés d'eau libre des eaux de surface. Cette variable, qui n'a pas été directement mesurée pendant cette étude, pourrait faire l'objet d'études futures dans la même zone d'étude.

Il a été montré que les virus sont une cause majeure de mortalité dans les océans (Suttle 2005) et ils ont été détectés dans le passé dans les eaux de surface de la zone d'étude, au printemps et à l'été (Payet et Suttle 2008). Dans la présente étude, je n'ai pas évalué si les microalgues étaient infectées par des virus. Je pense qu'il sera très important de déterminer s'il y a un lien entre la présence de virus, l'infection virale et la mort cellulaire, particulièrement suite à la détection du virus DsRNA infectant la pico-prasinophyte *Micromonas pusilla* (Brussaard et al. 2004), puisque cette espèce picoeucaryote est très abondante dans les eaux couvertes de glace et les eaux libres de l'Arctique (e.g. Lovejoy et al. 2007).

Ma thèse améliore énormément les connaissances sur la viabilité cellulaire, la performance photosynthétique et les mécanismes de photoprotection des algues de glace et du phytoplancton dans les eaux couvertes de glace et les eaux libres de l'Arctique. Elle est la première étude évaluant la viabilité des algues de glace dans les régions polaires et du phytoplancton dans les eaux de surface de la mer de Beaufort. Elle constitue aussi la première étude sur la composition pigmentaire des algues de glace dans le Haut-Arctique. Les marqueurs pigmentaires chémo-taxonomiques (avec le logiciel CHEMTAX) ont été utilisés pour évaluer la contribution des divers groupes d'algues de glace à la biomasse totale de chlorophylle *a* pendant les différents stades du bloom printanier des algues de glace.

Les impacts du changement climatique sont actuellement en train d'affecter l'Arctique d'une façon probablement irréversible. Les producteurs primaires jouent un rôle prédominant sur les cycles biogéochimiques. Il est donc important d'améliorer les connaissances sur la réponse des communautés algales aux changements présentement en cours des conditions du milieu.

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