NNT/NL : 2022AIXM0047/004ED251

THÈSE DE DOCTORAT

Soutenue à Aix-Marseille Université

le 18 mars 2022 par

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Etude de la dégradation des algues de glace et du phytoplancton d'eau libre en zone Arctique : impact de l'état de stress des bactéries associées à ce matériel sur sa préservation et sa contribution aux sédiments

Discipline
Sciences de l'Environnement

Spécialité
Océanographie

École doctorale
Sciences de l'Environnement (ED251)

Laboratoire/Partenaires de recherche
Institut Méditerranéen d'Océanologie (MIO) - Marseille

Composition du jury

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Brivaëla MORICEAU *LEMAR*	Rapporteuse
Sylvie COLLIN *METIS*	Examinatrice
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Patricia BONIN *MIO*	Co-directrice de thèse

Affidavit

Je soussigné, Christopher Burot, déclare par la présente que le travail présenté dans ce manuscrit est mon propre travail, réalisé sous la direction scientifique de Jean-François Rontani et Patricia Bonin, dans le respect des principes d'honnêteté, d'intégrité et de responsabilité inhérents à la mission de recherche. Les travaux de recherche et la rédaction de ce manuscrit ont été réalisés dans le respect à la fois de la charte nationale de déontologie des métiers de la recherche et de la charte d'Aix-Marseille Université relative à la lutte contre le plagiat.

Ce travail n'a pas été précédemment soumis en France ou à l'étranger dans une version identique ou similaire à un organisme examinateur.

Fait à Marseille, le 13 décembre 2021

Liste de publications et participation aux conférences

1. Liste des publications[1] réalisées dans le cadre du projet de thèse :

   1.1. Amiraux, R., Burot, C., Bonin, P., Guasco, S., Babin, M., & Rontani, J.-F. (2020). *Stress factors resulting from the Arctic vernal sea ice melt: Impact on the viability of the bacterial communities associated to sympagic algae*. Elementa Science of the Anthropocene 8 (1): 076. https://doi.org/10.1525/elementa.076

   1.2. Burot, C., Amiraux, R., Bonin, P., Guasco, S., Babin, M., Joux, F., Marie, D., Vilgrain, L., Heipieper, H.J., Rontani, J.-F., 2021. *Viability and stress state of bacteria associated with primary production or zooplankton-derived suspended particulate matter in summer along a transect in Baffin Bay (Arctic Ocean)*. Science of The Total Environment 770, 145252. https://doi.org/10.1016/j.scitotenv.2021.145252

   1.3. Amiraux R, Bonin P, Burot C, Rontani. 2021 *Preservation of sympagic algae in Canadian Arctic sediments: A process controlled by the physiological state of their attached bacteria*. Microorganisms *9*(12), 2626. https://doi.org/10.3390/microorganisms9122626

2. Participation aux conférences[2] et écoles d'été au cours de la période de thèse :
   2.1. C. Burot, R. Amiraux, P. Bonin, S. Guasco, M. Babin, H. J. Heipieper, F. Joux , D. Marie, J-F. Rontani. *Viability and stress state of bacteria associated to suspended particulate matter in summer along a transect across the Baffin Bay*. GreenEdge project Final meeting – 2019 – Nice (présentation orale)

   2.2. C. Burot, R. Amiraux, P. Bonin, G. Massé, S. Guasco, M. Babin, F. Joux, D. Marie, L. Vilgrain, H.J. Heipieper, F. Vaultier, J-F. Rontani 2021. *Stress State and Viability of Bacteria Associated with Sympagic Algae in Ice and Particulate Matter During the 2016 Melting Season in Baffin Bay (Arctic Ocean)*. World Microbe Forum 20, (poster en ligne). https://wmf2021-asm.ipostersessions.com/default.aspx?s=B8-D8-CB-3B-23-E3-2F-21-93-2C-0C-D7-13-7D-55-B0&guestview=true

   2.3. Burot, C., Amiraux, R., Bonin, P., Guasco, S., Babin, M., Joux, F., Marie, D., Vilgrain, L., Heipieper, H.J., Rontani, J.-F., 2021. *Viability and stress state of bacteria associated with primary production or zooplankton-derived suspended particulate matter in summer along a transect in Baffin Bay (Arctic Ocean)*. 28ème Congrès des doctorants de l'ED 251 (présentation orale).

[1] Cette liste comprend les articles publiés, les articles soumis à publication et les articles en préparation ainsi que les livres, chapitres de livre et/ou toutes formes de valorisation des résultats des travaux propres à la discipline du projet de thèse. La référence aux publications doit suivre les règles standards de bibliographie et doit être conforme à la charte des publications d'AMU.

[2] Le terme « conférence » est générique. Il désigne à la fois « conférence », « congrès », « workshop », « colloques », « rencontres nationales et/ou internationales » … etc.

Indiquer si vous avez fait une présentation orale ou sous forme de poster.

Remerciements

Et bien il est arrivé le temps des remerciements. Cela fait de nombreuses semaines que j'y pense, il y'a tant de personnes impliquées de près ou de loin dans cette thèse j'espère n'oublier personne. D'avance, j'adresse mes plus sincères remerciements à toutes celles et ceux que j'aurais pu oublier.

Je tiens premièrement à remercier les membres de mon jury : monsieur Fabien Joux, madame Brivaëla Moriceau, madame Sylvie Collin et monsieur Philippe Cuny pour le temps et l'attention que vous prendrez à évaluer mon travail de ces 3 dernières années.

Cette thèse s'est déroulée sous la direction de Jean-François Rontani et Patricia Bonin, mes deux Grands Chefs. Je vous remercie pour votre accompagnement, votre encadrement et votre patience à mon égard. Je n'ai certainement pas été le doctorant parfait (existe-t 'il seulement ?) et je vous suis reconnaissant d'avoir toujours été pédagogues et bienveillant. Tout cela malgré ma difficulté à parler, à poser des questions et mes gaffes à répétition. Ce fut un honneur d'être encadré par de tels chercheurs, passer ces 3 années sous votre direction m'a fait grandir, encore une fois merci.

Cela va bientôt faire 4 ans au sein du MIO, que de personnes rencontrées ici, de collègues devenus amis et de belles rencontres. Je pense en premier à vous, ceux que j'ai envie d'appeler la « Dream team », nous qui nous appelions les « 4 Fantastiques », je pense bien évidemment à Léa (je pourrais dire la plus belle, mais je ferai des jaloux), Maud et Djou (même si ou a fin' barrer aster pess ti makro). Merci à vous 3 pour tout, c'est grandement grâce à vous que j'ai réussi à tenir le coup pendant les moments difficiles. Merci pour votre bonne humeur infaillible, pour votre soutien moral et votre motivation. Merci aussi de toujours me laisser vous taxer des petits gâteaux éhé. Force à toi Maud pour la fin, si moi j'ai pu y arriver, tu arriveras au bout de ta thèse les yeux fermés !

Depuis mon arrivée en stage de M2 au MIO, j'ai été immédiatement à l'aise et me suis senti intégré, je remercie donc tous les membres de l'équipe MEB, plus particulièrement, Cécile, Philippe, Sophie, Yannick, Sylvain, Laure (pas MEB mais faisons une exception). Merci aussi à toi Morgane, pour m'avoir hébergé maintes fois, bon courage à toi et Greg pour cette aventure qu'est la parentalité. Merci aussi à toi Aude, grâce à qui j'ai « presque »

lu plus de livres que d'articles pendant ces 3 ans. Bref, un grand merci pour tous ces bons moments partagés à table ou autour d'une petite mousse.

Un véritable IMMENSE merci à Laurie, Corinne et Gwénola, particulièrement pour votre aide et votre acharnement sur ce dernier sprint final. C'est en très grande partie grâce à vous, votre expertise et vos qualités en biomol que ce dernier chapitre a pu s'écrire. Je vous suis extrêmement reconnaissant pour tout ce que vous avez réalisé pour cette thèse ces dernières semaines. Faisant également partie de l'équipe CEM, je remercie Cathy pour ta bonne humeur communicative, ainsi que Fred, pour ton humour (douteux cela dit), ton aide et ces cafés partagés tout au long de ces 3 années.

Je remercie également tous les doctorants que j'ai eu la chance de côtoyer au MIO : Chloé, Cathy, Marie, Caro, Floriane, Térence, je vous souhaite à toutes et tous de la réussite et un avenir radieux (même si pour toi Cathy c'est déjà bien parti).

Merci à la liste sport, grâce à qui j'ai réussi à me bouger un peu les fesses et prendre du plaisir à pratiquer une activité physique. Je pense à la team Bad, avec Nagib, François, Olivier, Floriane (encore !), Guillaume, Fabrice, peut être qu'un jour vous aurez des lignes tracées au SAM, qui sait ? Un grand merci aussi à Vincent, qui a réussi l'exploit de me faire apprécier la course, j'en ai bavé au début mais ces petits tours à Sugiton ou au mont Puget entre midi et 2 m'ont fait le plus grand bien.

Merci à toi aussi cher Rémi, véritable aventurier de l'Arctique. Merci pour ta présence tout au long de 3 ans, merci pour tes réponses et ton aide sur ce manuscrit et les articles. J'espère que tu trouveras le poste de tes rêves !

La fin de cette thèse marque aussi la fin de l'expérience marseillaise. Que de rencontres dans cette belle cité Phocéenne depuis mon arrivée ici en L3. J'aimerai remercier tous les collègues de Licence et de Master, un grand merci à Ari, Robin, Florian, Paulo, Héloïse (force à toi pour la fin de ta thèse d'ailleurs), Marie (ma chère grognasse) Cimane et tous les autres, ces années ont été les plus belles grâce à vous. Tant que j'y suis, je remercie mon bon Jim et ta cave « Chez Malt » (23 Rue des trois Mages, 13001 Marseille, si jamais je peux te faire un peu de pub), je ne compte plus les passages chez toi en rentrant du labo, je t'en ai acheté des bières, et la réussite de cette thèse est en partie grâce à toi, oui oui.

Ces 3 années de thèse se sont passées entre Marseille et Avignon, je remercie d'ailleurs un peu malgré moi la SNCF, grâce à qui, après 3 ans d'aller-retours quasi quotidiens, j'ai fait pratiquement 5 fois le tour du monde sur les rails (c'est approximatif, vous ne m'en voudrait pas). Aussi j'aimerai remercier toutes les merveilleuses personnes rencontrées

dans la Cité des Papes qui sont maintenant des amis. Je pense à Alice, Louise (et ton humour légendaire), Valentin (le pur BG), Elodie, Enrico, Dario, Léo, Pierre et tous les autres. J'ai aussi une pensée pour ma chère collègue doctorante croate Antonija, je sais que cette thèse est aussi difficile pour toi, mais hang on, tu peux le faire. Merci à toi Aurel, pour ton fier établissement le Beer District qui m'a accueilli parfois plus que de raison, c'est sans aucun doute chez toi que m'est venue l'inspiration pour ce manuscrit. J'en profite pour remercier les gens super rencontrés autour d'une mousse dans ton établissement, je pense à Toni, Quentin, Rémy, Charlotte et les autres. Dans le même ton et les mêmes raisons je te remercie Théo et ton Beer Garden, vous avez su étancher ma soif.

Nous voilà bientôt à la fin, mais il reste quelques personnes à remercier qui ne rentrent dans aucune case. Je remercie bien évidement mes parents et toute ma famille : Maman, Papa, André, Yannick, Solène, Hang. Vous m'avez accompagné et soutenu pendant toutes ces années. Merci pour la liberté que vous m'avez laissée et votre compréhension. Merci aussi à mes beaux-parents Phillipe et Dominique, pour votre soutient infaillible (et aussi pour ce super ordinateur portable qui a grandement servi à la rédaction de ce manuscrit).

J'aimerai aussi grandement remercier ma chère voisine, Maha, pour tes apparitions spontanées sur le palier lors de ces 3 dernières années, les discussions infinies, le bon vin et la bonne bouffe, je te souhaite tout ce qu'il y'a de meilleur.

Merci chère Aurélie, pour être une des meilleures collègues de bureau possible. Grâce à toi j'ai démarré cette thèse sur les bons rails. Je te souhaite a meilleure fin de thèse qui soit !

Merci à toi Charlène, d'avoir amené ton soleil dans mon bureau à un moment difficile, que l'avenir te soit radieux.

J'ai une pensée particulière pour mes années passées à la Réunion. Nombreuses personnes me viennent en tête quand je pense à ces années, mais quelqu'un a initié quelque chose et je me dois de la poursuivre ! J'ai donc une pensée pour mes compagnons de musique, je pense évidemment aux membres de Blackskull : Robin, Fredo, Chloé, Amandine et Samuel. Ces expériences de scène et d'album sont des souvenirs qui me sont chers, et j'y repenserai toujours avec une douce mélancolie.

Je remercie également la belle brochette de joyeux lurons que sont le groupe des « beaux mecs » (ou des « beaufs mecs », ça dépend des jours), Pierrick, Maxime, Rémy,

Benjamin, Matthieu, Jean-Charles, Mickaël. Merci pour ces discussions, toutes ces bières bues, pardon ces « dégustations » de liquides maltés et savamment houblonnés. A la prochaine IRL !

Je te remercie également, mon cher Nicolas, pour être le meilleur ami que l'on puisse avoir. Merci pour ces parenthèses que représentaient ces week-ends à Sanary, il me tarde de te revoir. Merci aussi pour toutes ces soirées, toutes les fois où tu m'as hébergé quand j'avais cours (ton canapé lit remporte d'ailleurs le prix du meilleur canapé lit que j'ai pu squatter pendant ces 3 ans). J'espère que tu resteras longtemps mon Poto.

Et enfin, "last but not least" comme on dit, merci à toi Marie, toi qui partages ma vie depuis plus de 8 ans. Merci de m'avoir soutenu pendant toutes ces années, de m'avoir poussé vers le haut et motivé quand j'étais au plus bas. Je te revois en L3 nous faire des programmes de révision pour les partiels, je ne serai jamais arrivé jusqu'ici sans son soutient infaillible. Pour ça, pour tout le reste et pour tout ce qu'il nous reste, merci, je t'aime.

J'espère n'avoir omis personne, si c'est le cas je m'en excuse, mais sachez que je ne vous oublie pas. Je n'ai pas vraiment quelqu'un à qui dédier cette thèse, c'est donc à vous tous, vous qui avez cru en moi et vous qui m'avez soutenu que je dédie ces travaux. Du fond du cœur, encore merci.

Bon allez, je pense qu'il faut y aller maintenant. Bonne lecture !

Résumé

L'océan Arctique et les perturbations de son fonctionnement liées au réchauffement global constituent une préoccupation majeure de la communauté scientifique mondiale. A la base du fonctionnement de l'Océan Arctique se trouve la production primaire, assurée par des organismes sympagiques appelés algues de glace. Ces dernières peuvent assurer dans certaines régions arctiques jusqu'à 60% de la production primaire totale. Etant donné la relative faible activité de dégradation de ce matériel par le bacterioplancton dans ces régions (entre 25 et 80% du bacterioplancton étant mort ou inactif) et la capacité du matériel sympagique à chuter rapidement vers les sédiments, les algues de glace contribuent fortement à l'export de carbone atmosphérique vers les fonds marins. La faible activité de dégradation du bacterioplancton peut être attribuée à : (i) un stress osmotique dans les canaux de saumure hypersalés de la glace, (ii) un stress chimique lié à la production de composés bactéricides par les algues de glace ou (iii) un stress photooxydatif.

L'objectif de cette thèse est donc de mieux comprendre l'impact de ces différents stress sur l'état physiologique des bactéries associées au matériel sympagique et sur la préservation de ce dernier le long de sa chute vers les sédiments. L'effet des stress halins et chimiques a pu être mis en évidence *via* l'analyse de nombreux échantillons de particules en suspension, en sédimentation ou de sédiments récoltés lors de diverses campagnes de prélèvement dans l'Arctique Canadien. Ces analyses ont démontré l'importance du stress halin sur les bactéries associées aux algues sympagiques, notamment au début du cycle de fonte, qui est à l'origine d'une activité de biodégradation moindre par des bactéries associées. L'importance du stress lié à la production des acides gras libres par les algues de glace, notamment en cas de forte irradiation de ce matériel a également été mis en évidence. En effet, les quantités d'acide palmitoléique libre mesurées dans nos échantillons (allant jusqu'à 4,8 $mg.L^{-1}$) semblent être à l'origine de la forte mortalité (pouvant aller jusqu'à 75%) des bactéries associées observée dans la glace et dans les particules. L'importance du stress photochimique sur les bactéries sympagiques ainsi que la résistance accrue des bactéries pigmentées à ce stress ont aussi pu être démontrées. Ces résultats peuvent en partie expliquer la forte occurrence de bactéries pigmentées dans les communautés de bacterioplancton en fin de blooms phytoplanctoniques.

Abstract

The Arctic Ocean and the expected disruption of its functioning due to global warming is a major concern for the scientific community. The root of the Arctic Ocean functioning is primary production, covered by sympagic (ice-associated) organisms called sea-ice algae. These sea-ice algae provide up to 60% of the annual primary production, depending on the region. Given the apparent low degradation activity of the bacteria associated to this material in Arctic regions (between 25-80% of dead or inactive bacterioplankton), and the high sinking rates of sympagic algae, it is expected that sea-ice algae contribute significantly to the export of atmospheric carbon to the seafloor. This weak activity may be attributed to: (i) osmotic stress in hypersaline brine channels of ice, (ii) chemical stress linked to the production of bactericidal compounds by sympagic algae, or (iii) photochemical stress.

The main objective of this thesis is to better understand the impact of the stresses on the physiological state and the degradation capacities of bacteria associated to sympagic material, and the subsequent preservation of the latter along its fall to the sediments. The impact of saline and chemical stress has been highlighted through the analysis of numerous samples of suspended or sedimenting particles and surface sediments collected in the Canadian Arctic. These analyses have demonstrated the significance of saline stress, especially during the early stages of the melting season on bacteria associated with sympagic material, resulting in a very low to no degradation activity. The importance of stress related to the production of free fatty acids, especially in cases of strong irradiation of sea-ice algae has also been put forward. Indeed, the high concentrations of free palmitoleic acid (up to 4,8 $mg.L^{-1}$) measured in ice might be at the origin of the high mortality of associated bacteria measured (up to 75%). Finally, the importance of bacterial photochemical stress in ice and the better resistance of pigmented bacteria to this stress were also demonstrated. These results may partly explain the high occurrence of pigmented bacteria in bacterioplankton communities at the end of bloom events.

Liste des figures

Liste des tableaux

Liste des abréviations (par ordre alphabétique)

DOX : Dioxygénase

^{1}Chl : Chlorophylle (état singulet)

^{3}Chl : Chlorophylle (état triplet)

$^{1}O_2$: Oxygène singulet / singlet oxygen

8-oxodG/^{O}G : 8-oxo-7,8dihydro2'-deoxyguanosine

AAPs : Aerobic Anoxygenic Phototrophs

AGs : Acides gras

AMOC : Atlantic Meridional Overturning Circulation

BA : Bacterial Abundance

BChl a : Bactériochlorophylle a

BER : Base Excision Repair

BP : Bacterial Production

BSTFA : N,O-Bis(trimethylsilyl)trifluoroacetamide

CAT : Catalase

Chl : Chlorophylle

CPPI : Chlorophyll Phytyl side chain Photodegradation Index

CTI : Cis/Trans Isomerase

DCM : Dichloromethane

DMSP : Dimethyldisulfopropionate

DOC : Dissolved Organic Carbon

ECF : Extracytoplasic Function

EI : Electron Ionisation

EPS : Extra Polymeric Substances

FAMEs : Fatty Acid Methyl Esters

FFAs : Free Fatty Acids

GC-MS : Gas Chromatography-Mass Spectrometry

GC-QTOF : Gas Chromatography-Quadrupole Time Of Flight

GF/F : Glass Fiber Filter

HBIs : Highly Branched Isoprenoids

IC : Irminger Current

IP_{25} : Ice Proxy 25

ITCZ : Inter Tropical Convergence Zone

LOX : Lipoxygénase

M.O : Matière Organique

MIZ : Marginal Ice Zone

MMR : Mismatch Repair

MRM : Multiple Reaction Monitor

MUFAs : Monounsaturated Fatty Acids

NER : Nucleotide Excision Repair

OGA : Ocean Gene Atlas

P.P : Production Primaire / Primary Production

PAR : Photosynthetically Available Radiations

PCR : Polymerase Chain Reaction

PFTBA : Perfluorotributylamine

PMA : Propidium Mono Azide

POC : Particulate Organic Carbon

POM : Particulate Organic Matter

PUFAs : Polyunsaturated Fatty Acids

qPCR : Quantitative Polymerase Chain Reaction

ROS : Reactive Oxigen Species

SOD : Super Oxide Dismutase

SPM : Suspended Particulate Matter

T/C : ratio *trans/cis*

TAGs : Triacylglycerols

TLEs : Total Lipid Extracts

TOF : Time Of Flight

UVP : Underwater Vision Profiler

WGC : West Greenland Curent

Table des matières

Chapitre I

Etat de l'art

Sommaire

1.1 Les écosystèmes Arctiques

1.1.1 Géographie

Les écosystèmes arctiques sont assez difficiles à définir, du fait des différentes utilisations du terme (Nutall & Callaghan, 2000). L'Arctique peut se définir par l'inclinaison de la Terre par rapport au soleil, ou par la courbe isotherme des 10°C au mois de juillet (limite permettant la présence d'arbres), mais la délimitation la plus admise est celle du cercle polaire Arctique (Figure 1.1), au-delà de la latitude 66.7°N (Callaghan, 2001). Les milieux arctiques sont caractérisés par un régime d'ensoleillement très particulier, avec des étés à ensoleillement quasi constants (soleil au-dessus de l'horizon à minuit) et des hivers froids sans lumière (soleil sous l'horizon à midi). En pratique les climats vont varier en fonction de la longitude tout au long du cercle polaire sous l'influence des courants marins. Les courants chauds de l'Atlantique Nord vont donner des climats plus cléments en Norvège où l'on va retrouver des forêts et terres cultivables à 68°N, alors que les courants froids qui longent les côtes du Canada expliquent la présence de toundra dès 51°N (Callaghan, 2001).

L'océan Arctique, vaste de près de 14 millions de km^2, se compose de deux principaux bassins de 3000 et 5000m de profondeur, entourés de plateaux continentaux très étendus (près de 50% des fonds), de ce fait, il a une profondeur moyenne nettement plus faible que les océans qui l'entourent (Jakobsson, 2002).

1.1.2 Caractérisation physico-chimique

Une autre caractéristique majeure de l'océan Arctique est qu'il se retrouve partiellement ou totalement recouvert de glace en suivant un régime saisonnier. Cette couche de glace constitue un nouvel habitat particulier, véritable frontière entre les compartiments atmosphérique et aquatique. Lorsque les températures baissent, de minces cristaux de glace dite « frazil » vont commencer à se former (voir Figure 1.2). L'apparition de ces cristaux, ressemblant à de fines aiguilles, est favorisée lors de mers agitées, qui facilitent leur formation (Kivisild, 1970 ; Martin, 1981). Ces cristaux vont pouvoir par la suite s'agglomérer pour donner des disques plus grands et plus solides appelés « pancake ice » (Doble *et al.,* 2003), qui vont eux même se regrouper pour donner de vastes étendues solides et plus ou moins homogènes (Petrich & Eicken, 2016 ; Wadhams *et al.,* 1987). Au sein même de la matrice de glace, se trouve un écosystème particulier, propre au milieu arctique. Lors de la formation de la glace, les sels contenus

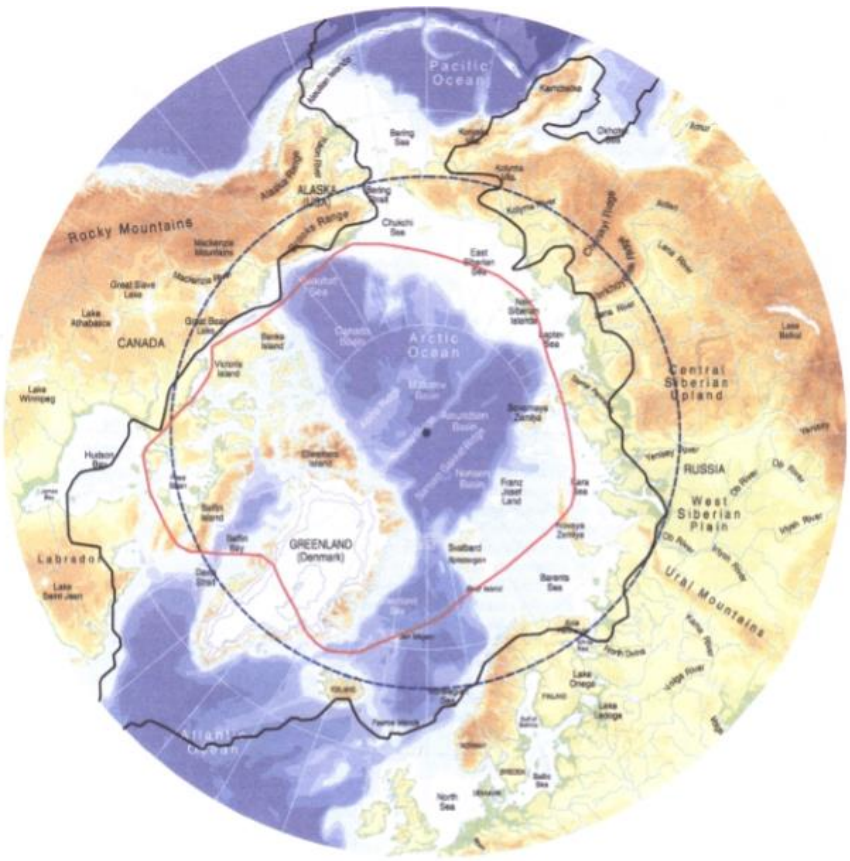


Figure 1.1 : Régions Arctiques. Le cercle en pointillé bleus représente le cercle polaire Arctique, la ligne noire continue représente la limite du bas Arctique, la ligne en pointillés rouge représente la limite du haut Arctique. D'après Nutall & Callaghan, 2000

dans l'eau de mer se retrouvent concentrés en saumure dans de minces canaux. Ces canaux forment un vaste réseau interconnecté, dans lequel se développent de nombreux organismes (algues, bactéries, virus…) qui ont été piégés dans la glace lors de sa formation. Ces canaux de saumure, appelées brines, vont être lessivés par de l'eau douce au début du printemps avec la fonte de la neige sur la glace.

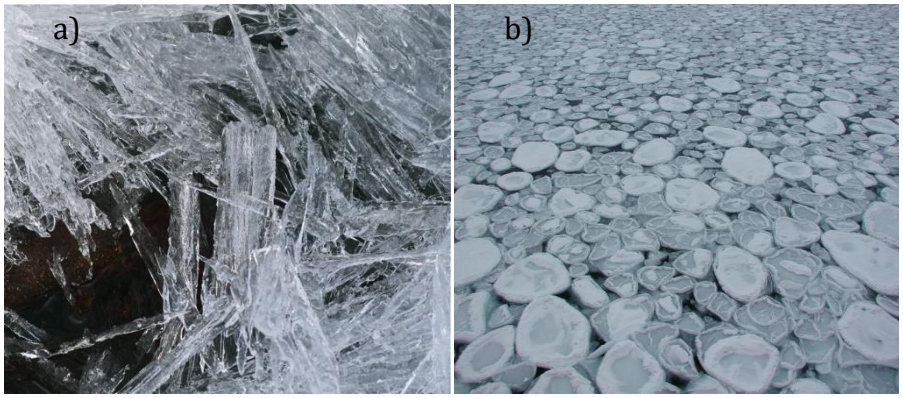


Figure 1.2 : a) Cristaux de glace constituant la glace « frasil » ; b) exemple de "pancake" ici à Svalbard. Crédits photo : Katherine Akey

Dans les océans arctiques et polaires est différenciée la glace saisonnière et poly-annuelle. La glace saisonnière est plutôt fine (1 à 2m d'épaisseur) et ne recouvre qu'en hiver les mers marginales (on parle ainsi de Marginal Ice Zone : MIZ), qui se retrouvent libres de glace en été ; alors que la glace pluriannuelle, plus épaisse (entre 3 et 7m) recouvre les zones maritimes centrales de l'Arctique. Ces différents types de glace, ainsi que la quantité non-négligeable de neige qui les recouvre, vont exercer une influence notable sur la quantité de lumière et d'énergie arrivant dans les différentes couches d'eau et donc sur le fonctionnement des écosystèmes (Kwok, 2004).

En effet, le régime radiatif de l'Arctique est très particulier avec beaucoup de variations spatiales et temporelles : soleil à minuit en été et nuit constante en hiver. Ce régime particulier associé à la présence de glace et de neige va générer des variations de la quantité d'énergie arrivant dans l'eau et donc disponible pour les organismes photosynthétiques. De plus, à de telles latitudes la fraction de radiation disponible pour la photosynthèse (Photosynthetically Active Radiation ou PAR, comprises entre 400 et 700nm) est significativement inférieure à celle d'autres zones de l'hémisphère Nord (Figure 1.3).

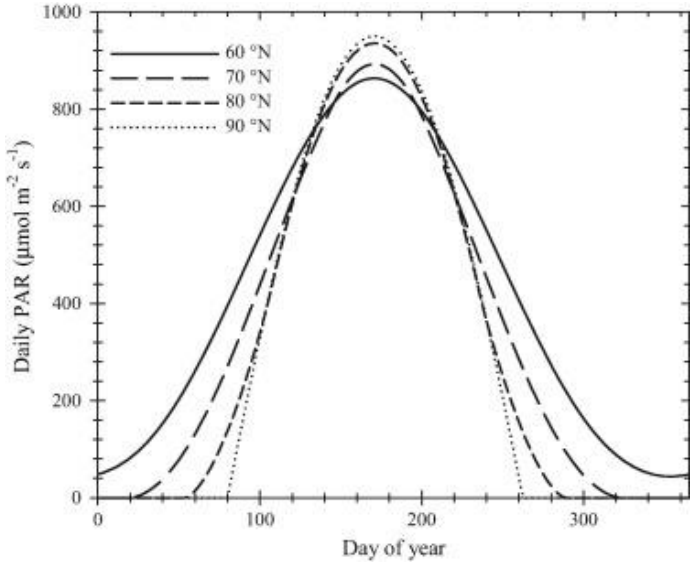


Figure 1.3 : Quantité de PAR journalière dans la haute atmosphère calculée à différentes latitudes de l'hémisphère Nord, d'après Leu *et al.,* 2015)

Si la glace ne filtre que très peu les radiations solaires, la neige cependant les attenue fortement (Weller & Bowling, 1972 ; Maykut & Grenfell, 1975 ; Hamre *et al.,* 2004). La couche de neige et de glace pouvant atténuer entre 0,2 et 5% des radiations PAR présentes en surface (Sakshaug, 2004). Si la glace et la neige peuvent absorber les radiations lumineuses, elles ont également un impact sur la réflectance de la lumière et l'albedo de surface. En effet, si la neige fraîche réfléchit intensément la lumière, la vieille neige va elle

absorber plus d'énergie (Hanesiak *et al.,* 2001). Il existe ainsi une relation complexe entre la présence de neige sur la glace et l'albedo de celle-ci (Curry *et al.,* 1995). L'état et l'épaisseur de la couche de neige vont donc grandement influencer la production primaire en Arctique. Au début du printemps la production de biomasse algale sera négativement influencée par la présence d'un couvert neigeux sur la glace (Leu *et al.,* 2015). Cette relation s'inverse en été, où la présence de neige va retarder la fonte de la glace et par conséquence la fin du bloom (Leu *et al.,* 2015). Durant cette période, la présence de neige peut également limiter les phénomènes de photo-inhibition (diminution volontaire de l'efficacité de la photosynthèse pour limiter les dommages des radiations solaires sur le photosystème II). En effet, l'exposition à de trop fortes doses lumineuses peut induire des dommages cellulaires sur les organismes photosynthétiques. Des études ont démontré qu'une sur-irradiation peut induire des pertes de biomasse algale (Campbell *et al.,* 2015 ; Juhl & Krembs 2010). Il est toutefois possible pour les algues de s'acclimater à une sur-irradiation (Juhl & Krembs, 2010). En Arctique les algues sympagiques (*i.e* se développant à l'intérieur de la matrice de glace, dans les premiers centimètres à l'interface avec l'eau) ont ainsi évolué et acquis différentes stratégies de défense face aux radiations intenses, comme la production de pigments photo-protecteurs tels que les caroténoïdes (Brunet *et al.,* 2011).

1.1.3 Grands enjeux environnementaux

Ces dernières décennies, le climat a subi des modifications importantes entraînant des dommages considérables dans les écosystèmes de hautes latitudes (Kauko *et al.,* 2017). On peut par exemple constater que les températures de surface augmentent plus vite en Arctique qu'aux latitudes plus basses (Screen & Simmonds, 2010). En Arctique, la diminution importante de la couverture de glace est une des conséquences principales du réchauffement global (voir Figure 1.4).

Les prévisions données par différents modèles annoncent une absence totale de glace estivale d'ici la fin du XXI^{ème} siècle (Wang & Overland, 2009 ; Stroeve *et al.,* 2012). Ces prévisions annoncent également une augmentation des précipitations, notamment des pluies lors de la période estivale (Deser *et al.,* 2010). La perte importante de couvert de glace va entraîner des répercussions à large échelle : jusqu'aux tropiques et même jusqu'au pôle sud (Deser *et al.,* 2015). En effet, du fait de son rôle de « refroidisseur », l'Arctique est à l'origine de nombreux courants marins.

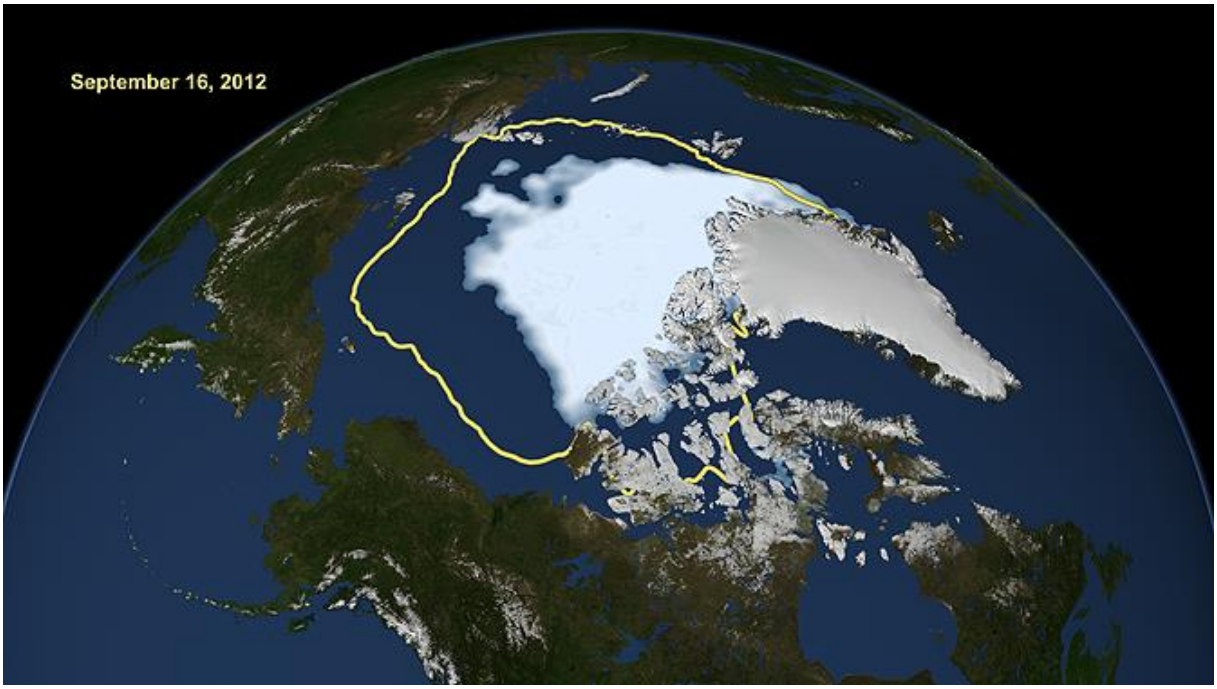


Figure 1.4 : Image satellite du record minimal de l'étendue de glace en Arctique en 2012 *vs* l'étendue moyenne de ces trente dernières années (ligne jaune). Crédit : NASA/Goddard Scientific Visualization Studio

La diminution importante du couvert de glace va s'accompagner d'un apport important d'eau douce et d'énergie arrivant à la surface (Sun *et al.,* 2018). Ces apports pourront grandement perturber des courants comme le AMOC (Atlantic Meridional Overturning Circulation), sensible aux pertes de glace (Tomas *et al.,* 2016 ; Oudar *et al.,* 2017). Les impacts du réchauffement global pourraient ainsi également impacter les océans tropicaux et plus particulièrement la Zone de Convergence InterTropicale (ITCZ) (Deser *et al.,* 2015).

Le réchauffement global en Arctique et surtout la fonte accélérée du couvert de glace peuvent entraîner également des répercussions sur le compartiment biologique. En effet la fonte avancée de la glace va perturber les cycles biogéochimiques notamment du carbone et le transfert d'énergie le long de la chaîne trophique. Les blooms algaux auront lieu plus tôt et dans des laps de temps plus courts (Meier *et al.,* 2014), En raison de la modification du temps de croissance des organismes photosynthétiques, l'intensité de ces blooms sera également (Wassmann & Reigstad, 2011). Il est donc crucial d'étudier l'impact du réchauffement global en Arctique, afin de prévoir quelles en seront les conséquences climatiques, biologiques et biogéochimiques.

1.2 Fonctionnement des écosystèmes Arctiques

1.2.1 Production primaire

En Arctique, la production primaire (P.P) est assurée par le phytoplancton d'eau libre ainsi que par les algues de glace dites sympagiques. Les organismes photosynthétiques sont broutés par des herbivores (principalement du zooplancton), eux-mêmes consommés par des poissons, source de nourriture des carnivores au sommet de la chaîne trophique (voir Figure 1.5).

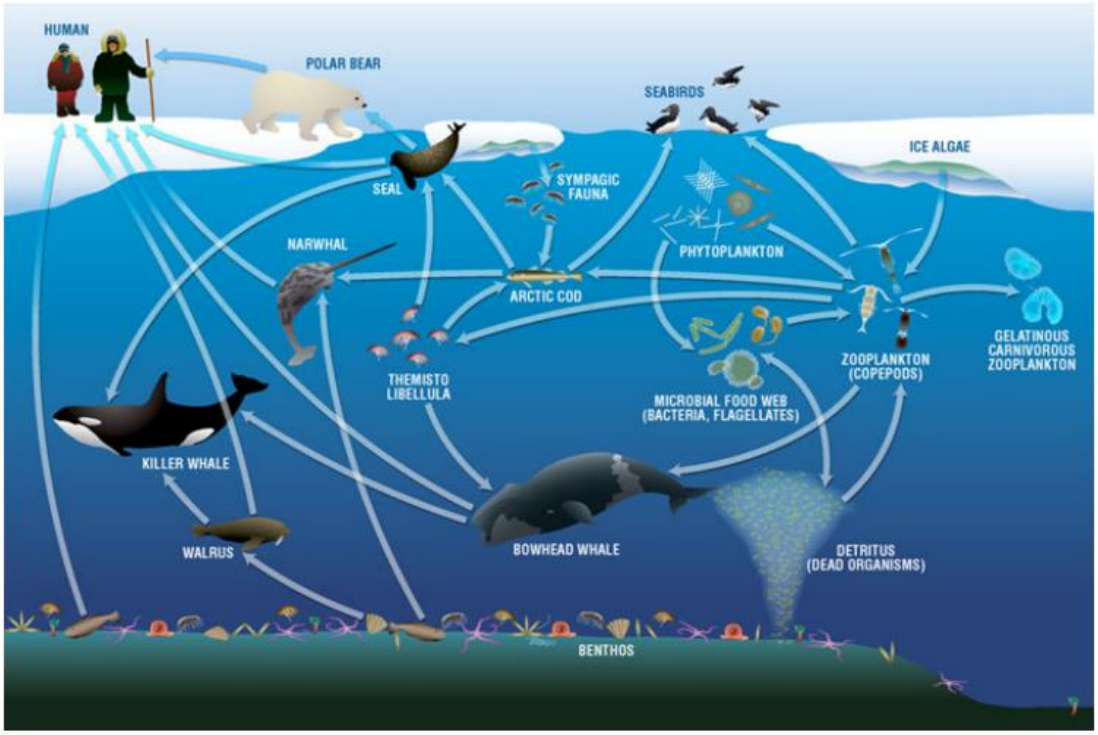


Figure 1.5 : Représentation schématique de la chaîne alimentaire Arctique canadienne. Transition d'un écosystème côtier à océanique de gauche à droite. D'après Darnis *et al.,* 2012

Il est à noter que, comme dans la majorité des chaînes trophiques, on observe entre chaque maillon d'importantes pertes en matière organique (de l'ordre de 75 à 80% : Sakshaug, 2004). Ces pertes résultent principalement de la respiration des organismes eux-mêmes, de la dégradation microbienne de la matière organique (M.O) dissoute, des restes cellulaires et des pelotes fécales, résultant en la production de CO_2 et de métabolites (Sakshaug, 2004). De ce fait, uniquement une petite fraction de la M.O produite atteint le fond.

Les algues de glace et le phytoplancton sont des organismes unicellulaires qui se multiplient par fission binaire. Dans les mers arctiques on va majoritairement retrouver

des diatomées, des chrysophytes, des dinoflagellés ainsi que des prymnesiophytes. La majorité des diatomées présentes en eau libre en Arctique appartiennent aux genres ubiquistes Arctiques Chaetoceros et *Thalassiosirale* (Rytter Hasle & Riddervold Heimdal 1998 ; Sukhanova, 1999). Parmi les espèces de diatomées sympagiques, on observe principalement les genres *Fragilariopsis* et *Navicula*, ou encore l'espèce *Nitzschia frigia* (Figure 1.6B), souvent très abondante au printemps (Syvertsen, 1991 ; von Quillfeldt, 1997). On peut également retrouver sous la glace d'épaisses (jusqu'à 0.5m) couches de cellules de la diatomée épiphytique *Melosira arctica* (voir Figure 1.6A), phénomène observé uniquement dans l'Arctique (Gradinger, 1999). On recense dans la glace plus de 300 espèces différentes de diatomées et de dinoflagellés, certaines pouvant se développer également en eau libre (Booth & Horner, 1997).

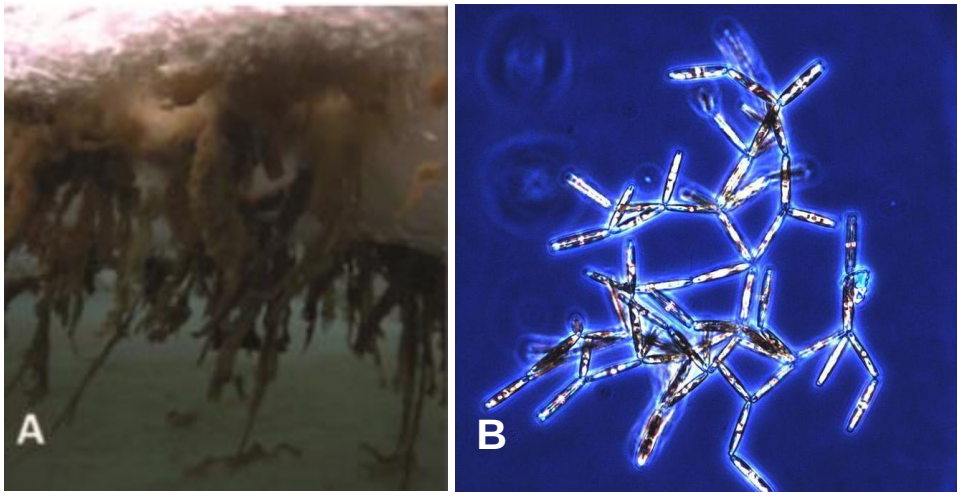


Figure 1.6 : A) photographie d'agrégats de cellules de *Melosira arctica* dans le haut Arctique, d'après Boetius *et al.,* 2013. B) photographie au microscope de cellules de *Nitzschia frigida* (Crédits : H. Höglander)

Dans les régions arctiques, la période de croissance des organismes photosynthétiques est réduite, généralement de mars à juin pour les organismes sympagiques (Cota *et al.,* 1987), avec des variations importantes en fonction du couvert de glace et de neige (Nicolaus *et al.,* 2010, 2012). Les blooms algaux (croissance très rapide d'une ou plusieurs espèces dans un temps relativement court) débutent en général lors de l'augmentation de l'irradiation solaire et utilisent les nutriments produits lors de la période hivernale. La période de croissance se termine à la fin de l'été ou au début de l'automne, avec la reformation de la glace (Hegseth, 1997). Les algues sympagiques, incorporées dans la glace lors de sa formation, se développent dans les canaux de saumure formés lors de la congélation de l'eau de mer. Dans ces canaux, où l'eau se trouve sous forme liquide, la circulation des nutriments est possible permettant ainsi aux organismes d'y vivre

(Becquevort *et al.,* 2009). Lorsque la neige et la glace commencent à fondre, de l'eau douce va percoler dans ces canaux et lessiver leur contenu, expulsant les algues de glace dans la mer. La majorité des organismes sympagiques ne se déplacent pas (Round *et al.,* 2007), ainsi leur survie dépend fortement de leurs capacités de résistance aux importantes variations de température et de salinité auxquelles ils sont exposés. En effet, au sein même des brines, la salinité peut varier entre 230 et 30 et les températures entre -2°C et -30°C (Ewert & Deming, 2013). La croissance des algues sympagiques, acclimatées aux conditions de vie particulières de la glace, débute à de faibles irradiations, jusqu'à la fonte du substrat. La glace et la neige s'y trouvant jouent donc un rôle crucial sur la production primaire en servant d'habitat pour les algues sympagiques et en atténuant les radiations solaires (Smetacek & Nicol, 2005). Les algues sympagiques laissent ensuite leur place au phytoplancton d'eau libre. Se faisant, on observe une discontinuité temporelle (Figure 1.7) entre bloom sympagique et pélagique (Søreide *et al.,* 2010).

La période de croissance des algues, sympagiques comme pélagiques, peut être limitée par de nombreux facteurs, tels que la lumière, la température et la présence de certains nutriments. En général, les nutriments azotés et phosphorés présents dans la zone euphotique vont être les facteurs limitants (chez les diatomées, possédant un squelette siliceux, la silice peut également être limitante ; Droop, 1974), si toutefois la lumière est suffisante pour permettre la photosynthèse. Une espèce ou une souche donnée va également avoir son propre optimum de croissance en termes de radiations ou de températures. Il est donc difficile en pratique de définir un seul facteur limitant dans ce genre de communautés composées de différentes espèces ayant des besoins en énergie lumineuse et en nutriments variés.

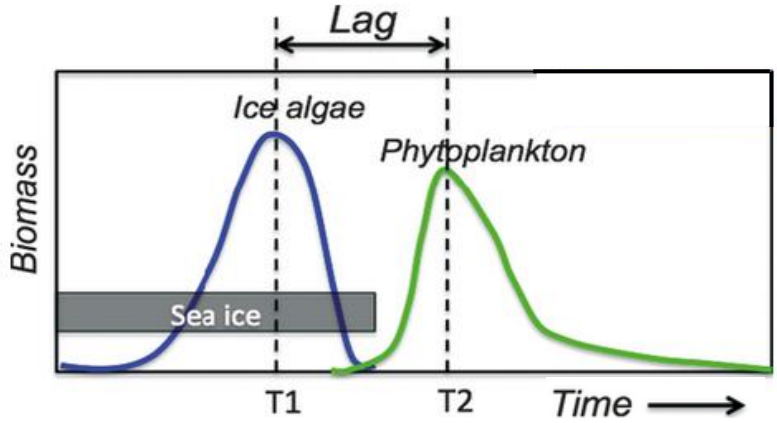


Figure 1.7 : Représentation schématique du timing du bloom sympagique et planctonique. Les temps T1 et T2 représentent les pics de biomasse des algues de glace et d'eau libre, respectivement. Le "lag" se défini comme le temps de latence entre ces deux pics. D'après Ji & Varpe, 2013

Dans les mers arctiques, les organismes phototrophes sympagiques et planctoniques sont les organismes assurant la P.P, l'importance relative de chaque groupe va dépendre de la saison et de la région considérée (Stein & MacDonald, 2004). Il a toutefois été démontré en Arctique que les algues sympagiques peuvent assurer entre 4 et 57% de la P.P totale (Fernández-Méndez *et al.,* 2014), représenter parfois 80% de la biomasse algale (Gosselin *et al.,* 1997), et contribuer de manière non-négligeable à l'export de carbone des eaux de surface, avec des taux variants entre 1 à 9g C m^{-2} an^{-1} (Boetius *et al.,* 2013). Cette production sympagique représente également une ressource nutritive au début du printemps et à la fin de l'automne, quand les autres sources de nourriture sont rares (Bradstreet & Cross, 1982). Une des particularités des algues sympagiques des mers arctiques est de produire une quantité importante (environ 10 fois plus que d'autres espèces pélagiques ; Riedel *et al.,* 2006) de mucus ou d'exo-polysaccharides (EPS), lors de la phase de sénescence (Kiørboe *et al.,* 1990). Cette production d'EPS va entraîner la formation d'agrégats qui vont chuter rapidement le long de la colonne d'eau ; ces organismes agrégés vont ainsi grandement contribuer à la pompe biologique marine (Jackson *et al.,* 2005). Des agrégats d'algues ont en effet été observés sur les fonds marins de plateaux océaniques (Ambrose *et al.,* 2005) et des zones centrales (Boetius *et al.,* 2013) arctiques, attestant ainsi de la vitesse de chute rapide de ce matériel lors de la fonte de la glace.

1.2.2 Devenir de la production primaire

La production primaire (P.P), qui dépend de la quantité de lumière disponible pour les organismes photosynthétiques (PAR), va donc être fortement dépendante de la présence ou de l'absence de glace en mer. Sur les 30 dernières années, la quantité de glace de mer en Arctique a diminué de 9% par décennie (Perovich & Richter-Menge, 2009). D'autres observations vont dans le même sens : sur la période 1998 – 2009, certains auteurs ont observé une glace (généralement d'un an) moins concentrée, plus fine et qui fond rapidement, ainsi qu'une augmentation du nombre de jours d'eau libre (+45 jours ; Arrigo & van Dijken, 2011). La réponse de la P.P arctique à cette réduction du couvert de glace est sans appel : +20% sur la période 1998 – 2009, passant de 441 à 585 Tg C. an^{-1} (Arrigo & van Dijken, 2011). Certaines zones, notamment l'Est Arctique, semblent plus enclines à de fortes augmentations de P.P, avec +135% pour la Sibérie Arctique (Arrigo & van Dijken, 2011). Sur la base des prévisions actuelles d'absence de glace en mer en période estivale avant la fin du XXIème siècle, la P.P arctique totale pourrait y atteindre 730 Tg C. an^{-1}. Ces

changements dans les caractéristiques physico-chimiques du milieu Arctique vont également impacter la sédimentation du matériel fortement agrégé. Il a en effet été démontré que l'augmentation des radiations lumineuses va augmenter la formation de bulles d'air (produites par la photosynthèse) dans la matrice d'EPS, et donc augmenter la flottabilité de ces agrégats (Fernández-Méndez *et al.,* 2014). L'étude de la P.P arctique est donc très compliquée, tant certains mécanismes demeurent incompris et que le milieu change rapidement.

Une étude approfondie de la problématique est importante, notamment des différents flux de nutriments, afin de déterminer si ces derniers pourront soutenir une P.P si importante ces prochaines années. Ces modifications vont aussi grandement impacter les organismes phototrophes, notamment leur distribution, leur productivité ainsi que leurs cycles de vie (Li *et al.,* 2009 ; Comeau *et al.,* 2011 ; Slagstad *et al.,* 2011 ; Brown & Arrigo, 2012 ; Ji & Varpe, 2013).

1.2.3 Dégradation de la POM

Ne représentant qu'une petite partie du carbone organique total (TOC), le carbone organique particulaire (POC) représente toutefois la fraction ayant le plus de potentiel de chute vers les sédiments, et ainsi de piégeage du CO_2 atmosphérique. Tout au long de sa chute à travers la colonne d'eau, le TOC peut être dégradé de différentes façons et reminéralisé et/ou recyclé afin d'alimenter le cycle du carbone (Neilson & Allard, 2007). Dans les sections suivantes les différentes voies de dégradation du TOC seront abordées, qu'elles soient biotiques (c'est-à-dire induites par le zooplancton, les bactéries ou les virus) ou abiotique (impliquant principalement des processus d'auto- ou de photo-oxydation).

1.2.3.1 Dégradation biotique

- Zooplancton

Dans les mers arctiques, le zooplancton herbivore va exercer une forte pression sur les organismes photosynthétiques (Tremblay *et al.,* 2006). En broutant intensivement les organismes photosynthétiques, le zooplancton transfère le carbone dans la chaîne trophique des brouteurs, qui passera ensuite aux poissons et enfin aux animaux supérieurs. En termes de biomasse, le zooplancton est généralement présent en plus grandes quantités que le phytoplancton, avec par exemple en mer de Barents, plus de 30g C m^{-2} de zooplancton contre 20g C m^{-2} d'algues (Sakshaug *et al.,* 1994). Le zooplancton

remonte donc vers la surface au début du printemps (début de la période de croissance), et broute intensément les algues, il va ensuite redescendre vers des couches plus profondes où il va rester en diapause pendant les mois hivernaux (Hirche, 1997). Une grande partie du carbone (entre 66 et 79%, Forest *et al*., 2011) produit par les algues est immédiatement respiré, le reste est stocké dans les lipides de réserve (Hirche, 1997 ; Auel *et al.,* 2003). Le zooplancton de petite taille, ne réalisant que peu de migrations verticales pendant son développement (Fortier, 2001), contribue fortement au recyclage du POC en surface du fait de la continuité de son activité et de sa capacité à brouter de petites particules (Hopcroft *et al.,* 2005).

Dans les mers arctiques et subarctiques, plus de 260 espèces de zooplancton ont été dénombrées (Zenkevich, 1963). Parmi les espèces de zooplancton les plus emblématiques on va principalement retrouver différents copépodes ou amphipodes, parmi lesquelles le genre *Pseudocalanus*, et les espèces de copépodes suivantes : *Calanus finmarchius*, *C. hyperboreus*, *C. glacialis*, ou encore *Oithona similis* (Falk-Petersen *et al.,* 1999).

- Bactéries hétérotrophes

Dans les océans, il est admis que les bactéries hétérotrophes jouent un rôle important dans le recyclage et la mobilisation de la P.P. En effet, dans le milieu marin, les bactéries hétérotrophes sont très abondantes, très diversifiées et présentent une forte activité métabolique (Yager *et al.,* 2001 ; Azam & Malfatti, 2007). Une grande fraction de la P.P se retrouve sous forme dissoute (DOC) et est ainsi aisément accessible pour les bactéries (Azam *et al.,* 1983 ; Ducklow & Carlson, 1992). Les bactéries vont également, *via* l'activité de leurs enzymes extracellulaires, hydrolyser la POM en de plus petites molécules assimilables (Cho & Azam, 1988 ; Piontek *et al.,* 2014). Dans la colonne d'eau, les bactéries sont présentes sous forme libre adoptant un mode de vie pélagique, ou attachées à des particules (i.e. neige marine, agrégats phytoplanctoniques ou sympagiques). Il a été démontré que ces dernières ont une plus grande activité que leurs homologues libres (Karner & Herndl, 1992), possiblement en raison de la proximité avec leur source de nourriture. Si les bactéries hétérotrophes sont pratiquement les seuls organismes à pouvoir utiliser directement les grandes quantités de DOM disponibles, leur contribution au recyclage du carbone est faible en Arctique (Piontek *et al.,* 2014). En effet, les bactéries arctiques respirent moins de carbone (25%) que celles d'autres mers aux latitudes plus basses (50%) (Cota *et al.,* 1996 ; Kirchman *et al.,* 2009 ; Rich *et al.,* 1997). Cette faible activité des bactéries arctiques a déjà été confirmé par Howard-Jones *et al.* (2002) qui ont effectué des mesures de respiration bactérienne. Il a en effet été démontré qu'une fraction

importante (15-80%) du bacterioplancton arctique de la mer de Barents est dans un état de dormance ou d'inactivité. Si cette faible activité a été attribuée par Pomeroy & Diebel (1986) aux températures très faibles de l'Arctique, des interactions plus complexes entre activité bactérienne et températures semblent être à l'œuvre (Rivkin *et al.,* 1996 ; Pomeroy & Wiebe, 2001). Ainsi l'activité métabolique des bactéries arctiques reste encore mal comprise.

La communauté bactérienne arctique est similaire à celle d'autres océans du globe, on retrouve principalement procaryotes affiliés à l'échelle du phyla aux *alpha, gamma* et, *beta* -*Protéobacteria*, des aux *Actinobactéria* et *Bacteroidetes* (Bano & Hollibaugh, 2002 ; Murray & Grzymski, 2007). On note toutefois la faible représentation des cyanobactéries dans ces eaux (Zubkov *et al.,* 1998). On note également la présence de bactéries dites AAPs (Aerobic Anoxygenic Phototroph), au métabolisme photo-hétérotrophe, notamment le clade des *Roseobacter* (Selje *et al.,* 2004).

- Virus

Les virus jouent également un rôle important dans le transfert de carbone et la régulation des microorganismes. Etant très abondants dans les milieux marins (entre 10^6 et 10^9 virus ml^{-1} ; Stuttle *et al.,* 1990), ils sont responsables d'une grande partie de la mortalité bactérienne (Stuttle *et al.,* 1990), induisant entre 20 et 50% de la mortalité bactérienne marine (Fuhrman *et al.,* 1995). Les virus vont également intervenir directement sur la production primaire, en s'attaquant à certains organismes phototrophes comme les diatomées, les cryptophytes et certaines cyanobactéries (Stuttle *et al.,* 1990).

1.2.3.2 Dégradation abiotique

- Photo-oxydation

Dans les océans, l'énergie lumineuse arrivant à la surface de l'eau va contribuer à la production primaire, mais également être à l'origine de nombreux processus de photo-oxydation de la matière organique. Cette photo-oxydation peut intervenir directement (action directe des radiations sur des composés organiques) ou indirectement, via des composés transférant cette énergie à d'autres ne pouvant l'absorber directement, appelés photo-sensibilisateurs. Dans l'environnement marin, les réactions de photo-oxydation directe sont plutôt rares, en raison du faible nombre de molécule organiques capables d'être excitées par les radiations solaires (Zafiriou *et al.,* 1984).

En milieu marin, les réactions de photo-oxydation indirectes sont communes et permettent la dégradation de composés résistants aux réactions de photo-oxydation directes (Zafiriou *et al.,* 1984). Elles font intervenir des photo-sensibilisateurs (tels que les pigments ou hydrocarbures aromatiques), qui vont transmettre cette énergie à d'autres composés. Ces photo-sensibilisateurs se retrouvent sous deux états d'excitation : singulet (^{1}sens) ou triplet (^{3}sens) (Foote, 1976) ; il est à noter qu'une grande partie des réactions de photo-oxydation indirectes font intervenir un photo-sensibilisateur dans son état triplet (Gollnick, 1968). On va distinguer deux types de photo-oxydation indirecte : (i) la photo-oxydation de type I faisant réagir le ^{3}sens directement avec d'autres molécules, après transfert d'un atome d'hydrogène ou d'un électron (Figure 1.8) et (ii) la photo-oxydation de type II faisant réagir le ^{3}sens avec les molécules d'oxygène qui l'entourent, produisant ainsi de l'oxygène dans un état excité singulet : $^1O_2$ (voir Figure 1.8). Dans une moindre mesure, le transfert d'électron de ^{3}sens à l'oxygène peut également entraîner la formation de l'anion superoxyde $O_2^{\cdot-}$ (Figure 1.8) (Gollnick, 1968). Lors de ces dernières années, il a pu être démontré que les processus photo-sensibilisés de type II faisant intervenir l'$^1O_2$ jouaient un rôle majeur dans la photo-oxydation des composants insaturés des algues planctoniques (pour des revues voir Rontani, 2001, 2012, ; Rontani & Belt 2020). Il est important de noter que du fait de l'appariement des deux électrons libres de l'$^1O_2$, les réactions impliquant cette forme excitée de l'oxygène font intervenir un doublet d'électrons et ne sont donc pas radicalaires.

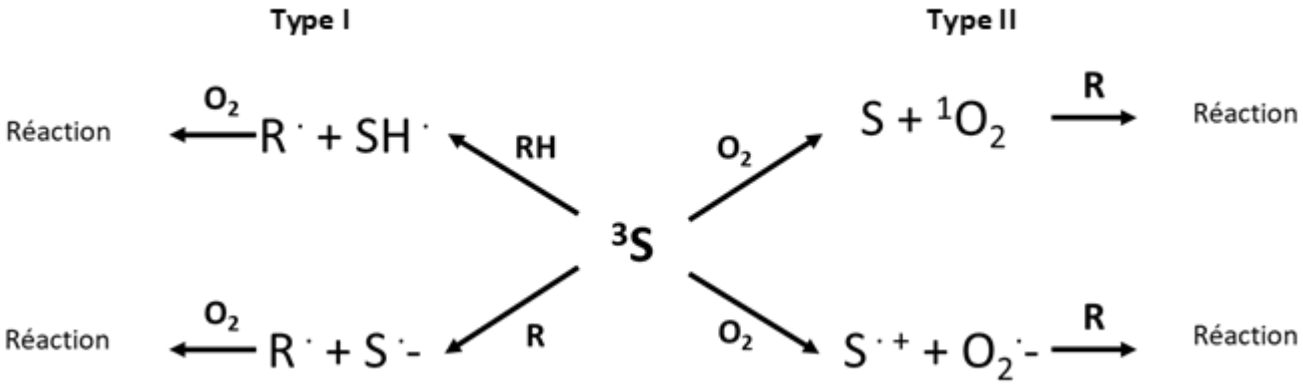


Figure 1.8 : Réactions de photo-oxydation indirectes d'un photo-sensibilisateur dans son état triplet, d'après Rontani (2001)

Dans le milieu marin, la chlorophylle (Chl) est le photo-sensibilisateur le plus abondant (Foote, 1976). Cette molécule est à la base de la photosynthèse : lorsqu'elle absorbe un quantum d'énergie lumineuse, la Chl passe dans un état excité singulet (^{1}Chl), qui va

rapidement transmettre cette énergie au reste de la chaîne photosynthétique (voir Figure 1.9A). Lors de cette réaction chez des cellules saines, une infime fraction (< 0.1%) de ^{1}Chl peut subir une « conversion intersystème » et se retrouver dans son état triplet (^{3}Chl) (Knox & Dodge, 1985). Cette ^{3}Chl peut être à l'origine de réactions de photo-oxydation de type I et II (Figure 1.8). Ces deux voies sont dommageables pour la cellule, notamment via la production de ROS (Reactive Oxygen Species) délétères et notamment d'oxygène singulet ($^1O_2$). Afin d'éviter ces dommages oxydatifs, les organismes photosynthétiques sont équipés de nombreux moyens de piégeage (quenching) de ces ROS. On peut citer les caroténoïdes, l'acide ascorbique ou les tocophérols qui sont des désactivateurs efficaces de l'$^1O_2$. L'enzyme SOD (Super Oxide Dismutase) peut également piéger l'anion superoxyde ($O_2^{\cdot}$-).

Si ces mécanismes de protection sont efficaces dans des cellules en bonne santé, ce n'est pas le cas dans des cellules sénescentes, chez qui la fonction photosynthétique n'est plus assurée. En effet, dans ce cas, la ^{1}Chl, qui est toujours produite par excitation lumineuse, n'est plus consommée dans les réactions photosynthétiques (Figure 1.9B). Cela induit une formation accélérée de ^{3}Chl et surtout de $^1O_2$ qui sature rapidement les systèmes photoprotecteurs de la cellule et résulte en une oxydation intense des composants insaturés membranaires (effet photo-dynamique, Nelson, 1993). Il est important de noter que l'environnement immédiat dans lequel est produit l'$^1O_2$ (membrane des thylakoïdes riche en lipides) induit une durée de vie à ce dernier bien plus longue que dans des milieux aqueux, ce qui favorise donc les dommages qu'il peut créer (Suwa *et al.,* 1977). Ainsi, dans les cellules des organismes photosynthétiques sénescents, les processus de photooxydation de type II sont intenses, et impactent principalement les composants lipidiques insaturés tels que les stérols, les acides gras insaturés, mais également les protéines (Morgan *et al.,* 2004) ou encore l'ADN (Agnez-Lima *et al.,* 1999 ; Ravanat *et al.,* 2000).

L'$^1O_2$ s'attaque également à la chlorophylle conduisant ainsi à une diminution de ses capacités photosensibilisatrices (photobleaching, Figure 1.9B). Le photobleaching de la chlorophylle et l'effet photodynamique sont donc des processus compétitifs. Il est intéressant de noter que l'efficacité de ces processus de photo-oxydation sensibilisés de Type II (ratio effet photodynamique/photobleaching de la chlorophylle) augmente fortement aux hautes latitude (Rontani *et al.,* 2021, Figure 1.10). Ce phénomène a été attribué aux faibles radiations solaires présentes sous la glace qui limitent le

photobleaching de la chlorophylle (Amiraux *et al.,* 2016) favorisant ainsi l'effet photodynamique.

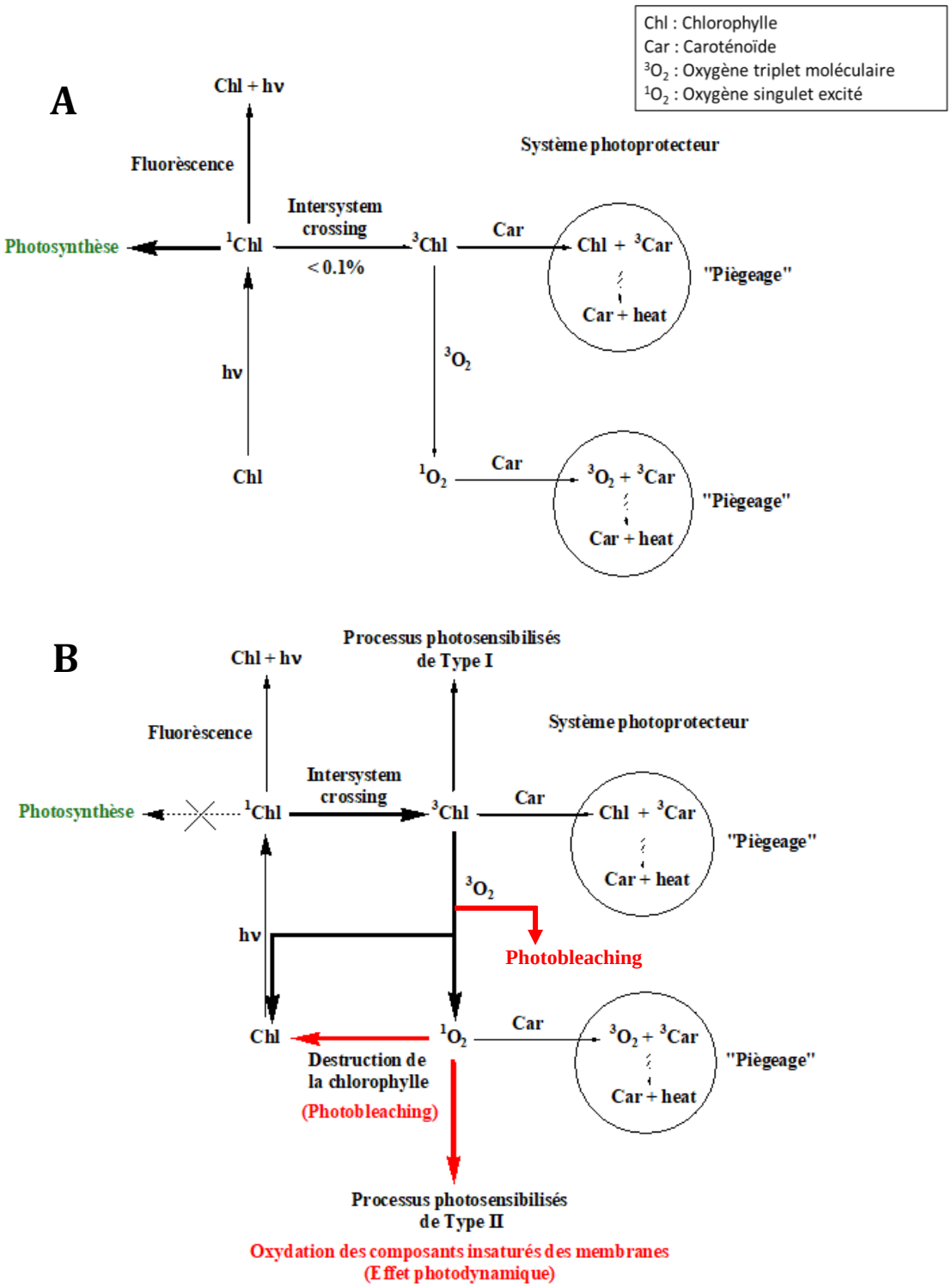


Figure 1.9 : Devenir de la chlorophylle photo-excitée dans une cellule phytoplanctonique (A) saine et (B) sénescente (schéma simplifié ne montrant que l'effet de l'oxygène singulet et le piégeage par les caroténoïdes, d'après Rontani & Belt, 2020).

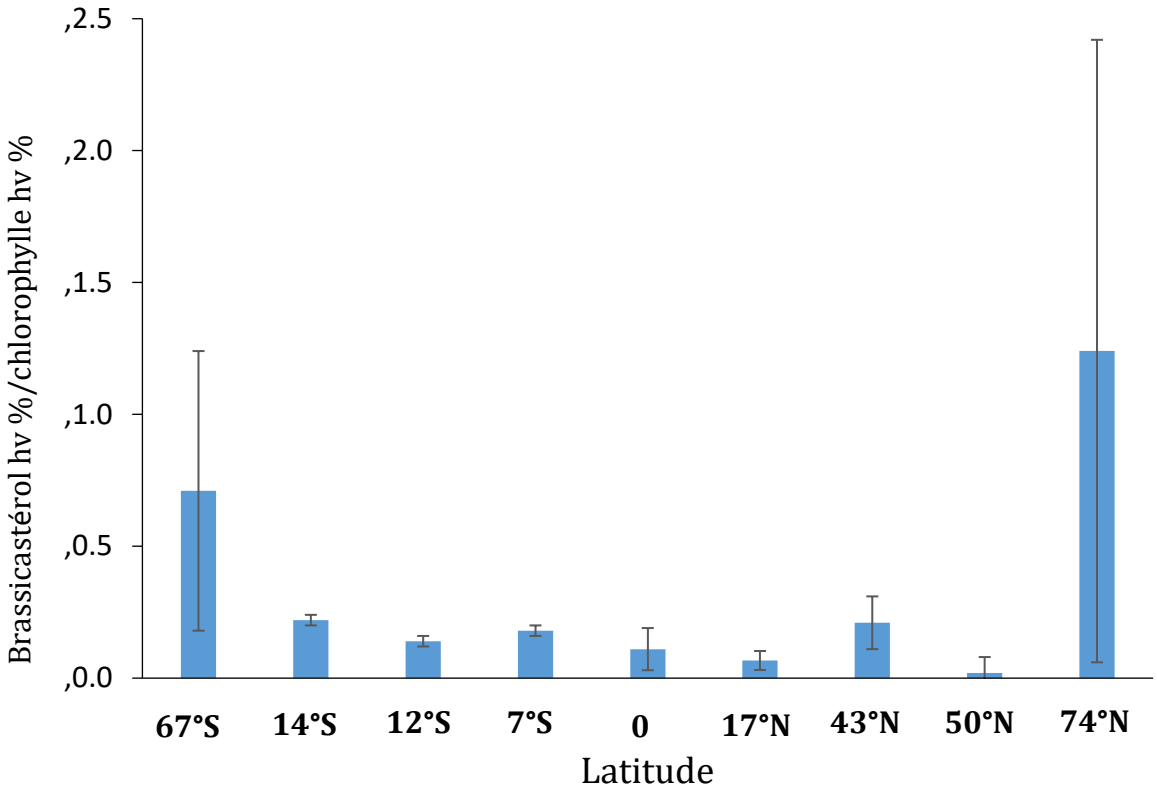


Figure 1.10 : Effet de la latitude sur l'efficacité des processus d'oxydation photosensibilisée de Type II (rapport effet photodynamique/photobleaching) dans le cas du brassicastérol. (Valeurs moyennes obtenues à partir de nombreuses données (> 100) publiées (Rontani *et al.,* 2011 ; 2012a ;2014 ;2019) et non-publiées.

- Auto-oxydation

L'auto-oxydation est un processus naturel durant lequel l'oxygène moléculaire présent dans le milieu réagit avec des molécules organiques dans des conditions douces. L'oxygène moléculaire se trouve naturellement sous la forme d'un état triplet ($^3O_2$) stable. Du fait de l'absence d'appariement de ses deux électrons libres, il va agir comme un biradical libre et induire des réactions d'oxydation radicalaires faisant intervenir essentiellement des réactions d'addition sur des doubles liaisons et d'arrachement d'atomes d'hydrogène. L'efficacité de cet arrachement est souvent dictée par la stabilité des radicaux formés ; il s'effectue donc en général en α d'une double liaison (position allylique), en α d'un atome d'oxygène ou sur des atomes de carbone tertiaires.

Lors d'une réaction d'auto-oxydation, trois phases se succèdent : une phase d'initiation, une phase de propagation et une phase de terminaison (Figure 1.11). Dans les phytodétritus ou les cellules phytoplanctoniques sénescentes, on considère que la phase d'initiation (formation d'un radical) résulte souvent du clivage homolytique des hydropéroxydes formés lors de processus photosensibilisés de Type II (Girotti, 1998 ;

Rontani *et al.,* 1998). Ce clivage peut être initié par : (i) certaines enzymes (lipoxygénases), (ii) certains ions métalliques induisant des réactions d'oxydo-réduction à un électron (Ex : Fe^{2+}, Cu^{2+}, Zn^{2+}, V^{2+}, Fe^{3+}…), (iii) la chaleur ou encore (iv) la lumière (Sheldon & Kochi, 1976 ; Schaich, 2005) (Figure 1.11). Les radicaux ainsi formés vont réagir avec l'oxygène moléculaire à son état fondamental et conduire à la formation de radicaux péroxyles, puis finalement à des hydropéroxydes. Cette réaction en chaîne s'arrête lorsque les radicaux libres entrent en collision et échangent des électrons pour former une nouvelle liaison, les rendant ainsi non-réactifs.

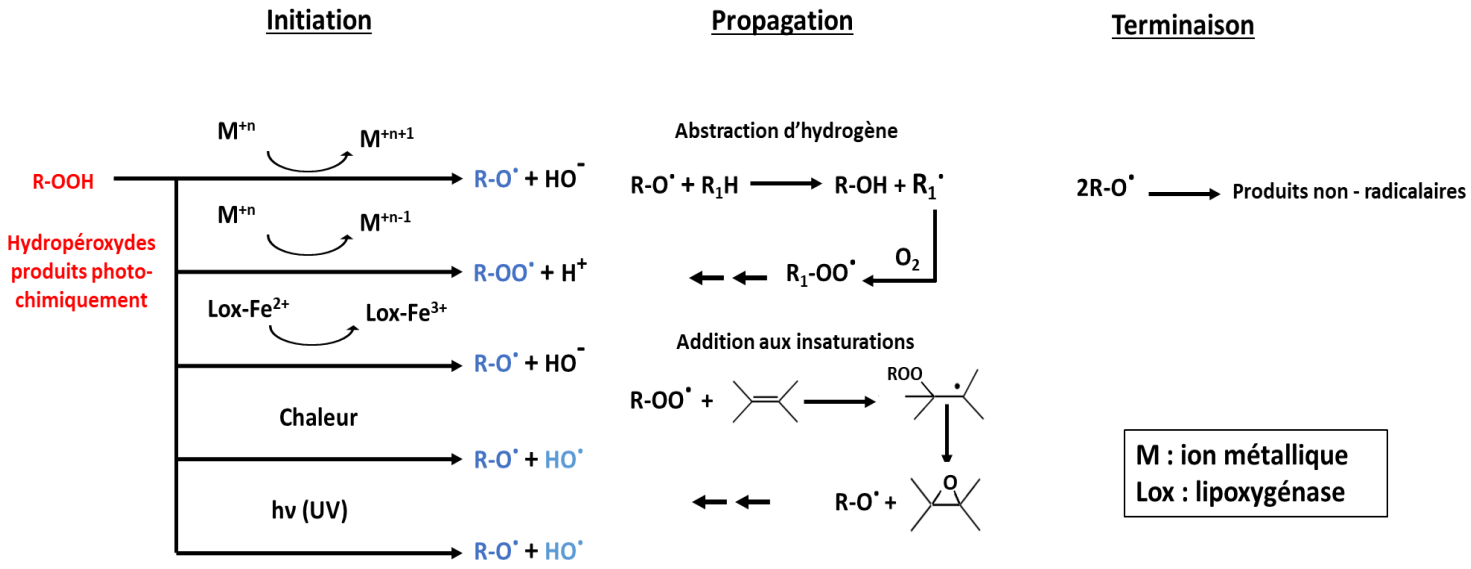


Figure 1.11 : Induction de processus d'auto-oxydation dans des cellules phytoplanctoniques sénescentes, d'après Rontani & Belt, 2020.

Grâce à l'utilisation de nouveaux marqueurs spécifiques de ce processus (Rontani *et al.,* 2003 ; Rontani & Aubert, 2005), le rôle de l'autooxydation dans la dégradation de la MO peut maintenant être prise en compte (Rontani *et al.,* 2006 ; Christodoulou *et al.,* 2009 ; Rontani *et al.,* 2011).

1.3 Lipides & produits d'oxydation

1.3.1 Intérêt en biogéochimie

Afin de pouvoir suivre le devenir et la composition de la MO dans le temps et l'espace, il est capital de développer l'utilisation de biomarqueurs stables et spécifiques pouvant

témoigner de l'origine et du ou des processus de dégradation subis par cette matière organique.

Les lipides constituent un groupe assez vaste de molécules organiques comprenant deux grandes catégories : les lipides à base d'acides gras (AGs) et les lipides à base d'isoprène (lipides polyisoprépniques tels que les stéroïdes, les terpénoïdes et les caroténoïdes). Dans la catégorie des lipides à base d'acides gras on distingue couramment les lipides simples – composés uniquement de carbone, d'hydrogène et d'oxygène (ex : glycérides) – et les lipides complexes, qui renferment également de l'azote, du phosphore ou du soufre. Ils assurent différentes fonctions physiologiques comme la réserve d'énergie, la structuration des cellules, et participent aussi activement au métabolisme (Bergé & Barnathan, 2005). Ils sont ainsi porteurs de beaucoup d'informations concernant l'état métabolique des organismes, leur biomasse et leur état de dégradation.

L'analyse des lipides et de leurs produits d'oxydation est très utile pour étudier la composition de la matière organique (phytoplancton, zooplancton ou bactérie) et son devenir. En effet, ces composés constituent une part importante (16-26%, Jónasdóttir, 2019) de la matière organique phytoplanctonique. Ils sont également moins labiles que les protéines ou les carbohydrates, et sont donc souvent utilisés comme traceurs de source (Volkman, 2003) et d'état de dégradation de la matière organique (Wakeham, 1995). Les lipides, du fait de leur abondance dans le pool de carbone marin, de leur diversité et de leur stabilité chimique, constituent donc de bons marqueurs lors des études de biogéochimie organique.

Afin de fournir une information robuste, les produits d'oxydation lipidiques doivent être relativement spécifiques (du processus de dégradation considéré) et stables en conditions naturelles. Il apparaît que ces critères sont respectés pour les produits d'oxydation de la chaîne phytyle de la chlorophylle, des Δ^5 – stérols, et des acides gras monoinsaturés (Pour une revue récente voir Rontani & Belt, 2020). Si les produits de dégradation de certains lipides (ex : ceux de certains hydrocarbures isoprénoïdes polybranchés (HBIs)) ne permettent pas de donner une information quantitative, en raison de leur instabilité ou de leur manque de spécificité, l'information qualitative qu'ils fournissent peut être très utile lors d'études paléoclimatiques (Rontani & Belt, 2020).

1.3.2 Acides gras

Les acides gras (AGs) sont des acides carboxyliques à chaîne plus ou moins longue, comprenant la plupart du temps un nombre pair d'atomes de carbone (entre 4 et 36 pour les plus longs). Ils fonctionnent comme de vrais « blocs de construction » pour les autres classes de lipides (triacylgycerols, galactolipides, phospholipides ; Jónasdóttir, 2019) Leur structure pouvant varier, ils sont classés en différents catégories (O'Keefe, 2002) :

- Les AGs saturés où toutes les liaisons carbone - carbone sont simples
- Les AGs insaturés, comprenant au moins une double liaison carbone – carbone est double. Si au moins deux liaisons sont doubles on parle d'AG polyinsaturé.
- Les AGs *cis* et *trans* : les AGs contenant une double liaison peuvent se trouver sous forme de deux conformations : *E (trans)*, observée chez certaines bactéries et ruminants, et *Z (cis)*, qui est la plus courante dans la plupart des organismes vivants.
- Les AGs peuvent également avoir une structure plus complexe ; ils peuvent être ramifiés ou encore cycliques et posséder certains groupes fonctionnels (hydroxyl-, époxy…).

Les AGs poly- ou mono-insaturés (respectivement PUFAs et MUFAs) sont très abondants chez les algues (Volkman *et al.,* 1989) où ils peuvent représenter entre 45 – 85% du total des acides gras (Jónasdóttir, 2019). Ces composés insaturés sont susceptibles de subir des réactions de photo-oxydation de type II lors de la sénescence algale (Heath & Packer, 1968). Le taux de photo-oxydation des AGs augmente logiquement en fonction de leur degré d'insaturation (Frankel, 1980). Les PUFAs sont donc très sensibles à ces processus de dégradation. Toutefois, en raison de : (i) l'instabilité des produits d'oxydation formés, et (ii) l'intervention de réactions intermoléculaires conduisant à la formation de macromolécules non indentifiables par chromatographie en phase gazeuse (Neff *et al.,* 1988), des photo-produits de PUFAs susceptibles de jouer le rôle de marqueurs n'ont pu être mis en évidence jusqu'ici dans des échantillons naturels.

1.3.2.1 Photo-oxydation des MUFAs

La réaction de photo-oxydation sensibilisée de Type II d'un MUFA fait intervenir ce que l'on nomme une « ene addition » (Frimer, 1979), c'est-à-dire l'addition de l'$^1O_2$ sur une extrémité de la double liaison et la migration concertée de celle-ci. Cette addition conduit

à la formation d'hydropéroxydes à chaque bout de la double liaison originelle (Figure 1.12), Frankel *et al.,* 1979). Ces hydropéroxydes (possédant une double liaison allylique en position *trans* : Frankel, 1980) peuvent ensuite subir un réarrangement allylique stéréosélectif, formant deux nouveaux isomères possédant également une double liaison *trans* (Figure 1.12), Porter *et al.,* 1995). Les hydroxyacides obtenus après réduction au $NaBH_4$ de ces photo-produits (trop fragiles pour être directement analysés en chromatographie en phase gazeuse) constituent de bons traceurs de processus de photo-oxydation de type II en milieu naturel (Rontani & Belt, 2020).

1.3.2.2 Auto-oxydation des MUFAs

L'auto-oxydation des MUFAs fait intervenir l'abstraction d'un atome d'hydrogène en position allylique et l'oxydation du radical allylique ainsi formé, conduisant ainsi à la formation de six hydropéroxyacides isomères. Dans le cas des MUFAs possédant une double liaison en Δ^9, l'auto-oxydation produit un mélange d'hydropéroxyacides [8-*cis*], [8-*trans*], [9-*trans*], [10-*trans*], [11-*trans*] et [11-*cis*] (voir Figure 1.12). Après réduction au $NaBH_4$ de ces isomères, la quantification des produits résultant de la photo- et de l'auto-oxydation des MUFAs est possible, en se basant sur la proportion des hydroxyacides de conformation *cis* (spécifiques de l'auto-oxydation ; Frankel, 1980 ; Porter *et al.,* 1995) et la température au moment du prélèvement (°C). Dans le cas d'un MUFA possédant une double liaison en Δ^9, la proportion d'hydroxyacides *trans* résultant des processus auto-oxydatifs peut être calculée à l'aide des équations (1) à (4), (Marchand & Rontani, 2001). La proportion des hydroxyacides d'origine photo-oxydative peut ensuite être obtenue par différence.

$$(1) \qquad \frac{[8-cis]+[11-cis]}{[9-trans]} = -0.0138\mathrm{T} + 1.502$$

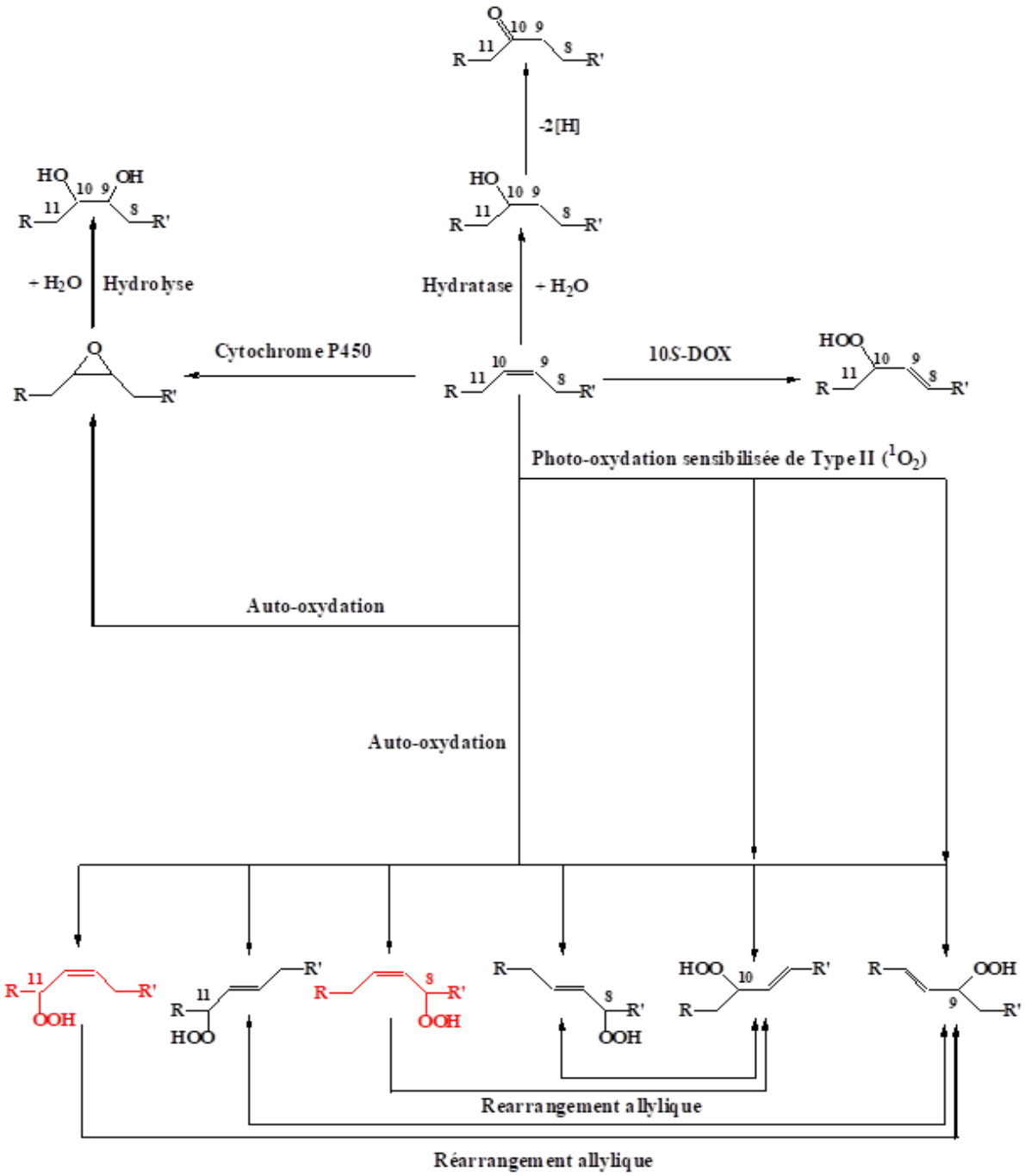
$$(2) \qquad \frac{[8-cis]+[11-cis]}{[10-trans]} = -0.0144\mathrm{T} + 1.553$$

$$(3) \qquad \frac{[8-cis]}{[8-cis]+[8-trans]} = -0.0055\mathrm{T} + 0.627$$

$$(4) \qquad \frac{[11-cis]}{[11-cis]+[11-trans]} = -0.0058\mathrm{T} + 0.635$$

1.3.2.3 Dégradation biotique des acides gras

Les acides gras sont susceptibles d'être biodégradés par de nombreuses enzymes, nommées en fonction de la position de leur attaque de la chaîne acyle (α-, β- ou ω-oxydases, Harwood & Russell, 1984). Cette dégradation conduit généralement à une minéralisation totale des AGs. La diversité de ces enzymes ne sera pas détaillée lors de cet exposé bibliographique.



R = -$(CH_2)_4$-CH_3

R' = -$(CH_2)_8$-COOH

Figure 1.12 : Processus de bio-, auto- et photo-dégradation des MUFAs & traceurs associés dans une cellule phytoplanctonique sénescente.

Les MUFAs et les PUFAs peuvent être également oxygénés en milieu de chaîne par des cytochromes P450, des lipoxygénases (LOXs) ou des hydratases. La superfamille des cytochromes P450 représente un ensemble très diversifié de protéines contenant une structure hémique ; elles sont présentes dans les bactéries, les champignons, les animaux et les plantes. Ces enzymes sont des monooxygénases qui peuvent catalyser un large éventail de réactions. Dans le cas des AGs insaturés, elles permettent l'hydroxylation spécifique de certaines positions de la chaîne carbonée (notamment des positions allyliques, Oliw *et al.,* 1996) et l'époxydation des doubles liaisons (Guengerich, 1991).

Les LOXs sont présentes généralement chez les eucaryotes (Garreta *et al.,* 2013), mais aussi chez quelques procaryotes (Porta & Rocha-Sosa, 2002 ; An *et al.,* 2018). Ces enzymes contenant du fer (non hémique) catalysent la dioxygénation des acides gras polyinsaturés contenant une structure 1(*Z*),4*(Z*)-pentadiène. Le produit formé est un hydropéroxyacide possédant une structure 1-hydropéroxy-2(*E*),4(*Z*)-pentadiène (Porta & Rocha-Sosa, 2002). L'oxydation des AGs par les LOXs peut jouer un rôle important dans l'étape clef des réactions d'auto-oxydation à savoir leur initiation (Figure 1.12). En effet, le cycle catalytique des LOXs produit des radicaux alkoxyles (Fuchs & Spiteller, 2000 ; Spiteller & Afzal, 2014).

Comme leur nom l'indique, les hydratases sont capables d'hydrater, stéréospécifiquement, les doubles liaisons des AGs insaturés. Dans le cas des acides gras insaturés Δ^9, cette hydratation résulte en la formation de 10-hydroxyacides (El-Sharkawy *et al.,* 1992 ; Yang *et al.,* 1993 ; Hou, 1994), qui sont souvent déshydrogénés par la suite en 10-cétoacides (El-Sharkawy *et al.,* 1992). Il est intéressant de noter que l'hydratation des acides gras insaturés peut constituer un mécanisme de détoxification chez certaines bactéries. Une telle détoxification peut être essentielle à la colonisation bactérienne et à la survie dans des environnements riches en acides gras libres bactéricides tels que les blooms d'algues de glace (Falk-Petersen *et al.,* 1998).

La production d'acide 10-hydropéroxyoctadec-8(*E*)-ènoïque a été observée lors de la croissance de *Pseudomonas aeruginosa sp.* 42A2 sur l'acide oléique (Guerrero *et al.,* 1997 ; Busquets *et al.,* 2004). Cette transformation a été attribuée initialement à une LOX, puis plus récemment à une dioxygénase (10*S*-DOX) appartenant à la sous famille des cytochromes C di-hémiques (Estupiñán *et al.,* 2015). Cette oxydation est liée à une activité diol synthase produisant de l'acide 7,10-dihydroxyoctadec-8(*E*)-enoïque (Martínez *et al.,* 2010). L'activité de cette 10*S*-DOX est particulièrement forte dans le cas des AGs

possédant une double liaison en Δ^9. Elle convertit ainsi l'acide palmitoléique ($C_{16:1\omega9}$) en acide 10-hydroperoxyhexadec-8(*E*)-énoïque. La présence de cette 10*S*-DOX a été récemment démontrée chez des bactéries des genres *Pseudoalteromonas*, *Shewanella* et *Aeromonas* (Shoja Chaghervand, 2019). Une dominance de l'acide 10-hydropéroxyhexadec-8(*E*)-ènoïque parmi les produits d'oxydation de l'acide palmitoléique résultant de cette activité 10*S*-DOX a été observée récemment dans la banquise et dans du matériel particulaire dans l'Arctique canadien (Amiraux *et al.,* 2017 ; Rontani *et al.,* 2018), mais aussi dans des estuaires de diverses latitudes (Galeron *et al.,* 2018). Martínez *et al.* (2010) ont précédemment suggéré que les AGs se lient à la 10*S*-DOX avec leur groupe carboxyle à une position fixe par rapport au site catalytique. Cette enzyme localisée dans le périplasme (Martínez *et al.,* 2013) est de ce fait principalement active sur les acides gras libres. Elle pourrait donc contribuer à la détoxification de ces acides gras délétères (Monfort *et al.,* 2000 ; Desbois et Smith, 2010) dans l'environnement bactérien (Martínez *et al.,* 2010).

1.3.3 Δ^5-Stérols

Les Δ^5-stérols sont des composants importants des membranes phytoplanctoniques où ils jouent un rôle capital dans la stabilisation de la bicouche lipidique membranaire (Piepho *et al.,* 2012). Ce sont des composés insaturés, qui représentent entre 2 et 8% du pool lipidique algal (Jónasdóttir, 2019). Certains d'entre eux ne sont produits que par un nombre très limité d'organismes (Volkman, 1986, 2003), ce qui a permis leur utilisation comme traceurs de sources lors d'études de la diversité des populations phytoplanctoniques (Véron *et al.,* 1998 ; Taipale *et al.,* 2016). Ne synthétisant pas de Δ^5-stérols, les bactéries ne peuvent être suivies à l'aide de ces traceurs.

1.3.3.1 Photo-oxydation des Δ^5- stérols

Etant insaturés et présents dans les membranes, les Δ^5-stérols sont donc susceptibles de subir des processus de photo-oxydation sensibilisée de Type II (Christodoulou *et al.,* 2009 ; Rontani *et al.,* 2009). Cette oxydation produit différents composés, principalement des Δ^6-5α-hydropéroxydes et des Δ^4-6α/β-hydropéroxydes, en plus faibles proportions (Figure 1.13, Nickon & Bagli, 1961 ; Kulig & Smith, 1973). Si les Δ^6-5α-hydropéroxydes sont produits majoritairement, ils sont très instables et sont rapidement convertis en 7β-hydropéroxydes par réarrangement allylique (Smith, 1980). Bien que produits en plus petites quantités, les Δ^4-6α/β-hydropéroxydes sont plus stables et spécifiques. Après

réduction au $NaBH_4$, les Δ^4-stera-6α/β-diols formés constituent de bons traceurs de la photo-oxydation des Δ^5-stérols (Christodoulou *et al.,* 2009 ; Rontani *et al.,* 2009). Il est possible d'estimer un % de photo-oxydation du Δ^5-stérol parent en se basant sur le % de Δ^4-stera-6α/β-diols détecté et en utilisant l'équation (5) (Christodoulou *et al.,* 2009).

$$(5) \quad \%\ de\ photo-oxydation\ des\ \text{stérols} = ((\Delta^4\ stera-6\alpha/\beta-\text{diols}\ \%)\ \text{x}\ (1+0.3)\ /\ 0.3)$$

1.3.3.2 Auto-oxydation des Δ⁵-stérols

L'oxydation des Δ^5-stérols par des radicaux libres (auto-oxydation) va produire majoritairement des 7α- et 7β-hydropéroxydes, ainsi que des 5α-/β,6α-/β-époxystérols et des 3β,5α,6β-trihydroxystérols en plus faibles proportions (Figure 1.13, Smith, 1980). Etant peu spécifiques et/ou instables, les 7β-hydropéroxydes et les 5α-/β,6α-/β-époxystérols ne peuvent être considérés comme de bons traceurs (Christodoulou *et al.,* 2009). Le taux d'auto-oxydation des Δ^5-stérols dans un échantillon naturel va donc être estimé en utilisant la concentration en 3β,5α,6β-trihydroxystérols et l'équation (6), proposée par Rontani *et al*. (2009) et basée sur des constantes de vitesse d'auto-oxydation calculées par Morrissey & Kiely (2006).

$$(6) \quad \%\ d'auto-oxydation\ des\ stérols = (3\beta,5\alpha,6\beta-trihydroxystérols\ \%)\ x\ 2.4$$

1.3.3.3 Biodégradation des Δ⁵-stérols

Les Δ^5-stérols peuvent être métabolisés par des bactéries de nombreux genres (Owen *et al.,* 1983 ; Naghibi *et al.,* 2002), aussi bien en conditions aérobies qu'anaérobies. La dégradation microbienne aérobie des Δ^5-stérols peut être effectuée via deux processus : à savoir par métabolisme de leur chaîne latérale ramifiée et/ou par l'ouverture de leurs cycles (Rostoniec *et al.,* 2009). La dégradation est amorcée par oxydation de la fonction hydroxyle 3β- et par l'isomérisation de la double liaison Δ^5 en position Δ^4 (Sojo *et al.,* 1997). Les premières étapes de cette dégradation donnent donc généralement des stér-4-en-3ones, des 5α(H)-stanones et des 5α(H)-stanols (Figure 1.13, Gagosian *et al.,* 1982 ; De Leeuw & Baas, 1986 ; Wakeham, 1989). Ces cétones et stanols sont des indicateurs très utiles pour suivre l'attaque bactérienne des Δ^5-stérols *in situ*. Il est toutefois important de

noter que ces traceurs ne permettent d'obtenir que des indications qualitatives concernant les processus de dégradation biotique agissant sur le Δ^5-stérol considéré.

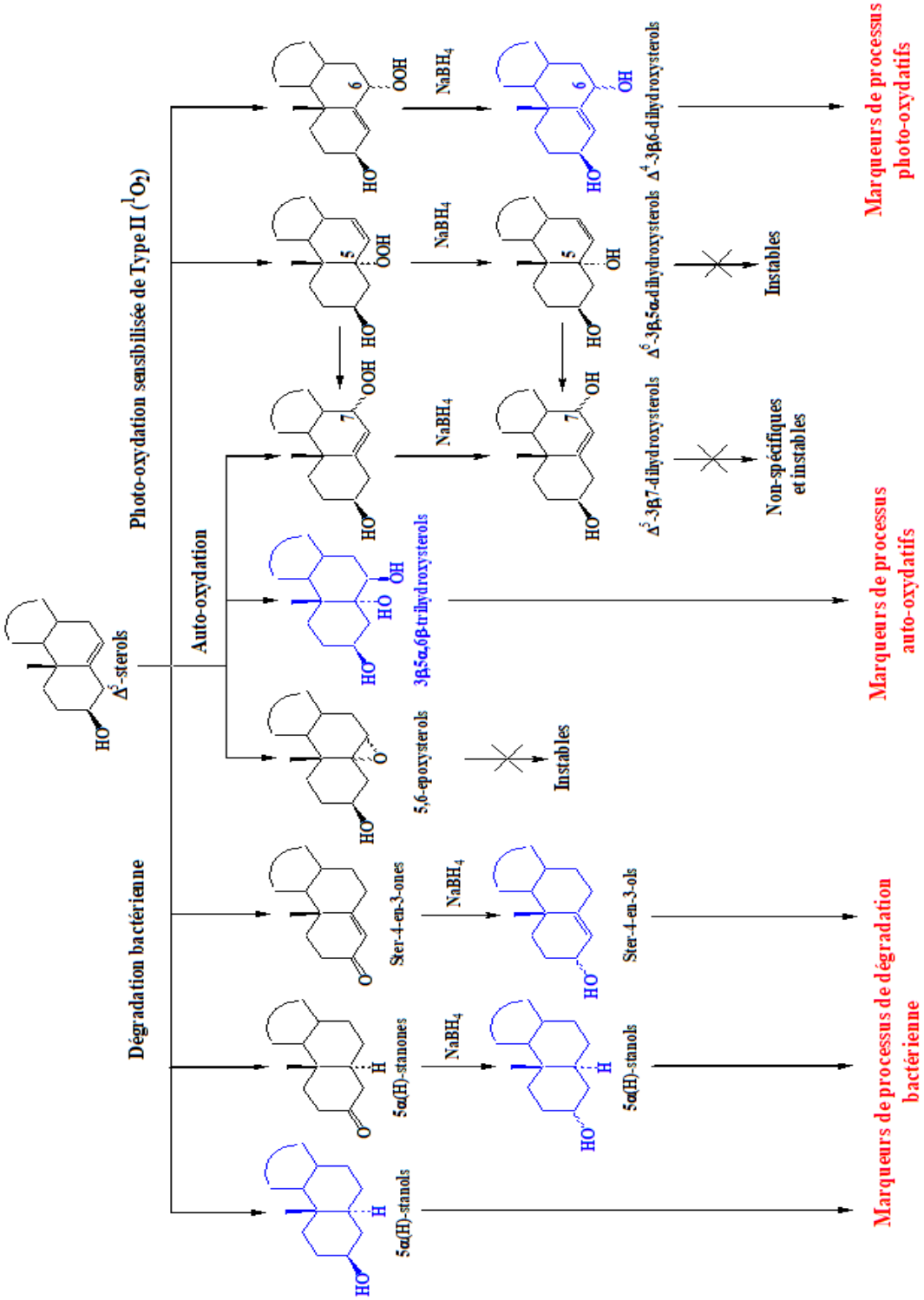


Figure 1.13 : Processus de bio-, auto- et photo-dégradation des stérols & traceurs associés dans une cellule phytoplanctonique sénescente.

1.3.4 Chlorophylle

La chlorophylle est le pigment le plus important des cellules photosynthétiques (Jeffrey & Hallegraeff, 1987). Lors de la sénescence, la chlorophylle se dégrade rapidement sous l'effet de l'$^1O_2$ qu'elle a contribué à produire *via* les processus photosensibilisés de Type II (Figure 1.9B ; Nelson, 1993 ; Rontani *et al.,* 1995 ; Christodoulou *et al.,* 2010). Si des produits de dégradation spécifiques et stables de la structure tétrapyrrolique de la chlorophylle n'ont pas pu être encore identifiés, l'oxydation de la chaîne phytyle de cette dernière (qui se dégrade 3 à 5 fois moins vite que la structure tétrapyrrolique, Cuny *et al.,* 1999 ; Christodoulou *et al.,* 2010) permet un bon suivi de la photo-dégradation de la chlorophylle.

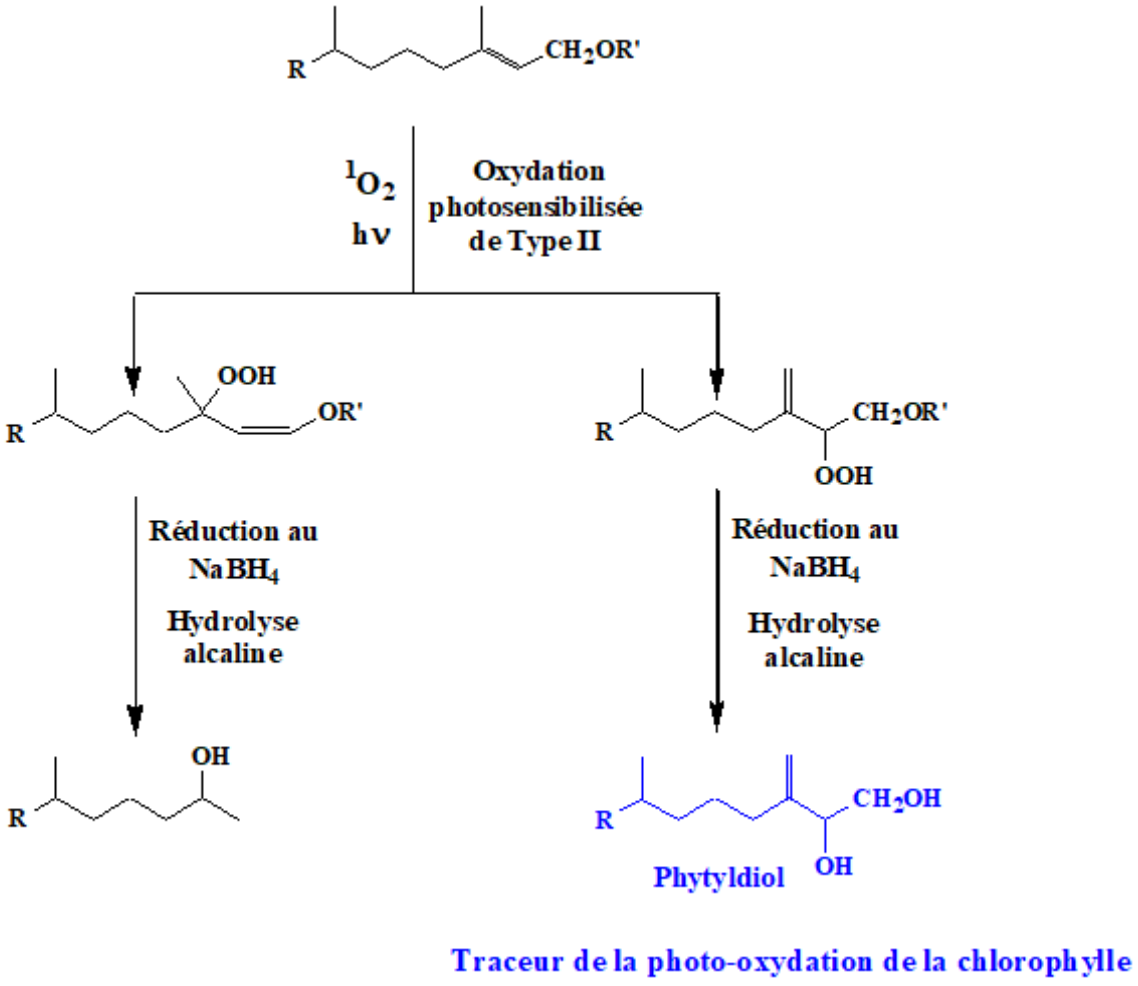


Figure 1.14 : Photo-oxydation sensibilisée de Type II de la chaîne phytyle de la chlorophylle, d'après Rontani & Belt, 2020)

L'$^1O_2$ réagit avec la chaîne phytyle pour former (après réduction au $NaBH_4$ et hydrolyse alcaline) un marqueur stable et spécifique de ce processus, le 3-méthylidène-7,11,15-triméthylhexadecan-1,2-diol ou phytyldiol (Figure 1.14 ; Rontani *et al.,* 1994). Cette molécule, ubiquiste en milieu marin, est spécifique à la photo-dégradation de la chlorophylle et a été proposée comme traceur de ce processus (Rontani *et al.,* 1994, 1996 ; Cuny & Rontani, 1999). En utilisant la rapport molaire phytyldiol/phytol appelé CPPI (Chlorophyll Phytyl side chain Photodegradation Index) et l'équation (7) le pourcentage de photo-dégradation de la chlorophylle peut être estimé (Cuny *et al.,* 1999)

(7) $\%\ de\ photo-dégradation\ de\ la\ chlorophylle = (1 - \ (CPPI + 1)^{-18.5}) x\ 100$

1.3.5 Alcène isoprénoïdes polybranchés (HBIs) et IP_{25}

Les alcènes isoprénoïdes sont des biomarqueurs marins retrouvés dans de nombreux sédiments (Robson & Rowland, 1988 ; Rowland & Robson, 1990 ; Damste, 2004) ; ces molécules possèdent en général des squelettes carbonés à 20, 25 ou 30 atomes de carbone et un nombre d'insaturation variant le plus souvent de 1 à 5 (Rowland & Robson, 1990 ; Volkman *et al.,* 1994 ; Wraige *et al.,* 1999 ; Belt *et al.,* 2001 ; Grossi *et al.,* 2004). Ces dernières années, la production d'alcènes HBIs par de nombreuses diatomées a été bien documentée. (Volkman *et al.,* 1994 ; Belt *et al.,* 2001a ; Grossi *et al.,* 2004).

Parmi la diversité d'HBIs, un alcène monoinsaturé à 25 atomes de carbone présent en Arctique est particulièrement bien conservé dans les sédiments (Belt *et al.,* 2007 ; Vare *et al.,* 2009). Cet isomère, produit par certaines diatomées de glace denomé « ice proxy with 25 carbon atoms » ou IP_{25} est un traceur robuste du couvert de glace en Arctique très souvent utilisé lors d'études paléoenvironnementales (pour une review voir Belt & Müller, 2013).

Récemment, il a pu être démontré que les processus photosensibilisés de Type II affectaient très fortement les alcènes HBIs polyinsaturés possédant au moins une double liaison trisubstituée (Rontani *et al.,* 2011, 2014a). Les processus auto-oxydatifs quant à eux agissent plus particulièrement sur les alcènes HBIs polyinsaturés possédant des positions bis-allyliques permettant la formation de radicaux très stables lors de l'étape d'arrachement d'atomes d'hydrogène (Rontani *et al.,* 2014b). Malheureusement il n'a pas été à ce jour possible de caractériser des photoproduits ou des produits d'oxydation de ces composés suffisamment stables pour tracer ces processus.

Du fait de leur faible réactivité avec les radicaux, l'IP_{25} et certains HBIs di-insaturés ne sont pas affectés par les processus photo- et auto-oxydatifs dans la colonne d'eau. Ils peuvent toutefois être auto-oxydés dans la couche oxique des sédiments arctiques (Rontani *et al.,* 2018, 2019, 2020). En effet, la durée de séjour de ces composés dans cet environnement oxique peut atteindre un siècle et permettre une oxydation significative de ces composés en dépit de la lenteur du processus. Cette dégradation potentielle devra être prise en compte lors d'études paléo-environnementales basées sur ces traceurs.

1.4 Etat de stress et réponse des bactéries arctiques

Dans les milieux arctiques et plus particulièrement dans la glace, des conditions physico-chimiques (température et salinité notamment, Collins *et al.,* 2010) extrêmes et très variables en fonction des saisons règnent et peuvent induire différents stress chez les organismes qui y vivent.

Dans cette partie seront traités les différents stress halins, chimiques et photo-oxydatifs subis par les bactéries arctiques, ainsi que leurs réponses métaboliques.

1.4.1 Stress halin

Les organismes vivants dans les canaux de saumure à l'intérieur de la glace de mer sont soumis à de fortes variations de salinité. En effet, lors de la formation de glace en automne, les sels et solutés se concentrent dans ces canaux, où la salinité peut atteindre 150. En été, lors de l'initiation de la fonte, ces canaux sont lessivés par percolation et les organismes sont alors exposés à des salinités proches de celle de l'eau douce et sont ensuite rejetés dans la colonne d'eau, à une salinité homogène marine. Ces fluctuations importantes peuvent induire des pertes importantes de la population bactérienne de la glace (Krembs *et al.,* 2002 ; Collins *et al.,* 2008). Pour faire face à ces variations de salinité intenses et parfois très rapides (quelques heures), les bactéries arctiques ont développé différentes stratégies adaptatives telles que : (i) la production et l'accumulation de composés osmocompatibles ; (ii) des processus enzymatiques ; ou (iii) la production de substances extrapolymériques (EPS) (Ewert & Deming, 2013).

- Production et accumulation de composés osmocompatibles & EPS

Afin de supporter l'osmolarité très forte des canaux de saumure, les bactéries peuvent produire et accumuler des molécules dites osmocompatibles ou osmoprotectrices (de charge généralement neutre chez les bactéries, Galinski, 1995), cela sans interférer avec les fonctions cellulaires (Roberts, 2005). Parmi ces osmolytes on retrouve par exemple la glycine, la bétaïne ou encore la proline (Roberts, 2005 ; Empadinhas & Viete-Vallejo, 2008 ; Wargo, 2013). Ces osmolytes, synthétisés dans la cellule ou incorporés à partir du milieu, vont permettre à la cellule de maintenir sa pression de turgescence, son volume et sa concentration en électrolytes ; éléments importants pour la division et la prolifération cellulaire. Les organismes arctiques se développant dans la glace ont également la particularité de produire de grandes quantités d'EPS (Krembs & Engel, 2001 ; Meiners *et al.,* 2003). Ces polymères permettent une meilleure adhésion au substrat et peuvent également constituer une protection contre le froid. Dans le cas d'un stress halin, la production d'EPS peut aussi servir de barrière de diffusion et ainsi protéger les bactéries contre la forte pression osmotique dans les canaux de saumure (Krembs & Deming, 2008).

- Maintien de la fluidité membranaire *via* la *cis*/*trans* isomérase

Un autre moyen pour les bactéries arctiques de tolérer les fortes variations de salinité est de maintenir la fluidité de leurs membranes *via* un processus de remodelage des lipides, appelé « adaptation homéovisqueuse » (Sinensky, 1974). Cette adaptation se traduit physiologiquement par la conversion enzymatique des AGs membranaires de conformation *cis* en *trans* (Loffeld & Keweloh, 1996 ; Heipieper *et al.,* 2003). Ce changement de conformation est généralement réalisé à l'aide d'enzymes appelées *cis*/*trans* isomérases (CTIs, Figure 1.15).

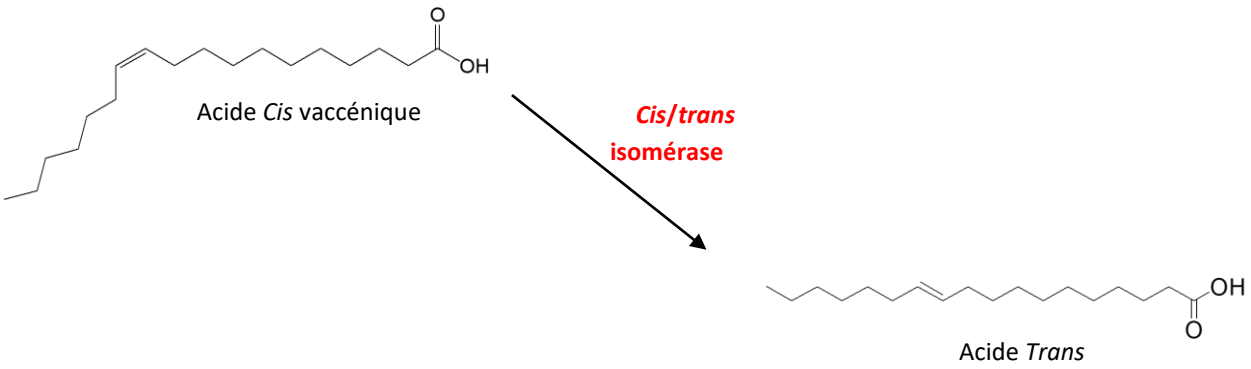


Figure 1.15 : Action de la *cis/trans* isomérase sur l'acide vaccénique

L'isomérisation des AGs membranaires permet ainsi aux bactéries de « rigidifier » leurs membranes et ainsi de prévenir les risques de lyse cellulaire (Heipieper *et al.,* 2003). Le ratio des AG *trans*/*cis* (T/C) témoigne également de la présence d'un stress halin. On peut noter qu'un ratio T/C d'un échantillon environnemental > 0.1 est souvent utilisé comme indicateur d'un état de stress bactérien (Guckert *et al.,* 1986).

Il est important de noter que l'isomérisation des AGs membranaires est, chez les bactéries, un phénomène de protection relativement rapide (Heipieper *et al.,* 2007). Lorsque le stress osmotique disparait, le ratio T/C retrouve une valeur normale (Fischer *et al.,* 2010). La réaction inverse de la CTI (conversion des AG *trans* en AG *cis*) n'est toutefois pas catalysée (Eberlein *et al.,* 2018), ainsi le retour à un ratio T/C normal ne s'obtient que par une synthèse *de novo* d'AG de conformation *cis*.

1.4.2 Stress chimique

Les AG libres (ou Free Fatty Acids : FFAs) sont des AGs non estérifiés, qui ne sont donc pas rattachés à une autre molécule (sucre, glycérol, phosphate etc…), ils sont produits de manière significative par les diatomées sympagiques (Falk-Petersen *et* al., 1998 ; Parrish *et* al., 2005) en réponse à un stress radiatif (Hu *et* al., 2008), de prédation (Jüttner, 2001) ou en fin de bloom (Smith *et* al., 1993). Les bactéries se développant dans les canaux de saumure peuvent subir un stress chimique, lié à la sécrétion des FFAs par les diatomées sympagiques. Ces FFAs sont rapidement excrétés hors de la cellule dans le milieu *via* des enzymes lipolytiques (Jüttner, 2001 ; Wichard *et al.,* 2007). Ces enzymes, majoritairement des lipases (Desbois & Smith, 2010) vont séparer l'AG de la molécule à laquelle il est rattaché *via* hydrolyse pour donner un AG libre. Le pH à l'intérieur des canaux de saumure, qui est compris entre 8.0 et 8.5 (Hare *et al.,* 2013), favorise la solubilisation des FFAs (Parrish *et al.,* 2013). Les bactéries associées aux diatomées dans ces milieux sont donc fortement exposées à ces composés.

Les FFAs possèdent de nombreuses propriétés bactéricides et/ou bactériostatiques (Desbois & Smith, 2010) selon différents modes d'action (pour une revue détaillée, voir Desbois & Smith, 2010). De manière générale, les FFAs insaturés ont une plus grande activité antibactérienne que leurs homologues saturés (Desbois *et al.,* 2009), de même les FFAs monoinsaturés montrant les plus fortes activités antibactériennes ont en général entre 14 et 16 atomes de carbone (Feldlaufer *et al.,* 1993).

Les FFAs vont d'abord fortement détériorer les lipides membranaires, en y créant des failles de tailles variables, conduisant à des fuites de composants cellulaires, à la perturbation des protéines membranaires et à terme, la lyse cellulaire. Ils peuvent également avoir un fort impact sur la chaîne de transports d'électrons perturbant ainsi la production d'ATP et donc l'apport en énergie de la cellule. Les FFAs sont également des inhibiteurs de diverses enzymes membranaires ou du cytosol, pouvant inhiber jusqu'à la synthèse d'AGs *in vivo* (Zheng *et al.,* 2005) et ainsi conduire à l'altération ou à la perméabilité des membranes (Desbois & Smith, 2010). Enfin, ils vont également empêcher la cellule d'accéder à des nutriments essentiels comme les acides aminés, probablement suite à des perturbations du transport actif de nutriments. Cependant, le mode d'action des FFAs à ce niveau n'est pas connu.

Les bactéries ont différentes stratégies pour tenter de se protéger des FFAs. La première est de renforcer leurs membranes, ces structures étant en contact direct avec ces composés. En isomérisant leurs membranes, les bactéries les rendent plus difficiles à pénétrer et limitent ainsi les effets antibactériens des FFAs (Kenny *et al.,* 2009). Ce mécanisme de défense va également modifier la polarité des membranes et les rendre moins hydrophobes. Par conséquent les AGs libres vont être moins attirés par les membranes cellulaires et ainsi moins les pénétrer (Clarke *et al.,* 2007 ; Kenny *et al.,* 2009). La présence de pigments membranaires, comme des caroténoïdes, peut également aider à diminuer la fluidité membranaire et ainsi éviter le passage des FFAs (Chamberlain *et al.,* 1991).

Enfin, les bactéries sont également capables, au-delà d'une simple protection contre les FFAs, de détoxifier ces derniers *via* des processus enzymatiques. En effet, il a été démontré que l'hydratation d'AGs libres par des hydratases est un mécanisme efficace de détoxification (Volkov *et al.,* 2010 ; Joo *et al.,* 2012) permettant aux bactéries évoluant dans un environnement riche en FFAs de survivre.

1.4.3 Stress photo-oxydatif

Comme il a été exposé précédemment, la photo-oxydation (principalement de type II), faisant intervenir un photo-sensibilisateur dans un état excité (^{3}sens) est un mécanisme induisant indirectement de nombreux dommages dans les organismes photosynthétiques sénescents, *via* la production de ROS. En effet chez ces derniers, les différents systèmes de protection contre les dommages ne sont plus suffisamment performants pour

« quencher » efficacement les ROS produits naturellement, ce qui entraîne une accumulation de ces composés.

Du fait de l'absence de photo-sensibilisateurs efficaces chez la majorité des bactéries, l'effet des processus photo-oxidatifs y est beaucoup plus limité. Toutefois, des proportions significatives de produits de photo-oxydation de l'acide *cis*-vaccénique (AG typique des bactéries Gram négatives : Sicre *et al.,* 1988 ; Keweloh & Heipieper, 1996) ont été observées *in situ* (Rontani *et al.,* 2011 ; Rontani, 2012) et après irradiation de phytodétritus non-axéniques (Petit *et al.,* 2015). Ces observations surprenantes ont été attribuées à : (i) la présence de bactéries photohétérotrophes (Aerobic Anoxygenic Phototrophs : AAPs, Uchoa *et al.,* 2008 ; Berghoff *et al.,* 2011) possédant de la bactériochlorophylle a (BChl *a*) ou (ii) un transfert de ROS des cellules phytoplanctoniques sénescentes aux bactéries qui leur sont attachées.

1.4.3.1 Photo-oxydations des AAPs

Les AAPs, largement distribués dans les environnements marins, possèdent de la bacterio-chlorophylle a (BChl a) qui leur permet de produire de l'ATP dans certaines conditions via un processus appelé photosynthèse anoxygénique. La BChl a étant une molécule photo-sensibilisatrice, une photo-oxidation des AAPs initiée par la BChl a est donc envisageable. L'irradiation de cellules mortes de deux AAPs isolés du milieu marin (*Erythrobacter* sp. NAP1 et *Roseobacter* sp. COL2P) (contenant de fortes proportions d'acide vaccénique) n'a pas permis de mettre en évidence d'attaque significative de la chaîne phytyle de la Bchl a et de cet acide par l'$^1O_2$ (Rontani *et al.,* 2003a). L'oxydation très lente de l'acide vaccénique observée résultait essentiellement de processus radicalaires. Cette absence de processus photosensibilisés de Type II a été attribuée à la présence de fortes proportions de caroténoïdes (piégeant l'$^1O_2$) dans ces bactéries pigmentées.

1.4.3.2 Transfert des ROS aux bactéries

L'action délétère des ROS et plus particulièrement de l'$^1O_2$ ne se limite pas à l'environnement immédiat des chloroplastes (où ils sont générés). En effet, Il a été observé dans l'appareil photosynthétique de *Chlamydomonas reinhardtii* que l'$^1O_2$ produit dans les membranes thylakoïdes des chloroplastes dans des conditions de forte luminosité est capable d'atteindre le cytoplasme ou même le noyau (Fisher *et al.,* 2007). Il est important de savoir que l'$^1O_2$ a une durée de vie plus longue dans les membranes biologiques (14 µs) que dans les milieux aqueux (3.5 µs). Dans ces membranes, il a donc une distance de

diffusion théorique de l'ordre de 400 nm (Baier *et al.,* 2005). Cette distance est tout à fait suffisante pour lui permettre de migrer hors des cellules phytoplanctoniques sénescentes et ainsi induire des dégâts aux bactéries qui leur sont attachées (Rontani *et al.,* 2003a ; Christodoulou *et al.,* 2010). Cette hypothèse a pu être confirmée par Rontani *et al.* (2003a), qui ont irradié des cellules mortes : (i) de phytoplancton axénique, (ii) de bactéries hétérotrophes et (iii) du mélange des deux dans les mêmes conditions et ont observé une photo-oxydation accélérée de l'acide vaccénique bactérien en présence de phytoplancton. Certains microorganismes étant mieux équipés que d'autres (présence de caroténoïdes) pour faire face à ce stress, l'exposition à l'$^1O_2$ pourrait donc être un facteur de sélection dans les environnements marins pouvant modifier la composition de la communauté bactérienne (Glaeser *et al.,* 2010 ; Petit *et al.,* 2015).

Les phytodétritus constituent donc des environnements hydrophobes privilégiés permettant l'allongement de la durée de vie de l'$^1O_2$ et sa diffusion hors des cellules. Il a récemment été démontré que les écosystèmes arctiques sont très propices à la production et la diffusion d'$^1O_2$ en raison : (i) des faibles irradiances présentes sous la glace, limitant la dégradation des sensibilisateurs (photobleaching, Figure 1.9B) et allongeant ainsi la durée de production de l'$^1O_2$ et (ii) des faibles températures y régnant, qui ralentissent la diffusion de l'$^1O_2$ au travers des membranes, y induisant donc plus de dégâts (Amiraux *et al.,* 2016). Du fait de l'absence de système photoprotecteur et anti-oxydant suffisamment efficace chez la plupart des bactéries hétérotrophes associées aux phytodétritus, les dommages photo-oxydatifs liés à l'$^1O_2$ doivent donc y être conséquents (Garcia-Pichel, 1994).

Il est toutefois important de noter que le transfert d'oxygène singulet des phytodétritus aux bactéries associées dépend grandement du morphotype de la cellule phytoplanctonique. En effet la présence d'une frustule de silice inhibe totalement le transfert de l'$^1O_2$ aux cellules attachées, contrairement aux coccolithes carbonatés qui n'inhibent pas ce transfert (Petit *et al.,* 2015a).

1.4.3.3 Dommages oxydants sur la cellule

Comme il a été exposé précédemment, les processus photo-oxydatifs de Type II faisant intervenir l'$^1O_2$ peuvent induire une oxydation très intense des composés insaturés de la cellule. De plus, la réaction entre l'$^1O_2$ et les lipides membranaires conduit à la production d'hydropéroxydes, pouvant eux même induire (après clivage homolytique) des réactions

d'oxydation en chaîne (peroxydation lipidique) qui dégradent rapidement les membranes (Glaeser *et al.,* 2011).

Parmi les cibles privilégiées de l'$^1O_2$ dans la cellule autres que les lipides insaturés on peut noter certains acides aminés, et plus particulièrement la guanine, avec qui il réagit pour donner un produit stable, la 8-oxo-7,8dihydro2'-deoxyguanosine (8-oxodG), ou ^OG (Cadet *et al.,* 2006, 2010). Cette oxydation se traduit par l'introduction d'un groupement -oxo en C-8 et d'un atome d'hydrogène en C-7 (van Loon *et al.,* 2010 ; Figure 1.16). Les autres bases peuvent également être oxydées, mais la guanine possède une plus forte affinité en raison de son faible potentiel redox (Steenken & Jovanovic, 1997). La principale conséquence de cette oxydation de la guanine est bien évidemment la forte occurrence de mutations (Menck *et al.,* 1993). La mutation la plus couramment observée est une transversion G → T (Mascio *et al.,* 1990 ; De Oliveira *et al.,* 1992 ; Agnez-Lima *et al.,* 1999). On note également l'occurrence moins importante de la transversion G → C (McBride *et al.,* 1991 ; van den Akker *et al.,* 1994 ; Wagner & Fuchs, 1997), et des délétions de bases (Decuyper-Debergh *et al.,* 1987 ; Shibutani *et al.,* 1991). Les conséquences de l'exposition à l'$^1O_2$ sont variées mais les mutations induites mènent généralement à la mort de la cellule. Il est difficile de déterminer précisément les causes, tant l'$^1O_2$ réagit avec différentes éléments cellulaires (membranes, protéines, ADN…), mais on a pu noter la perturbation d'enzymes indispensables au métabolisme primaire, comme les ADN polymérases, dont la progression est bloquée par l'^OG empêchant ainsi la réplication de l'ADN (Piette & Moore, 1982).

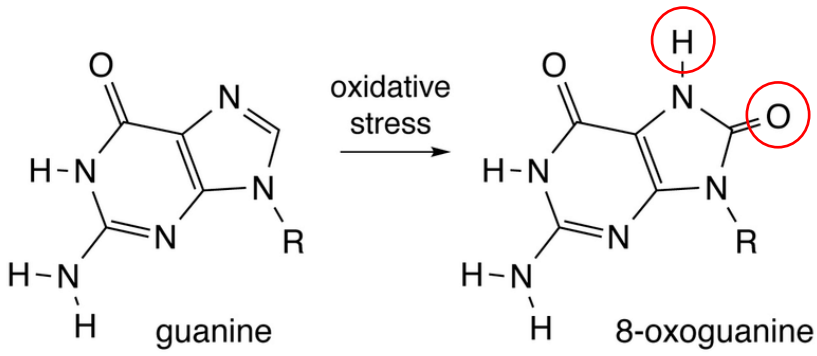


Figure 1.16 : Oxydation de la guanine formant la base 8-oxodG (d'après Lanier & Williams, 2017)

- Mécanismes de réparation et de protection

Les bactéries possèdent différentes stratégies pour se protéger ou limiter l'impact des forts dégâts oxydatifs de l'$^1O_2$. Ceux-ci peuvent être préventifs (permettant d'éviter l'occurrence des dommages cellulaires) ou curatifs. Des enzymes comme la Super Oxide

Dismutase (SOD) ou la catalase (CAT) peuvent avoir un effet protecteur contre l'$^1O_2$ (Glaeser *et al.,* 2011 ; Kim *et al.,* 2002) ; la présence d'anti-oxydants comme des pigments (caroténoïdes) peuvent également bloquer les molécules photosensibilisatrices ou l'$^1O_2$ lui-même.

Trois principales voies de réparation de l'ADN sont impliquées dans la réparation des lésions liées à l'$^1O_2$, à savoir la Nucleotide Excision Repair (NER), la Base Excision Repair (BER) et la Mismatch Repair (MMR), toutefois la BER est la voie la plus utilisée pour la réparation de l'ADN ayant subi des dommages oxydatifs (Mitra *et al.,* 2001 ; Agnez-Lima *et al.,* 2012). La réparation de ces lésions passe généralement par la reconnaissance de la base oxydée et par son excision par des endonucléases (Czeczot *et al.,* 1991 ; Schulz *et al.,* 2000). Chez les procaryotes, le système de réparation de l'ADN contre l'effet mutagène de l'^0G est appelé « GO system » (Michaels & Miller 1992) et se compose de trois protéines : MutM, MutY et MutT, codées par les gènes *mutM, mutY* et *mutT*, respectivement. L'enzyme MutM ou FPG, est une glycosylase qui retire la 8-oxodG quand elle est associée à la cytosine, le site abasique résultant de l'action de cette enzyme est également retiré, *via* des réactions de β- et δ-élimination (Figure 1.17; Boiteux *et al.,* 1992 ; Tchou *et al.,* 1991). La protéine MutY est elle aussi une glycosylase, qui va elle retirer l'adénine, quand elle est associée à une 8-oxodG (Figure 1.17; Michaels *et al.,* 1992). Enfin, la protéine MutT a un autre mode d'action, cette enzyme est une nucléotide tri-phosphatase, qui va « détoxifier » le pool de nucléotides en hydrolysant les 8-oxodGTP en 8-oxoGMP, empêchant ainsi l'incorporation de la base dans les brins d'ADN (Figure 1.17; Maki & Sekigushi, 1992).

Chez les bactéries phototrophes ou hétérotrophes, la réponse moléculaire face à l'$^1O_2$ est régulée par des facteurs sigma, qui vont activer ou réprimer l'expression des gènes de défense et par conséquent la production d'enzymes de protection ou de réparation de l'ADN. Les facteurs sigma sont de petites protéines capables de reconnaître une séquence nucléotidique particulière et de s'y fixer en amont, au niveau des promoteurs. La reconnaissance de ces facteurs sigma par les ARN polymérases permet de débuter la transcription par le bon brin et le bon nucléotide.

Les facteurs sigma ECF sont une grande famille de facteurs sigma impliqués dans la perception d'un stimulus et dans l'activation de la réponse moléculaire adaptée (Mascher, 2013). L'ECF (ExtraCytoplasmic Function) facteur sigma RpoE est un facteur sigma de type IV, qui est maintenu inactif par son anti-facteur sigma ChrR (Newman *et al.,* 2001 ; Anthony *et al.,* 2005 ; Campbell *et al.,* 2007). Pour que le facteur sigma RpoE soit activé et qu'il se fixe sur l'ARN, il doit être libéré de son anti-facteur sigma ChrR. Il a été démontré

que lors de l'exposition des cellules à l'$^1O_2$, le complexe RpoE:ChrR se dissocie, permettant à RpoE d'induire l'expression des gènes de défense et de réparation de l'ADN (Anthony *et al.,* 2005, 2004). Il existe également le facteur sigma RpoH (possédant deux paralogues, $RpoH_I$ et $RpoH_{II}$), dépendant directement de l'action de RpoE (Nuss *et al.,* 2009). Les facteurs sigma $RpoH_I$ et $RpoH_{II}$ sont impliqués notamment dans la réponse face à un choc thermique, mais également face à l'$^1O_2$. Ces deux facteurs sigma (RpoE et RpoH) sont ainsi les principaux régulateurs des gènes de protection et de réparation face au stress photo-oxydatif.

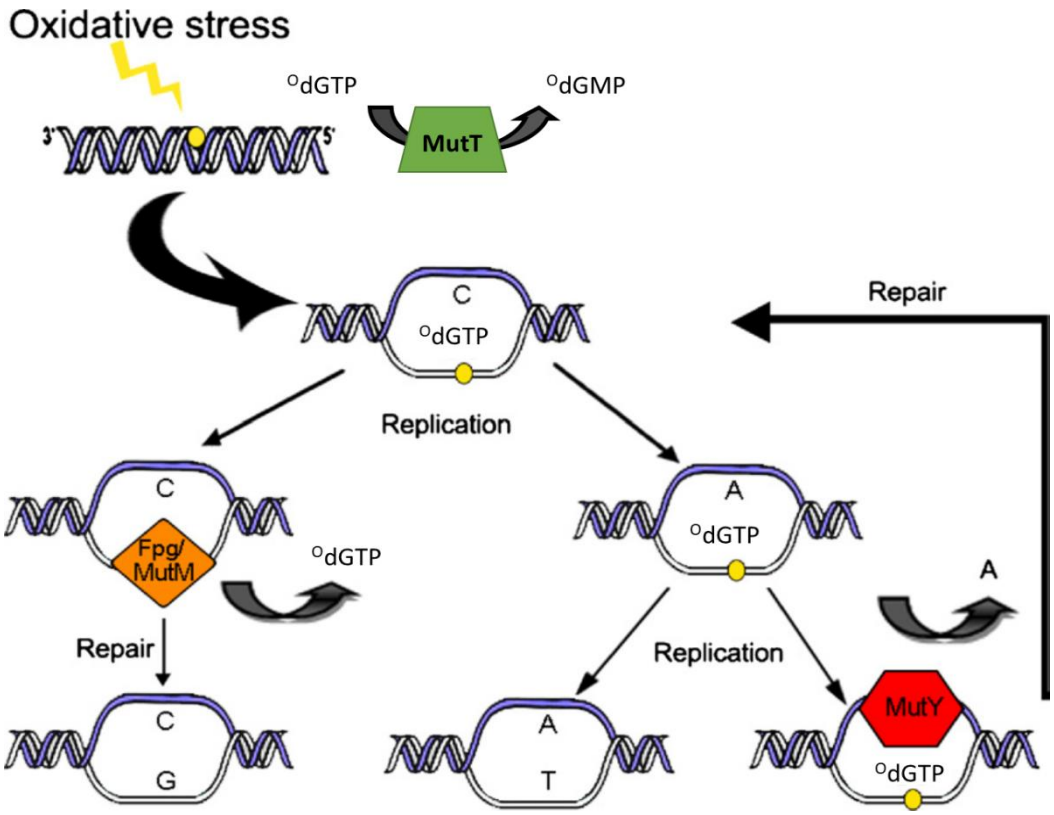


Figure 1.17 : Le « GO system » en cas de stress oxydatif de l'ADN. Différentes voies sont possibles. Premièrement MutT peut hydrolyser l'^{8}dGTP en ^{8}dGMP, inactivant la base dans le pool de nucléotide. Si l'^{8}dGTP est incorporé dans l'ADN, soit MutM excise la base et le site abasique est remplacé par un dGTP, soit la réplication à lieu. Dans ce cas, une adénine peut être associée avec la ^{8}dGTP et être retirée par MutY. Si MutY ne retire pas l'adénine, celle-ci sera la cause d'une transversion G→T. D'après Agnez-Lima *et al.,* 2012, modifié.

1.5 Objectifs et organisation de la thèse

Du fait des perturbations climatiques liées au changement global en termes de couvert de glace, d'intensité et d'abondance de précipitations ou d'augmentation des températures, l'étude des écosystèmes arctiques constitue un challenge pour la communauté scientifique. Sur la base des estimations alarmantes annonçant des diminutions drastiques du couvert de glace (absence totale de glace pluriannuelle d'ici la

fin du siècle, diminution de près de 75% du couvert d'ici 2050, Overland & Wang, 2007 ; Kwok *et al*., 2009), il est urgent d'étudier de plus près le fonctionnement de ces écosystèmes afin de prédire le plus précisément possible l'impact de ces perturbations sur cette zone stratégique.

La production primaire (P.P) arctique, assurée par les organismes photosynthétiques sympagiques (de glace) ou pélagiques, constitue le moteur toute la chaîne alimentaire et est à la base du cycle du carbone dans ces écosystèmes (Boras *et al.,* 2010 ; Boetius *et al.,* 2015). De par leur capacité à s'agréger, les algues sympagiques ont une vitesse de chute rapide, qui leur permet de contribuer fortement à l'export de carbone vers les sédiments arctiques (Riebesell *et al.,* 1991 ; Azetsu-Scott & Passow, 2004). Le devenir de la production primaire et sa chute vers les sédiments dépend grandement de l'activité de minéralisation bactérienne. Si la boucle microbienne consomme près de la moitié de la P.P dans les océans de basse latitude (Ducklow, 2000), il a été démontré que cette dernière est bien moins efficace dans les eaux en hautes latitudes (Howard-Jones *et al*., 2002).

Différents paramètres peuvent expliquer cette faible activité du bacterioplancton arctique. En effet, les conditions physico-chimiques régnant dans ces milieux particuliers peuvent induire des stress impactant l'état physiologique des bactéries. La salinité élevée présente dans les canaux de saumure de la glace (avec des valeurs de salinité pouvant atteindre 150) expose les bactéries sympagiques à un stress halin important, pouvant être un des facteurs limitant leur activité (Amiraux *et al*., 2017). Un autre facteur stressant pour les bactéries dans la glace peut également provenir de substances bactéricides produites par les algues. En effet comme il a déjà été démontré, les algues sympagiques peuvent produire des acides gras sous forme libre (Desbois & Smith, 2010), notamment en cas de stress lumineux, ces composés ayant une forte activité bactéricide. Enfin, les conditions des milieux arctiques favorisent également les réactions de photo-oxydation de type II dans le matériel algal, l'impact de ces réactions pouvant être transféré aux bactéries qui lui sont associées (Christodoulou *et al.,* 2010).

Dans ce contexte, il est capital de bien comprendre l'impact de ces différents stress sur l'activité des bactéries associées au matériel sympagique et pélagique et sur la préservation de ce matériel dans les sédiments. Une meilleure compréhension de ces phénomènes pourrait permettre d'affiner les différents modèles quantifiant les flux de matière dans la glace et la colonne d'eau dans cette zone particulière.

Cette thèse vise ainsi à mesurer l'impact des stress halin, chimique et photochimique sur les bactéries associées au matériel algal et sur leur efficacité de dégradation. Pour mener à bien ces objectifs, elle s'articule en quatre chapitres, basés sur deux approches bien distinctes.

Approche *In Vivo* :

Les trois premiers chapitres de cette thèse vont s'intéresser à l'état de stress des bactéries associées au matériel algal arctique en s'appuyant sur de nombreux échantillons de glace ou de matière organique récoltés dans l'Arctique Canadien, principalement dans le cadre du projet de recherche GreenEdge, dont l'objectif principal est de mieux comprendre la dynamique du bloom algal printanier en Arctique. Le premier chapitre concernera l'analyse d'échantillons de particules en sédimentation récoltés à une station fixe localisée à Qikiqtarjuaq lors de la saison estivale (mai à juillet) de 2016. Des analyses lipidiques seront réalisées sur ces échantillons afin d'étudier l'état de stress halin et chimique des bactéries associées, ainsi que leur mortalité grâce à l'utilisation du Propidium Mono-Azide (PMA). Le second chapitre de cette thèse portera sur l'étude de particules en suspension récoltées lors de la même campagne GreenEdge de 2016, cette fois sur un transect allant de la glace à l'eau libre. Des analyses similaires à celles mises en place dans le 1er chapitre seront menées, afin d'estimer l'état de stress et la mortalité des bactéries associées à ce matériel. Enfin, le 3ème chapitre de cette approche *In Vivo* se portera sur l'étude de nombreux échantillons de sédiments de surface récoltés lors de différentes campagnes d'échantillonnage en baie de Baffin et en mer de Beaufort. Dans ce chapitre, les analyses lipidiques réalisées se focaliseront sur la préservation du matériel algal issu de la glace, en suivant différents traceurs de dégradation d'origine bactérienne.

Approche *In Vitro* :

Dans le 4ème et dernier chapitre de cette thèse, sera abordé en détail la question du stress photochimique de type II sur les bactéries associées au matériel algal sénescent. Deux souches bactériennes modèles (une souche ubiquiste marine non pigmentée et une souche bactérienne possédant des caroténoïdes) seront mises en culture avec des cellules algales sénescentes, afin de mesurer l'impact du transfert de l'oxygène singulet de ces algues à leurs bactéries associées sur l'oxydation des lipides membranaires bactériens ainsi que sur l'expression de gènes de réparation de l'ADN spécifique à ce stress. Lors de cette étude, nous nous sommes proposés de : (i) déterminer d'une part si ce transfert est favorisé dans la glace où les algues sont exposées durant de longues périodes à un

éclairement relativement intense et (ii) d'autre part de vérifier si la présence de caroténoïdes à fort pouvoir antioxydant dans les membranes de certaines bactéries peut limiter significativement les effets délétères de l'oxygène singulet.

Bibliographie

Agnez-Lima, L. F., Mascio, P. D., Napolitano, R. L., Fuchs, R. P., & Menck, C. F. M. (1999). Mutation Spectrum Induced by Singlet Oxygen in Escherichia coli Deficient in Exonuclease III. *Photochemistry and Photobiology*, *70*(4), 505–511. https://doi.org/10.1111/j.1751-1097.1999.tb08245.x

Agnez-Lima, L. F., Melo, J. T. A., Silva, A. E., Oliveira, A. H. S., Timoteo, A. R. S., Lima-Bessa, K. M., Martinez, G. R., Medeiros, M. H. G., Di Mascio, P., Galhardo, R. S., & Menck, C. F. M. (2012). DNA damage by singlet oxygen and cellular protective mechanisms. *Mutation Research/Reviews in Mutation Research*, *751*(1), 15–28. https://doi.org/10.1016/j.mrrev.2011.12.005

Ambrose, W. G., Quillfeldt, C. von, Clough, L. M., Tilney, P. V. R., & Tucker, T. (2005). The sub-ice algal community in the Chukchi sea: Large- and small-scale patterns of abundance based on images from a remotely operated vehicle. *Polar Biology*, *28*(10), 784–795. https://doi.org/10.1007/s00300-005-0002-8

Amiraux, R., Belt, S. T., Vaultier, F., Galindo, V., Gosselin, M., Bonin, P., & Rontani, J.-F. (2017). Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers. *Marine Chemistry*, *194*, 89–99. https://doi.org/10.1016/j.marchem.2017.05.006

Amiraux, R., Jeanthon, C., Vaultier, F., & Rontani, J.-F. (2016). Paradoxical effects of temperature and solar irradiance on the photodegradation state of killed phytoplankton. *Journal of Phycology*, *52*(3), 475–485. https://doi.org/10.1111/jpy.12410

An, J.-U., Hong, S.-H., & Oh, D.-K. (2018). Regiospecificity of a novel bacterial lipoxygenase from Myxococcus xanthus for polyunsaturated fatty acids. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1863*(8), 823–833. https://doi.org/10.1016/j.bbalip.2018.04.014

Anthony, J. R., Newman, J. D., & Donohue, T. J. (2004). Interactions Between the Rhodobacter sphaeroides ECF Sigma Factor, σE, and its Anti-sigma Factor, ChrR. *Journal of Molecular Biology*, *341*(2), 345–360. https://doi.org/10.1016/j.jmb.2004.06.018

Anthony, J. R., Warczak, K. L., & Donohue, T. J. (2005). A transcriptional response to singlet oxygen, a toxic byproduct of photosynthesis. *Proceedings of the National Academy of Sciences*, *102*(18), 6502–6507. https://doi.org/10.1073/pnas.0502225102

Arrigo, K. R., & Dijken, G. L. van. (2011). Secular trends in Arctic Ocean net primary production. *Journal of Geophysical Research: Oceans*, *116*(C9). https://doi.org/10.1029/2011JC007151

Auel, H., Klages, M., & Werner, I. (2003). Respiration and lipid content of the Arctic copepod Calanus hyperboreus overwintering 1 m above the seafloor at 2,300 m water depth in the Fram Strait. *Marine Biology*, *143*(2), 275–282. https://doi.org/10.1007/s00227-003-1061-4

Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., & Thingstad, F. (1983). The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology Progress Series*, *10*, 257–263. https://doi.org/10.3354/meps010257

Azam, F., & Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nature Reviews Microbiology*, *5*(10), 782–791. https://doi.org/10.1038/nrmicro1747

Azetsu-Scott, K., & Passow, U. (2004). Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the upper ocean. *Limnology and Oceanography*, *49*(3), 741–748. https://doi.org/10.4319/lo.2004.49.3.0741

Baier, J., Maier, M., Engl, R., Landthaler, M., & Bäumler, W. (2005). Time-Resolved Investigations of Singlet Oxygen Luminescence in Water, in Phosphatidylcholine, and in Aqueous Suspensions of Phosphatidylcholine or HT29 Cells. *The Journal of Physical Chemistry B*, *109*(7), 3041–3046. https://doi.org/10.1021/jp0455531

Bano, N., & Hollibaugh, J. T. (2002). Phylogenetic Composition of Bacterioplankton Assemblages from the Arctic Ocean. *Applied and Environmental Microbiology*, *68*(2), 505–518. https://doi.org/10.1128/AEM.68.2.505-518.2002

Becquevort, S., Dumont, I., Tison, J.-L., Lannuzel, D., Sauvée, M.-L., Chou, L., & Schoemann, V. (2009). Biogeochemistry and microbial community composition in sea ice and underlying seawater off East Antarctica during early spring. *Polar Biology*, *32*(6), 879–895. https://doi.org/10.1007/s00300-009-0589-2

Belt, S. T., Allard, W. G., Johns, L., König, W. A., Massé, G., Robert, J.-M., & Rowland, S. (2001). Variable stereochemistry in highly branched isoprenoids from diatoms. *Chirality*, *13*(8), 415–419. https://doi.org/10.1002/chir.1053

Belt, S. T., Allard, W. G., Massé, G., Robert, J.-M., & Rowland, S. J. (2001a). Structural characterisation of C30 highly branched isoprenoid alkenes (rhizenes) in the marine diatom Rhizosolenia setigera. *Tetrahedron Letters*, *42*(32), 5583–5585. https://doi.org/10.1016/S0040-4039(01)01063-2

Belt, S. T., Massé, G., Rowland, S. J., Poulin, M., Michel, C., & LeBlanc, B. (2007). A novel chemical fossil of palaeo sea ice: IP25. *Organic Geochemistry*, *38*(1), 16–27. https://doi.org/10.1016/j.orggeochem.2006.09.013

Belt, S. T., & Müller, J. (2013). The Arctic sea ice biomarker IP25: A review of current understanding, recommendations for future research and applications in palaeo sea ice reconstructions. *Quaternary Science Reviews*, *79*, 9–25. https://doi.org/10.1016/j.quascirev.2012.12.001

Bergé, J.-P., & Barnathan, G. (2005). Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Economical Aspects. In R. Ulber & Y. Le Gal (Eds.), *Marine Biotechnology I* (Vol. 96, pp. 49–125). Springer Berlin Heidelberg. https://doi.org/10.1007/b135782

Berghoff, B. A., Glaeser, J., Nuss, A. M., Zobawa, M., Lottspeich, F., & Klug, G. (2011). Anoxygenic photosynthesis and photooxidative stress: A particular challenge for Roseobacter. *Environmental Microbiology*, *13*(3), 775–791. https://doi.org/10.1111/j.1462-2920.2010.02381.x

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernandez-Mendez, M., Hendricks, S., Katlein, C., Lalande, C., Krumpen, T., Nicolaus, M., Peeken, I., Rabe, B., Rogacheva, A., Rybakova, E., Somavilla, R., Wenzhofer, F., & RV Polarstern ARK27-3-Shipboard Science Party. (2013). Export of Algal Biomass from the Melting Arctic Sea Ice. *Science*, *339*(6126), 1430–1432. https://doi.org/10.1126/science.1231346

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J. A., & Rapp, J. Z. (2015). Microbial ecology of the cryosphere: Sea ice and glacial habitats. *Nature Reviews Microbiology*, *13*(11), 677–690. https://doi.org/10.1038/nrmicro3522

Boiteux, S., Gajewski, E., Laval, J., & Dizdaroglu, M. (1992). Substrate specificity of the Escherichia coli Fpg protein formamidopyrimidine-DNA glycosylase: Excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry*, *31*(1), 106–110. https://doi.org/10.1021/bi00116a016

Booth, B. C., & Horner, R. A. (1997). Microalgae on the arctic ocean section, 1994: Species abundance and biomass. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(8), 1607–1622. https://doi.org/10.1016/S0967-0645(97)00057-X

Boras, J. A., Sala, M. M., Arrieta, J. M., Sà, E. L., Felipe, J., Agustí, S., Duarte, C. M., & Vaqué, D. (2010). Effect of ice melting on bacterial carbon fluxes channelled by viruses and protists in the Arctic Ocean. *Polar Biology*, *33*(12), 1695–1707. https://doi.org/10.1007/s00300-010-0798-8

Bradstreet, M. S. W., & Cross, W. E. (1982). Trophic Relationships at High Arctic Ice Edges. *Arctic*, *35*(1), 1–12. JSTOR.

Brown, Z. W., & Arrigo, K. R. (2012). Contrasting trends in sea ice and primary production in the Bering Sea and Arctic Ocean. *ICES Journal of Marine Science*, *69*(7), 1180–1193. https://doi.org/10.1093/icesjms/fss113

Brunet, C., Johnsen, G., Lavaud, J., & Roy, S. (2011). Pigments and photoacclimation processes. In S. Roy, C. Llewellyn, E. S. Egeland, & G. Johnsen (Eds.), *Phytoplankton Pigments* (pp. 445–471). Cambridge University Press. https://doi.org/10.1017/CBO9780511732263.017

Busquets, M., Deroncelé, V., Vidal-Mas, J., Rodríguez, E., Guerrero, A., & Manresa, A. (2004). Isolation and characterization of a lipoxygenase from Pseudomonas 42A2 responsible for the biotransformation of oleic acid into (S)-(E)-10-hydroxy-8-octadecenoic acid. *Antonie van Leeuwenhoek*, *85*(2), 129–139. https://doi.org/10.1023/B:ANTO.0000020152.15440.65

Cadet, J., Douki, T., & Ravanat, J.-L. (2010). Oxidatively generated base damage to cellular DNA. *Free Radical Biology and Medicine*, *49*(1), 9–21. https://doi.org/10.1016/j.freeradbiomed.2010.03.025

Cadet, J., Ravanat, J.-L., Martinez, G. R., Medeiros, M. H. G., & Mascio, P. D. (2006). Singlet Oxygen Oxidation of Isolated and Cellular DNA: Product Formation and Mechanistic Insights. *Photochemistry and Photobiology*, *82*(5), 1219–1225. https://doi.org/10.1562/2006-06-09-IR-914

Callaghan, T. V. (2001). Arctic Ecosystems. In John Wiley & Sons, Ltd (Ed.), *Encyclopedia of Life Sciences* (p. a0003197). John Wiley & Sons, Ltd. https://doi.org/10.1038/npg.els.0003197

Campbell, E. A., Greenwell, R., Anthony, J. R., Wang, S., Lim, L., Das, K., Sofia, H. J., Donohue, T. J., & Darst, S. A. (2007). A Conserved Structural Module Regulates Transcriptional Responses to Diverse Stress Signals in Bacteria. *Molecular Cell*, *27*(5), 793–805. https://doi.org/10.1016/j.molcel.2007.07.009

Campbell, K., Mundy, C. J., Barber, D. G., & Gosselin, M. (2015). Characterizing the sea ice algae chlorophyll a–snow depth relationship over Arctic spring melt using transmitted irradiance. *Journal of Marine Systems*, *147*, 76–84. https://doi.org/10.1016/j.jmarsys.2014.01.008

Chamberlain, N. R., Mehrtens, B. G., Xiong, Z., Kapral, F. A., Boardman, J. L., & Rearick, J. I. (1991). Correlation of carotenoid production, decreased membrane fluidity, and resistance to oleic acid killing in Staphylococcus aureus 18Z. *Infection and Immunity*, *59*(12), 4332–4337. https://doi.org/10.1128/IAI.59.12.4332-4337.1991

Cho, B. C., & Azam, F. (1988). Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature*, *332*(6163), 441–443. https://doi.org/10.1038/332441a0

Christodoulou, S., Joux, F., Marty, J.-C., Sempéré, R., & Rontani, J.-F. (2010). Comparative study of UV and visible light induced degradation of lipids in non-axenic senescent cells of Emiliania huxleyi. *Marine Chemistry*, *119*(1), 139–152. https://doi.org/10.1016/j.marchem.2010.01.007

Christodoulou, S., Marty, J.-C., Miquel, J.-C., Volkman, J. K., & Rontani, J.-F. (2009). Use of lipids and their degradation products as biomarkers for carbon cycling in the northwestern Mediterranean Sea. *Marine Chemistry*, *113*(1), 25–40. https://doi.org/10.1016/j.marchem.2008.11.003

Clarke, S. R., Mohamed, R., Bian, L., Routh, A. F., Kokai-Kun, J. F., Mond, J. J., Tarkowski, A., & Foster, S. J. (2007). The Staphylococcus aureus Surface Protein IsdA Mediates Resistance to Innate Defenses of Human Skin. *Cell Host & Microbe*, *1*(3), 199–212. https://doi.org/10.1016/j.chom.2007.04.005

Collins, R. E., Carpenter, S. D., & Deming, J. W. (2008). Spatial heterogeneity and temporal dynamics of particles, bacteria, and pEPS in Arctic winter sea ice. *Journal of Marine Systems*, *74*(3), 902–917. https://doi.org/10.1016/j.jmarsys.2007.09.005

Collins, R. E., Rocap, G., & Deming, J. W. (2010). Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. *Environmental Microbiology*, *12*(7), 1828–1841. https://doi.org/10.1111/j.1462-2920.2010.02179.x

Comeau, A. M., Li, W. K. W., Tremblay, J.-É., Carmack, E. C., & Lovejoy, C. (2011). Arctic Ocean Microbial Community Structure before and after the 2007 Record Sea Ice Minimum. *PLoS ONE*, *6*(11), e27492. https://doi.org/10.1371/journal.pone.0027492

Cota, G. F., Prinsenberg, S. J., Bennett, E. B., Loder, J. W., Lewis, M. R., Anning, J. L., Watson, N. H. F., & Harris, L. R. (1987). Nutrient fluxes during extended blooms of Arctic ice algae. *Journal of Geophysical Research: Oceans*, *92*(C2), 1951–1962. https://doi.org/10.1029/JC092iC02p01951

Cota, G., Pomeroy, L., Harrison, W., Jones, E., Peters, F., Sheldon, W., & Weingartner, T. (1996). Nutrients, primary production and microbial heterotrophy in the southeastern Chukchi Sea:Arctic summer nutrient depletion and heterotrophy. *Marine Ecology Progress Series*, *135*, 247–258. https://doi.org/10.3354/meps135247

Cuny, P., Romano, J.-C., Beker, B., & Rontani, J.-F. (1999). Comparison of the photodegradation rates of chlorophyll chlorin ring and phytol side chain in phytodetritus: Is the phytyldiol versus phytol ratio (CPPI) a new biogeochemical index? *Journal of Experimental Marine Biology and Ecology*, *237*(2), 271–290. https://doi.org/10.1016/S0022-0981(99)00010-6

Cuny, P., & Rontani, J.-F. (1999). On the widespread occurrence of 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol in the marine environment: A specific isoprenoid marker of chlorophyll photodegradation. *Marine Chemistry*, *65*(3), 155–165. https://doi.org/10.1016/S0304-4203(98)00093-0

Curry, J. A., Schramm, J. L., & Ebert, E. E. (1995). Sea Ice-Albedo Climate Feedback Mechanism. *Journal of Climate*, *8*(2), 240–247. https://doi.org/10.1175/1520-0442(1995)008<0240:SIACFM>2.0.CO;2

Czeczot, H., Tudek, B., Lambert, B., Laval, J., & Boiteux, S. (1991). Escherichia coli Fpg protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus visible light in vivo and in vitro. *Journal of Bacteriology*, *173*(11), 3419–3424. https://doi.org/10.1128/JB.173.11.3419-3424.1991

Damste, J. S. S. (2004). The Rise of the Rhizosolenid Diatoms. *Science*, *304*(5670), 584–587. https://doi.org/10.1126/science.1096806

Darnis, G., Robert, D., Pomerleau, C., Link, H., Archambault, P., Nelson, R. J., Geoffroy, M., Tremblay, J.-É., Lovejoy, C., Ferguson, S. H., Hunt, B. P. V., & Fortier, L. (2012). Current state and trends in Canadian Arctic marine ecosystems: II. Heterotrophic food web, pelagic-benthic coupling, and biodiversity. *Climatic Change*, *115*(1), 179–205. https://doi.org/10.1007/s10584-012-0483-8

De Leeuw, J. W., & Baas, M. (1986). Early-stage diagenesis of steroids. *Early-Stage Diagenesis of Steroids*, *24*, 101–123.

De Oliveira, R. C., Riberio, D. T., Nigro, R. G., Di Mascio, P., & Menck, C. F. M. (1992). Singlet Oxygen induced mutation spectrum in mammalian cells. *Nucleic Acids Research*, *20*(16), 4319–4323. https://doi.org/10.1093/nar/20.16.4319

Decuyper-Debergh, D., Piette, J., & Vorst, A. V. de. (1987). Singlet oxygen-induced mutations in M13 lacZ phage DNA. *The EMBO Journal*, *6*(10), 3155–3161. https://doi.org/10.1002/j.1460-2075.1987.tb02626.x

Desbois, A. P., Mearns-Spragg, A., & Smith, V. J. (2009). A Fatty Acid from the Diatom Phaeodactylum tricornutum is Antibacterial Against Diverse Bacteria Including Multi-resistant Staphylococcus aureus (MRSA). *Marine Biotechnology*, *11*(1), 45–52. https://doi.org/10.1007/s10126-008-9118-5

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, *85*(6), 1629–1642. https://doi.org/10.1007/s00253-009-2355-3

Deser, C., Tomas, R. A., & Sun, L. (2015). The Role of Ocean–Atmosphere Coupling in the Zonal-Mean Atmospheric Response to Arctic Sea Ice Loss. *Journal of Climate*, *28*(6), 2168–2186. https://doi.org/10.1175/JCLI-D-14-00325.1

Deser, C., Tomas, R., Alexander, M., & Lawrence, D. (2010). The Seasonal Atmospheric Response to Projected Arctic Sea Ice Loss in the Late Twenty-First Century. *Journal of Climate*, *23*(2), 333–351. https://doi.org/10.1175/2009JCLI3053.1

Doble, M. J., Coon, M. D., & Wadhams, P. (2003). Pancake ice formation in the Weddell Sea. *Journal of Geophysical Research: Oceans*, *108*(C7). https://doi.org/10.1029/2002JC001373

Droop, M. R. (1974). The nutrient status of algal cells in continuous culture. *Journal of the Marine Biological Association of the United Kingdom*, *54*(4), 825–855. https://doi.org/10.1017/S002531540005760X

Ducklow, H. (2000). Bacterial Production and Biomass in the Oceans. In *Microbial Ecology of the Oceans* (Vol. 1, pp. 85–120). Wiley & Sons.

Ducklow, H. W., & Carlson, C. A. (1992). Oceanic Bacterial Production. In K. C. Marshall (Ed.), *Advances in Microbial Ecology* (Vol. 12, pp. 113–181). Springer US. https://doi.org/10.1007/978-1-4684-7609-5_3

Eberlein, C., Baumgarten, T., Starke, S., & Heipieper, H. J. (2018). Immediate response mechanisms of Gram-negative solvent-tolerant bacteria to cope with environmental stress: Cis-trans isomerization of unsaturated fatty acids and outer membrane vesicle secretion. *Applied Microbiology and Biotechnology*, *102*(6), 2583–2593. https://doi.org/10.1007/s00253-018-8832-9

El-Sharkawy, S. H., Yang, W., Dostal, L., & Rosazza, J. P. (1992). Microbial oxidation of oleic acid. *Applied and Environmental Microbiology*, *58*(7), 2116–2122. https://doi.org/10.1128/AEM.58.7.2116-2122.1992

Empadinhas, N., & Viete-Vallejo, O. (2008). Osmoadaptation mechanisms in prokaryotes: Distribution of compatible solutes. *International Microbiology*, *11*, 151–161. https://doi.org/10.2436/20.1501.01.55

Estupiñán, M., Álvarez-García, D., Barril, X., Diaz, P., & Manresa, A. (2015). In Silico/In Vivo Insights into the Functional and Evolutionary Pathway of Pseudomonas aeruginosa Oleate-Diol Synthase. Discovery of a New Bacterial Di-Heme Cytochrome C Peroxidase Subfamily. *PLOS ONE*, *10*(7), e0131462. https://doi.org/10.1371/journal.pone.0131462

Ewert, M., & Deming, J. (2013). Sea Ice Microorganisms: Environmental Constraints and Extracellular Responses. *Biology*, *2*(2), 603–628. https://doi.org/10.3390/biology2020603

Falk-Petersen, S. (1999). Spatial distribution and life-cycle timing of zooplankton in the marginal ice zone of the Barents Sea during the summer melt season in 1995. *Journal of Plankton Research*, *21*(7), 1249–1264. https://doi.org/10.1093/plankt/21.7.1249

Falk-Petersen, S., Sargent, J. R., Henderson, J., Hegseth, E. N., Hop, H., & Okolodkov, Y. B. (1998). Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. *Polar Biology*, *20*(1), 41–47. https://doi.org/10.1007/s003000050274

Feldlaufer, M. F., Knox, D. A., Lusby, W. R., & Shimanuki, H. (1993). Antimicrobial activity of fatty acids against Bacillus larvae, the causative agent of American foulbrood disease. *Apidologie*, *24*(2), 95–99. https://doi.org/10.1051/apido:19930202

Fernández-Méndez, M., Wenzhöfer, F., Peeken, I., Sørensen, H. L., Glud, R. N., & Boetius, A. (2014). Composition, Buoyancy Regulation and Fate of Ice Algal Aggregates in the Central Arctic Ocean. *PLoS ONE*, *9*(9), e107452. https://doi.org/10.1371/journal.pone.0107452

Fischer, B. B., Krieger-Liszkay, A., Hideg, É., Šnyrychová, I., Wiesendanger, M., & Eggen, R. I. L. (2007). Role of singlet oxygen in chloroplast to nucleus retrograde signaling in Chlamydomonas reinhardtii. *FEBS Letters*, *581*(29), 5555–5560. https://doi.org/10.1016/j.febslet.2007.11.003

Fischer, J., Schauer, F., & Heipieper, H. J. (2010). The trans/cis ratio of unsaturated fatty acids is not applicable as biomarker for environmental stress in case of long-term contaminated habitats. *Applied Microbiology and Biotechnology*, *87*(1), 365–371. https://doi.org/10.1007/s00253-010-2544-0

Foote, C. (1976). Photosensitized oxidation and singlet oxygen: Consequences in biological systems. In *Free radicals in biology* (p. 133). Academic Press.

Forest, A., Galindo, V., Darnis, G., Pineault, S., Lalande, C., Tremblay, J.-É., & Fortier, L. (2011). Carbon biomass, elemental ratios (C:N) and stable isotopic composition (δ13C, δ15N) of dominant calanoid copepods during the winter-to-summer transition in the Amundsen Gulf (Arctic Ocean). Journal of Plankton Research, 33(1), 161–178. https://doi.org/10.1093/plankt/fbq103

Fortier, M. (2001). Visual predators and the diel vertical migration of copepods under Arctic sea ice during the midnight sun. *Journal of Plankton Research*, *23*(11), 1263–1278. https://doi.org/10.1093/plankt/23.11.1263

Frankel, E. N. (1980). *Lipid oxidation*. https://pubag.nal.usda.gov/catalog/25421

Frankel, E. N. (1998). Lipid oxidation. *Progress in Lipid Research*, *19*(1–2), 1–22. https://doi.org/10.1016/0163-7827(80)90006-5

Frankel, E. N., Neff, W. E., & Bessler, T. R. (1979). Analysis of autoxidized fats by gas chromatography-mass spectrometry: V. Photosensitized oxidation. *Lipids*, *14*(12), 961–967. https://doi.org/10.1007/BF02533431

Frimer, A. A. (1979). The reaction of singlet oxygen with olefins: The question of mechanism. *Chemical Reviews*, *79*(5), 359–387. https://doi.org/10.1021/cr60321a001

Fuchs, C., & Spiteller, G. (2000). Iron Release from the Active Site of Lipoxygenase. *Zeitschrift Für Naturforschung C*, *55*(7–8), 643–648. https://doi.org/10.1515/znc-2000-7-825

Fuhrman, J. A., & Noble, R. T. (1995). Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnology and Oceanography*, *40*(7), 1236–1242. https://doi.org/10.4319/lo.1995.40.7.1236

Gagosian, R. B., Zafiriou, O. C., Peltzer, E. T., & Alford, J. B. (1982). Lipids in aerosols from the tropical North Pacific: Temporal variability. *Journal of Geophysical Research: Oceans*, *87*(C13), 11133–11144. https://doi.org/10.1029/JC087iC13p11133

Galeron, M.-A., Radakovitch, O., Charrière, B., Vaultier, F., Volkman, J. K., Bianchi, T. S., Ward, N. D., Medeiros, P. M., Sawakuchi, H. O., Tank, S., Kerhervé, P., & Rontani, J.-F. (2018). Lipoxygenase-induced autoxidative degradation of terrestrial particulate organic matter in estuaries: A widespread process enhanced at high and low latitude. *Organic Geochemistry*, *115*, 78–92. https://doi.org/10.1016/j.orggeochem.2017.10.013

Galinski, E. A. (1995). Osmoadaptation in Bacteria. In R. K. Poole (Ed.), *Advances in Microbial Physiology* (Vol. 37, pp. 273–328). Academic Press. https://doi.org/10.1016/S0065-2911(08)60148-4

Garcia-Pichel, F. (1994). A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnology and Oceanography*, *39*(7), 1704–1717. https://doi.org/10.4319/lo.1994.39.7.1704

Garreta, A., Val-Moraes, S. P., García-Fernández, Q., Busquets, M., Juan, C., Oliver, A., Ortiz, A., Gaffney, B. J., Fita, I., Manresa, À., & Carpena, X. (2013). Structure and interaction with phospholipids of a prokaryotic lipoxygenase from Pseudomonas aeruginosa. *The FASEB Journal*, *27*(12), 4811–4821. https://doi.org/10.1096/fj.13-235952

Girotti, A. W. (1998). *Lipid hydroperoxide generation, turnover, and effector action in biological systems*. *39*(8), 1529–1542.

Glaeser, J., Nuss, A. M., Berghoff, B. A., & Klug, G. (2011). Singlet Oxygen Stress in Microorganisms. In *Advances in Microbial Physiology* (Vol. 58, pp. 141–173). Elsevier. https://doi.org/10.1016/B978-0-12-381043-4.00004-0

Glaeser, S. P., Grossart, H.-P., & Glaeser, J. (2010). Singlet oxygen, a neglected but important environmental factor: Short-term and long-term effects on bacterioplankton composition in a humic lake. *Environmental Microbiology*, *12*(12), 3124–3136. https://doi.org/10.1111/j.1462-2920.2010.02285.x

Gollnick, K. (1968). Type II Photooxygenation Reactions in Solution. In W. A. Noyes, G. S. Hammond, & J. N. Pitts (Eds.), *Advances in Photochemistry* (pp. 1–122). John Wiley & Sons, Inc. https://doi.org/10.1002/9780470133361.ch1

Gosselin, M., Levasseur, M., Wheeler, P. A., Horner, R. A., & Booth, B. C. (1997). New measurements of phytoplankton and ice algal production in the Arctic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(8), 1623–1644. https://doi.org/10.1016/S0967-0645(97)00054-4

Gradinger, R. (1999). *Vertical fine structure of the biomass and composition of algal communities in Arctic pack ice*. *133*(4), 745–754.

Grossi, V., Beker, B., Geenevasen, J. A. J., Schouten, S., Raphel, D., Fontaine, M.-F., & Sinninghe Damsté, J. S. (2004). C25 highly branched isoprenoid alkenes from the marine benthic diatom Pleurosigma strigosum. *Phytochemistry*, *65*(22), 3049–3055. https://doi.org/10.1016/j.phytochem.2004.09.002

Guckert, J. B. (1986). Phospholipid Ester-Linked Fatty Acid Profile Changes during Nutrient Deprivation of Vibrio cholerae: Increases in the trans/cis Ratio and Proportions of Cyclopropyl Fatty Acids. *Applied Environmental Microbiology*, *52*, 8.

Guengerich, F. P. (1991). *Reactions and significance of cytochrome P-450 enzymes*. *266*(16), 10019–10022.

Guerrero, A., Casals, I., Busquets, M., Leon, Y., & Manresa, A. (1997). Oxydation of oleic acid to (E)-10-hydroperoxy-8-octadecenoic and (E)-10-hydroxy-8-octadecenoic acids by Pseudomonas sp. 42A2. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *1347*(1), 75–81. https://doi.org/10.1016/S0005-2760(97)00056-8

Hamre, B., Winther, J.-G., Gerland, S., Stamnes, J. J., & Stamnes, K. (2004). Modeled and measured optical transmittance of snow-covered first-year sea ice in Kongsfjorden, Svalbard. *Journal of Geophysical Research: Oceans*, *109*(C10). https://doi.org/10.1029/2003JC001926

Hanesiak, J. M., Barber, D. G., Abreu, R. A. D., & Yackel, J. J. (2001). Local and regional albedo observations of arctic first-year sea ice during melt ponding. *Journal of Geophysical Research: Oceans*, *106*(C1), 1005–1016. https://doi.org/10.1029/1999JC000068

Hare, A. A., Wang, F., Barber, D., Geilfus, N.-X., Galley, R. J., & Rysgaard, S. (2013). PH evolution in sea ice grown at an outdoor experimental facility. *Marine Chemistry*, *154*, 46–54. https://doi.org/10.1016/j.marchem.2013.04.007

Harwood, J. L., & Russell, N. J. (1984). Distribution of lipids. In J. L. Harwood & N. J. Russell (Eds.), *Lipids in Plants and Microbes* (pp. 35–70). Springer Netherlands. https://doi.org/10.1007/978-94-011-5989-0_3

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: II. Role of electron transfer. *Archives of Biochemistry and Biophysics*, *125*(3), 850–857. https://doi.org/10.1016/0003-9861(68)90523-7

Hegseth, E. N. (1997). Phytoplankton of the Barents Sea—The end of a growth season. *Polar Biology*, *17*(3), 235–241. https://doi.org/10.1007/s003000050127

Heipieper, H. J., Meinhardt, F., & Segura, A. (2003). The cis–trans isomerase of unsaturated fatty acids in Pseudomonas and Vibrio: Biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism. *FEMS Microbiology Letters*, *229*(1), 1–7. https://doi.org/10.1016/S0378-1097(03)00792-4

Heipieper, H. J., Neumann, G., Cornelissen, S., & Meinhardt, F. (2007). Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Applied Microbiology and Biotechnology*, *74*(5), 961–973. https://doi.org/10.1007/s00253-006-0833-4

Hirche, H.-J. (1997). Life cycle of the copepod Calanus hyperboreus in the Greenland Sea. *Marine Biology*, *128*(4), 607–618. https://doi.org/10.1007/s002270050127

Hopcroft, R. R., Clarke, C., Nelson, R. J., & Raskoff, K. A. (2005). Zooplankton communities of the Arctic's Canada Basin: The contribution by smaller taxa. *Polar Biology*, *28*(3), 198–206. https://doi.org/10.1007/s00300-004-0680-7

Hou, C. T. (1994). Production of 10-Ketostearic Acid from Oleic Acid by Flavobacterium sp. Strain DS5 (NRRL B-14859). *Applied and Environmental Microbiology*, *60*(10), 3760–3763. https://doi.org/10.1128/AEM.60.10.3760-3763.1994

Howard-Jones, M. H., Ballard, V. D., Allen, A. E., Frischer, M. E., & Verity, P. G. (2002). Distribution of bacterial biomass and activity in the marginal ice zone of the central Barents Sea during summer. *Journal of Marine Systems*, *38*(1–2), 77–91. https://doi.org/10.1016/S0924-7963(02)00170-7

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., & Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and

advances. *The Plant Journal*, *54*(4), 621–639. https://doi.org/10.1111/j.1365-313X.2008.03492.x

Jackson, G. A., Waite, A. M., & Boyd, P. W. (2005). Role of algal aggregation in vertical carbon export during SOIREE and in other low biomass environments. *Geophysical Research Letters*, *32*(13). https://doi.org/10.1029/2005GL023180

Jakobsson, M. (2002). Hypsometry and volume of the Arctic Ocean and its constituent seas: HYPOSYMETRY AND VOLUME OF ARCTIC OCEAN. *Geochemistry, Geophysics, Geosystems*, *3*(5), 1–18. https://doi.org/10.1029/2001GC000302

Jeffrey, S., & Hallegraeff, G. (1987). Chlorophyllase distribution in ten classes of phytoplankton: A problem for chlorophyll analysis. *Marine Ecology Progress Series*, *35*, 293–304. https://doi.org/10.3354/meps035293

Ji, R., Jin, M., & Varpe, Ø. (2013). Sea ice phenology and timing of primary production pulses in the Arctic Ocean. *Global Change Biology*, *19*(3), 734–741. https://doi.org/10.1111/gcb.12074

Jónasdóttir, S. (2019). Fatty Acid Profiles and Production in Marine Phytoplankton. *Marine Drugs*, *17*(3), 151. https://doi.org/10.3390/md17030151

Joo, Y.-C., Seo, E.-S., Kim, Y.-S., Kim, K.-R., Park, J.-B., & Oh, D.-K. (2012). Production of 10-hydroxystearic acid from oleic acid by whole cells of recombinant Escherichia coli containing oleate hydratase from Stenotrophomonas maltophilia. *Journal of Biotechnology*, *158*(1), 17–23. https://doi.org/10.1016/j.jbiotec.2012.01.002

Juhl, A. R., & Krembs, C. (2010). Effects of snow removal and algal photoacclimation on growth and export of ice algae. *Polar Biology*, *33*(8), 1057–1065. https://doi.org/10.1007/s00300-010-0784-1

Jüttner, F. (2001). Liberation of 5,8,11,14,17-Eicosapentaenoic Acid and Other Polyunsaturated Fatty Acids from Lipids as a Grazer Defense Reaction in Epilithic Diatom Biofilms. *Journal of Phycology*, *37*(5), 744–755. https://doi.org/10.1046/j.1529-8817.2001.00130.x

Karner, M., & Herndl, G. J. (1992). *Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria*. 7.

Kauko, H. M., Taskjelle, T., Assmy, P., Pavlov, A. K., Mundy, C. J., Duarte, P., Fernández-Méndez, M., Olsen, L. M., Hudson, S. R., Johnsen, G., Elliott, A., Wang, F., & Granskog, M. A. (2017). Windows in Arctic sea ice: Light transmission and ice algae in a refrozen lead. *Journal of Geophysical Research: Biogeosciences*, *122*(6), 1486–1505. https://doi.org/10.1002/2016JG003626@10.1002/(ISSN)2169-9291.NICE1

Kenny, J. G., Ward, D., Josefsson, E., Jonsson, I.-M., Hinds, J., Rees, H. H., Lindsay, J. A., Tarkowski, A., & Horsburgh, M. J. (2009). The Staphylococcus aureus Response to Unsaturated Long Chain Free Fatty Acids: Survival Mechanisms and Virulence Implications. *PLOS ONE*, *4*(2), e4344. https://doi.org/10.1371/journal.pone.0004344

Keweloh, H., & Heipieper, H. J. (1996). Trans unsaturated fatty acids in bacteria. *Lipids*, *31*(2), 129–137. https://doi.org/10.1007/BF02522611

Kim, S.-Y., Kim, E.-J., & Park, J.-W. (2002). Control of Singlet Oxygen-induced Oxidative Damage in Escherichia coli. *BMB Reports*, *35*(4), 353–357. https://doi.org/10.5483/BMBRep.2002.35.4.353

Kiørboe, T., Andersen, K. P., & Dam, H. G. (1990). Coagulation efficiency and aggregate formation in marine phytoplankton. *Marine Biology*, *107*(2), 235–245. https://doi.org/10.1007/BF01319822

Kirchman, D. L., Morán, X. A. G., & Ducklow, H. (2009). Microbial growth in the polar oceans—Role of temperature and potential impact of climate change. *Nature Reviews Microbiology*, *7*(6), 451–459. https://doi.org/10.1038/nrmicro2115

Kivisild, H. R. (1970). *River and lake ice terminology*.

Knox, J. P., & Dodge, A. D. (1985). Singlet oxygen and plants. *Phytochemistry*, *24*(5), 889–896. https://doi.org/10.1016/S0031-9422(00)83147-7

Krembs, C., & Deming, J. W. (2008). The Role of Exopolymers in Microbial Adaptation to Sea Ice. In R. Margesin, F. Schinner, J.-C. Marx, & C. Gerday (Eds.), *Psychrophiles: From Biodiversity to Biotechnology* (pp. 247–264). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-74335-4_15

Krembs, C., Eicken, H., Junge, K., & Deming, J. W. (2002). High concentrations of exopolymeric substances in Arctic winter sea ice: Implications for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep Sea Research Part I: Oceanographic Research Papers*, *49*(12), 2163–2181. https://doi.org/10.1016/S0967-0637(02)00122-X

Krembs, C., & Engel, A. (2001). Abundance and variability of microorganisms and transparent exopolymer particles across the ice/water interface of melting first-year sea ice in the Laptev Sea (Arctic). *Marine Biology*, *138*, 1370–1377.

Kulig, M. J., & Smith, L. L. (1973). Sterol metabolism. XXV. Cholesterol oxidation by singlet molecular oxygen. *The Journal of Organic Chemistry*, *38*(20), 3639–3642. https://doi.org/10.1021/jo00960a050

Kwok, R. (2004). Annual cycles of multiyear sea ice coverage of the Arctic Ocean: 1999–2003. *Journal of Geophysical Research: Oceans*, *109*(C11). https://doi.org/10.1029/2003JC002238

Kwok, R., & Rothrock, D. A. (2009). Decline in Arctic sea ice thickness from submarine and ICESat records: 1958–2008. *Geophysical Research Letters*, *36*(15). https://doi.org/10.1029/2009GL039035

Lanier, K. A., & Williams, L. D. (2017). The Origin of Life: Models and Data. *Journal of Molecular Evolution*, *84*(2–3), 85–92. https://doi.org/10.1007/s00239-017-9783-y

Leu, E., Mundy, C. J., Assmy, P., Campbell, K., Gabrielsen, T. M., Gosselin, M., Juul-Pedersen, T., & Gradinger, R. (2015). Arctic spring awakening – Steering principles behind the phenology of vernal ice algal blooms. *Progress in Oceanography*, *139*, 151–170. https://doi.org/10.1016/j.pocean.2015.07.012

Loffeld, B., & Keweloh, H. (1996). Cis/trans isomerization of unsaturated fatty acids as possible control mechanism of membrane fluidity inPseudomonas putida P8. *Lipids*, *31*(8), 811–815. https://doi.org/10.1007/BF02522976

Li, W. K. W., McLaughlin, F. A., Lovejoy, C., & Carmack, E. C. (2009). Smallest Algae Thrive As the Arctic Ocean Freshens. *Science*, *326*(5952), 539-539. https://doi.org/10.1126/science.1179798

Maki, H., & Sekiguchi, M. (1992). MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*, *355*(6357), 273–275. https://doi.org/10.1038/355273a0

Marchand, D., & Rontani, J.-F. (2001). Characterisation of photo-oxidation and autoxidation products of phytoplanktonic monounsaturated fatty acids in marine particulate matter and recent sediments. *Organic Geochemistry*, *32*(2), 287–304. https://doi.org/10.1016/S0146-6380(00)00175-3

Martin, S. (1981). Frazil Ice in Rivers and Oceans. *Annual Review of Fluid Mechanics*, *13*(1), 379–397. https://doi.org/10.1146/annurev.fl.13.010181.002115

Martínez, E., Estupiñán, M., Pastor, F. I. J., Busquets, M., Díaz, P., & Manresa, A. (2013). Functional characterization of ExFadLO, an outer membrane protein required for exporting oxygenated long-chain fatty acids in Pseudomonas aeruginosa. *Biochimie*, *95*(2), 290–298. https://doi.org/10.1016/j.biochi.2012.09.032

Martínez, E., Hamberg, M., Busquets, M., Díaz, P., Manresa, A., & Oliw, E. H. (2010). Biochemical Characterization of the Oxygenation of Unsaturated Fatty Acids by the Dioxygenase and Hydroperoxide Isomerase of *Pseudomonas aeruginosa* 42A2. *Journal of Biological Chemistry*, *285*(13), 9339–9345. https://doi.org/10.1074/jbc.M109.078147

Mascher, T. (2013). Signaling diversity and evolution of extracytoplasmic function (ECF) σ factors. *Current Opinion in Microbiology*, *16*(2), 148–155. https://doi.org/10.1016/j.mib.2013.02.001

Mascio, P. D., Devasagayam, T. P. A., Kaiser, S., & Sies, H. (1990). Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. *Biochemical Society Transactions*, *18*(6), 1054–1056. https://doi.org/10.1042/bst0181054

Maykut, G. A., & Grenfell, T. C. (1975). The spectral distribution of light beneath first-year sea ice in the Arctic Ocean. *Limnology and Oceanography*, *20*(4), 554–563. https://doi.org/10.4319/lo.1975.20.4.0554

McBride, T. J., Preston, B. D., & Loeb, L. A. (1991). Mutagenic spectrum resulting from DNA damage by oxygen radicals. *Biochemistry*, *30*(1), 207–213. https://doi.org/10.1021/bi00215a030

Meier, W. N., Hovelsrud, G. K., Oort, B. E. H. van, Key, J. R., Kovacs, K. M., Michel, C., Haas, C., Granskog, M. A., Gerland, S., Perovich, D. K., Makshtas, A., & Reist, J. D. (2014). Arctic sea ice in transformation: A review of recent observed changes and impacts on biology and human activity. *Reviews of Geophysics*, *52*(3), 185–217. https://doi.org/10.1002/2013RG000431

Meiners, K., Gradinger, R., Fehling, J., Civitarese, G., & Spindler, M. (2003). Vertical distribution of exopolymer particles in sea ice of the Fram Strait (Arctic) during autumn. *Marine Ecology Progress Series*, *248*, 1–13. https://doi.org/10.3354/meps248001

Menck, C. F. M., Di Mascio, P., Agnez-Lima, L. F., Ribeiro, D. T., & Costa de Oliveira, R. (1993). *Genetic deleterious effects of singlet oxygen*. *16*, 328–336.

Michaels, M. L., Cruz, C., Grollman, A. P., & Miller, J. H. (1992). Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. *Proceedings of the National Academy of Sciences*, *89*(15), 7022–7025. https://doi.org/10.1073/pnas.89.15.7022

Michaels, M. L., & Miller, J. H. (1992). The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *Journal of Bacteriology*, *174*(20), 6321–6325.

Mitra, S., Boldogh, I., Izumi, T., & Hazra, T. K. (2001). Complexities of the DNA base excision repair pathway for repair of oxidative DNA damage. *Environmental and Molecular Mutagenesis*, *38*(2–3), 180–190. https://doi.org/10.1002/em.1070

Monfort, P., Demers, S., & Levasseur, M. (2000). Bacterial dynamics in first year sea ice and underlying seawater of Saroma-ko Lagoon (Sea of Okhotsk, Japan) and Resolute Passage (High Canadian Arctic): Inhibitory effects of ice algae on bacterial dynamics. *Canadian Journal of Microbiology*, *46*(7), 623–632. https://doi.org/10.1139/w00-024

Morgan, P. E., Dean, R. T., & Davies, M. J. (2004). Protective mechanisms against peptide and protein peroxides generated by singlet oxygen. *Free Radical Biology and Medicine*, *36*(4), 484–496. https://doi.org/10.1016/j.freeradbiomed.2003.11.021

Morrissey, P. A., & Kiely, M. (2006). Oxysterols: Formation and Biological Function. In P. F. Fox & P. L. H. McSweeney (Eds.), *Advanced Dairy Chemistry Volume 2 Lipids* (pp. 641–674). Springer US. https://doi.org/10.1007/0-387-28813-9_18

Murray, A. E., & Grzymski, J. J. (2007). Diversity and genomics of Antarctic marine micro-organisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1488), 2259–2271. https://doi.org/10.1098/rstb.2006.1944

Naghibi, F., Yazdi, M. T., Sahebgharani, M., & Daloii, M. R. N. (2002). *Microbial transformation of cholesterol by Mycobacterium Smegmatis*. *13*(2), 4.

Neff, W. E., Frankel, E. N., & Fujimoto, K. (1988). Autoxidative dimerization of methyl linolenate and its monohydroperoxides, hydroperoxy epidioxides and dihydroperoxides. *Journal of the American Oil Chemists' Society*, *65*(4), 616–623. https://doi.org/10.1007/BF02540690

Neilson, A. H., & Allard, A.-S. (2007). *Environmental Degradation and Transformation of Organic Chemicals*. CRC Press.

Nelson, J. R. (1993). Rates and possible mechanism of light-dependent degradation of pigments in detritus derived from phytoplankton. *Journal of Marine Research*, *51*(1), 155–179. https://doi.org/10.1357/0022240933223837

Newman, J. D., Anthony, J. R., & Donohue, T. J. (2001). The importance of zinc-binding to the function of Rhodobacter sphaeroides ChrR as an anti-sigma factor11Edited by M. Gottesman. *Journal of Molecular Biology*, *313*(3), 485–499. https://doi.org/10.1006/jmbi.2001.5069

Nickon, A., & Bagli, J. F. (1961). Reactivity and Geometry in Allylic Systems. I. Stereochemistry of Photosensitized Oxygenation of Monoölefins [1,2]. *Journal of the American Chemical Society*, *83*(6), 1498–1508. https://doi.org/10.1021/ja01467a049

Nicolaus, M., Gerland, S., Hudson, S. R., Hanson, S., Haapala, J., & Perovich, D. K. (2010). Seasonality of spectral albedo and transmittance as observed in the Arctic Transpolar Drift in 2007. *Journal of Geophysical Research: Oceans*, *115*(C11). https://doi.org/10.1029/2009JC006074

Nicolaus, M., Katlein, C., Maslanik, J., & Hendricks, S. (2012). Changes in Arctic sea ice result in increasing light transmittance and absorption. *Geophysical Research Letters*, *39*(24). https://doi.org/10.1029/2012GL053738

Nuss, A. M., Glaeser, J., & Klug, G. (2009). RpoHII Activates Oxidative-Stress Defense Systems and Is Controlled by RpoE in the Singlet Oxygen-Dependent Response in Rhodobacter sphaeroides. *Journal of Bacteriology*, *191*(1), 220–230. https://doi.org/10.1128/JB.00925-08

Nuttall, M., & Callaghan, T. (2000). *Arctic: Environment, People, Policy*. CRC Press.

O'Keefe, S. F. (2002). Nomenclature and Classification of Lipids. In *Food Lipids: Chemistry, Nutrition, and Biotechnology, Second Edition* (pp. 1–40). CRC Press.

Oliw, E. H., Bylund, J., & Herman, C. (1996). Bisallylic hydroxylation and epoxidation of polyunsaturated fatty acids by cytochrome P450. *Lipids*, *31*(10), 1003–1021. https://doi.org/10.1007/BF02522457

Oudar, T., Sanchez-Gomez, E., Chauvin, F., Cattiaux, J., Terray, L., & Cassou, C. (2017). Respective roles of direct GHG radiative forcing and induced Arctic sea ice loss on the Northern Hemisphere atmospheric circulation. *Climate Dynamics*, *49*(11–12), 3693–3713. https://doi.org/10.1007/s00382-017-3541-0

Overland, J. E., & Wang, M. (2007). Future regional Arctic sea ice declines. *Geophysical Research Letters*, *34*(17). https://doi.org/10.1029/2007GL030808

Owen, R. W., Mason, A. N., & Bilton, R. F. (1983). *The degradation of cholesterol by Pseudomonas sp. NCIB 10590 under aerobic conditions*. 12.

Parrish, C. C. (2013). Lipids in Marine Ecosystems. *ISRN Oceanography*, *2013*, e604045. https://doi.org/10.5402/2013/604045

Parrish, C., Thompson, R., & Deibel, D. (2005). Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment. *Marine Ecology Progress Series*, *286*, 57–68. https://doi.org/10.3354/meps286057

Perovich, D. K., & Richter-Menge, J. A. (2009). Loss of Sea Ice in the Arctic. *Annual Review of Marine Science*, *1*(1), 417–441. https://doi.org/10.1146/annurev.marine.010908.163805

Petit, M., Bonin, P., Amiraux, R., Michotey, V., Guasco, S., Armitano, J., Jourlin-Castelli, C., Vaultier, F., Méjean, V., & Rontani, J.-F. (2015). Dynamic of bacterial communities attached to lightened phytodetritus. *Environmental Science and Pollution Research*, *22*(18), 13681–13692. https://doi.org/10.1007/s11356-015-4209-0

Petit, M., Suroy, M., Sempéré, R., Vaultier, F., Volkman, J. K., Goutx, M., & Rontani, J.-F. (2015a). Transfer of singlet oxygen from senescent irradiated phytoplankton cells to attached

heterotrophic bacteria: Effect of silica and carbonaceous matrices. *Marine Chemistry*, *171*, 87–95. https://doi.org/10.1016/j.marchem.2015.02.007

Petrich, C., & Eicken, H. (2016). Overview of sea ice growth and properties. In D. N. Thomas (Ed.), *Sea Ice* (pp. 1–41). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118778371.ch1

Piepho, M., Martin-Creuzburg, D., & Wacker, A. (2012). Phytoplankton sterol contents vary with temperature, phosphorus and silicate supply: A study on three freshwater species. *European Journal of Phycology*, *47*(2), 138–145. https://doi.org/10.1080/09670262.2012.665484

Piette, J., & Moore, P. D. (1982). DNA synthesis on øx174 template damaged by proflavine and light treatment. *Photochemistry and Photobiology*, *35*(5), 705–708. https://doi.org/10.1111/j.1751-1097.1982.tb02633.x

Piontek, J., Sperling, M., Nöthig, E.-M., & Engel, A. (2014). Regulation of bacterioplankton activity in Fram Strait (Arctic Ocean) during early summer: The role of organic matter supply and temperature. *Journal of Marine Systems*, *132*, 83–94. https://doi.org/10.1016/j.jmarsys.2014.01.003

Pomeroy, L. R., & Deibel, D. (1986). Temperature Regulation of Bacterial Activity During the Spring Bloom in Newfoundland Coastal Waters. *Science*, *233*(4761), 359–361. https://doi.org/10.1126/science.233.4761.359

Pomeroy, L., & Wiebe, W. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology*, *23*, 187–204. https://doi.org/10.3354/ame023187

Porta, H., & Rocha-Sosa, M. (2002). Plant Lipoxygenases. Physiological and Molecular Features. *Plant Physiology*, *130*(1), 15–21. https://doi.org/10.1104/pp.010787

Porter, N. A., Caldwell, S. E., & Mills, K. A. (1995). Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, *30*(4), 277–290. https://doi.org/10.1007/BF02536034

Ravanat, J.-L., Di Mascio, P., Martinez, G. R., Medeiros, M. H. G., & Cadet, J. (2000). Singlet Oxygen Induces Oxidation of Cellular DNA. *Journal of Biological Chemistry*, *275*(51), 40601–40604. https://doi.org/10.1074/jbc.M006681200

Rich, J., Gosselin, M., Sherr, E., Sherr, B., & Kirchman, D. L. (1997). High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(8), 1645–1663. https://doi.org/10.1016/S0967-0645(97)00058-1

Riebesell, U., Schloss, I., & Smetacek, V. (1991). Aggregation of algae released from melting sea ice: Implications for seeding and sedimentation. *Polar Biology*, *11*(4), 239–248. https://doi.org/10.1007/BF00238457

Riedel, A., Michel, C., & Gosselin, M. (2006). Seasonal study of sea-ice exopolymeric substances on the Mackenzie shelf: Implications for transport of sea-ice bacteria and algae. *Aquatic Microbial Ecology*, *45*, 195–206. https://doi.org/10.3354/ame045195

Rivkin, R., Anderson, M., & Lajzerowicz, C. (1996). Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. *Aquatic Microbial Ecology*, *10*, 243–254. https://doi.org/10.3354/ame010243

Roberts, M. F. (2005). Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline Systems*, *1*(1), 5. https://doi.org/10.1186/1746-1448-1-5

Robson, J. N., & Rowland, S. J. (1988). Biodegradation of highly branched isoprenoid hydrocarbons: A possible explanation of sedimentary abundance. *Organic Geochemistry*, *13*(4–6), 691–695. https://doi.org/10.1016/0146-6380(88)90090-3

Rontani, J. (2001). Visible light-dependent degradation of lipidic phytoplanktonic components during senescence: A review. *Phytochemistry*, *58*(2), 187–202. https://doi.org/10.1016/S0031-9422(01)00202-3

Rontani, J. (2012). Photo- and free radical-mediated oxidation of lipid components during the senescence of phototrophic organisms. In *Senescence* (IntechOpen, pp. 3–31). Nagata.

Rontani, J., Amiraux, R., Lalande, C., Babin, M., Kim, H.-R., & Belt, S. T. (2018). Use of palmitoleic acid and its oxidation products for monitoring the degradation of ice algae in Arctic waters and bottom sediments. *Organic Geochemistry*, *124*, 88–102. https://doi.org/10.1016/j.orggeochem.2018.06.002

Rontani, J., & Aubert, C. (2005). Characterization of isomeric allylic diols resulting from chlorophyll phytyl side-chain photo- and autoxidation by electron ionization gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, *19*(5), 637–646. https://doi.org/10.1002/rcm.1835

Rontani, J., Beker, B., Raphel, D., & Baillet, G. (1995). Photodegradation of chlorophyll phytyl chain in dead phytoplanktonic cells. *Journal of Photochemistry and Photobiology A: Chemistry*, *85*(1), 137–142. https://doi.org/10.1016/1010-6030(94)03891-W

Rontani, J., & Belt, S. T. (2020). Photo- and autoxidation of unsaturated algal lipids in the marine environment: An overview of processes, their potential tracers, and limitations. *Organic Geochemistry*, *139*, 103941. https://doi.org/10.1016/j.orggeochem.2019.103941

Rontani, J., Belt, S. T., Brown, T. A., & Aubert, C. (2014a). Electron ionization mass spectrometry fragmentation pathways of trimethylsilyl derivatives of isomeric allylic alcohols derived from HBI alkene oxidation. *Rapid Communications in Mass Spectrometry*, *28*(17), 1937–1947. https://doi.org/10.1002/rcm.6974

Rontani, J., Belt, S. T., Vaultier, F., Brown, T. A., & Massé, G. (2014b). Autoxidative and Photooxidative Reactivity of Highly Branched Isoprenoid (HBI) Alkenes. *Lipids*, *49*(5), 481–494. https://doi.org/10.1007/s11745-014-3891-x

Rontani, J., Charriere, B., Petit, M., Vaultier, F., Heipieper, H. J., Link, H., Chaillou, G., & Sempéré, R. (2012). Degradation state of organic matter in surface sediments from the Southern Beaufort Sea: A lipid approach. *Biogeosciences*, *9*(9), 3513–3530. https://doi.org/10.5194/bg-9-3513-2012

Rontani, J., Charrière, B., Sempéré, R., Doxaran, D., Vaultier, F., Vonk, J. E., & Volkman, J. K. (2014). Degradation of sterols and terrigenous organic matter in waters of the Mackenzie Shelf, Canadian Arctic. *Organic Geochemistry*, *75*, 61–73. https://doi.org/10.1016/j.orggeochem.2014.06.002

Rontani, J., Cuny, P., & Grossi, V. (1996). Photodegradation of chlorophyll phytyl chain in senescent leaves of higher plants. *Phytochemistry*, *42*(2), 347–351. https://doi.org/10.1016/0031-9422(95)00872-1

Rontani, J., Cuny, P., & Grossi, V. (1998). Identification of a "pool" of lipid photoproducts in senescent phytoplanktonic cells. *Organic Geochemistry*, *29*(5), 1215–1225. https://doi.org/10.1016/S0146-6380(98)00073-4

Rontani, J., Grossi, V., Faure, R., & Aubert, C. (1994). "Bound" 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol: A new isoprenoid marker for the photodegradation of chlorophyll-a in seawater. *Organic Geochemistry*, *21*(2), 135–142. https://doi.org/10.1016/0146-6380(94)90150-3

Rontani, J.-F., Koblížek, M., Beker, B., Bonin, P., & Kolber, Z. S. (2003a). On the origin of cis-vaccenic acid photodegradation products in the marine environment. *Lipids*, *38*(10), 1085-1092. https://doi.org/10.1007/s11745-006-1164-z

Rontani, J., Marty, J.-C., Miquel, J.-C., & Volkman, J. K. (2006). Free radical oxidation (autoxidation) of alkenones and other microalgal lipids in seawater. *Organic Geochemistry*, *37*(3), 354–368. https://doi.org/10.1016/j.orggeochem.2005.10.007

Rontani, J., Rabourdin, A., Marchand, D., & Aubert, C. (2003). Photochemical oxidation and autoxidation of chlorophyll phytyl side chain in senescent phytoplanktonic cells: Potential

sources of several acyclic isoprenoid compounds in the marine environment. *Lipids*, *38*(3), 241–254. https://doi.org/10.1007/s11745-003-1057-1

Rontani, J., Smik, L., & Belt, S. T. (2019). Autoxidation of the sea ice biomarker proxy IPSO25 in the near-surface oxic layers of Arctic and Antarctic sediments. *Organic Geochemistry*, *129*, 63–76. https://doi.org/10.1016/j.orggeochem.2019.02.002

Rontani, J., Smik, L., & Belt, S. T. (2020). Electron ionization mass spectrometric fragmentation and detection of autoxidation products of 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl)-pentadec-5-ene in Arctic sediments. *Rapid Communications in Mass Spectrometry*, *34*(15), e8816. https://doi.org/10.1002/rcm.8816

Rontani, J.-F., Amiraux, R., Smik, L., Wakeham, S. G., Paulmier, A., Vaultier, F., Sun-Yong, H., Jun-oh, M., & Belt, S. T. (2021). Type II photosensitized oxidation in senescent microalgal cells at different latitudes: Does low under-ice irradiance in polar regions enhance efficiency? *Science of The Total Environment*, *779*, 146363. https://doi.org/10.1016/j.scitotenv.2021.146363

Rontani, J., Zabeti, N., & Wakeham, S. G. (2009). The fate of marine lipids: Biotic vs. abiotic degradation of particulate sterols and alkenones in the Northwestern Mediterranean Sea. *Marine Chemistry*, *113*(1), 9–18. https://doi.org/10.1016/j.marchem.2008.11.001

Rontani, J., Zabeti, N., & Wakeham, S. G. (2011). Degradation of particulate organic matter in the equatorial Pacific Ocean: Biotic or abiotic? *Limnology and Oceanography*, *56*(1), 333–349. https://doi.org/10.4319/lo.2011.56.1.0333

Rostoniec, K. Z., Wilbrink, M. H., Capyk, J. K., Mohn, W. W., Ostendorf, M., van der Geize, R., Dijkhuizen, L., & Eltis, L. D. (2009). Cytochrome P450 125 (CYP125) catalyses C26-hydroxylation to initiate sterol side-chain degradation in *Rhodococcus jostii* RHA1. *Molecular Microbiology*, *74*(5), 1031–1043. https://doi.org/10.1111/j.1365-2958.2009.06915.x

Round, F. E., Crawford, R. M., & Mann, D. G. (2007). *Diatoms: Biology and Morphology of the Genera*. Cambridge University Press.

Rowland, S. J., & Robson, J. N. (1990). The widespread occurrence of highly branched acyclic C20, C25 and C30 hydrocarbons in recent sediments and biota—A review. *Marine Environmental Research*, *30*(3), 191–216. https://doi.org/10.1016/0141-1136(90)90019-K

Rytter Hasle, G., & Riddervold Heimdal, B. (1998). The net phytoplankton in Kongsfjorden, Svalbard, July 1988, with general remarks on species composition of Arctic phytoplankton. *Polar Research*, *17*(1), 31–52. https://doi.org/10.3402/polar.v17i1.6605

Sakshaug, E. (2004). Primary and Secondary Production in the Arctic Seas. In R. Stein & R. W. MacDonald (Eds.), *The Organic Carbon Cycle in the Arctic Ocean* (pp. 57–81). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-18912-8_3

Sakshaug, E., Bjørge, A., Gulliksen, B., Loeng, H., & Mehlum, F. (1994). Structure, biomass distribution, and energetics of the pelagic ecosystem in the Barents Sea: A synopsis. *Polar Biology*, *14*(6). https://doi.org/10.1007/BF00240261

Schaich, K. M. (2005). Lipid Oxidation: Theoretical Aspects. In *Bailey's Industrial Oil and Fat Products*. American Cancer Society. https://doi.org/10.1002/047167849X.bio067

Schulz, I., Mahler, H.-C., Boiteux, S., & Epe, B. (2000). Oxidative DNA base damage induced by singlet oxygen and photosensitization: Recognition by repair endonucleases and mutagenicity. *Mutation Research/DNA Repair*, *461*(2), 145–156. https://doi.org/10.1016/S0921-8777(00)00049-5

Screen, J. A., & Simmonds, I. (2010). The central role of diminishing sea ice in recent Arctic temperature amplification. *Nature*, *464*(7293), 1334–1337. https://doi.org/10.1038/nature09051

Selje, N., Simon, M., & Brinkhoff, T. (2004). A newly discovered Roseobacter cluster in temperate and polar oceans. *Nature*, *427*(6973), 445–448. https://doi.org/10.1038/nature02272

Sheldon, R. A., & Kochi, J. K. (1976). Metal-Catalyzed Oxidations of Organic Compounds in the Liquid Phase: A Mechanistic Approach. In *Advances in Catalysis* (Vol. 25, pp. 272–413). Elsevier. https://doi.org/10.1016/S0360-0564(08)60316-8

Shibutani, S., Takeshita, M., & Grollman, A. P. (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, *349*(6308), 431–434. https://doi.org/10.1038/349431a0

Shoja Chaghervand, S. (2019). *Characterization of the enzymes involved in the diolsynthase pathway in Pseudomonas aeruginosa.* University of Barcelona.

Sicre, M.-A., Paillasseur, J.-L., Marty, J.-C., & Saliot, A. (1988). Characterization of seawater samples using chemometric methods applied to biomarker fatty acids. *Organic Geochemistry*, *12*(3), 281–288. https://doi.org/10.1016/0146-6380(88)90265-3

Sinensky, M. (1974). Homeoviscous Adaptation—A Homeostatic Process that Regulates the Viscosity of Membrane Lipids in Escherichia coli. *Proceedings of the National Academy of Sciences*, *71*(2), 522–525. https://doi.org/10.1073/pnas.71.2.522

Slagstad, D., Ellingsen, I. H., & Wassmann, P. (2011). Evaluating primary and secondary production in an Arctic Ocean void of summer sea ice: An experimental simulation

approach. *Progress in Oceanography*, *90*(1), 117–131. https://doi.org/10.1016/j.pocean.2011.02.009

Smetacek, V., & Nicol, S. (2005). Polar ocean ecosystems in a changing world. *Nature*, *437*(7057), 362–368. https://doi.org/10.1038/nature04161

Smith, L. L. (1980). The Autoxidation of Cholesterol. In M. G. Simic & M. Karel (Eds.), *Autoxidation in Food and Biological Systems* (pp. 119–132). Springer US. https://doi.org/10.1007/978-1-4757-9351-2_7

Smith, R. E. H., Cavaletto, J. F., Eadie, B. J., & Gardner, W. S. (1993). Growth and lipid composition of high Arctic ice algae during the spring bloom at Resolute, Northwest Territories, Canada. *Marine Ecology Progress Series*, *97*(1), 19–29. JSTOR.

Sojo, M., Bru, R., Lopez-Molina, D., Garcia-Carmona, F., & Argüelles, J.-C. (1997). Cell-linked and extracellular cholesterol oxidase activities from Rhodococcus erythropolis. Isolation and physiological characterization. *Applied Microbiology and Biotechnology*, *47*(5), 583–589. https://doi.org/10.1007/s002530050977

Søreide, J. E., Leu, E., Berge, J., Graeve, M., & Falk-Petersen, S. (2010). Timing of blooms, algal food quality and Calanus glacialis reproduction and growth in a changing Arctic. *Global Change Biology*, *16*(11), 3154–3163. https://doi.org/10.1111/j.1365-2486.2010.02175.x

Spiteller, G., & Afzal, M. (2014). The Action of Peroxyl Radicals, Powerful Deleterious Reagents, Explains Why Neither Cholesterol Nor Saturated Fatty Acids Cause Atherogenesis and Age-Related Diseases. *Chemistry – A European Journal*, *20*(46), 14928–14945. https://doi.org/10.1002/chem.201404383

Steenken, S., & Jovanovic, S. V. (1997). How Easily Oxidizable Is DNA? One-Electron Reduction Potentials of Adenosine and Guanosine Radicals in Aqueous Solution. *Journal of the American Chemical Society*, *119*(3), 617–618. https://doi.org/10.1021/ja962255b

Stein, R., & MacDonald, R. W. (Eds.). (2004). *The Organic Carbon Cycle in the Arctic Ocean*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-18912-8

Stroeve, J. C., Kattsov, V., Barrett, A., Serreze, M., Pavlova, T., Holland, M., & Meier, W. N. (2012). Trends in Arctic sea ice extent from CMIP5, CMIP3 and observations. *Geophysical Research Letters*, *39*(16). https://doi.org/10.1029/2012GL052676

Stuttle, C. A., Chan, A. M., & Cottrell, M. T. (1990). Infection of phytoplankton by viruses and reduction of primary productivity. *Nature*, *347*(6292), 467–469.

Sukhanova, I. N. (1999). Spatial distribution and temporal variability of phytoplankton in the Bering Sea. *Dynamics of the Bering Sea*, 453–483.

Sun, L., Alexander, M., & Deser, C. (2018). Evolution of the Global Coupled Climate Response to Arctic Sea Ice Loss during 1990–2090 and Its Contribution to Climate Change. *Journal of Climate*, *31*(19), 7823–7843. https://doi.org/10.1175/JCLI-D-18-0134.1

Suwa, K., Kimura, T., & Schaap, A. P. (1977). Reactivity of singlet molecular oxygen with cholesterol in a phospholipid membrane matrix. A model for oxidative damage of membranes. *Biochemical and Biophysical Research Communications*, *75*(3), 785–792. https://doi.org/10.1016/0006-291X(77)91541-8

Syvertsen, E. E. (1991). Ice algae in the Barents Sea: Types of assemblages, origin, fate and role in the ice-edge phytoplankton bloom. *Polar Research*, *10*(1), 277–288. https://doi.org/10.3402/polar.v10i1.6746

Taipale, S. J., Hiltunen, M., Vuorio, K., & Peltomaa, E. (2016). Suitability of Phytosterols Alongside Fatty Acids as Chemotaxonomic Biomarkers for Phytoplankton. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00212

Tchou, J., Kasai, H., Shibutani, S., Chung, M. H., Laval, J., Grollman, A. P., & Nishimura, S. (1991). 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proceedings of the National Academy of Sciences*, *88*(11), 4690–4694. https://doi.org/10.1073/pnas.88.11.4690

Tomas, R. A., Deser, C., & Sun, L. (2016). The Role of Ocean Heat Transport in the Global Climate Response to Projected Arctic Sea Ice Loss. *Journal of Climate*, *29*(19), 6841–6859. https://doi.org/10.1175/JCLI-D-15-0651.1

Tremblay, J.-É., Hattori, H., Michel, C., Ringuette, M., Mei, Z.-P., Lovejoy, C., Fortier, L., Hobson, K. A., Amiel, D., & Cochran, K. (2006). Trophic structure and pathways of biogenic carbon flow in the eastern North Water Polynya. *Progress in Oceanography*, *71*(2), 402–425. https://doi.org/10.1016/j.pocean.2006.10.006

Uchoa, A. F., Knox, P. P., Turchielle, R., Seifullina, N. Kh., & Baptista, M. S. (2008). Singlet oxygen generation in the reaction centers of Rhodobacter sphaeroides. *European Biophysics Journal*, *37*(6), 843–850. https://doi.org/10.1007/s00249-008-0287-y

van den Akker, E., Lutgerink, J. T., Lafleur, M. V. M., Joenje, H., & Retèl, J. (1994). The formation of one-G deletions as a consequence of singlet-oxygen-induced DNA dmage. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *309*(1), 45–52. https://doi.org/10.1016/0027-5107(94)90041-8

van Loon, B., Markkanen, E., & Hübscher, U. (2010). Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. *DNA Repair*, *9*(6), 604–616. https://doi.org/10.1016/j.dnarep.2010.03.004

Vare, L. L., Massé, G., Gregory, T. R., Smart, C. W., & Belt, S. T. (2009). Sea ice variations in the central Canadian Arctic Archipelago during the Holocene. *Quaternary Science Reviews*, *28*(13), 1354–1366. https://doi.org/10.1016/j.quascirev.2009.01.013

Véron, B., Dauguet, J.-C., & Billard, C. (1998). Sterolic Biomarkers in Marine Phytoplankton. Ii. Free and Conjugated Sterols of Seven Species Used in Mariculture. *Journal of Phycology*, *34*(2), 273–279. https://doi.org/10.1046/j.1529-8817.1998.340273.x

Volkman, J. (2003). Sterols in microorganisms. *Applied Microbiology and Biotechnology*, *60*(5), 495–506. https://doi.org/10.1007/s00253-002-1172-8

Volkman, J. K. (1986). A review of sterol markers for marine and terrigenous organic matter. *Organic Geochemistry*, *9*(2), 83–99. https://doi.org/10.1016/0146-6380(86)90089-6

Volkman, J. K., Barrett, S. M., & Dunstan, G. A. (1994). C25 and C30 highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Organic Geochemistry*, *21*(3–4), 407–414. https://doi.org/10.1016/0146-6380(94)90202-X

Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I., & Garland, C. D. (1989). Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, *128*(3), 219–240. https://doi.org/10.1016/0022-0981(89)90029-4

Volkov, A., Liavonchanka, A., Kamneva, O., Fiedler, T., Goebel, C., Kreikemeyer, B., & Feussner, I. (2010). Myosin Cross-reactive Antigen of *Streptococcus pyogenes* M49 Encodes a Fatty Acid Double Bond Hydratase That Plays a Role in Oleic Acid Detoxification and Bacterial Virulence. *Journal of Biological Chemistry*, *285*(14), 10353–10361. https://doi.org/10.1074/jbc.M109.081851

von Quillfeldt, C. H. (1997). Distribution of diatoms in the Northeast Water Polynya, Greenland. *Journal of Marine Systems*, *10*(1–4), 211–240. https://doi.org/10.1016/S0924-7963(96)00056-5

Wadhams, P., Lange, M. A., & Ackley, S. F. (1987). The ice thickness distribution across the Atlantic sector of the Antarctic Ocean in midwinter. *Journal of Geophysical Research: Oceans*, *92*(C13), 14535–14552. https://doi.org/10.1029/JC092iC13p14535

Wagner, J., & Fuchs, R. P. P. (1997). Frameshift Mutagenesis Induced in *Escherichia coli* after *in Vitro* Treatment of Double-Stranded DNA with Methylene Blue plus White Light: Evidence for the Involvement of Lesion(s) Other than 8-Oxo-7,8-dihydro-2‘-deoxyguanosine. *Chemical Research in Toxicology*, *10*(5), 568–574. https://doi.org/10.1021/tx960169j

Wakeham, S. G. (1989). Reduction of stenols to stanols in particulate matter at oxic–anoxic boundaries in sea water. *Nature*, *342*(6251), 787–790. https://doi.org/10.1038/342787a0

Wakeham, S. G. (1995). Lipid biomarkers for heterotrophic alteration of suspended particulate organic matter in oxygenated and anoxic water columns of the ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, *42*(10), 1749–1771. https://doi.org/10.1016/0967-0637(95)00074-G

Wang, M., & Overland, J. E. (2009). A sea ice free summer Arctic within 30 years? *Geophysical Research Letters*, *36*(7). https://doi.org/10.1029/2009GL037820

Wargo, M. J. (2013). Homeostasis and Catabolism of Choline and Glycine Betaine: Lessons from Pseudomonas aeruginosa. *Applied and Environmental Microbiology*, *79*(7), 2112–2120. https://doi.org/10.1128/AEM.03565-12

Wassmann, P., & Reigstad, M. (2011). Future Arctic Ocean Seasonal Ice Zones and Implications for Pelagic-Benthic Coupling. *Oceanography*, *24*(3), 220–231. https://doi.org/10.5670/oceanog.2011.74

Weller, G., & Bowling, S. A. (1972). *Studies of the Solar and Terrestrial Radiation Fluxes over Arctic Pack Ice*. 75.

Wichard, T., Gerecht, A., Boersma, M., Poulet, S. A., Wiltshire, K., & Pohnert, G. (2007). Lipid and Fatty Acid Composition of Diatoms Revisited: Rapid Wound-Activated Change of Food Quality Parameters Influences Herbivorous Copepod Reproductive Success. *ChemBioChem*, *8*(10), 1146–1153. https://doi.org/10.1002/cbic.200700053

Wraige, E. J., Johns, L., Belt, S. T., Massé, G., Robert, J.-M., & Rowland, S. (1999). Highly branched C25 isoprenoids in axenic cultures ofHaslea ostrearia. *Phytochemistry*, *51*(1), 69–73. https://doi.org/10.1016/S0031-9422(98)00694-3

Yager, P. L., Connelly, T. L., Mortazavi, B., Wommack, K. E., Bano, N., Bauer, J. E., Opsahl, S., & Hollibaugh, J. T. (2001). Dynamic bacterial and viral response to an algal bloom at subzero temperatures. *Limnology and Oceanography*, *46*(4), 790–801. https://doi.org/10.4319/lo.2001.46.4.0790

Yang, W., Dostal, L., & Rosazza, J. P. N. (1993). Stereospecificity of Microbial Hydrations of Oleic Acid to 10-Hydroxystearic Acid. *Applied and Environmental Microbiology*, *59*(1), 281–284. https://doi.org/10.1128/AEM.59.1.281-284.1993

Zafiriou, O. C., Joussot-Dubien, J., Zepp, R. G., & Zika, R. G. (1984). Photochemistry of natural waters. *Environmental Science & Technology*, *18*(12), 358A-371A. https://doi.org/10.1021/es00130a711

Zenkevich, L. A. (1963). Biologiya morey SSSR [Biology of the Seas of the USSR]. *Academy Sciences of the USSR, Moscow, USSR*, *739*.

Zheng, C. J., Yoo, J.-S., Lee, T.-G., Cho, H.-Y., Kim, Y.-H., & Kim, W.-G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters*, *579*(23), 5157–5162. https://doi.org/10.1016/j.febslet.2005.08.028

Zubkov, M. V., Sleigh, M. A., Tarran, G. A., Burkill, P. H., & Leakey, R. J. G. (1998). Picoplanktonic community structure on an Atlantic transect from 50°N to 50°S. *Deep Sea Research Part I: Oceanographic Research Papers*, *45*(8), 1339–1355. https://doi.org/10.1016/S0967-0637(98)00015-6

Chapitre II

Facteurs de stress résultant de la fonte vernale de la glace de mer dans l'Arctique : Impact sur la viabilité des communautés bactériennes associées aux algues sympagiques

Sommaire

2.1 Avant-propos

Les milieux arctiques ont un fonctionnement soumis à une forte saisonnalité, notamment en ce qui concerne la production primaire (Tremblay *et al*., 2012). Si le phytoplancton assure la majeure partie de cette production tout au long de l'année (Gosselin *et al*., 1997), les algues sympagiques en assurent également une portion non négligeable (allant de 1 à 60% selon les zones considérées, Loose *et al.,* 2011 ; Dupont, 2012 ; Fernández-Méndez *et al.,* 2015). La contribution des algues sympagiques à la pompe biologique dépend de l'intensité de leur dégradation (biotique et abiotique) dans la glace et tout au long de leur chute dans la colonne d'eau.

La faible activité bactérienne dans les eaux arctiques (entre 25 et 80% de bactéries inactives ou mortes, Howard-Jones *et al*., 2002) peut s'expliquer par différents facteurs, comme le fort gradient de salinité (passage d'eaux hyper- à hyposalines) dans la glace au cours d'un cycle de fonte. En effet, des marqueurs de stress halin ont déjà été observés en Arctique par Amiraux *et al*., (2017) dans du matériel particulaire récolté à l'aide de trappes à sédiments durant la période de fonte. Cette même étude a mis en avant l'état physiologique plutôt dégradé des bactéries associées au matériel sympagique, et ainsi sa bonne préservation tout au long de sa chute vers les sédiments.

Le but de ce chapitre est ainsi d'utiliser différents traceurs lipidiques de stress bactériens et des mesures de viabilité afin d'évaluer l'état de stress et la mortalité des bactéries associées aux algues de glace dans des échantillons de glace et de particules en sédimentation collectées au camp de glace du projet GreenEdge (situé à Qikiqtarjuaq) en 2016 lors de la saison de fonte.

Les résultats de cette étude ont montré que l'impact de la salinité sur les bactéries associées aux algues de glace est limité aux premiers stades de fonte de la banquise. Ils ont également mis en avant l'importance des acides gras libres excrétés par les algues de glace lors du sur éclairement résultant de la fonte de la neige dans la mortalité et/ou inactivité du bacterioplancton. Une forte mortalité bactérienne a en effet été mesurée dans la glace et dans les particules chutant sous celle-ci en fin de fonte lors de l'expulsion des algues de glace. Ces résultats vont dans le sens d'une dégradation relativement faible et d'une bonne préservation du matériel algal sympagique lors de sa chute vers le fond.

- Contribution au chapitre

La collecte des échantillons et certains traitements devant être effectués sur place (PMA) n'ont pas été réalisés dans le cadre de cette thèse. J'ai effectué les extractions lipidiques, le traitement au DMDS de ces derniers, ainsi que les analyses à l'aide de couplages chromatographes en phase gazeuse – spectromètre de masse (CPG-MS). J'ai également contribué à l'obtention des résultats de viabilité cellulaire ainsi qu'à la rédaction et relecture de l'article publié dans la revue *Elementa: Science of the Anthropocene*.

2.2 Stress factors resulting from the Arctic vernal sea-ice melt: Impact on the viability of bacterial communities associated with sympagic algae

2.2.1 Résumé en français

Lors de la fonte de la glace de mer en Arctique, la production primaire des algues sympagiques (algues de glace) peut être exportée efficacement vers les fonds marins si la vitesse de chute du matériel est rapide et si l'activité des bactéries hétérotrophes associées à ce matériel est limitée. Le stress salin, dû à la fonte de la glace a été suggéré pour expliquer une telle faible activité bactérienne. Dans le cadre du projet GreenEdge, nous avons ici étudié cette hypothèse en analysant divers échantillons de glace de mer et de particules en chute collectés du 18 Mai au 29 Juin 2016, à l'Ouest de la Baie de Baffin. Nous avons utilisé une méthode inédite e, région polaire : PCR quantitative couplée à de l'ADN traité au propidium monoazide, afin d'évaluer la viabilité des bactéries associées aux algues de glace et en chute. Nous avons également mesuré l'activité de la *Cis/Trans isomérase*, connue pour témoigner de la réponse rapide des bactéries à un stress halin, ainsi que la production d'acide gras libres, produits par les algues avec de fortes propriétés bactéricides. La viabilité des bactéries associées au matériel sympagique était forte en mai (environ 10% de mortalité des bactéries totales) et plutôt faible en Juin (mortalité moyenne de 43%, avec un maximum à 75%). Un stress à court terme, comme en témoigne l'activité de la *Cis/Trans isomérase* n'a été observé que dans les échantillons de particule en chute collectés au plus tôt de la série temporelle. Toutefois en juin, suite à la fonte de la neige et la saturation en radiations disponibles pour la photosynthèse, nous avons observé une augmentation de la production de composés bactéricides par les algues de glace (ici acides gras libres, jusqu'à 4,8 $mg.L^{-1}$). Nous suggérons donc que la protection du matériel sympagique en chute contre la dégradation bactérienne résulte, en début de fonte : de la faible activité bactérienne due au stress halin, alors que plus tard dans la saison, la production de composés bactéricides par les algues induit une mortalité bactérienne conséquente. Ainsi la succession de ces facteurs de stress bactériens pendant une saison de fonte en Arctique nous aide à expliquer l'export efficace du matériel sympagique jusqu'aux fonds marins.

2.2.2 Article

Stress factors resulting from the Arctic vernal sea-ice melt: Impact on the viability of bacterial communities associated with sympagic algae

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Article publié dans *Elementa, Science of the Anthropocene* en octobre 2020

Abstract

During sea-ice melt in the Arctic, primary production by sympagic (sea-ice) algae can be exported efficiently to the seabed if sinking rates are rapid and activities of associated heterotrophic bacteria are limited. Salinity stress due to melting ice has been suggested to account for such low bacterial activity. We further tested this hypothesis by analyzing samples of sea ice and sinking particles collected from May 18 to June 29, 2016, in western Baffin Bay as part of the Green Edge project. We applied a method not previously used in polar regions—quantitative PCR coupled to the propidium monoazide DNA-binding method—to evaluate the viability of bacteria associated with sympagic and sinking algae. We also measured cis-trans isomerase activity, known to indicate rapid bacterial response to salinity stress in culture studies, as well as free fatty acids known to be produced by algae as bactericidal compounds. The viability of sympagic associated bacteria was strong in May (only approximately 10% mortality of total bacteria) and weaker in June (average mortality of 43%; maximum of 75%), with instances of elevated mortality in sinking particle samples across the time series (up to 72%). Short-term stress reflected by cis-trans isomerase activity was observed only in samples of sinking particles collected early in the time series. Following snow melt, however, and saturating levels of photosynthetically active radiation in June, we observed enhanced ice-algal production of bactericidal compounds (free palmitoleic acid; up to 4.8 mg L–1). We thus suggest that protection of sinking sympagic material from bacterial degradation early in a melt season results from low bacterial activity due to salinity stress, while later in the season, algal production of bactericidal compounds induces bacterial mortality. A succession of bacterial stressors during Arctic ice melt helps to explain the efficient export of sea-ice algal material to the seabed.

1. Introduction

Primary production in the Arctic Ocean is characterized by short-lived, springtime blooms of sympagic (ice-associated) algae and phytoplankton, which are the major sources of autochthonous organic carbon for the Arctic food web (Horner and Schrader, 1982; Gosselin *et al.,* 1997; Pabi *et al.,* 2008; Wassmann *et al.,* 2011). For both types of microalgae, light availability is the principal factor controlling bloom onset (e.g., Cota, 1985; Lavoie *et al.,* 2005; Lee and Whitledge, 2005; Popova *et al.,* 2010; Campbell *et al.,* 2016), while nutrient availability is the key factor determining its magnitude, duration, and taxonomic composition. The contribution of sympagic algae to total primary production varies depending on the season and the region (from <1% to 60%; e.g., Loose *et al.,* 2011; Dupont, 2012; Fernández-Méndez *et al.,* 2015), but these ice algae represent a crucial food source for the marine food web (Søreide *et al.,* 2010). In sea ice, microalgae are largely concentrated near the ice–water interface within the skeletal layer of the congelation ice, where light is sufficient, the water is relatively rich in nutrients at the start of the bloom season, and physico-chemical conditions (e.g., pH, salinity, temperature) are relatively stable. During the melting season, the high concentrations of extracellular polymeric substances (EPS) they have produced while inhabiting the ice (Ewert and Deming, 2013) favor their agglomeration (Riebesell *et al.,* 1991) and therefore their descent to the seafloor and contribution to sediments. Other scenarios are possible, however, as EPS may also enhance the buoyancy of particles (Riedel *et al.,* 2006; Assmy *et al.,* 2013; Belt *et al.,* 2018).

The fate of algal material in the water column during its sinking depends not only on zooplankton grazing but also on the hydrolytic and remineralization activity of bacteria associated with the sinking particles (whether by sea-ice or water-column bacteria). Howard-Jones et al. (2002) suggested that significant fractions (25%–80%) of Arctic bacterioplankton (including the particle-associated ones) are dormant or express very low activity, based on measurements made in the water column of the Barents Sea marginal ice zone, where the percentage of active bacteria varied by measurement method. Early studies suggested that heterotrophic bacterial remineralization was generally low in perennially cold environments like the Arctic (Pomeroy and Deibel, 1986), allowing organic matter to be exported from productive to oligotrophic regions or to be preserved long enough for use during times of low primary productivity. Subsequent studies have pointed to more complex interactions between temperature and

heterotrophic bacterial production and other aspects of bacterial metabolism (Rivkin *et al.,* 1996; Yager and Deming, 1999; Pomeroy and Wiebe, 2001). The effect of temperature on Arctic bacterial activity remains unclear (Kirchman *et al.,* 2005).

In sea ice, some substances produced by ice algae have the potential to inhibit bacterial growth; for example, acrylic acid, which can form during intensive blooms as dimethylsulfoniopropionate (DMSP) is degraded (Monfort *et al.,* 2000), and free fatty acids (FFAs), as produced by some diatoms (Desbois and Smith, 2010). Viral infection may also play a role in the mortality of bacteria in sea ice and the underlying water column (e.g., Maranger *et al.,* 1994; Deming and Collins, 2017). Virus-to-bacteria ratios measured in the bottommost layer of Arctic Sea ice are among the highest reported in natural samples (Maranger *et al.,* 1994; Collins and Deming, 2011).

Recently, we suggested that at least for sea-ice bacteria, their limited activity in the Arctic could also result from other stress factors such as salinity (Amiraux *et al.,* 2017). During the early stage of ice melting in spring, brine inclusions become interconnected in channels and experience expulsion from the sea ice into the underlying seawater (Wadhams and Martin, 2001; Ewert and Deming, 2013), which occurs over relatively short timescales (e.g., hours). To cope with the stress of salinity shifts that may be experienced in this ecosystem as a result of brine drainage, bacteria appear to have developed various strategies. Previous work on the microstructure of sea ice raised the possibility that EPS, observed to fill sea-ice pores and coat individual cells inhabiting the brine phase, may provide a physical buffer against extreme salinities (Krembs *et al.,* 2011). Bacteria in sea-ice brines are also able to import or export compatible solutes such as proline, glycine betaine, or choline that can act as osmoprotectants (Ewert and Deming, 2014; Firth *et al.,* 2016). Another major adaptive response of many microorganisms, including bacteria, is to maintain membrane fluidity through "homeoviscous adaptation" (Sinensky, 1974). The shifts in fatty acid composition of membrane lipids, most notably by the enzymatic conversion of cis- to trans-unsaturated fatty acids (Loffeld and Keweloh, 1996; Heipieper *et al.,* 2003) through the activity of cis–trans isomerases (CTI), can constitute an important bacterial mechanism to modify membrane fluidity. To our knowledge, this mechanism has not been studied in the sea-ice environment; however, in other environments, membrane trans/cis ratios >0.1 have been suggested to indicate bacterial stress (Guckert *et al.,* 1986).

Within the framework of the Green Edge project, originally designed to investigate the dynamics of the spring bloom in ice-impacted Baffin Bay (Massicotte *et al.,* 2020), we

found relatively strong CTI activity (high trans/cis ratios) in sea ice and sinking particles collected in the water column during the 2015 vernal melting period, which suggested the occurrence of salinity stress during the early stages of ice melt (Amiraux *et al.,* 2017). The relative stability of these high ratios with depth also suggested that (1) sinking particles were not strongly colonized by pelagic bacteria (unstressed, with low trans/cis ratios) and (2) bacterial communities associated with sinking sympagic algae were not growing (as growth is associated with changing trans/cis ratios). These two hypotheses are linked, because in the absence of other forms of stress, the trans/cis ratio of bacteria decreases to a basic level (Fischer *et al.,* 2010), a decrease that requires de novo synthesis of cis fatty acids (Eberlein *et al.,* 2018) and thus bacterial growth. The implication of marine trans/cis ratios associated with sinking material remaining at high levels is that growth is not occurring. Nongrowing bacteria, however, are not necessarily inactive metabolically (Kjelleberg *et al.,* 1987), and other forms of stress besides salinity may be involved.

In the 2015 field study (Amiraux *et al.,* 2017), a specific oxidation of the fatty acid palmitoleic acid at position 10 (resulting in the production of 10S-hydroxyhexadec-8(trans)-enoic acid) was also observed in sea ice and sinking material. This oxidation was attributed to the involvement of a bacterial 10S-DOX-like lipoxygenase, initially isolated from Pseudomonas 42A2 (Guerrero *et al.,* 1997; Busquets *et al.,* 2004); more recently, the activity of this lipoxygenase has also been observed in members of the genera Pseudoalteromonas, Shewanella, and Aeromonas (Shoja Chaghervand, 2019). The lipoxygenase enzyme, active mainly on free unsaturated fatty acids (Martínez *et al.,* 2010), could play a role in bacterial survival and colonization of environments rich in free fatty acids, such as sympagic algae (Amiraux *et al.,* submitted).

Owing to their high sinking rates and the inferred nongrowing state of their associated bacterial communities, sympagic algae should be relatively preserved during their descent to the seabed and thus contribute perhaps strongly to the benthos and to carbon sequestration at depth. However, the estimation of the bacterial physiological state in the studies cited above was based only on indirect evidence. Here, we evaluated the potential occurrence of the bacterial stressors of salinity and bactericidal FFAs and their impacts on bacterial viability in sea ice and sinking particles during the 2016 vernal melting season of the Green Edge project. To assess comparative bacterial viability between samples, we used a method not previously applied to these environments—the PMA method, which uses propidium monoazide (PMA), a photoreactive dye that binds to DNA,

coupled with quantitative PCR (qPCR) to estimate the percentage of viable and dead bacteria.

2. Materials and methods

2.1. Study site and sampling

Sampling was conducted every 48 h from May 18 to July 8, 2016, at a landfast ice station located near Broughton Island (67° 28.766′N, 63° 47.579′W; water depth of 350 m; Figure 2.) in Davis Strait within the framework of the Green Edge project. Due to long processing times, samples for the targeted parameters of this study (e.g., bacterial viability and specific lipid content) were taken from sea ice and the water column (sinking particles) on 8 of the 23 sampling dates, from May 18 to June 29. However, these parameters are contextualized by core parameters (e.g., ice and snow thickness, brine salinity and volume, photosynthetically active radiation, chlorophyll a concentration, bacterial abundance, common fatty acid content) that were collected on each sampling day. Many of these core parameters have been published elsewhere (Amiraux *et al.,* 2019; Oziel *et al.,* 2019; Massicotte *et al.,* 2020) and are cited specifically in later sections.

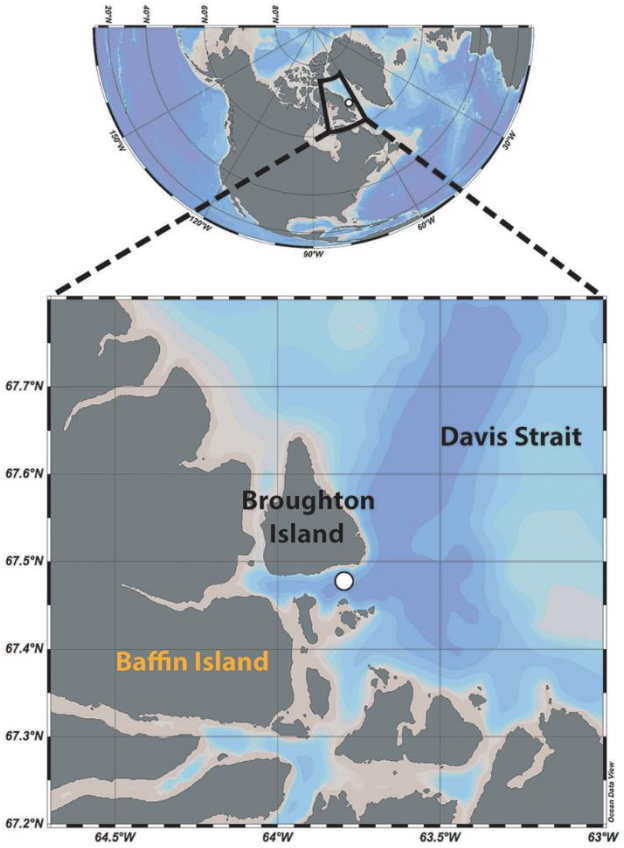

Figure 2.1: Map of the study area with location of the station investigated in Davis Strait. The white circle on the enlarged map of western Davis Strait indicates the sampling location.

2.1.1. Sea ice

On each sampling date, two sets of sea-ice samples for biota were collected using a Kovacs Mark V 14-cm diameter corer and focusing on the bottommost 10 cm of sea ice where the bulk of ice biota are typically found (Smith *et al.,* 1990). The first set of samples were subsectioned into two intervals, 0–3 and 3–10 cm, while the second set focused on the bottommost 0–1 cm of the ice. To compensate for biomass heterogeneity, common in sea ice (Gosselin *et al.,* 1986), 3 or 4 equivalent core sections were pooled for each sampling set on each sampling day and held in isothermal containers until processing. Analyses for chlorophyll a (Chl a), lipids, and bacterial viability were carried out on the bottom 3-cm sections of the 0–3 and 3–10 cm sampling sets (as well as on the 3–10 cm section for some lipids), while the photosynthetic parameters of sympagic algae were investigated in the 0–1 cm set. At the shore-site laboratory, the pooled sets of the thicker (3 cm) sea-ice sections were melted in dark isothermal containers in filtered seawater (FSW, using 0.2-µm Whatman nucleopore hydrophilic membrane; 3 parts FSW to 1 part ice, vol:vol) to minimize osmotic stress to the microbial community during melting (<12 h; Bates and Cota, 1986; Garrison and Buck, 1987). The pooled sets of the thinner sea-ice sections were melted in dark isothermal containers with a larger relative volume of FSW (ca. 38 parts FSW to 1 part ice, vol: vol) in order to reduce the melting time, which can impact algal physiological photoresponses.

Two additional full ice cores were sampled as described in Miller et al. (2015) to measure vertical profiles of temperature and bulk salinity (Oziel *et al.,* 2019). Ice temperature was measured at 10-cm intervals using a high-precision thermometer (Testo 720; ± 0.1 °C). For ice salinity, the ice core was cut with a handsaw into 10-cm sections, which were stored in plastic containers (Whirl-Pak bags) and later melted at room temperature. Bulk (practical) salinity of the melted ice sections was determined using a conductivity probe (Thermo Scientific Orion portable salinometer model WP-84TPS) that was calibrated every sampling day with seawater standard (35) and MilliQ water (0). Brine salinity and volume fraction (%) were calculated for each 10-cm section using the ice temperature and bulk salinity following Cox and Weeks (1983). Salinity of the seawater at the interface with sea ice was obtained using a Sea-Bird Electronics 19plus V2 CTD system (factory calibrated prior to the expedition). The data were post-processed according to the standard procedures recommended by the manufacturer.

Photosynthetically active radiation (PAR) below the sea ice was estimated at 1.3 m using the multispectral data collected with a compact-optical profiling system (C-OPS; version IcePRO; Biospherical instruments, Inc.; see Oziel *et al.,* 2019). To reduce the effects of sea-ice surface heterogeneity on irradiance measurements (e.g., Katlein *et al.,* 2015), the vertical attenuation coefficients of PAR were calculated by fitting a single exponential function on PAR profiles between 10 and 50 m, then used to estimate PAR at 1.3 m (for more details; see Massicotte *et al.,* 2018). Note that 1.3 m corresponds to the average ice thickness measured during the field campaign and thus to the first measure under the ice (mean = 129 cm; standard deviation [SD] = 11 cm, n = 23).

2.1.2. Sinking particles

Short-term sediment traps were deployed unfilled and without preservative on a mooring line to collect sinking particles at 2 m and 25 m from below sea ice. Sediment traps were immersed for approximately 48 h and recovered at the same frequency as for the sea-ice sampling sets. Sediment traps were made in-house of polyvinyl chloride (PVC) and had an aperture diameter of 15 cm for a height-to-diameter ratio of 6:7 with a collection cup at the base. Approximately 300 ml was removed from the collection cup on each trap recovery day and subsampled for the different parameters analyzed.

2.2. Chlorophyll a

At the shore-site laboratory, and within 24 h of sampling, duplicate samples of sea ice and sediment trap samples were filtered through Whatman GF/F glass fiber filters. The concentration of Chl a retained on the filters was measured using a TD-700 Turner Design fluorimeter, after 18–24 h extraction in 90% acetone at 4 °C in the dark (Parsons *et al.,* 1984). The fluorometer was calibrated with a commercially available Chl a standard (Anacystis nidulans, Sigma).

2.3. Photosynthetic parameters

To determine photosynthetic parameters, pooled melted 0–1 cm sea-ice samples were incubated at different irradiance levels in the presence of ^{14}C-labeled sodium bicarbonate using a method derived from Babin et al. (1994). Inorganic 14C ($NaH^{14}CO_3$) was added to the samples to achieve a final concentration of approximately 0.7 µCi mL^{-1}. Total initial activity was determined by adding 20-µL aliquots (in triplicate) of the ^{14}C-amended sample to 50 µL of an organic base (ethanolamine), 1 ml of distilled water and 10 ml of

scintillation cocktail (EcoLume, MPBiomedicals, San Diego, USA) in glass scintillation vials. Aliquots of the the ^{14}C-amended sample were dispensed into 50-ml cell culture flasks and exposed to a gradient of irradiance provided by LED bulbs (Phillips 9W 6500K dimmable). The incubation system was cooled by circulation of in situ seawater. Scalar PAR irradiance was measured at each position in the incubation chamber prior to the incubation using an irradiance quantum meter (Biospherical QSL-2100, USA) equipped with a 4π spherical collector. After incubating for 120 min, the samples were filtered through 0.2-µm pore-size isopore membrane filters (Milipore, Burlington, USA). Filters were placed in plastic scintillation vials and acidified for 4 h with 500 µl of HCl (6N) to remove excess inorganic carbon. Finally, 10 ml of scintillation cocktail were added to each vial prior to counting in a liquid scintillation counter (Tri-Carb, PerkinElmer, Boston, USA). The carbon fixation rate was then estimated according to Parsons et al. (1984). Photosynthetic parameters, including the saturation parameter (Ek), were estimated from P versus E curves by fitting nonlinear models based on the original definition of Platt and Gallegos (1980).

2.4. Bacterial abundance and viability

Bacterial abundance in the bottom 0–3 cm sea-ice sections was measured by flow cytometry. Samples of the melted ice sections (1.5 ml) were preserved at the shore-site laboratory with a mix of glutaraldehyde and Pluronic F-68 (Gibco; Marie *et al.,* 2014) and stored at –80 °C. Samples were analyzed on a FACS Canto flow cytometer (Becton Dickinson) in the laboratory at the Station Biologique de Roscoff. Bacterial abundance was determined based on the fluorescence of SYBR green-stained DNA (Marie *et al.,* 1997) and corrected for the melt dilution. Measurements of bacterial abundance in the sediment trap samples were not possible to obtain.

For both sea ice and sinking particles, samples for bacterial viability were collected in quadruplicate and treated at the shore-site laboratory. To estimate bacteria viability, we employed the PMA method, adapted from Nocker et al. (2009). The principle is based on the assumption that PMA (phenanthridium, 3-amino-8-azido-5-[3-(diethylmethylammonio)-propyl]-6-phenyl dichloride; Biotium, Inc., Hayward, California, dissolved in 20% dimethyl sulfoxide), when added to a mixture of living and dead (membrane-compromised) cells, selectively enters only the dead cells. Once inside the cells, PMA binds with DNA and can be covalently crosslinked to it by light exposure. PCR

amplification of such modified DNA is strongly inhibited. Thus, the comparison of quantitative PCR (qPCR) results obtained for subsamples treated and not treated with PMA provides an estimate of the viability of cells in the sample, quantified as the ratio of living to dead bacteria.

2.4.1. PMA crosslinking

At the shore-site laboratory, sea-ice and sinking particle samples (ca. 10–40 ml), in quadruplicate, were concentrated for the purpose of PMA crosslinking. Samples were pelleted at 5,000 × g for 5 min, and the pellets homogenized in 1 ml of 0.2-µm FSW (that had also been autoclaved 1 h at 120 °C after filtration). Two (of four) samples were designated for no treatment and stored at –80 °C, while the other two samples were treated by adding PMA at a final concentration of 100 µM. Following an incubation period of 5 min in the dark at 4 °C with occasional mixing., these samples (1 ml) were filtered through 0.8-µm Whatman nucleopore polycarbonate filters (24 mm, autoclaved 1 h at 110 °C prior to use) to retain attached bacteria on the filters (Bidle and Azam, 1999; Ghiglione *et al.,* 2007). Filters were placed in a Petri dish TM (Millipore ®) and exposed to light for 2 min using a 650W halogen light source (FAD 120V, 3200K; Osram, light spectrum: 350–800 nm). The samples were placed about 20 cm from the light source and laid horizontally on ice (to avoid excessive heating).

2.4.2. DNA isolation

DNA was extracted from the filter previously obtained (treated or not treated with PMA from sample volumes of 10–40 ml) using DNeasy Blood and Tissue Mini Kits (Qiagen) according to the manufacturer's instructions. The resulting DNA was kept frozen at –20 °C until further use to enumerate bacteria by quantitative PCR, as described in the next section.

2.4.3 Quantitative PCR

Quantification of the bacterial SSU ribosomal RNA (rRNA) gene was carried out by qPCR with SsoAdvancedTM Sybr Green Supermix on a CFX96 real-time system (C1000 Thermal Cycler, Bio-Rad Laboratories, CA, USA) according to the procedure described in Fernandes et al. (2016). The primers used were DGGE300F (GCCTACGGGAGGCAGCAG; Michotey *et al.,* 2012) and Univ516R sets (GTDTTACCGCGGCKGCTGRCA; Takai and Horikoshi, 2000). Before amplification, an initial denaturation step of 2 min at 98 °C was performed to activate the polymerase. The real-time PCR cycles consisted of a

denaturation step of 5s at 98 °C, a hybridization step of 10s at 55 °C, and an elongation step of 12s at 72 °C for 30 cycles. PCR amplification efficiency was between 96% and 99%. For standard curve construction, a gammaproteobacterial fragment was cloned after purification into pGEMT vector (Promega, WI, USA). After purification, using the Wizard® Plus SV Minipreps Start-Up Kit (Promega), the concentration of plasmids was determined using a NanoDropTM 1000 spectrophotometer (Thermo Fisher Scientific, DE, USA). The target abundance for standards was calculated using the following formula: gene abundance = 6.023×10^{23} (copies mol^{-1}) × standard concentration (g mL^{-1}) / molecular mass (g mol^{-1}), assuming that double-stranded DNA has a molecular mass of 660 Da. Standards for bacterial ribosomal gene corresponded to pGEMT plasmids (Promega), harboring one copy of gammaproteobacterial SSU rRNA gene fragment. Data were analyzed by comparative starting quantity of the targeted genes (Table S2.1) The regression equation was calculated on the graph, plotted between the CT value and the copy number of the standard ranging from 5.78×10^6 to 5.78×10^2 copies in the reaction (Figure S2.1). At the end of the PCR reactions, the specificity of the amplification was checked from the first derivative of their melting curves and also analyzed by agarose gel electrophoresis.

2.5. Lipid analysis

Due to the number of replicates required to determine bacterial viability (Section 2.4), samples for lipid analysis could not be collected in duplicate. Lipid samples were obtained by filtration through pre-weighed Whatman glass fiber filters (nominal porosity 0.7 µm, 47 mm, combusted for 4 h at 450 °C), with the filters kept frozen (<–20 °C). Owing to the porosity of the filters, these analyses concerned mainly algae (and likely EPS; Meiners *et al.,* 2008) and their attached bacteria (Bidle and Azam, 1999; Ghiglione *et al.,* 2007).

2.5.1. Lipid extraction

Filtered samples were reduced in MeOH (25 ml, 30 min) with excess $NaBH_4$, to reduce labile hydroperoxides (resulting from 10S-DOX oxidation) to alcohols, more amenable to analysis using gas chromatography-mass spectrometry (GC-MS). Water (25 ml) and KOH (2.8 g) were then added, and the resulting mixture saponified by refluxing (2 h). After cooling, the mixture was acidified (HCl, 2N) to pH 1 and extracted with dichloromethane (DCM; 3 × 20 ml). The combined DCM extracts were dried over anhydrous Na_2SO_4 and

filtered and concentrated by rotary evaporation at 40 °C to give total lipid extracts (TLEs). Aliquots of TLEs were either silylated and analyzed by gas chromatography-electron impact quadrupole time-of-flight mass spectrometry (GC-QTOF) for lipid quantification, or methylated, then treated with dimethyldisulphide (DMDS) and analyzed by GC-MS/MS for the determination of monounsaturated fatty acid double-bond stereochemistry, as previously described by Amiraux et al. (2017). Cis and trans isomers of monounsaturated fatty acid methyl esters react with DMDS stereospecifically to form threo and erythro adducts, which exhibit similar mass spectra but are readily separated by gas chromatography, allowing unambiguous double-bond stereochemistry determination (Buser *et al.,* 1983).

2.5.2. Gas chromatography/tandem mass spectrometry

GC-MS and GC-MS/MS analyses were performed using an Agilent 7890A/7010A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis—ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert) (30 m × 0.25 mm, 0.25-µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270 °C. The oven temperature was ramped from 70 °C to 130 °C at 20 °C min^{-1}, then to 250 °C at 5 °C min^{-1} and then to 300 °C at 3 °C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69 × 10^5 Pa until the end of the temperature program and then ramped from 0.69 × 10^5 Pa to 1.49 × 10^5 Pa at 0.04 × 10^5 Pa min^{-1}. The following mass spectrometric conditions were used: electron energy 70 eV, source temperature 230 °C, quadrupole 1 temperature 150 °C, quadrupole 2 temperature 150 °C, collision gas (N_2) flow 1.5 ml min^{-1}, quench gas (He) flow 2.25 ml min^{-1}, mass range 50–700 Daltons, cycle time 313 ms. Quantification of DMDS derivatives was carried out in multiple reaction monitoring (MRM) mode. Precursor ions were selected from the more intense ions (and specific fragmentations) observed in electron ionization (EI) mass spectra. Trans/cis ratios were obtained directly from peak area measurement (performed several times) of threo and erythro DMDS adducts.

2.5.3. Gas chromatography–EI quadrupole time of flight mass spectrometry

Accurate mass measurements were carried out in full scan mode using an Agilent 7890B/7200 GC/QTOF System (Agilent Technologies, Parc Technopolis—ZA

Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Macherey-Nagel; OPTIMA-5MS Accent; 30 m × 0.25 mm, 0.25-µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270 °C. The oven temperature was ramped from 70 °C to 130 °C at 20 °C min^{-1} and then to 300 °C at 5 °C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69 × 105 Pa until the end of the temperature program. Instrument temperatures were 300 °C for the transfer line and 230 °C for the ion source. Nitrogen (1.5 ml min^{-1}) was used as collision gas. Accurate mass spectra were recorded across the range m/z 50–700 at 4 GHz with the collision gas opened. The QTOF-MS instrument provided a typical resolution ranging from 8,009 to 12,252 from m/z 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was used for daily MS calibration. Quantification of the different lipids (sterols, fatty acids and 10-hydroxyhexadec-8(trans)-enoic acid) involved extraction of specific accurate fragment ions, peak integration and determination of individual response factors using external standards (SD ± 5%).

3. Results

3.1. Sea ice conditions

During the period investigated, snow thickness decreased from 20 cm to 2 cm and ice thickness, from 142 cm to 105 cm (Figure 2.2A). A particularly notable decrease in snow thickness was seen from June 3, ending in its disappearance on June 17. Brine salinity (calculated using the whole sea ice core) decreased throughout the study period from 42.9 to 17.2, while brine salinity in the bottommost 10 cm of sea ice remained relatively constant (mean ± SD = 29.4 ± 2.6, n = 21; Figure 2.2B). Depth profiles of brine salinity and of temperature and bulk salinity of the ice are provided in Figure S2.2. Salinity of the seawater at the interface with sea ice was relatively constant from May 18 to June 10 (mean ± SD = 32.1 ± 0.3, n = 12), then decreased from June 13 to July 06 (mean ± SD = 13.5 ± 7.0, n = 10; Figure 2.2B), corresponding with the temporal evolution of bulk salinity in the ice (Figure S2.2). These patterns indicate that release of hypersaline brines from sea ice through brine channels would have occurred prior to June 13, as hypothesized by Amiraux et al. (2019), while the downward percolation of meltwater (hyposaline) took place from ca. June 13 until the end of sampling.

The concentrations of Chl a and palmitoleic acid in the bottom 0–3 cm of sea ice were quantifiable throughout the sampling period, with values ranging from 0.9 to 317.3 µg L^{-1} (mean ± SD = 123.0 ± 85.2 µg L^{-1}, n = 23) and 0.0 to 36.3 mg L^{-1} (mean ± SD = 7.1 ± 8.9

mg L^{-1}, n = 22) (Figure 2.2C), respectively. The lowest sea-ice concentrations of Chl a were observed on the last three sampling dates (<4 µg L^{-1}), while the two maxima were observed on June 1 and June 13 (317.3 and 306.9 µg L^{-1}, respectively). The concentration of palmitoleic acid in the bottom 0–3 cm of sea ice, which did not correlate with Chl a concentration (r = 0.1, P > 0.5, n = 22), increased from about June 10 and reached a maximum (32.2 mg L^{-1}) on June 20, with a second lesser peak on June 29 (Figure 2.2C). The maximum occurred 3 days after the disappearance of snow (Figure 2.2A), which occurred simultaneously with the first maximum of PAR, as estimated under the ice cover at 1.3 m (Figure 2.3).

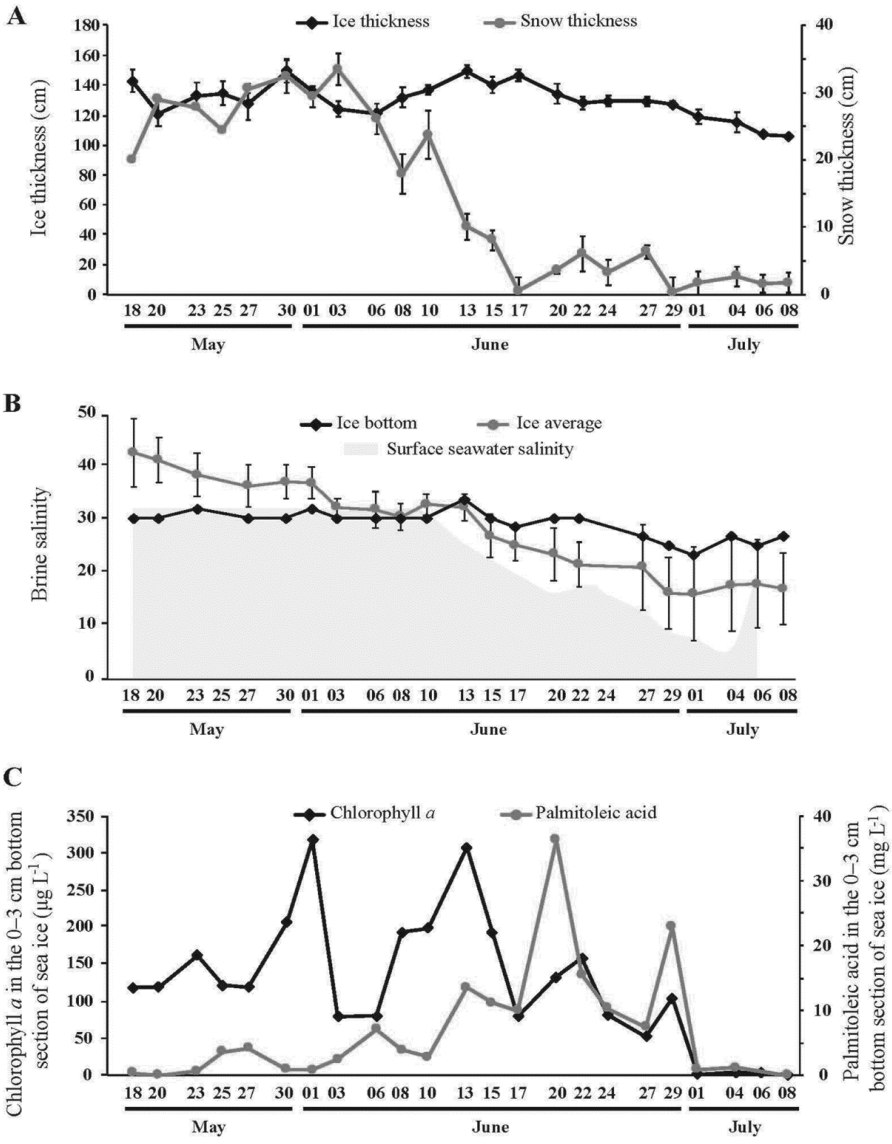

Figure 2.2: Time series of core parameters and biomarkers in sea ice. Time series of (A) snow (n = 8–17) and ice thickness (n = 8–22), where error bars are standard deviation (SD) of the mean; (B) brine salinity in bottom ice (0–10 cm, black diamonds) and averaged for the whole sea ice core (gray circles), where error bars are SD of the mean (n = 10–15) and surface seawater salinity is represented in watermark (adapted from Amiraux *et al.,* 2019); and (C) concentrations of Chl a and palmitoleic acid in the bottom 0–3 cm sea ice section (3–4 sections pooled prior to analysis) from May 18 to June 08, 2016, at the sampling location in Davis Strait (Figure 2.1).

This under-ice PAR increased from May 18 to June 17 (from 1.6 to 39.3 µE m^{-2} s^{-1}) and again from June 24 to July 08 (from 19.2 to 56.7 µE m^{-2} s^{-1}). Although PAR was relatively low through the beginning of the sampling period, the PAR value required to reach saturating irradiance (Ek) in the 0–1 cm bottommost section of sea ice was measured by

June 8 and increased from that date onward to reach Ek values of 93.4 and 104.5 μE m^{-2} s^{-1} on June 20 and July 8, respectively.

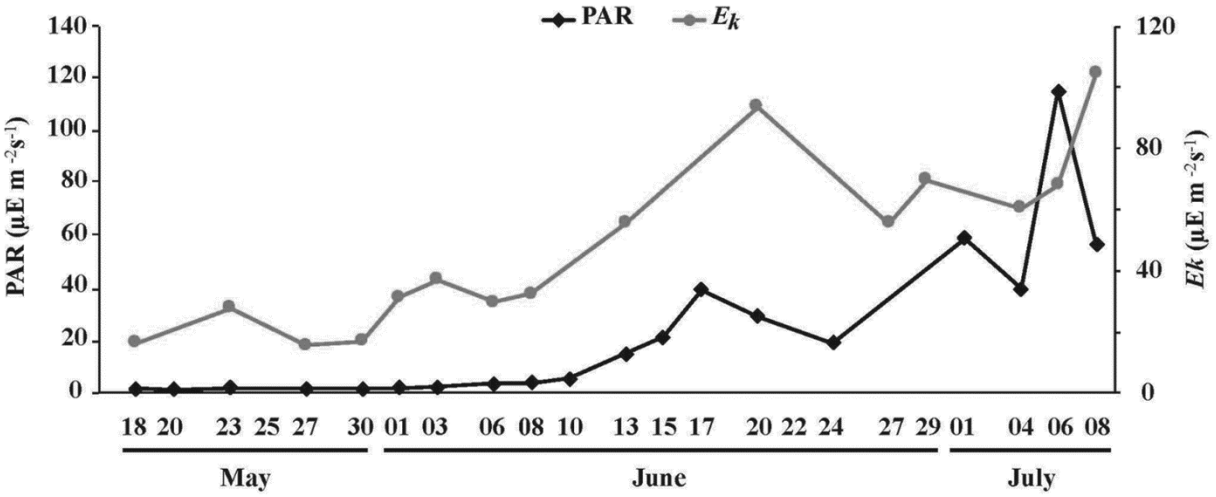

Figure 2.3: Time series of light parameters in sea ice. Time series of (A) photosynthetically active radiation (PAR, black diamonds) at 1.3 m, underneath the ice (see text); and (B) sympagic algae saturating irradiance (Ek, gray circles) in the bottom (0–1 cm) sea-ice section from May 18 to July 08, 2016, at the sampling location in Davis Strait (Figure 2.1).

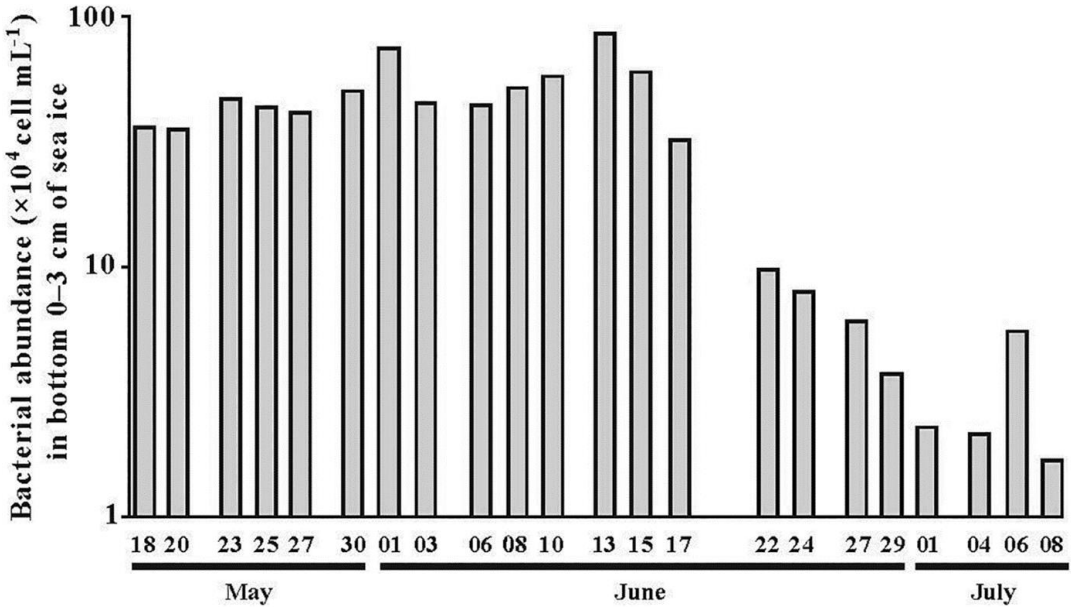

Figure 2.4: Time series of bacterial abundance in sea ice. Bacterial abundance in the bottom (0–3 cm) sea-ice section from May 18 to June 29, 2016, at the sampling location in Davis Strait (Figure 2.1), where the value for each date represents 3–4 pooled sea-ice sections. Chlorophyll peaks occurred on 1 and June 15, 2016, and concentrations of bactericidal palmitoleic acid increased thereafter.

3.2. Bacterial abundance, stress signals, and viability

Bacterial abundances in the bottommost 3 cm of sea ice increased slowly during May and most of June, with peaks of 7.7 × 10^5 and 9.0 × 10^5 bact mL^{-1} on June 1 and June 13, respectively (Figure 2.4), corresponding to the two maxima observed in Chl a

concentration (Figure 2.2C). Thereafter, bacterial abundance dropped relatively rapidly to 0.4 × 10^4 bact mL^{-1} by the end of the sampling period (Figure 2.4). Overall, bacterial abundance correlated well with Chl a concentration ($r = 0.86$, $P < 0.001$).

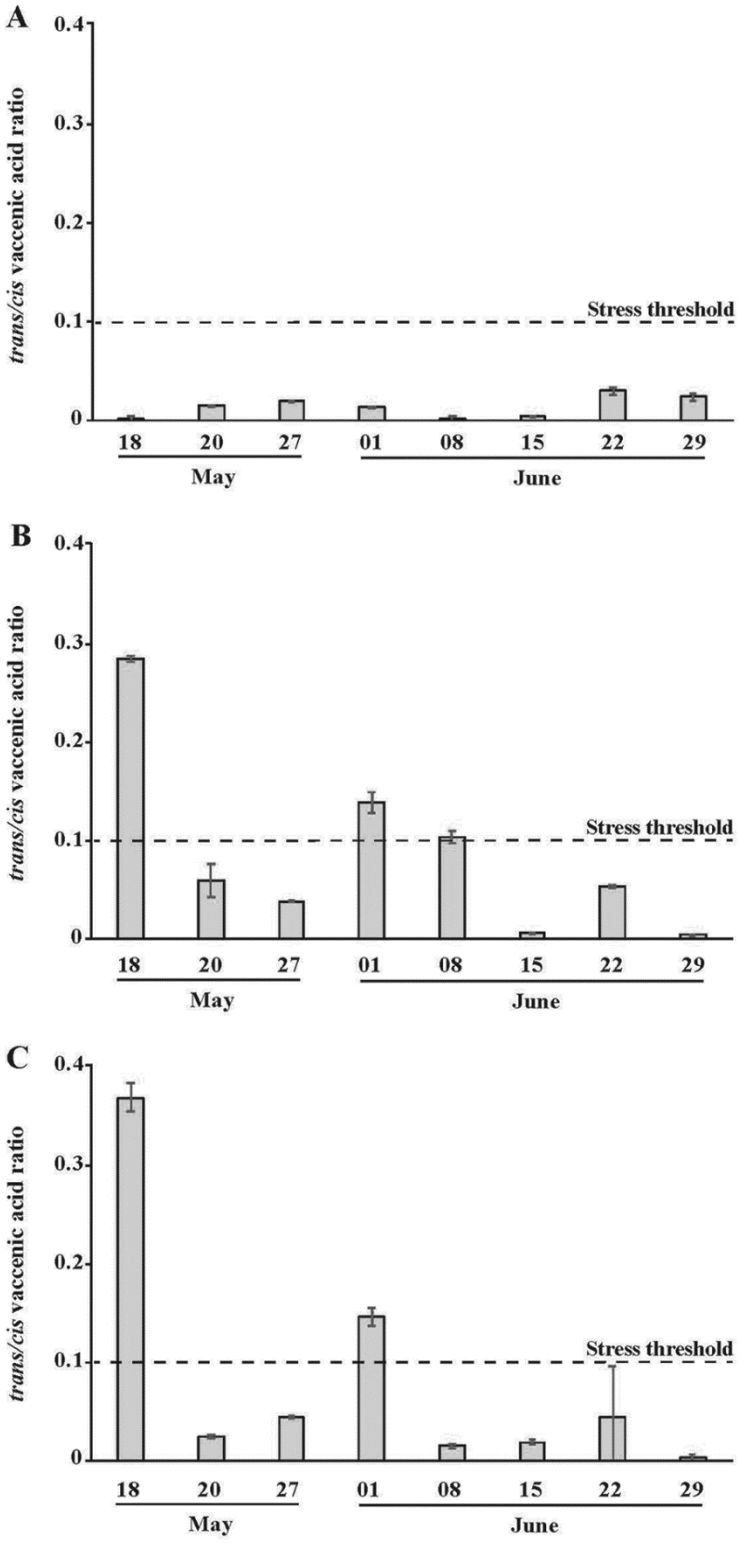

Figure 2.5: Time series of bacterial osmotic stress signals in sea ice and sinking particles. Time series of trans/cis ratios in (A) the bottom (0–3 cm) sea-ice section, and sinking particles collected at (B) 2 m and (C) 25 m from May 18 to June 29, 2016, at the sampling location in Davis Strait (Figure 2.1). The dashed line indicates the threshold stress value of 0.1 as defined by Guckert et al. (1986). Error bars correspond to standard deviation of the dimethyldisulphide (DMDS) derivative peak integration (see text; n = 3 for A, B, and C).

The trans/cis vaccenic acid ratios measured in the bottommost 3 cm of sea ice across the study period fell well below the threshold stress value of 0.1 (mean ± SD = 0.01 ± 0.01,

n = 8; Figure 2.5A), indicating no stress. In contrast, ratios measured for sinking particles collected at 2 m and 25 m included values from two sampling dates that were clearly above the threshold stress value: 0.29 and 0.36, respectively, on May 18, and 0.15 and 0.15, respectively, on June 1 (Figure 2.5B, C).

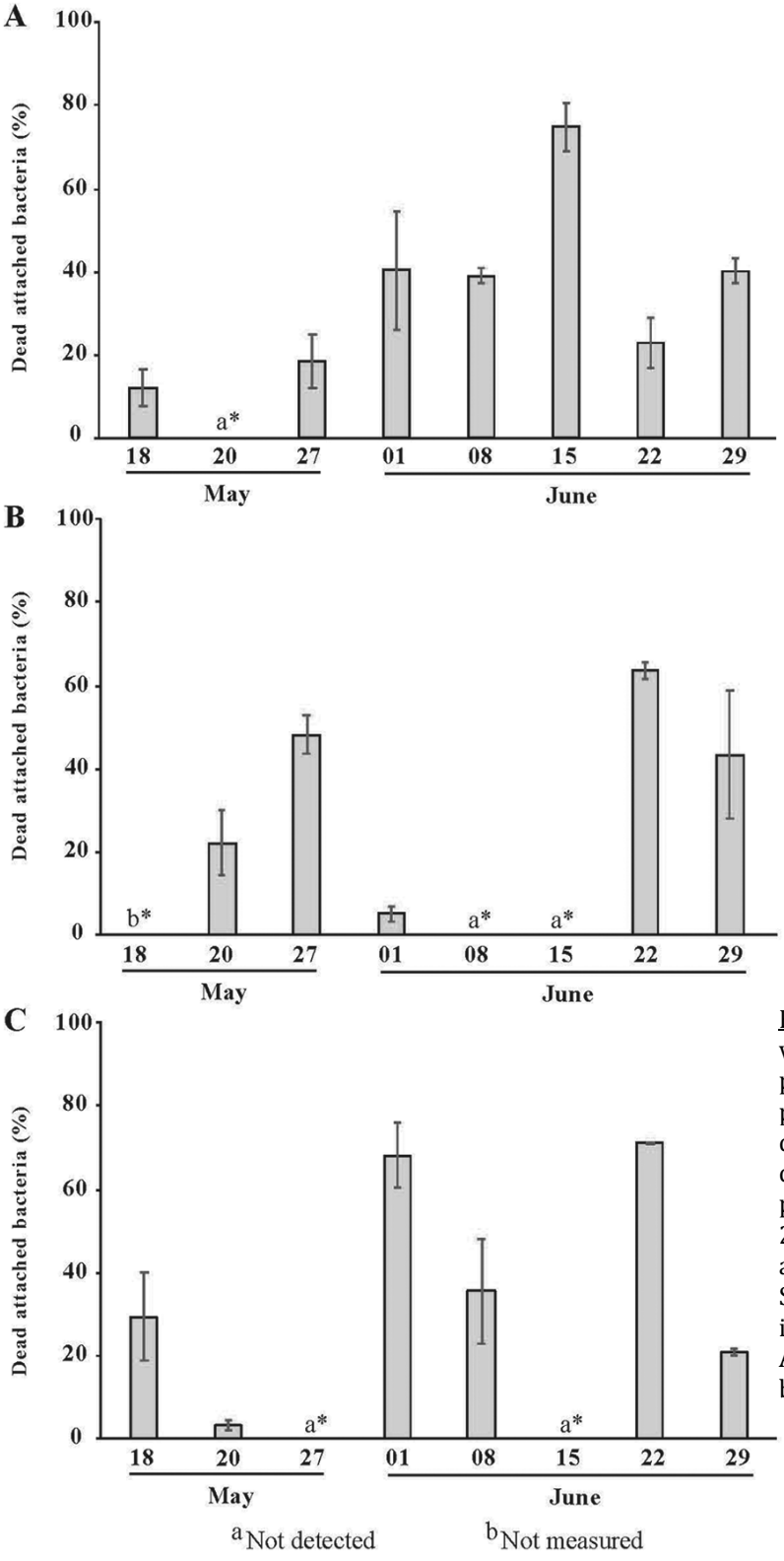

Figure 2.6: Time series of bacterial viability in sea ice and sinking particles. Time series of the dead percentage of attached bacterial communities in (A) the bottom (0–3 cm) sea-ice section, and in sinking particles collected at (B) 2 m and (C) 25 m from May 18 to June 29, 2016, at the sampling location in Davis Strait (Figure 2.1). Error bars indicate standard deviation (n = 2 for A, B, and C); a* indicates not detected; b* indicates not measured.

The viability of the attached bacterial community inhabiting sea ice appeared to be strong from May 18 to May 27, with only 0%–19% mortality, but weaker in June, with 23%–75% mortality (mean of 43.5 ± 17.0%, n = 10; Figure 2.6A; Table S2.1). In the 2-m and 25-m sediment trap samples, bacterial viability was highly variable across the sampling period, with mortality ranges of 0%–64% and 0%–71% at the 2-m and 25-m depths, respectively; Figure 2.6B; Table S2.1).

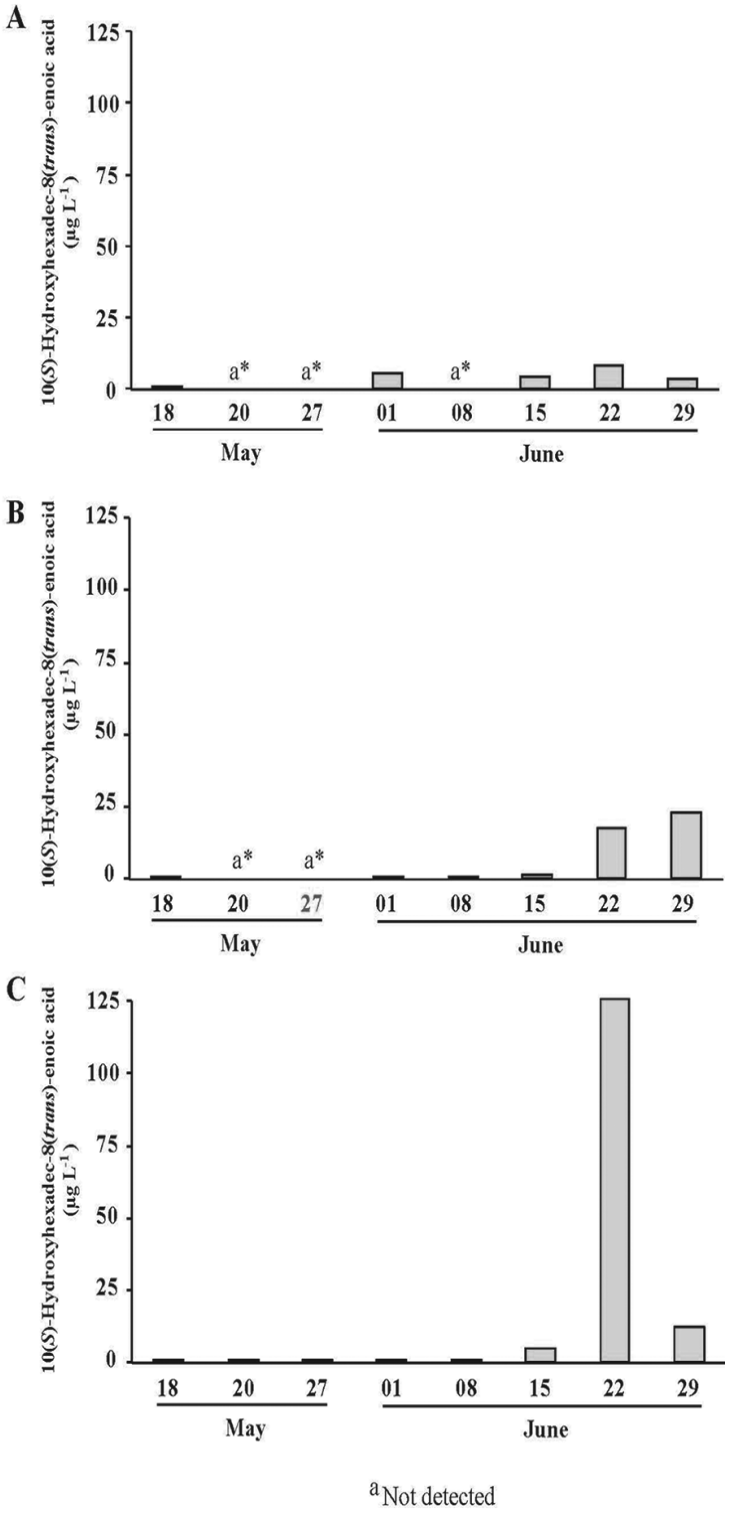

Figure 2.7: Time series of free fatty acid stress signals in sea ice and sinking particles. Time series of 10(S)-Hydroxyhexadec-8(trans)-enoic acid concentration in (A) the bottom (0–3 cm) sea-ice section and in sinking particles collected at (B) 2 m and (C) 25 m from May 18 to June 29, 2016, at the sampling location in Davis Strait (Figure 2.1); a* indicates not detected.

The fatty acid 10(S)-hydroxyhexadec-8(E)-enoic acid was quantifiable in most of the samples, whether of sea ice or sinking particles (Figure 2.7). In the sediment trap samples, higher values were observed at the end of the sampling period, on 22 and June 29. The highest concentration 10(S)-hydroxyhexadec-8(trans)-enoic acid was measured in the deepest (25 m) trap sample on June 22

3.3. Lipid composition

Fatty acid profiles of the sea-ice samples were dominated by $C_{16:1\Delta9}$ (palmitoleic) and $C_{16:0}$ (palmitic) acids; they also exhibited smaller proportions of $C_{14:0}$, $C_{18:1\Delta9}$, C18:0, and $C_{20:5}$ acids (Table S2.2). Similar fatty acids were also detected in the sediment trap samples, but in different proportions (Table S2.2). The palmitoleic/palmitic acid ratio was used to estimate the contribution of diatoms to the sinking material (Pedersen *et al.,* 1999; Reuss and Poulsen, 2002). Low values were observed at the beginning of sampling before increasing from June 8 onward, with similar values and temporal patterns observed for the two sampling depths (Figure 2.8). We noted that chromatograms of the lipid fraction of the sinking particles collected at 2 m (Figure S2.4) and 25 m on June 15 showed the presence of significant proportions of $C_{20:1\,\Delta\,11}$ and $C_{22:1\,\Delta\,11}$ alcohols and acids.

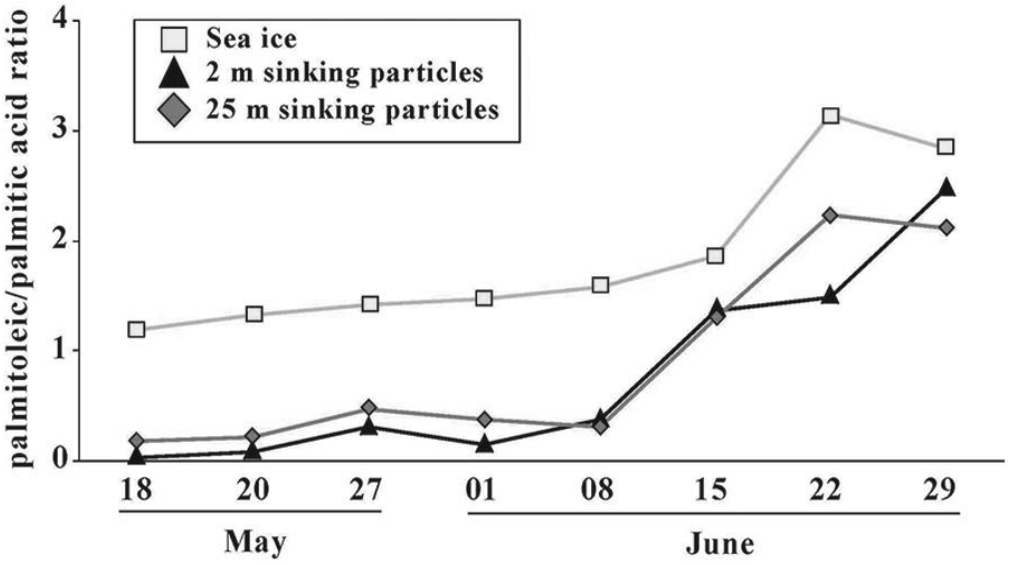

Figure 2.8: Time series of diatom biomarker in sea ice and sinking particles. Time series of palmitoleic/palmitic acid ratio in (A) the bottom (0–3 cm) sea-ice section and in sinking particles collected at (B) 2 m and (C) 25 m from May 18 to June 29, 2016, at the sampling location in Davis Strait (Figure 2.1).

The sterol compositions of the different samples were compared to evaluate spatial and temporal changes in algal diversity. We excluded cholesterol from this comparison because of its lack of specificity (Volkman, 1986, 2003) and the possible inputs of this

compound during collection and treatment of the samples (Grenacher and Guerin, 1994). Sea-ice samples appeared to be dominated by cholesta-5,24-dien-3β-ol (desmosterol), 24-methylcholesta-5,22(E)-dien-3β-ol (brassicasterol), and 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol); they also contained smaller proportions of 24-norcholesta-5,22(E)-dien-3β-ol, 24-norcholest-5-en-3β-ol, 24-ethylcholest-5-en-3β-ol (sitosterol), and cholesta-5,22(E)-dien-3β-ol (Figure 2.9A). These seven sterols were also present in sinking particles, but in different proportions (Figure 2.9B, C).

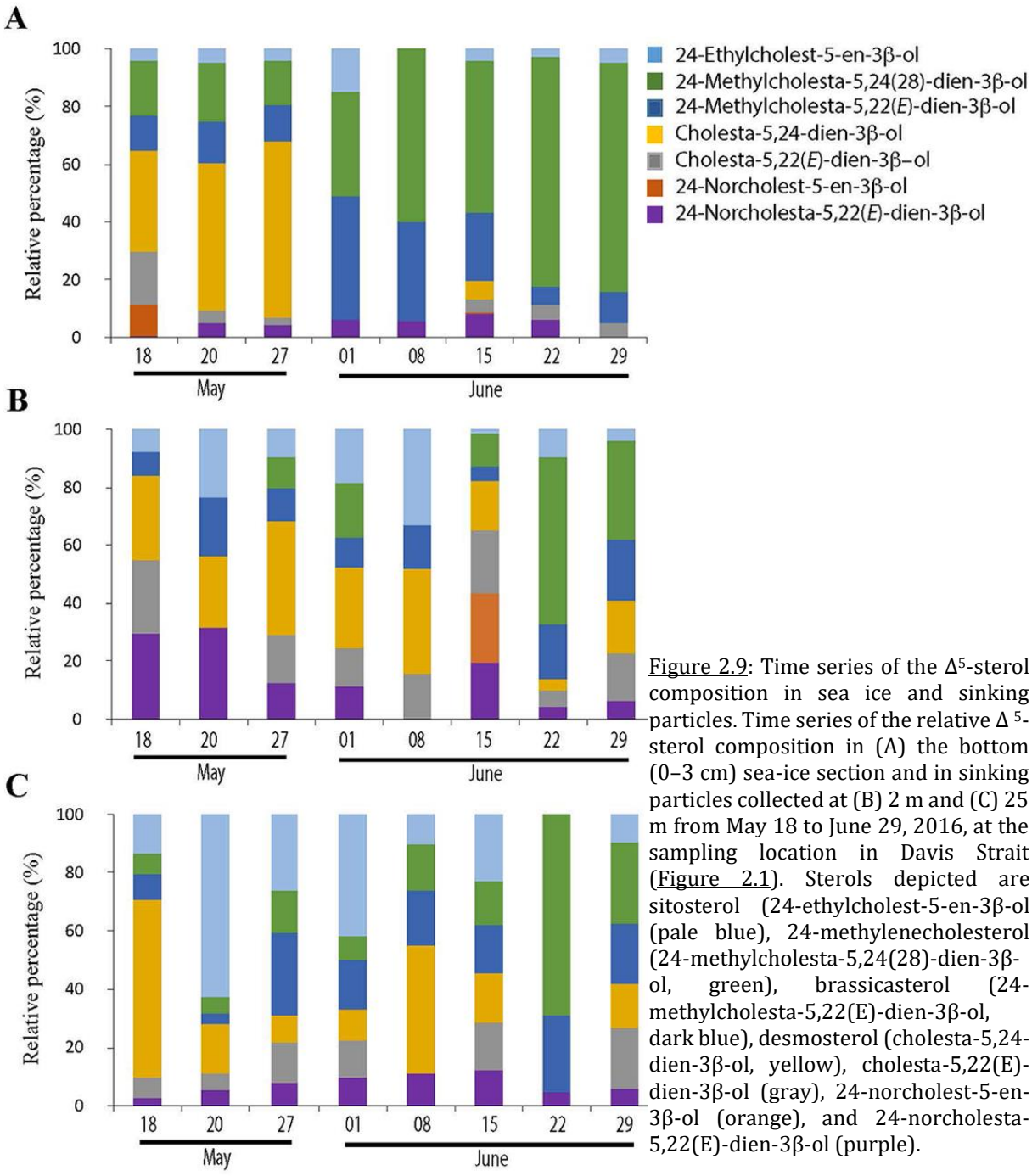

Figure 2.9: Time series of the Δ^5-sterol composition in sea ice and sinking particles. Time series of the relative Δ^5-sterol composition in (A) the bottom (0–3 cm) sea-ice section and in sinking particles collected at (B) 2 m and (C) 25 m from May 18 to June 29, 2016, at the sampling location in Davis Strait (Figure 2.1). Sterols depicted are sitosterol (24-ethylcholest-5-en-3β-ol (pale blue), 24-methylenecholesterol (24-methylcholesta-5,24(28)-dien-3β-ol, green), brassicasterol (24-methylcholesta-5,22(E)-dien-3β-ol, dark blue), desmosterol (cholesta-5,24-dien-3β-ol, yellow), cholesta-5,22(E)-dien-3β-ol (gray), 24-norcholest-5-en-3β-ol (orange), and 24-norcholesta-5,22(E)-dien-3β-ol (purple).

4. Discussion

4.1. Photoacclimation of sympagic algae

During the sampling period, the bottommost 3 cm of sea ice exhibited two periods of particularly high Chl a concentration centered on June 1 and 13 (Figure 2.2C; Amiraux *et al.,* 2019). These peaks occurred during a period of relatively stable salinity conditions in the bottommost centimeters of sea ice (Figure 2.2B and Figure S2.3). Light and nutrient availability are commonly accepted as the principal factors determining the onset, magnitude, and duration of blooms (Lavoie *et al.,* 2005; Campbell *et al.,* 2016). In light of our results, however, we suggest that the stability of sea-ice salinity may represent another important factor to consider. Although hyposaline conditions are known to significantly impact the sympagic algae (Gosselin *et al.,* 1986; Ralph *et al.,* 2007), the final release of sympagic algae in our study took place in July, well after the release of hyposaline meltwater had begun (Figure 2.2B) and was most likely due to melting sea ice (Figure 2.2A). The occurrence of the second chlorophyll maximum during an advanced snow melting stage (Figure 2.2A) is not surprising, as sympagic algae accumulate biomass rapidly when light conditions improve during snowmelt (Mock and Junge, 2007).

Along with Chl a concentrations, palmitoleic acid, the major fatty acid of sea-ice diatoms (Fahl and Kattner, 1993; Leu *et al.,* 2010), was also quantified throughout the sampling period. Unlike Chl a concentrations, however, palmitoleic acid remained relatively low through May and into June, with order-of-magnitude increases recorded only in the latter part of June, when a lag between the peaks of Chl a and palmitoleic acid was evident (Figure 2.2C). Although we cannot reject the possibility that spatial heterogeneity explains these results (the different measurements were made on different cores), the results are consistent with the involvement of photoacclimation processes during this period. Cells acclimated to high light usually exhibit low Chl a to C ratios, whereas low-light adapted cells have high ratios because they accumulate pigments to enhance their light absorption efficiency per unit of C biomass (Johnsen and Sakshaug, 1993). Based on the rapid disappearance of snow during the first part of June (Figure 2.2A) and the increase in irradiance (PAR) under ice from below 5 to ca. 40 µE m^{-2} s^{-1} by June 17 (Figure 2.3), the sympagic algae represented by the second chlorophyll maximum in mid-June (Figure 2.2C) would be expected to undergo photoacclimation to higher light intensities. The temporal evolution of the sympagic saturating irradiance photosynthetic parameter (Ek) in the bottommost 0–1 cm of sea ice during this period strongly supports this expectation.

Because the bottommost 0–1 cm section of sea ice receives less light than upper sections (e.g., light-shading from ice algae; Perovich, 1996), the increase in Ek measured in the 0–1 cm section from mid-June until the end of sampling (Figure 2.3) suggests that Ek in the 0–3 cm section would have been even higher. The photoacclimation result is also consistent with the photoacclimation of sympagic algae to higher light intensities, and the consequent reduction of their Chl a content per cell is consistent with the observed mismatch between Chl a and palmitoleic acid peaks (Figure 2.2C).

In algae, increasing light intensities also induce a decrease in their total polar lipid content, with a concomitant increase in the amounts of neutral storage lipids, the triacylglycerols (TAGs), and free fatty acids (FFAs; Brown *et al.,* 1996; Khotimchenko and Yakovleva, 2005; Hu *et al.,* 2008). The high irradiance resulting from the disappearance of snow cover on June 17 should thus induce a concomitant decrease in Chl a content per cell due to photoacclimation and an accumulation of TAGs and FFAs, including palmitoleic acid, as observed in Figure 2.2C.

4.2. Stress and resulting viability of the attached bacterial community in sea ice

In this study, we defined the term of attached bacteria on the basis of filtration using a filter pore size of 0.8 µm. This operational definition allows retention of most particle aggregates and their attached bacteria. However, free-living bacteria may also be retained as a consequence of filter clogging, with the fraction of retained free-living bacteria generally assumed to increase with the volume of sample filtered. Here, sample volumes of less than 40 ml were filtered with no evidence of clogging, so we assume that free-living bacteria were a negligible component in the analysis of bacteria called "attached."

Regarding total bacterial abundance in the bottommost 3 cm of sea ice, limited increases were observed during the sampling period associated with the chlorophyll maxima (Figure 2.4), and bacterial production was relatively weak (F. Joux, personal communication; GreenEdge meeting, 2019). Although hinting at a degree of stress to the bacterial community, such bulk measurements are less likely to reveal stress responses than more specific measurements targeting a subset of the community, in particular the attached bacteria.

Bacteria inhabiting brine channels in ice are exposed to highly variable salinity, pH, and dissolved inorganic nutrient concentrations (Thomas and Papadimitriou, 2003). One of the adaptive responses of bacteria to changes in salinity is to keep their membrane fluidity at a constant value through "homeoviscous adaptation" (Heipieper *et al.,* 2003), a process

facilitated by conversion of cis to trans unsaturated fatty acids (Loffeld and Keweloh, 1996). Indeed, a trans/cis fatty acid ratio >0.1 was proposed previously as an indicator of bacterial stress (Guckert *et al.,* 1986). The very low values of the trans/cis ratio of vaccenic acid, a fatty acid specific to bacteria (Sicre *et al.,* 1988), observed in our sea-ice samples (Figure 2.5A) suggest that bacteria attached to sympagic algae in the bottommost sections of the ice were not experiencing osmotic stress during the sampling period. This interpretation is consistent with the stable brine salinities of that layer of sea ice throughout the sampling period (Figure 2.2B). Even higher in the ice, brine salinities were not extreme (maximum of ca. 43, on the first day of sampling; Figure 2.2B). Consequently, the high mortality measured during June (mean of 44%), using the PMA method coupled with qPCR (Figure 2.6A), must be attributable to a different stress.

A role for viruses in the mortality of sympagic bacteria cannot be excluded and may have been likely (Maranger *et al.,* 1994); however, the mortality we observed was based on the percentage of intact cells that were nonfunctional (dead) due to compromised membranes. Bacteria lysed (no longer intact) by viruses would not have been included in these percentages. Bacterial mortality associated with sympagic algae could also result from the algal release of toxic compounds such as acrylic acid (degradation product of DMSP; Monfort *et al.,* 2000) or FFAs (Desbois and Smith, 2010). However, concentrations of DMSP and dimethyl sulfide (DMS; degradation product of DMSP; Lizotte *et al.,* 2017) in sea ice are generally well correlated with Chl a concentrations (Tison *et al.,* 2010). An induction of acrylic acid-induced stress when Chl a concentration was low (Figure 2.2C) thus seems unlikely, nor did the presence of Phaeocystis, a known producer of copious amounts of DMSP, dimethylsulfide and acrylic acid, appear sufficient to influence the bacterial community (reaching only 1.5% and 3.6% of the protist community on June 20 and 29, respectively; M. Babin, personal communication; GreenEdge meeting, 2019).

Instead, the presence of FFAs, produced by sympagic algae as a significant proportion of their lipid content (Falk-Petersen *et al.,* 1998) which can be amplified during late bloom conditions (Smith *et al.,* 1993) or under high irradiation (Hu *et al.,* 2008), could be at the origin of this stress. FFAs are released from cellular lipids by host lipolytic enzymes (Jüttner, 2001; Wichard *et al.,* 2007). They are toxic for many bacteria owing to their deleterious effect on bacterial cellular membranes (Greenway and Dyke, 1979; Chamberlain *et al.,* 1991). They can also inhibit enzyme activity, disrupt electron transport chains, and uncouple oxidative phosphorylation (reviewed by Desbois and Smith, 2010).

In general, unsaturated FFAs tend to have greater antibacterial potency than saturated FFAs with the same carbon chain length (Desbois *et al.,* 2009), and the most potent usually have 14 or 16 carbon atoms (Feldlaufer *et al.,* 1993). Free palmitoleic acid, a monounsaturated 16-C FFA, displays strong bactericidal action against Gram-negative marine bacterial pathogens, as reported by Desbois et al. (2009). Sympagic algal production of this FFA (stimulated by increasing irradiance) could thus contribute to the high mortality (approximately 75%) of attached bacteria observed on June 15 (Figure 2.6A).

To test this hypothesis, we quantified free palmitoleic acid in samples collected on June 1 and 15 during the two chlorophyll peaks. The concentrations measured (0.3 and 4.8 mg L^{-1}, respectively) support elevated bactericidal activity during the second chlorophyll peak. Although the basic nature of seawater has been proposed to enhance the solubility and thus toxicity of FFAs (Parrish, 2013), the effect may be stronger in sea-ice brines where the pH varies between 8.0 and 8.5 (Hare *et al.,* 2013). The proximity of bacteria to sympagic algae cells (attached bacteria) would also make them particularly susceptible to the antibacterial activity of free palmitoleic acid. Indeed, FFAs released by host lipolytic enzymes from a microalgal cell generally act on bacterial pathogens in the local vicinity (Desbois and Smith, 2010).

4.3. Stress and resulting viability of attached bacteria in sinking particles

The algal material present in sinking particles collected by the sediment traps was less than the material present in bottom sea ice, based on Chl a concentrations (<1.2 μg L^{-1} until June 24 near the end of the time series, as reported by Amiraux *et al.,* 2019). The presence of diatoms, the dominant taxa of sympagic and planktonic blooms in the Arctic (Sakshaug, 2004; Mikkelsen *et al.,* 2008; Tremblay *et al.,* 2012), could be estimated using the palmitoleic/palmitic acid ratio, however, as employed by others to follow diatom blooms (Pedersen *et al.,* 1999; Reuss and Poulsen, 2002). Our results showed an increase in diatoms in sinking particles from early June onward (Figure 2.8). Concentrations of IP25, the specific tracer of sympagic diatoms (Belt and Müller, 2013; Belt, 2018) measured in the same samples (Amiraux *et al.,* 2019), supported sea ice as the source of this sinking material. Amiraux et al. (2019) suggested that the flush of sympagic algae into the water column resulted from shifts in the salinity of sea-ice brines likely induced by the downward percolation of melted snow and, from June 24, to melting of the sea-ice skeletal layer (Figure 2.2A, B). The resulting hyposaline stresses, known to reduce sympagic algal

growth and survival (Gosselin *et al.,* 1986), would favor their release from the ice as well as their potential agglomeration (Riebesell *et al.,* 1991).

High trans/cis vaccenic acid ratios were previously observed in sinking particles collected a year earlier during the 2015 GreenEdge campaign (Amiraux *et al.,* 2017). These high values were attributed to the release of sympagic algae with attached bacteria stressed by hypersaline brines during the early stages of sea ice melting. In the present study, the investigation of this ratio showed high values only on the first day of the time series (May 18; Figure 2.5B, C) when brine salinity was also highest (Figure 2.2B). The relatively high trans/cis ratios measured at the start of the time series in the 3–10 cm section of ice (approximately 0.15, above the threshold indicative of stress; Figure S2.5) suggests that sinking particles derived from brine channels during the beginning of the melting season. During the latter part of the time series (June 15–29) when most ice-algal material was released and settling, the trans/cis ratio remained very low, attesting to the lack of salinity stress (or other stressors). We thus attribute the high mortality of attached bacteria observed in sinking particles from the last two sampling dates in June (Figure 2.6B, C) to the presence of free palmitoleic acid at depth. Unfortunately, the fraction of sediment trap material available for lipid analyses was too low to allow quantification of FFAs, including palmitoleic acid. However, the likely presence of an elevated proportion of FFAs in the sinking material is supported by the increase in 10(S)-hydroxyhexadec-8(trans)-enoic acid concentration observed on June 22 (Figure 2.7), when most of the sinking material appeared to be composed of sympagic material (based on IP25; Amiraux *et al.,* 2019). Fatty acids are thought to bind to 10S-DOX-like lipoxygenase with their carboxyl groups at a fixed position relative to the catalytic site, allowing the enzyme to contribute to the detoxification of fatty acids in the bacterial environment (Martínez *et al.,* 2010). The increase in 10(S)-hydroxyhexadec-8(trans)-enoic acid concentration observed with depth (Figure 2.7), indicative of 10S-DOX-like lipoxygenase activity, is consistent with the proposed elevated proportions of free palmitoleic acid in sinking particles.

Because the highest sinking fluxes of sympagic algae were observed from June 15 onward (Amiraux *et al.,* 2019), we analyzed the samples from this period in greater depth. The mortality of attached bacteria in sinking particles collected on June 22 and 29 was clearly elevated (Figure 2.6B, C), yet no mortality was detected in the sediment trap samples collected on June 15. The high mortality in sea ice observed on this date was not

matched by enhanced mortality in sinking particles. This discrepancy could be attributed to (1) a bulk dilution of dead bacteria associated with sympagic algae by living bacterial inputs (i.e., bacteria associated with phytoplankton or copepod fecal pellets) or (2) a lag in sedimentation.

To estimate the relative contribution of sympagic algae, phytoplankton, and zooplankton to these samples, Δ^5-sterol compositions of corresponding sea-ice and sinking particle samples were compared (Figure 2.9). Three sterol profiles were observed in the sea ice during the time series. The first profile between May 18 and 27 was characterized by the dominance of desmosterol (Figure 2.9) and cholesterol (data not shown), suggesting the presence of sympagic amphipods (Harvey *et al.,* 1987) known to live in sea-ice brine channels (Macnaughton *et al.,* 2007). The second, observed from June 1 to 8, was mainly composed of pennate diatoms (dominated by Nitzschia frigida; B Queguiner, personal communication; GreenEdge meeting, 2019) as suggested by the dominance of brassicasterol and 24-methylenecholesterol (Rampen *et al.,* 2010). The third community, from June 15 to 29, showed an increasing contribution of centric diatoms (Melosira arctica), as suggested by the dominance of 24-methylenecholesterol (Rampen *et al.,* 2010). The similarities of sterol profiles observed in sea-ice and sediment traps collected on June 22 attest to the dominance of sympagic algae in sinking particles, as well as their rapid descent (Amiraux *et al.,* 2019).

In contrast, the sterol compositions of trap samples collected on June 15 and 29 differed from those of the corresponding sea-ice samples by their higher contents of 24-norcholesta-5,22(E)-dien-3β-ol, 24-norcholest-5-en-3β-ol, desmosterol and cholesta-5,22(E)-dien-3β-ol) (Figure 2.9). These differences suggested the presence of high and moderate proportions of phytoplankton in trap samples collected on June 15 and 29, respectively. On the basis of number of epiphytic bacteria per algal cell, generally one order of magnitude higher for ice diatoms (up to 25 in Arctic sea ice; Smith *et al.,* 1989) compared to phytoplankton (<2; Kaczmarska *et al.,* 2005), a bulk dilution of bacteria attached to sympagic algae by unstressed bacteria attached to phytoplankton seems unlikely on June 15. This conclusion is supported by the relatively high bacterial mortality observed in sinking particles collected later, on June 29, which were composed, at least in part, of phytoplankton (under-ice bloom from June 24; Amiraux *et al.,* 2019).

Lipid extracts of sinking particles collected on June 15 showed the presence of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alcohols and acids (Figure S2.4), known to occur in herbivorous copepods that undergo diapause (Graeve *et al.,* 1994), and a high proportion of sterols relative to fatty

acids (Figure 2.4), as often observed after copepod feeding on phytoplankton (Bradshaw *et al.,* 1991). These results suggest that the samples collected on June 15 contained copepod fecal pellets, a favorable environment for bacterial growth; indeed, incubation of copepods collected in the water column near this date exhibited maximum production of fecal pellets (Sampei *et al.,* submitted). Because fecal pellets increase the sedimentation rate of particulate matter (Small *et al.,* 1979), the lack of bacterial mortality in sinking particles collected on June 15 seems unlikely to be due to a lag in sedimentation. We thus attribute the lack of detectable bacterial mortality in sinking particles from this date to bulk dilution of stressed bacteria attached to sympagic algae by fecal pellet-colonizing bacteria.

4.4. Impact of bacterial stress on the preservation of sympagic material

Following the use of cis-trans isomerase activity as an urgent response to guarantee survival against stress, bacteria replace this short-term activity with other adaptive mechanisms (Heipieper *et al.,* 2007). Consequently, in the absence of osmotic stress in the water column (after release from exposure to any higher brine salinity in sea ice), the trans/cis ratio of salinity-stressed bacteria associated with sympagic algae should decrease to the basic level (as for other bacteria; Fischer *et al.,* 2010). If the conversion of trans to cis fatty acids is no longer being catalysed (Eberlein *et al.,* 2018), then recovery to the regularly low trans/cis ratio requires de novo synthesis of cis fatty acids, which is dependent on bacterial growth. The stability of the trans/cis ratio previously observed associated with sinking particles during the 2015 vernal melting period at our same sampling site was thus attributed to the nongrowing state of attached bacteria (Amiraux *et al.,* 2017). The high trans/cis ratios measured in sinking particles collected in this study on May 18, 2016 (0.29 and 0.36 at 2-m and 25-m depth, respectively; Figure 2.5 B, C), again points to the nongrowing state of bacteria attached to sinking material during the early stages of ice melt. Most of these attached bacteria remained viable (Figure 2.4), as PMA did not enter the cells to bind to DNA and thus inhibit PCR amplification (Nocker *et al.,* 2006).

In contrast, by the end of the melting season, the PMA technique showed that most of the bacteria associated with sympagic algae had disrupted membranes and were considered dead. Because osmotic stress appeared to be lacking at this time, free palmitoleic acid produced by the ice algae can explain the strongly altered membranes of

these bacteria. The insertion of FFAs into the inner bacterial membrane increases its permeability, allowing internal contents to leak from the cell, which can result in growth inhibition or death (Shin *et al.,* 2007).

Overall, our study suggests that the bacterial community associated with sympagic algae is exposed to multiple stresses during most of the vernal Arctic melting season. This exposure reduces their viability, as indicated by the nonreversible loss of membrane integrity, thereby limiting their potential to remineralize sympagic algal material. We hypothesize that the fate of sympagic algae during a complete melting cycle of the ice occurs in successive steps as follows: (1) During the beginning of ice melt, a limited amount of sympagic algae is discharged into the water column, and bacteria attached to the sinking algae are nongrowing due to osmotic stress from prior exposure to hypersaline sea-ice brine. (2) At the end of snow cover melt, sympagic algae bloom, followed by a photoacclimation phase that induces the production of bactericidal FFAs. (3) The downward percolation of melted snow generates hyposaline conditions that cause sympagic algae to aggregate. (4) The aggregated sympagic algae flush into the water column, with their attached bacteria strongly impacted by bactericidal FFAs, which may even increase with depth.

5. Conclusions

During this work, we evaluated the viability of bacteria in sea ice and in sinking particles over the course of a spring ice melt season, from May 18 to June 29, 2016, in Davis Strait, Canadian Arctic. In mid-June, just after the second of two chlorophyll peaks in the ice, an intense production of palmitoleic acid, mainly esterified in TAGs but also present in free form, was detected and attributed to the effects of excess irradiance induced by the complete disappearance of snow cover. The viability of the attached bacterial community in sea ice, quantified by the PMA approach coupled with qPCR, showed high mortality (up to 75%, mean of 44%) toward the end of ice melting. We attribute the mortality of these attached bacteria to the bactericidal properties of free palmitoleic acid released by sympagic algae under the effect of light stress. Osmotic stress to bacteria in sea-ice brines appeared to be limited to the beginning of ice melt, which could be tested in future by applying our suite of measurements to sea ice prior to the melting season. Due to their propensity for strong aggregation (shortening residence time within the water column) and the high mortality of their attached bacteria (limiting

remineralization), sympagic algae should contribute importantly to the export of carbon to Arctic sediments.

2.2.3 Informations supplémentaires

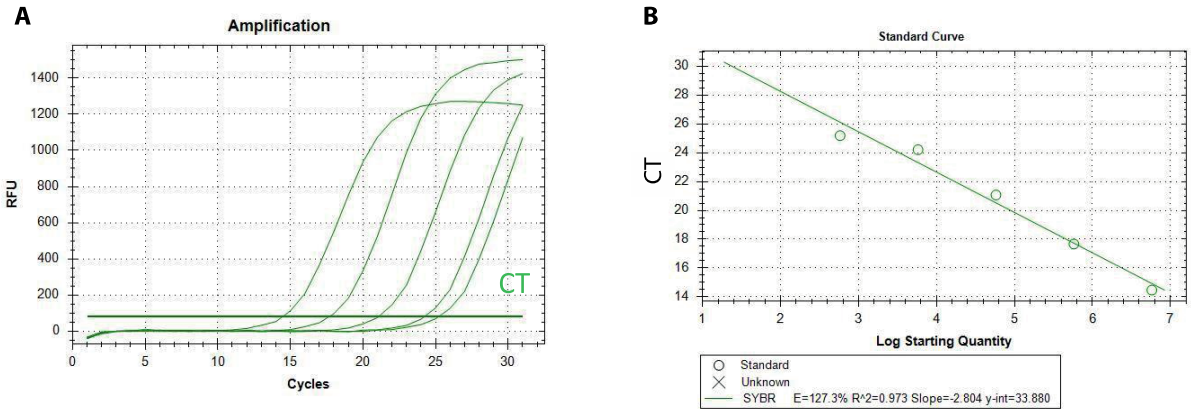

Figure S2.1: qPCR calibration curves for determining the number of bacterial 16S rDNA genes**.** Example of (A) relative fluorescence vs. cycle number. Amplification curves are created when the fluorescent signal from each sample is plotted against cycle number; therefore, amplification plots represent the accumulation of product over the duration of the real-time PCR experiment. The samples used to create the plots are a dilution series of the target DNA sequence. Example of (B) a standard curve of real-time PCR data. A standard curve shows threshold cycle (Ct) on the y-axis and the starting quantity (SQ) of DNA target on the x-axis. Slope, y-intercept, and correlation coefficient values are used to provide information about the performance of the reaction.

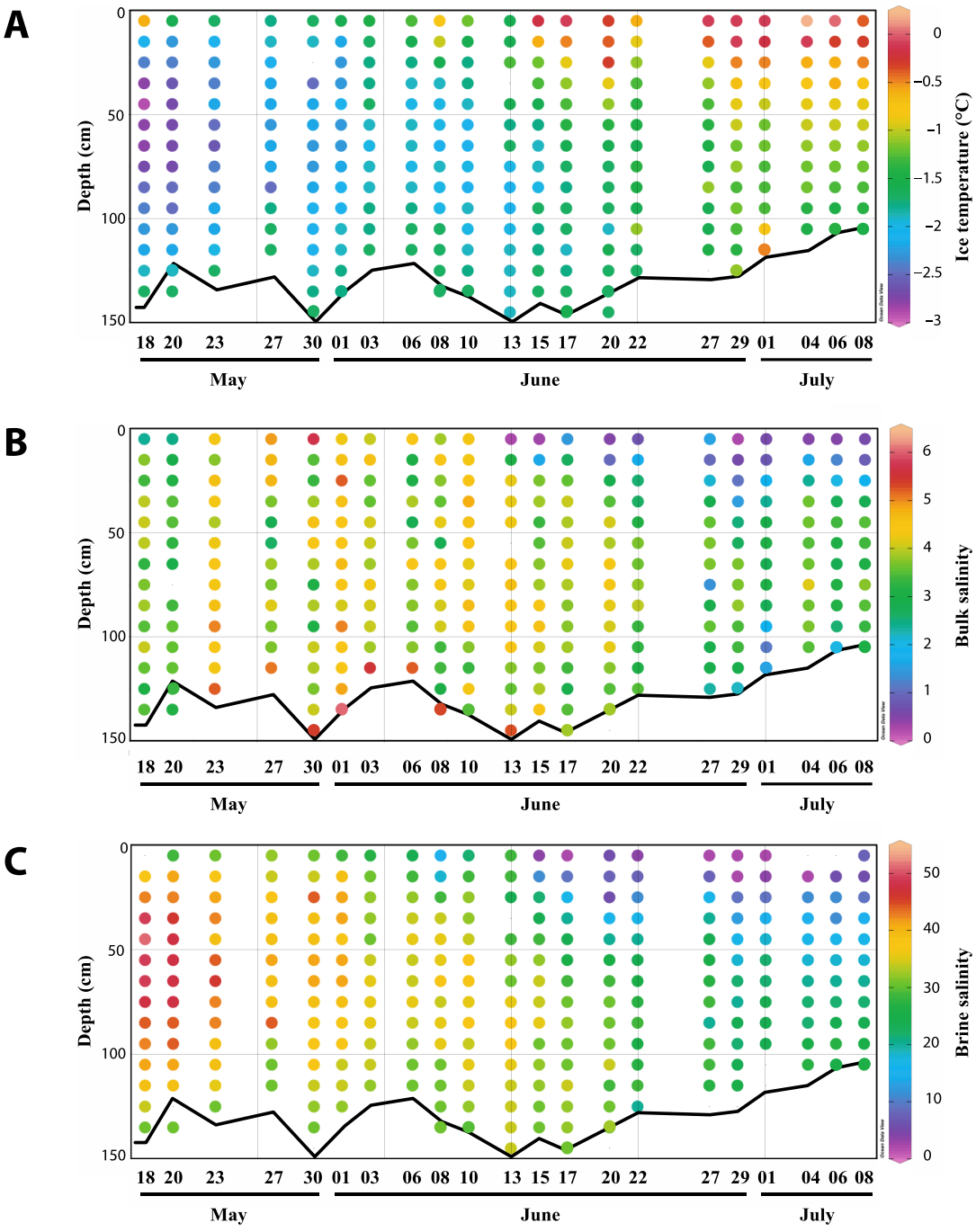

Figure S2.2: Temporal evolution of sea-ice properties. Time series of (A) sea-ice temperature, (B) bulk salinity, and (C) brine salinity as a function of depth, where mean sea-ice thickness is indicated by the black line (adapted from Oziel *et al.,* 2019).

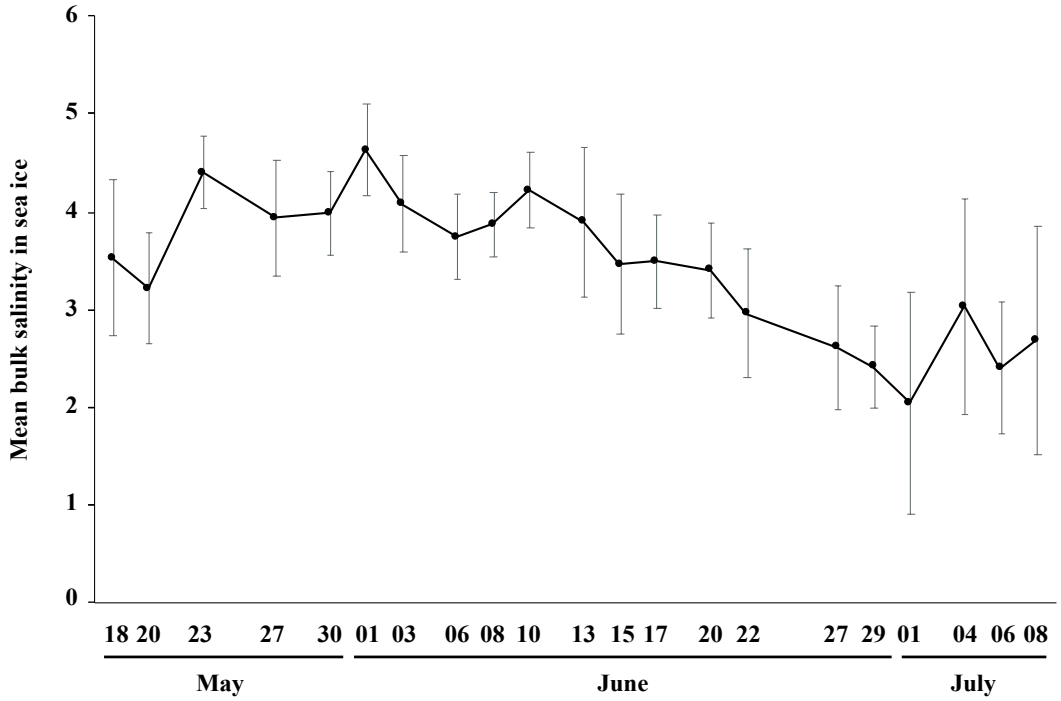

Figure S2.3: Temporal evolution of mean bulk salinity in sea ice. Time series of mean bulk salinity in the whole sea-ice core. Error bars are standard deviation of the mean (n = 11–15).

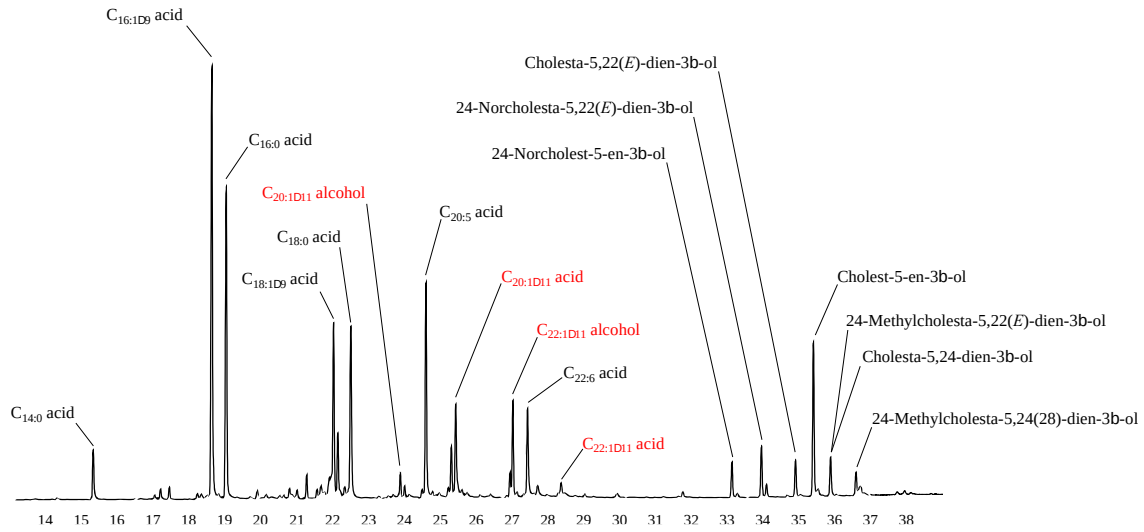

Figure S2.4: TIC chromatogram of the total lipid extract in sinking particles. TIC chromatogram of the total lipid extract of sinking particles collected at 2 m on 15 June, showing the presence of C20:1Δ11 and C22:1Δ11 alcohols and acids.

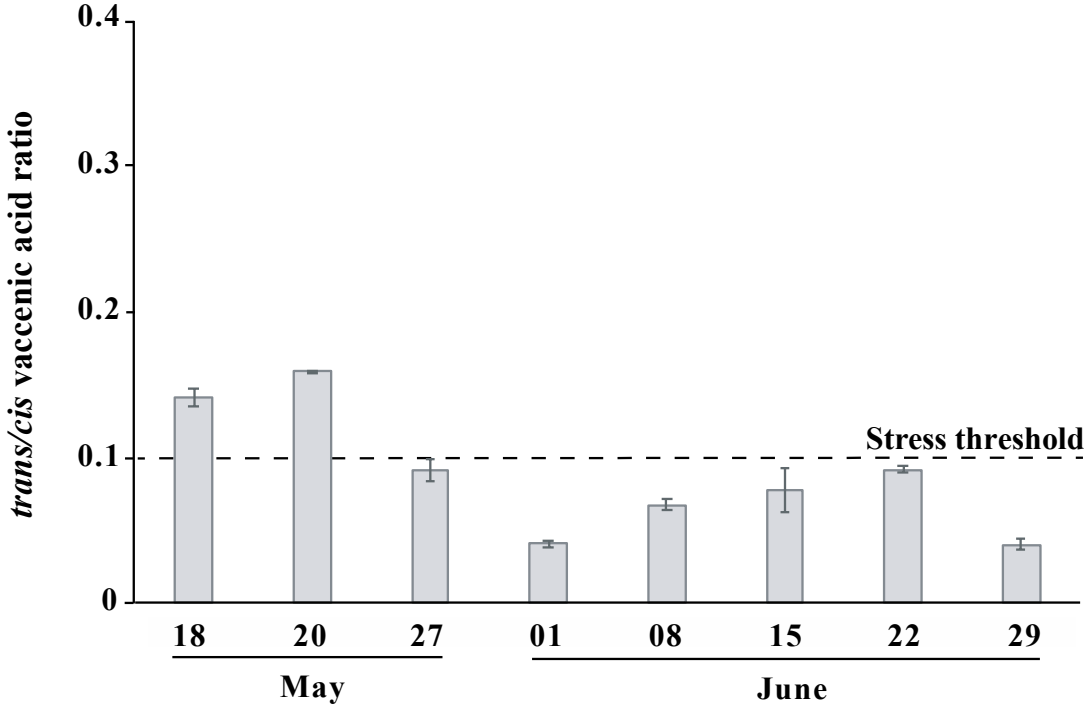

Figure S2.5: Time series of bacterial osmotic stress signals in the near-bottom 3–10 cm sea-ice section. Time series of *trans/cis* ratios in the near-bottom 3–10 cm sea-ice section from 18 May to 29 June 2016 at the sampling location in Davis Strait (Figure 2.1). The dashed horizontal line indicates the threshold stress value of 0.1 as defined by Guckert et al. (1986). Error bars represent the standard deviation of the dimethyldisulphide (DMDS) derivative peak integration (n = 3).

Table S2.1: qPCR raw data for determination of the percentage of dead bacteria.

Sample type	Date in 2016 (day/month)	SQ-PMA[a]	SQ-noPMA[b]	Dead bacteria (%)	Mean dead bacteria (%)	SD
Sea ice (bottom 0–3 cm)	18-mai	9.66×10^{1}	1.16×10^{2}	16.5	12.2	4.3
		2.84×10^{2}	3.09×10^{2}	7.9		
	20-mai	1.15×10^{2}	1.09×10^{2}	−5.5	−7.6[c]	2.1
		1.36×10^{2}	1.24×10^{2}	−9.6		
	27-mai	1.11×10^{2}	1.48×10^{2}	25.1	18.6	6.5
		5.96×10^{2}	6.79×10^{2}	12.1		
	01-juin	1.54×10^{2}	3.41×10^{2}	54.7	40.4	14.3
		1.91×10^{2}	2.58×10^{2}	26.1		
	08-juin	8.81×10^{1}	1.40×10^{2}	37.2	39.1	1.8
		9.08×10^{1}	1.54×10^{2}	40.9		
	15-juin	8.06×10^{1}	4.19×10^{2}	80.8	74.9	5.9
		8.38×10^{1}	2.70×10^{2}	69		
	22-juin	1.15×10^{2}	1.61×10^{2}	28.8	22.8	6
		1.16×10^{2}	1.40×10^{2}	16.8		
	29-juin	8.02×10^{1}	1.41×10^{2}	43.2	40.2	3
		1.70×10^{2}	2.70×10^{2}	37.2		
Sediment trap (2-m depth)	20-mai	2.22×10^{1}	3.18×10^{1}	30.1	22.2	7.9
		1.64×10^{1}	1.91×10^{1}	14.4		
	27-mai	1.93×10^{1}	4.11×10^{1}	53	48.3	4.7
		2.49×10^{1}	4.42×10^{1}	43.6		
	01-juin	3.83×10^{1}	3.96×10^{1}	3.3	5.2	1.8
		5.14×10^{1}	5.53×10^{1}	7		
	08-juin	1.51×10^{1}	1.40×10^{1}	−8.0	−5.0	2.9
		8.74×10^{0}	8.56×10^{0}	−2.1		
	15-juin	5.99×10^{1}	5.70×10^{1}	−5.1	−5.3	0.2
		1.15×10^{2}	1.09×10^{2}	−5.5		
	22-juin	1.10×10^{1}	2.88×10^{1}	61.9	63.9	2
		4.11×10^{1}	1.20×10^{2}	65.9		
	29-juin	1.50×10^{2}	3.66×10^{2}	58.9	43.5	15.4
		3.81×10^{1}	5.31×10^{1}	28.2		
Sediment trap (25-m depth)	18-mai	1.43×10^{1}	2.19×10^{1}	34.7	29.5	5.2
		3.66×10^{1}	4.84×10^{1}	24.3		
	20-mai	6.24×10^{0}	6.48×10^{0}	3.8	3.2	0.6
		2.19×10^{1}	2.25×10^{1}	2.6		
	27-mai	1.48×10^{1}	1.43×10^{1}	−3.3	−2.9	0.3
		2.60×10^{1}	2.53×10^{1}	−2.6		
	01-juin	2.80×10^{1}	1.01×10^{2}	72.2	68.3	3.9
		5.06×10^{1}	1.42×10^{2}	64.3		
	08-juin	6.08×10^{1}	1.05×10^{2}	41.8	35.6	6.3
		5.80×10^{1}	8.19×10^{1}	29.3		
	15-juin	2.22×10^{2}	2.16×10^{2}	−2.9	−2.2	0.7
		3.13×10^{2}	3.08×10^{2}	−1.5		
	22-juin	6.61×10^{0}	2.30×10^{1}	71.2	71.3	0.1
		3.09×10^{1}	1.08×10^{2}	71.3		
	29-juin	5.24×10^{1}	6.65×10^{1}	21.2	20.9	0.3
		7.07×10^{1}	8.90×10^{1}	20.6		

[a] SQ-PMA indicates the starting quantity (SQ) of the number of targeted genes in the PCR mix before amplification of DNA extracted from samples treated with propidium monoazide (PMA).
[b] SQ-noPMA indicates the SQ of the number of targeted genes in the PCR mix before amplification of DNA from samples not treated with PMA.
[c] Negative values are reported in the main text as 0%.

Table S2.2: Relative fatty acid composition of sea ice and sediment trap samples.

Date in 2016 (day/month)	Sample type[a]	Relative fatty acid composition (% of total)											
		$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:4}$	$C_{16:1w7}$	$C_{16:0}$	$C_{18:1w9}$	$C_{18:1w7}$	$C_{18:0}$	$C_{20:5}$	$C_{20:1}$	$C_{22:6}$
17/5	Ice 0–3 cm	2.9	17.9	2.1	–	30.3	33.9	4.5	1.5	5.1	1.8	–	–
	Trap 2 m	–[b]	1.1	–	–	1	26.4	5.5	1	65	–	–	–
	Trap 25 m	1	6.9	3	–	7.9	37.9	4.5	1.4	18.7	18.7	–	–
20/5	Ice 0–3 cm	1	11.1	1.3	2.7	36.5	26.6	4.4	1.6	0.6	13.3	–	0.9
	Trap 2 m	1.9	8.2	3.1	–	5.3	53.1	2.9	–	25.5	–	–	–
	Trap 25 m	1.3	6.4	3.6	–	8.7	38	7.8	1	28	5.2	–	–
27/5	Ice 0–3 cm	1.9	13.7	1.2	5.8	33.9	20.7	7	1.5	7.1	7.2	–	–
	Trap 2 m	1.1	6.2	1.6	–	14.9	42.3	6.1	0.9	26.9	–	–	–
	Trap 25 m	1.5	11	2	–	21.6	43.4	4.2	1.2	15.1	–	–	–
1/6	Ice 0–3 cm	–	6.3	–	–	39.2	32.3	–	–	–	19.6	–	2.6
	Trap 2 m	1.5	6.6	2	–	8	42.2	11.1	0.8	27.8	–	–	–
	Trap 25 m	1.6	8	3	–	15.6	40.1	11.5	3.7	16.5	–	–	–
8/6	Ice 0–3 cm	–	17.2	0.8	–	50.7	28.9	1.3	0.5	0.6	–	–	–
	Trap 2 m	1.6	8.4	2.2	–	18.1	45.9	7.6	0.8	15.4	–	–	–
	Trap 25 m	1.5	11.1	3.5	–	18.1	46.8	7.6	1.9	9.5	–	–	–
15/6	Ice 0–3 cm	–	13.3	1.2	–	50.6	23.8	4.4	0.6	2.1	4	–	–
	Trap 2 m	0.2	3	0.6	–	26.5	18.7	10.3	3.6	10.9	12.2	8.8	5.2
	Trap 25 m	0.8	17.8	1.9	–	41.1	30.5	3	1	3.4	–	0.5	–
22/6	Ice 0–3 cm	–	14.1	0.6	–	54.3	20.8	2.8	1.6	2.2	3.6	–	–
	Trap 2 m	–	7	1	–	54.2	37	0.6	–	0.2	–	–	–
	Trap 25 m	–	13.7	–	–	58.8	26.4	0.9	–	0.2	–	–	–
29/6	Ice 0–3 cm	–	9.7	0.8	–	60.2	24.6	1.6	1.4	1.7	–	–	–
	Trap 2 m	–	16.5	0.8	–	54.1	22.8	2.6	1	1.3	0.9	–	–
	Trap 25 m	–	12.9	0.8	–	54.3	27.9	2.1	0.8	1.2	–	–	–

[a] Sea ice samples from bottommost 0–3 cm; sediment trap samples from 2-m and 25-m depths

[b] Not detected

Bibliographie

Amiraux, R., Belt, S. T., Vaultier, F., Galindo, V., Gosselin, M., Bonin, P., & Rontani, J.-F. (2017). Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers. *Marine Chemistry*, *194*, 89–99. https://doi.org/10.1016/j.marchem.2017.05.006

Amiraux, R., Rontani, J.-F., Armougom, F., Frouin, E., Babin, M., Artigue, L., & Bonin, P. (2021). Bacterial diversity and lipid biomarkers in sea ice and sinking particulate organic material during the melt season in the Canadian Arctic. *Elementa: Science of the Anthropocene*, *9*(1). https://doi.org/10.1525/elementa.2019.040

Amiraux, R., Smik, L., Köseoğlu, D., Rontani, J.-F., Galindo, V., Grondin, P.-L., Babin, M., & Belt, S. T. (2019). Temporal evolution of IP_{25} and other highly branched isoprenoid lipids in sea ice and the underlying water column during an Arctic melting season. *Elementa Science of the Anthropocene*, *7*(1), 38. https://doi.org/10.1525/elementa.377

Assmy, P., Ehn, J. K., Fernández-Méndez, M., Hop, H., Katlein, C., Sundfjord, A., Bluhm, K., Daase, M., Engel, A., Fransson, A., Granskog, M. A., Hudson, S. R., Kristiansen, S., Nicolaus, M., Peeken, I., Renner, A. H. H., Spreen, G., Tatarek, A., & Wiktor, J. (2013). Floating Ice-Algal Aggregates below Melting Arctic Sea Ice. *PLOS ONE*, *8*(10), e76599. https://doi.org/10.1371/journal.pone.0076599

Babin, M., Morel, A., & Gagnon, R. (1994). An incubator designed for extensive and sensitive measurements of phytoplankton photosynthetic parameters. *Limnology and Oceanography*, *39*(3), 694–702. https://doi.org/10.4319/lo.1994.39.3.0694

Bates, S. S., & Cota, G. F. (1986). Fluorescence Induction and Photosynthetic Responses of Arctic Ice Algae to Sample Treatment and Salinity1. *Journal of Phycology*, *22*(4), 421–429. https://doi.org/10.1111/j.1529-8817.1986.tb02484.x

Belt, S. T. (2018). Source-specific biomarkers as proxies for Arctic and Antarctic sea ice. *Organic Geochemistry*, *125*, 277–298. https://doi.org/10.1016/j.orggeochem.2018.10.002

Belt, S. T., Brown, T. A., Smik, L., Assmy, P., & Mundy, C. J. (2018). Sterol identification in floating Arctic sea ice algal aggregates and the Antarctic sea ice diatom Berkeleya adeliensis. *Organic Geochemistry*, *118*, 1–3. https://doi.org/10.1016/j.orggeochem.2018.01.008

Belt, S. T., & Müller, J. (2013). The Arctic sea ice biomarker IP25: A review of current understanding, recommendations for future research and applications in palaeo sea ice

reconstructions. *Quaternary Science Reviews*, *79*, 9–25. https://doi.org/10.1016/j.quascirev.2012.12.001

Bidle, K. D., & Azam, F. (1999). Accelerated dissolution of diatom silica by marine bacterial assemblages. *Nature*, *397*(6719), 508–512. https://doi.org/10.1038/17351

Bradshaw, S. A., Eglinton, G., O'Hara, S. C. M., & Corner, E. D. S. (1991). Biogeochemical Changes in Lipids in a Model Marine Food Chain. In J. Berthelin (Ed.), *Developments in Geochemistry* (Vol. 6, pp. 49–58). Elsevier. https://doi.org/10.1016/B978-0-444-88900-3.50010-2

Brown, M. R., Dunstan, G. A., Norwood, Suzanne. J., & Miller, K. A. (1996). Effects of Harvest Stage and Light on the Biochemical Composition of the Diatom Thalassiosira Pseudonana. *Journal of Phycology*, *32*(1), 64–73. https://doi.org/10.1111/j.0022-3646.1996.00064.x

Buser, H. Rudolf., Arn, Heinrich., Guerin, Patrick., & Rauscher, Stefan. (1983). Determination of double bond position in mono-unsaturated acetates by mass spectrometry of dimethyl disulfide adducts. *Analytical Chemistry*, *55*(6), 818–822. https://doi.org/10.1021/ac00257a003

Busquets, M., Deroncelé, V., Vidal-Mas, J., Rodríguez, E., Guerrero, A., & Manresa, A. (2004). Isolation and characterization of a lipoxygenase from Pseudomonas 42A2 responsible for the biotransformation of oleic acid into (S)-(E)-10-hydroxy-8-octadecenoic acid. *Antonie van Leeuwenhoek*, *85*(2), 129–139. https://doi.org/10.1023/B:ANTO.0000020152.15440.65

Campbell, K., Mundy, C. J., Landy, J. C., Delaforge, A., Michel, C., & Rysgaard, S. (2016). Community dynamics of bottom-ice algae in Dease Strait of the Canadian Arctic. *Progress in Oceanography*, *149*, 27–39. https://doi.org/10.1016/j.pocean.2016.10.005

Chamberlain, N. R., Mehrtens, B. G., Xiong, Z., Kapral, F. A., Boardman, J. L., & Rearick, J. I. (1991). Correlation of carotenoid production, decreased membrane fluidity, and resistance to oleic acid killing in Staphylococcus aureus 18Z. *Infection and Immunity*, *59*(12), 4332–4337. https://doi.org/10.1128/IAI.59.12.4332-4337.1991

Collins, R. E., & Deming, J. W. (2011). Abundant dissolved genetic material in Arctic sea ice Part II: Viral dynamics during autumn freeze-up. *Polar Biology*, *34*(12), 1831–1841. https://doi.org/10.1007/s00300-011-1008-z

Cota, G. F. (1985). Photoadaptation of high Arctic ice algae. *Nature*, *315*(6016), 219–222. https://doi.org/10.1038/315219a0

Cox, G. F. N., & Weeks, W. F. (1983). Equations for Determining the Gas and Brine Volumes in Sea-Ice Samples. *Journal of Glaciology*, *29*(102), 306–316. https://doi.org/10.3189/S0022143000008364

Deming, J., & Collins, R. (2016). Sea ice as a habitat for Bacteria, Archaea and viruses. In *Sea Ice: Third Edition* (pp. 326–351). https://doi.org/10.1002/9781118778371.ch13

Desbois, A. P., Mearns-Spragg, A., & Smith, V. J. (2009). A Fatty Acid from the Diatom Phaeodactylum tricornutum is Antibacterial Against Diverse Bacteria Including Multi-resistant Staphylococcus aureus (MRSA). *Marine Biotechnology*, *11*(1), 45–52. https://doi.org/10.1007/s10126-008-9118-5

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, *85*(6), 1629–1642. https://doi.org/10.1007/s00253-009-2355-3

Dupont, F. (2012). Impact of sea-ice biology on overall primary production in a biophysical model of the pan-Arctic Ocean. *Journal of Geophysical Research: Oceans*, *117*(C8). https://doi.org/10.1029/2011JC006983

Eberlein, C., Baumgarten, T., Starke, S., & Heipieper, H. J. (2018). Immediate response mechanisms of Gram-negative solvent-tolerant bacteria to cope with environmental stress: Cis-trans isomerization of unsaturated fatty acids and outer membrane vesicle secretion. *Applied Microbiology and Biotechnology*, *102*(6), 2583–2593. https://doi.org/10.1007/s00253-018-8832-9

Ewert, M., & Deming, J. (2013). Sea Ice Microorganisms: Environmental Constraints and Extracellular Responses. *Biology*, *2*(2), 603–628. https://doi.org/10.3390/biology2020603

Ewert, M., & Deming, J. W. (2014). Bacterial responses to fluctuations and extremes in temperature and brine salinity at the surface of Arctic winter sea ice. *FEMS Microbiology Ecology*, *89*(2), 476–489. https://doi.org/10.1111/1574-6941.12363

Fahl, K., & Kattner, G. (1993). Lipid Content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). *Polar Biology*, *13*(6), 405–409. https://doi.org/10.1007/BF01681982

Falk-Petersen, S., Sargent, J. R., Henderson, J., Hegseth, E. N., Hop, H., & Okolodkov, Y. B. (1998). Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. *Polar Biology*, *20*(1), 41–47. https://doi.org/10.1007/s003000050274

Feldlaufer, M. F., Knox, D. A., Lusby, W. R., & Shimanuki, H. (1993). Antimicrobial activity of fatty acids against Bacillus larvae, the causative agent of American foulbrood disease. *Apidologie*, *24*(2), 95–99. https://doi.org/10.1051/apido:19930202

Fernandes, S. O., Javanaud, C., Michotey, V. D., Guasco, S., Anschutz, P., & Bonin, P. (2016). Coupling of bacterial nitrification with denitrification and anammox supports N removal

in intertidal sediments (Arcachon Bay, France). *Estuarine, Coastal and Shelf Science*, *179*, 39–50. https://doi.org/10.1016/j.ecss.2015.10.009

Fernández-Méndez, M., Katlein, C., Rabe, B., Nicolaus, M., Peeken, I., Bakker, K., Flores, H., & Boetius, A. (2015). Photosynthetic production in the central Arctic Ocean during the record sea-ice minimum in 2012. *Biogeosciences*, *12*(11), 3525–3549. https://doi.org/10.5194/bg-12-3525-2015

Firth, E., Carpenter, S. D., Sørensen, H. L., Collins, R. E., & Deming, J. W. (2016). Bacterial use of choline to tolerate salinity shifts in sea-ice brines. *Elementa: Science of the Anthropocene*, *4*(000120). https://doi.org/10.12952/journal.elementa.000120

Fischer, J., Schauer, F., & Heipieper, H. J. (2010). The trans/cis ratio of unsaturated fatty acids is not applicable as biomarker for environmental stress in case of long-term contaminated habitats. *Applied Microbiology and Biotechnology*, *87*(1), 365–371. https://doi.org/10.1007/s00253-010-2544-0

Garrison, D. L., Buck, K. R., & Fryxell, G. A. (1987). Algal Assemblages in Antarctic Pack Ice and in Ice-Edge Plankton. *Journal of Phycology*, *23*(4), 564–572. https://doi.org/10.1111/j.1529-8817.1987.tb04206.x

Ghiglione, J. F., Mevel, G., Pujo-Pay, M., Mousseau, L., Lebaron, P., & Goutx, M. (2007). Diel and Seasonal Variations in Abundance, Activity, and Community Structure of Particle-Attached and Free-Living Bacteria in NW Mediterranean Sea. *Microbial Ecology*, *54*(2), 217–231. https://doi.org/10.1007/s00248-006-9189-7

Gosselin, M., Legendre, L., Therriault, J.-C., Demers, S., & Rochet, M. (1986). Physical control of the horizontal patchiness of sea-ice microalgae. *Marine Ecology Progress Series*, *29*, 289–298. https://doi.org/10.3354/meps029289

Gosselin, M., Levasseur, M., Wheeler, P. A., Horner, R. A., & Booth, B. C. (1997). New measurements of phytoplankton and ice algal production in the Arctic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(8), 1623–1644. https://doi.org/10.1016/S0967-0645(97)00054-4

Graeve, M., Kattner, G., & Hagen, W. (1994). Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. *Journal of Experimental Marine Biology and Ecology*, *182*(1), 97–110. https://doi.org/10.1016/0022-0981(94)90213-5

Greenway, D. L. A., & Dyke, K. G. H. Y. 1979. (1979). Mechanism of the Inhibitory Action of Linoleic Acid on the Growth of Staphylococcus aureus. *Microbiology*, *115*(1), 233–245. https://doi.org/10.1099/00221287-115-1-233

Grenacher, S., & Guerin, P. M. (1994). Inadvertent introduction of squalene, cholesterol, and other skin products into a sample. *Journal of Chemical Ecology*, *20*(11), 3017–3025. https://doi.org/10.1007/BF02098406

Guckert, J. B. (1986). Phospholipid Ester-Linked Fatty Acid Profile Changes during Nutrient Deprivation of Vibrio cholerae: Increases in the translcis Ratio and Proportions of Cyclopropyl Fatty Acids. *APPL. ENVIRON. MICROBIOL.*, *52*, 8.

Guerrero, A., Casals, I., Busquets, M., Leon, Y., & Manresa, A. (1997). Oxydation of oleic acid to (E)-10-hydroperoxy-8-octadecenoic and (E)-10-hydroxy-8-octadecenoic acids by Pseudomonas sp. 42A2. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *1347*(1), 75–81. https://doi.org/10.1016/S0005-2760(97)00056-8

Hansen, B., & Bech, G. (1996). Bacteria associated with a marine planktonic copepod in culture. I. Bacterial genera in seawater, body surface, intestines and fecal pellets and succession during fecal pellet degradation. *Journal of Plankton Research*, *18*(2), 257–273. https://doi.org/10.1093/plankt/18.2.257

Hare, A. A., Wang, F., Barber, D., Geilfus, N.-X., Galley, R. J., & Rysgaard, S. (2013). PH evolution in sea ice grown at an outdoor experimental facility. *Marine Chemistry*, *154*, 46–54. https://doi.org/10.1016/j.marchem.2013.04.007

Harvey, H. R., Eglinton, G., O'Hara, S. C. M., & Corner, E. D. S. (1987). Biotransformation and assimilation of dietary lipids by Calanus feeding on a dinoflagellate. *Geochimica et Cosmochimica Acta*, *51*(11), 3031–3040. https://doi.org/10.1016/0016-7037(87)90376-0

Heipieper, H. J., Meinhardt, F., & Segura, A. (2003). The cis–trans isomerase of unsaturated fatty acids in Pseudomonas and Vibrio: Biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism. *FEMS Microbiology Letters*, *229*(1), 1–7. https://doi.org/10.1016/S0378-1097(03)00792-4

Heipieper, H. J., Neumann, G., Cornelissen, S., & Meinhardt, F. (2007). Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Applied Microbiology and Biotechnology*, *74*(5), 961–973. https://doi.org/10.1007/s00253-006-0833-4

Horner, R., & Schrader, G. C. (1982). Relative Contributions of Ice Algae, Phytoplankton, and Benthic Microalgae to Primary Production in Nearshore Regions of the Beaufort Sea. *ARCTIC*, *35*(4), 485–503. https://doi.org/10.14430/arctic2356

Howard-Jones, M. H., Ballard, V. D., Allen, A. E., Frischer, M. E., & Verity, P. G. (2002). Distribution of bacterial biomass and activity in the marginal ice zone of the central Barents Sea during summer. *Journal of Marine Systems*, *38*(1–2), 77–91. https://doi.org/10.1016/S0924-7963(02)00170-7

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., & Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. *The Plant Journal*, *54*(4), 621–639. https://doi.org/10.1111/j.1365-313X.2008.03492.x

Jacobsen, T. R., & Azam, F. (1984). Role of Bacteria in Copepod Fecal Pellet Decomposition: Colonization, Growth Rates and Mineralization. *Bulletin of Marine Science*, *35*(3), 495–502.

Johnsen, G., & Sakshaug, E. (1993). Bio-optical characteristics and photoadaptive responses in the toxic and bloom-forming dinoflagellates Gyrodinium aureolum, Gymnodinium galatheanum, and two strains of Prorocentrum minimum. *Journal of Phycology*, *29*(5), 627–642. https://doi.org/10.1111/j.0022-3646.1993.00627.x

Jüttner, F. (2001). Liberation of 5,8,11,14,17-Eicosapentaenoic Acid and Other Polyunsaturated Fatty Acids from Lipids as a Grazer Defense Reaction in Epilithic Diatom Biofilms. *Journal of Phycology*, *37*(5), 744–755. https://doi.org/10.1046/j.1529-8817.2001.00130.x

Kaczmarska, I., Ehrman, J. M., Bates, S. S., Green, D. H., Léger, C., & Harris, J. (n.d.). *Diversity and distribution of epibiotic bacteria on Pseudo-nitzschia multiseries in culture, and comparison with those on diatoms in native seawater*. 43.

Katlein, C., Arndt, S., Nicolaus, M., Perovich, D. K., Jakuba, M. V., Suman, S., Elliott, S., Whitcomb, L. L., McFarland, C. J., Gerdes, R., Boetius, A., & German, C. R. (2015). Influence of ice thickness and surface properties on light transmission through Arctic sea ice. *Journal of Geophysical Research: Oceans*, *120*(9), 5932–5944. https://doi.org/10.1002/2015JC010914

Khotimchenko, S., & Yakovleva, I. (2005). Lipid composition of the red alga Tichocarpus crinitus exposed to different levels of photon irradiance. *Phytochemistry*, *66*, 73–79. https://doi.org/10.1016/j.phytochem.2004.10.024

Kirchman, D. L., Malmstrom, R. R., & Cottrell, M. T. (2005). Control of bacterial growth by temperature and organic matter in the Western Arctic. *Deep Sea Research Part II: Topical Studies in Oceanography*, *52*(24), 3386–3395. https://doi.org/10.1016/j.dsr2.2005.09.005

Kjelleberg, S., Hermansson, M., Marden, P., & Jones, G. W. (1987). The Transient Phase Between Growth and Nongrowth of Heterotrophic Bacteria, with Emphasis on the Marine

Environment. *Annual Review of Microbiology*, *41*(1), 25–49. https://doi.org/10.1146/annurev.mi.41.100187.000325

Krembs, C., Eicken, H., & Deming, J. W. (2011). Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. *Proceedings of the National Academy of Sciences*, *108*(9), 3653–3658. https://doi.org/10.1073/pnas.1100701108

Lavoie, D., Denman, K., & Michel, C. (2005). Modeling ice algal growth and decline in a seasonally ice-covered region of the Arctic (Resolute Passage, Canadian Archipelago). *Journal of Geophysical Research: Oceans*, *110*(C11). https://doi.org/10.1029/2005JC002922

Lee, S. H., & Whitledge, T. E. (2005). Primary and new production in the deep Canada Basin during summer 2002. *Polar Biology*, *28*(3), 190–197. https://doi.org/10.1007/s00300-004-0676-3

Leu, E., Wiktor, J., Søreide, J., Berge, J., & Falk-Petersen, S. (2010). Increased irradiance reduces food quality of sea ice algae. *Marine Ecology Progress Series*, *411*, 49–60. https://doi.org/10.3354/meps08647

Lizotte, M., Levasseur, M., Law, C. S., Walker, C. F., Safi, K. A., Marriner, A., & Kiene, R. P. (2017). Dimethylsulfoniopropionate (DMSP) and dimethyl sulfide (DMS) cycling across contrasting biological hotspots of the New Zealand subtropical front. *Ocean Science*, *13*(6), 961–982. https://doi.org/10.5194/os-13-961-2017

Loffeld, B., & Keweloh, H. (1996). Cis/trans isomerization of unsaturated fatty acids as possible control mechanism of membrane fluidity inPseudomonas putida P8. *Lipids*, *31*(8), 811–815. https://doi.org/10.1007/BF02522976

Loose, B., Miller, L. A., Elliott, S., & Papakyriakou, T. (2011). Sea Ice Biogeochemistry and Material Transport Across the Frozen Interface. *Oceanography*, *24*(3), 202–218.

Macnaughton, M. O., Thormar, J., & Berge, J. (2007). Sympagic amphipods in the Arctic pack ice: Redescriptions of Eusirus holmii Hansen, 1887 and Pleusymtes karstensi (Barnard, 1959). *Polar Biology*, *30*(8), 1013–1025. https://doi.org/10.1007/s00300-007-0260-8

Maranger, R., Bird, D. F., & Juniper, S. K. (1994). Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, N.W.T., Canada. *Marine Ecology Progress Series*, *111*(1/2), 121–127.

Marie, D., Partensky, F., Jacquet, S., & Vaulot, D. (1997). Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology*, *63*(1), 186–193. https://doi.org/10.1128/aem.63.1.186-193.1997

Marie, D., Rigaut-Jalabert, F., & Vaulot, D. (2014). An improved protocol for flow cytometry analysis of phytoplankton cultures and natural samples. *Cytometry Part A*, *85*(11), 962–968. https://doi.org/10.1002/cyto.a.22517

Martínez, E., Hamberg, M., Busquets, M., Díaz, P., Manresa, A., & Oliw, E. H. (2010). Biochemical Characterization of the Oxygenation of Unsaturated Fatty Acids by the Dioxygenase and Hydroperoxide Isomerase of *Pseudomonas aeruginosa* 42A2. *Journal of Biological Chemistry*, *285*(13), 9339–9345. https://doi.org/10.1074/jbc.M109.078147

Massicotte, P., Amiraux, R., Amyot, M.-P., Archambault, P., Ardyna, M., Arnaud, L., Artigue, L., Aubry, C., Ayotte, P., Bécu, G., Bélanger, S., Benner, R., Bittig, H. C., Bricaud, A., Brossier, É., Bruyant, F., Chauvaud, L., Christiansen-Stowe, D., Claustre, H., … Babin, M. (2020). Green Edge ice camp campaigns: Understanding the processes controlling the under-ice Arctic phytoplankton spring bloom. *Earth System Science Data*, *12*(1), 151–176. https://doi.org/10.5194/essd-12-151-2020

Massicotte, P., Bécu, G., Lambert-Girard, S., Leymarie, E., & Babin, M. (2018). Estimating Underwater Light Regime under Spatially Heterogeneous Sea Ice in the Arctic. *Applied Sciences*, *8*(12), 2693. https://doi.org/10.3390/app8122693

Meiners, K., Krembs, C., & Gradinger, R. (2008). Exopolymer particles: Microbial hotspots of enhanced bacterial activity in Arctic fast ice (Chukchi Sea). *Aquatic Microbial Ecology*, *52*, 195–207. https://doi.org/10.3354/ame01214

Michotey, V., Guasco, S., Boeuf, D., Morezzi, N., Durieux, B., Charpy, L., & Bonin, P. (2012). Spatio-temporal diversity of free-living and particle-attached prokaryotes in the tropical lagoon of Ahe atoll (Tuamotu Archipelago) and its surrounding oceanic waters. *Marine Pollution Bulletin*, *65*(10), 525–537. https://doi.org/10.1016/j.marpolbul.2012.01.009

Mikkelsen, D., Rysgaard, S., & Glud, R. (2008). Microalgal composition and primary production in Arctic sea ice: A seasonal study from Kobbefjord (Kangerluarsunnguaq), West Greenland. *Marine Ecology Progress Series*, *368*, 65–74. https://doi.org/10.3354/meps07627

Miller, L. A., Fripiat, F., Else, B. G. T., Bowman, J. S., Brown, K. A., Collins, R. E., Ewert, M., Fransson, A., Gosselin, M., Lannuzel, D., Meiners, K. M., Michel, C., Nishioka, J., Nomura, D., Papadimitriou, S., Russell, L. M., Sørensen, L. L., Thomas, D. N., Tison, J.-L., … Zhou, J. (2015). Methods for biogeochemical studies of sea ice: The state of the art, caveats, and recommendations. *Elementa: Science of the Anthropocene*, *3*, 000038. https://doi.org/10.12952/journal.elementa.000038

Mock, T., & Junge, K. (2007). Psychrophilic diatoms. In *Algae and cyanobacteria in extreme environments* (Springer, pp. 343–364).

Monfort, P., Demers, S., & Levasseur, M. (2000). Bacterial dynamics in first year sea ice and underlying seawater of Saroma-ko Lagoon (Sea of Okhotsk, Japan) and Resolute Passage (High Canadian Arctic): Inhibitory effects of ice algae on bacterial dynamics. *Canadian Journal of Microbiology*, *46*(7), 623–632. https://doi.org/10.1139/w00-024

Nocker, A., Cheung, C.-Y., & Camper, A. K. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. Dead bacteria by selective removal of DNA from dead cells. *Journal of Microbiological Methods*, *67*(2), 310–320. https://doi.org/10.1016/j.mimet.2006.04.015

Nocker, A., Mazza, A., Masson, L., Camper, A. K., & Brousseau, R. (2009). Selective detection of live bacteria combining propidium monoazide sample treatment with microarray technology. *Journal of Microbiological Methods*, *76*(3), 253–261. https://doi.org/10.1016/j.mimet.2008.11.004

Oziel, L., Massicotte, P., Randelhoff, A., Ferland, J., Vladoiu, A., Lacour, L., Galindo, V., Lambert-Girard, S., Dumont, D., Cuypers, Y., Bouruet-Aubertot, P., Mundy, C.-J., Ehn, J., Bécu, G., Marec, C., Forget, M.-H., Garcia, N., Coupel, P., Raimbault, P., … Babin, M. (2019). Environmental factors influencing the seasonal dynamics of spring algal blooms in and beneath sea ice in western Baffin Bay. *Elementa: Science of the Anthropocene*, *7*(34). https://doi.org/10.1525/elementa.372

Pabi, S., van Dijken, G. L., & Arrigo, K. R. (2008). Primary production in the Arctic Ocean, 1998–2006. *Journal of Geophysical Research*, *113*(C8), C08005. https://doi.org/10.1029/2007JC004578

Parrish, C. C. (2013). Lipids in Marine Ecosystems. *ISRN Oceanography*, *2013*, e604045. https://doi.org/10.5402/2013/604045

Parsons, T., Maita, Y., & Lalli, C. (1984). A manual of chemical and biological methods for seawater analysis. *Marine Ecology Progress Series*, *199*, 43–53.

Pedersen, L., Jensen, H. M., Burmeister, A., & Winding, B. (1999). The significance of food web structure for the condition and tracer lipid content of juvenile snail fish (Pisces: Liparis spp.) along 65–72°N off West Greenland. *Journal of Plankton research*. 21: 1593–1611. DOI: https://doi.org/10.1093/plankt/21.9.1593.

Perovich, D. K. (1996). The optical properties of sea ice. *This Digital Resource Was Created in Microsoft Word and Adobe Acrobat*. https://erdc-library.erdc.dren.mil/jspui/handle/11681/2648

Platt, T., & Gallegos, C. L. (1980). Modelling Primary Production. In P. G. Falkowski (Ed.), *Primary Productivity in the Sea* (Vol. 19, pp. 339–362). Springer US. https://doi.org/10.1007/978-1-4684-3890-1_19

Pomeroy, L. R., & Deibel, D. (1986). Temperature Regulation of Bacterial Activity During the Spring Bloom in Newfoundland Coastal Waters. *Science*, *233*(4761), 359–361. https://doi.org/10.1126/science.233.4761.359

Pomeroy, L., & Wiebe, W. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology*, *23*, 187–204. https://doi.org/10.3354/ame023187

Popova, E. E., Yool, A., Coward, A. C., Aksenov, Y. K., Alderson, S. G., de Cuevas, B. A., & Anderson, T. R. (2010). Control of primary production in the Arctic by nutrients and light: Insights from a high resolution ocean general circulation model. *Biogeosciences*, *7*(11), 3569–3591. https://doi.org/10.5194/bg-7-3569-2010

Ralph, P. J., Ryan, K. G., Martin, A., & Fenton, G. (2007). Melting out of sea ice causes greater photosynthetic stress in algae than freezing in1. *Journal of Phycology*, *43*(5), 948–956. https://doi.org/10.1111/j.1529-8817.2007.00382.x

Rampen, S. W., Abbas, B. A., Schouten, S., & Sinninghe Damste, J. S. (2010). A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. *Limnology and Oceanography*, *55*(1), 91–105. https://doi.org/10.4319/lo.2010.55.1.0091

Reuss, N., & Poulsen, L. (2002). Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. *Marine Biology*, *141*(3), 423–434. https://doi.org/10.1007/s00227-002-0841-6

Riebesell, U., Schloss, I., & Smetacek, V. (1991). Aggregation of algae released from melting sea ice: Implications for seeding and sedimentation. *Polar Biology*, *11*(4), 239–248. https://doi.org/10.1007/BF00238457

Riedel, A., Michel, C., & Gosselin, M. (2006). Seasonal study of sea-ice exopolymeric substances on the Mackenzie shelf: Implications for transport of sea-ice bacteria and algae. *Aquatic Microbial Ecology*, *45*, 195–206. https://doi.org/10.3354/ame045195

Rivkin, R., Anderson, M., & Lajzerowicz, C. (1996). Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. *Aquatic Microbial Ecology*, *10*, 243–254. https://doi.org/10.3354/ame010243

Sakshaug, E. (2004). Primary and Secondary Production in the Arctic Seas. In R. Stein & R. W. MacDonald (Eds.), *The Organic Carbon Cycle in the Arctic Ocean* (pp. 57–81). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-18912-8_3

Sampei, M., Fortier, L., Raimbault, P., Matsuno, K., Abe, Y., Quéguiner, B., Lafond, A., Babin, M., & Hirawake, T. (2021). An estimation of the quantitative impacts of copepod grazing on an under sea-ice spring phytoplankton bloom in western Baffin Bay, Canadian Arctic. *Elementa: Science of the Anthropocene*, *9*(1), 00092. https://doi.org/10.1525/elementa.2019.00092

Shin, S. Y., Bajpai, V. K., Kim, H. R., & Kang, S. C. (2007). Antibacterial activity of bioconverted eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) against foodborne pathogenic bacteria. *International Journal of Food Microbiology*, *113*(2), 233–236. https://doi.org/10.1016/j.ijfoodmicro.2006.05.020

Shoja Chaghervand, S. (2019). *Characterization of the enzymes involved in the diolsynthase pathway in Pseudomonas aeruginosa* [Universitat de Barcelona]. http://diposit.ub.edu/dspace/handle/2445/139344

Sicre, M.-A., Paillasseur, J.-L., Marty, J.-C., & Saliot, A. (1988). Characterization of seawater samples using chemometric methods applied to biomarker fatty acids. *Organic Geochemistry*, *12*(3), 281–288. https://doi.org/10.1016/0146-6380(88)90265-3

Sinensky, M. (1974). Homeoviscous Adaptation—A Homeostatic Process that Regulates the Viscosity of Membrane Lipids in Escherichia coli. *Proceedings of the National Academy of Sciences*, *71*(2), 522–525. https://doi.org/10.1073/pnas.71.2.522

Small, L. F., Fowler, S. W., & Ünlü, M. Y. (1979). Sinking rates of natural copepod fecal pellets. *Marine Biology*, *51*(3), 233–241. https://doi.org/10.1007/BF00386803

Smith, R. E. H., Cavaletto, J. F., Eadie, B. J., & Gardner, W. S. (1993). Growth and lipid composition of high Arctic ice algae during the spring bloom at Resolute, Northwest Territories, Canada. *Marine Ecology Progress Series*, *97*(1), 19–29.

Smith, R. E. H., Clement, P., & Cota, G. F. (1989). Population Dynamics of Bacteria in Arctic Sea Ice. *Microbial Ecology*, *17*(1), 63–76.

Smith, S. D., Muench, R. D., & Pease, C. H. (1990). Polynyas and leads: An overview of physical processes and environment. *Journal of Geophysical Research: Oceans*, *95*(C6), 9461–9479. https://doi.org/10.1029/JC095iC06p09461

Søreide, J. E., Leu, E., Berge, J., Graeve, M., & Falk-Petersen, S. (2010). Timing of blooms, algal food quality and Calanus glacialis reproduction and growth in a changing Arctic. *Global Change Biology*, *16*(11), 3154–3163. https://doi.org/10.1111/j.1365-2486.2010.02175.x

Takai, K., & Horikoshi, K. (2000). Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes. *Applied and Environmental Microbiology*, *66*(11), 5066–5072. https://doi.org/10.1128/AEM.66.11.5066-5072.2000

Thomas, D., & Papadimitriou, S. (2003). Biogeochemistry of Sea ice. In *Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology* (pp. 267–332). Blackwell.

Tison, J.-L., Brabant, F., Dumont, I., & Stefels, J. (2010). High-resolution dimethyl sulfide and dimethylsulfoniopropionate time series profiles in decaying summer first-year sea ice at Ice Station Polarstern, western Weddell Sea, Antarctica. *Journal of Geophysical Research*, *115*(G4), G04044. https://doi.org/10.1029/2010JG001427

Tremblay, J.-É., Robert, D., Varela, D. E., Lovejoy, C., Darnis, G., Nelson, R. J., & Sastri, A. R. (2012). Current state and trends in Canadian Arctic marine ecosystems: I. Primary production. *Climatic Change*, *115*(1), 161–178. https://doi.org/10.1007/s10584-012-0496-3

Volkman, J. (2003). Sterols in microorganisms. *Applied Microbiology and Biotechnology*, *60*(5), 495–506. https://doi.org/10.1007/s00253-002-1172-8

Wadhams, P., & Martin, S. (2001). *Ice in the ocean* (2nd ed., Vol. 54). CRC press.

Wassmann, P., Duarte, C. M., Agustí, S., & Sejr, M. K. (2011). Footprints of climate change in the Arctic marine ecosystem. *Global Change Biology*, *17*(2), 1235–1249. https://doi.org/10.1111/j.1365-2486.2010.02311.x

Wichard, T., Gerecht, A., Boersma, M., Poulet, S. A., Wiltshire, K., & Pohnert, G. (2007). Lipid and Fatty Acid Composition of Diatoms Revisited: Rapid Wound-Activated Change of Food Quality Parameters Influences Herbivorous Copepod Reproductive Success. *ChemBioChem*, *8*(10), 1146–1153. https://doi.org/10.1002/cbic.200700053

Yager, P. L., Connelly, T. L., Mortazavi, B., Wommack, K. E., Bano, N., Bauer, J. E., Opsahl, S., & Hollibaugh, J. T. (2001). Dynamic bacterial and viral response to an algal bloom at subzero temperatures. *Limnology and Oceanography*, *46*(4), 790–801. https://doi.org/10.4319/lo.2001.46.4.0790

Chapitre III

Viabilité et état de stress des bactéries associées à des particules en suspension issues de l'activité zooplanctonique ou de la production primaire en Eté le long d'un transect en baie de Baffin (Océan Arctique)

Sommaire

3.1 Avant-propos

Ce nouveau chapitre s'inscrit dans la continuité du précédent, en abordant l'étude de la composition lipidique ainsi que de l'état physiologique des bactéries dans la glace et dans les particules en suspension. Les échantillons étudiés ici ont été collectés, toujours dans le cadre du projet GreenEdge, en Eté 2016 en baie de Baffin, le long d'un transect allant de la glace à l'eau libre.

L'analyse de la composition lipidique des échantillons a souligné l'importance de l'activité zooplanctonique, avec de fortes proportions de microzooplancton dans la glace et de copépodes dans la colonne d'eau en fin de fonte. Comme lors du chapitre précédent, une forte mortalité bactérienne probablement liée à la production d'acides gras libres par les algues sympagiques a été observée dans la glace. De fortes proportions de bactéries mortes ou inactives ont été également détectées dans des particules en suspension composées vraisemblablement d'agrégats d'EPS, relargués après les algues de glace en fin de fonte dans la banquise et concentrés du fait de leur faible densité dans les eaux de surface.

De forts signaux de stress osmotique ont été mesurés dans des particules en suspension collectées sous la glace, ils ont été attribués à l'ingestion de bactéries stressées par la salinité dans la glace par du microzooplancton bactérivore (ciliés), qui a la capacité d'intégrer les acides gras bactériens dans ses membranes et de les exporter par la suite dans la colonne d'eau

Si cette étude confirme le fort état de stress des bactéries sympagiques, elle met également en avant la complexité de ce réseau trophique particulier, et la nécessité de mieux le comprendre. En effet, si le matériel algal sympagique relargué de la glace semble associé à des bactéries majoritairement mortes ou inactives et donc peu aptes à le dégrader efficacement, sa capacité de préservation et donc d'export dépend également fortement du timing et de l'efficacité du broutage zooplanctonique.

- Contribution au chapitre

Comme pour le chapitre précédent, l'échantillonnage et les traitements au PMA qui devaient être effectués sur du matériel frais n'ont pas été réalisés lors de cette thèse. Là encore j'ai effectué les extractions lipidiques, le traitement au DMDS de ces derniers ainsi que les analyses en CPG/SM. J'ai également effectué les extractions d'ADN et les qPCRs

permettant d'obtenir les pourcentages de mortalité bactérienne. J'ai enfin contribué à la rédaction et la relecture de cet article, publié dans la revue *STOTEN: Science of the Total Environment.*

3.2 Viability and stress state of bacteria associated with primary production or zooplankton-derived suspended particulate matter in summer along a transect in Baffin Bay (Arctic Ocean)

3.2.1 Résumé en français

Dans le cadre du projet GreenEdge (dont l'objectif principal est de comprendre la dynamique du bloom printanier de phytoplancton dans l'océan Arctique), la composition lipidique, la viabilité et l'état de stress des bactéries ont été mesurés dans des échantillons de glace et de particules en suspension (SPM) collectés en 2016 le long d'un transect allant de la glace de mer à l'eau libre dans la Baie de Baffin (océan Arctique). Les analyses lipidiques ont confirmé la dominance de diatomées dans la plus basse couche de glace, suggéré (i) la forte proportion de micro-zooplancton dans les SPM collectées à l'Ouest du transect, au stations recouvertes de glace St 403 et St 409 et (ii) une forte proportion de macro-zooplancton (copépodes) dans les SPM collectées à l'Est du transect à la station recouverte de glace St 413 et la station d'eau libre St 418. L'utilisation du propidium monoazide (PMA) nous a permis de montrer une forte mortalité bactérienne dans la glace et dans les SPM collectées dans les eaux peu profondes des stations St 409 et St 418. Cette mortalité est attribuée au relargage d'acides gras libres bactéricides par les algues sympagiques en situation de stress dû à la lumière. Une forte isomérisation *cis-trans* des MUFAs bactériens a été observée dans les échantillons de SPM les plus profonds collectés aux stations St 403 et St 409. Ce résultat est attribué à l'ingestion de bactéries stressées par la salinité dans les canaux de saumure de la glace par du micro-zooplancton sympagique (ciliés), incorporant les acides gras *trans* de leurs proies avant d'être relargué dans la colonne d'eau lors de la fonte. La forte valeur des ratio *trans/cis* également observés dans les échantillons de SPM collectés dans les eaux peu profondes des stations St 413 et St 418 suggère la présence de particules riches en polymères extracellulaires (EPS) à flottabilité positive ou neutre, retenues dans la glace et rejetées (avec des bactéries stressées par la salinité) dans l'eau de mer après la libération initiale de la biomasse algale. De telles particules d'EPS, qui sont généralement considérés comme des vecteurs idéaux de distribution horizontale de bactéries en Arctique, semblent contenir de fortes proportions de bactéries morte ou non-actives.

3.2.2 Article

Viability and stress state of bacteria associated with primary production or zooplankton-derived suspended particulate matter in summer along a transect in Baffin Bay (Arctic Ocean)

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Article publié dans *Science of the Total Environment (STOTEN)* en janvier 2021

Abstract

In the framework of the GreenEdge Project (whose the general objective is to understand the dynamic of the phytoplankton spring bloom in Arctic Ocean), lipid composition and viability and stress state of bacteria were monitored in sea ice and suspended particulate matter (SPM) samples collected in 2016 along a transect from sea ice to open water in Baffin Bay (Arctic Ocean). Lipid analyses confirmed the dominance of diatoms in the bottommost layer of ice and suggested (i) the presence of a strong proportion of micro-zooplankton in SPM samples collected at the western ice-covered St 403 and St 409 and (ii) a high proportion of macro-zooplankton (copepods) in SPM samples collected at the eastern ice-covered St 413 and open water St 418. The use of the propidium monoazide (PMA) method allowed to show a high bacterial mortality in sea ice and in SPM material collected in shallower waters at St 409 and St 418. This mortality was attributed to the release of bactericidal free fatty acids by sympagic diatoms under the effect of light stress. A strong cis-trans isomerization of bacterial MUFAs was observed in the deeper SPM samples collected at the St 403 and St 409. It was attributed to the ingestion of bacteria stressed by salinity in brine channels of ice by sympagic bacterivorous microzooplankton (ciliates) incorporating trans fatty acids of their preys before to be released in the water column during melting. The high trans/cis ratios also observed in SPM samples collected in the shallower waters at St 413 and St 418 suggest the presence of positively or neutrally buoyant extracellular polymeric substances (EPS)-rich particles retained in sea ice and discharged (with bacteria stressed by salinity) in seawater after the initial release of algal biomass. Such EPS particles, which are generally considered as ideal vectors for bacterial horizontal distribution in the Arctic, appeared to contain a high proportion of dead and non-growing bacteria.

1. Introduction

Arctic sea ice shelters a huge diversity of organisms particularly well-adapted to the harsh living conditions in this ecosystem (Boetius *et al.,* 2015). They include bacteria, viruses, archaea and diatoms (von Quillfeldt *et al.,* 2003; Junge *et al.,* 2004; Sazhin *et al.,* 2019). One of the numerous ecosystem services that this particular biota fulfil is the production of organic matter (Boras *et al.,* 2010; Boetius *et al.,* 2015). Sympagic diatoms (diatoms inhabiting the ice matrix) are responsible for much of this production. Their contribution to annual primary production (PP) varies widely depending on the season and the region (<1–60%, e.g. Dupont, 2012; Fernández-Méndez *et al.,* 2015), but it represents a crucial food source for the marine foodweb, especially in winter (Søreide *et al.,* 2010).

It has been shown that Arctic sea ice contains large amounts of extracellular polymeric substances (EPSs) (Krembs *et al.,* 2002), one order of magnitude higher than in the surface water (Krembs and Engel, 2001; Meiners *et al.,* 2003). These substances are produced by both bacteria and algae, with sympagic diatoms being their primary source in sea ice (Meiners *et al.,* 2003; Mancuso Nichols *et al.,* 2004). EPSs can help protect cells against harsh environmental conditions (e.g. salinity fluctuations) and assist cell adhesion (Cooksey and Wigglesworth-Cooksey, 1995). The release of exopolymers by the sympagic biota further influences carbon cycling by (i) providing a carbon-rich substrate that will support bacterial production and metabolic activity (Simon *et al.,* 2002), (ii) bypassing microbially mediated POC production by the abiotic formation of large EPS-containing particles or aggregates (Passow, 2002; Thornton, 2002), (iii) directly contributing to the organic carbon pool, with concentrations potentially equivalent to those of particulate organic carbon (POC) in pelagic environments (Mari, 1999; Engel and Passow, 2001), and (iv) increasing the sympagic biota sedimentation rates through aggregation (Riebesell *et al.,* 1991; Azetsu-Scott and Passow, 2004). From their high sedimentation rates and their good preservation during their travel toward the seafloor (Boetius *et al.,* 2013; Rontani *et al.,* 2016; Amiraux *et al.,* 2017), it has been suggested that sympagic PP contributes significantly to the total PP source in sea waters, especially deep ones (Glud and Rysgaard, 2007; Krause-Jensen, 2007). The fate of sympagic biota in sea ice and as they sink in the water column depends mostly on grazing by zooplankton and mineralization by their attached bacteria. In the Arctic, it is estimated that the zooplankton graze about 66–79% of the new PP (including sympagic algae; Forest *et al.,* 2011), and since their fecal pellets

generally increase the sinking rates of their food, it is estimated that zooplankton probably form most of the PP export to the aphotic zone and seafloor (Forest *et al.,* 2011). By contrast, bacterial activity, which is higher for attached than for free bacteria (Hoppe, 1991; Karner and Herndl, 1992), allows cleavage of the POM into smaller pieces by extracellular enzymatic hydrolysis (Cho and Azam, 1988). Such processes enhance the further enzymatic digestion of the matter and ultimately reduce its potential to reach the seafloor. However, while heterotrophic bacteria and the rest of the microbial loop process about half of the P.P in low-latitude oceans (Ducklow, 2000), their contribution at higher latitudes is assumed to be smaller. Based on bacterial activity measurements, Howard-Jones et al. (2002) suggest that a significant fraction (25–80%) of Arctic bacterioplankton is dormant or inactive in the marginal ice zone of the Barents Sea.

Recently, Amiraux *et al*. (2017) suggested that the weaker activity of bacteria in the Arctic could result from the involvement of some stress factors in ice, such as salinity. During the early stage of ice melting in spring, brine inclusions (where salinity may reach up to 150 in some ice sections collected in early spring 2015 at the GreenEdge Ice Camp, Galindo, unpublished data) become interconnected in channels and are expelled from the sea ice into the underlying seawater (Wadhams and Martin, 2001). The ice algal bacterial community is therefore exposed to a salinity stress, which occurs over relatively short timescales (*e.g.* hours). In this ecosystem, prokaryotic cells subjected to high osmotic pressure have developed mechanisms to live in these extreme conditions. Various strategies are used: (i) implementation of active Na^+ and K^+ ion transport systems (Thompson and MacLeod, 1971), (ii) accumulation of osmocompatible compounds such as glycine betaine or proline (Piuri *et al.,* 2003) or (iii) production of EPSs, which can act as a diffusion barrier (Kim and Chong, 2017). Another major adaptive response of many microorganisms, including bacteria, is to maintain membrane fluidity through 'homeoviscous adaptation' (Sinensky, 1974). The shifts in fatty acid composition of membrane lipids, and most notably by enzymatic conversion of cis- to trans-unsaturated fatty acids (Loffeld and Keweloh, 1996; Heipieper *et al.,* 2003) through the activity of cis-trans isomerases (CTIs) can be an important bacterial mechanism for modifying membrane fluidity. It has been previously suggested that trans/cis ratios >0.1 in environmental samples may be indicative of bacterial stress (Guckert, 1986). Previous analyses of sea ice and sinking particles collected in the water column during the vernal melting period showed a relatively strong CTI activity, suggesting the occurrence of salinity stress during the early stages of ice melt (Amiraux *et al.,* 2017). The high trans/cis

ratios observed in sinking particles was attributed to the flush of bacteria associated with ice algae from internal hypersaline ice brines (Amiraux *et al.,* 2021b). The relative stability of these ratios with depth also suggested that bacterial communities associated with sinking sympagic algae were non-growing.

In a previous study, Amiraux et al. (2021a) studied the stress state of bacteria attached to sinking sympagic algae during a vernal melting season at a landfast ice station in Davis Strait. Their results emphasized the impact of salinity, limiting the growth state of attached bacteria at the beginning of sea ice melting, subsequently giving way to an intense free fatty acid (FFAs) stress. Indeed, the production of bactericidal FFAs by sympagic algae is enhanced by the increase in light transmittance through the ice (due to the advance melting of sea ice) resulting in a high bacterial mortality. If this study gave us valuable information on the interactions between sympagic algae and their associated bacteria in sinking samples, data on those interactions after ice melting and on suspended particles are still lacking

In the present work, we thus monitored the salinity stress and mortality of bacteria associated with sea ice and suspended particulate matter (SPM) samples collected in 2016 along a transect from sea ice to open water in Baffin Bay (Arctic Ocean). We intend to determine if the bacteria associated with these suspended particles are also weakly active or in a poor physiological state, thus impacting the preservation of this material.

2. Materials and methods

2.1 Sampling

Samples were taken at three ice stations (St 403, St 409 and St 413) and one open water station (St 418) (Figure 3.1) from the Canadian icebreaker CCGS Amundsen along a longitudinal transect from 68° 4′ 25.32″ N and 61° 36′ 30.54″ W to 68° 6′ 52.14″ N and 57° 46′ 7.14″ W between 25 and 28 June 2016 as part of the GreenEdge project. At the time of sampling, this transect was under the influence of the Arctic current from the North acting practically perpendicular to the transect (A. Randelhoff, personal communication). Consequently, advection along the transect (East-West) should be relatively limited.

The sea ice sampling was carried out using a Kovacs Mark V 14 cm diameter corer, focusing on the bottom-most 10 cm of sea ice (sub-sectioned into two further intervals:

0–3 and 3–10 cm) where most ice biota are found (Smith *et al.,* 1990). To compensate for biomass heterogeneity, common in sea ice (Gosselin *et al.,* 1986), three or four equivalent core sections were pooled for each sampling day in isothermal containers. Pooled sea ice sections were then melted in the dark with 0.2 µm filtered seawater (FSW; 3:1 v:v) to minimize osmotic stress on the microbial community during melting (Bates and Cota, 1986; Garrison and Buck, 1986).

Suspended particulate matter (SPM) samples were collected at seven depths in the first 100 m of the water column using large (20 L) Niskin bottles to accommodate any within-sample heterogeneity.

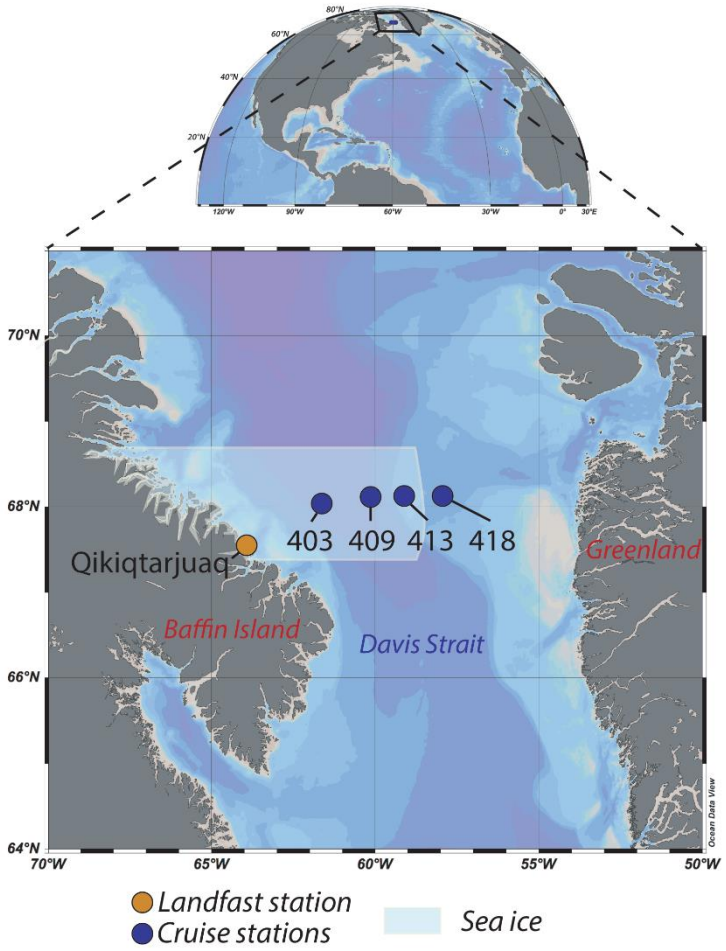

Figure 3.1: Map of the study area with location of the stations investigated in Baffin Bay. Blue circles on the enlarged map of western Baffin Bay indicate the stations investigated during the transect. The orange circle indicates the GreenEdge ice camp station investigated by Amiraux et al. (2019, 2021a). White color indicates the sea ice cover during the sampling.

For both sea ice and SPM, samples were collected in pentaplicate (a sample for lipid analyses and four for PMA analyses) as follows. Lipid, chlorophyll a, and total particulate carbon samples were obtained by filtration through pre-weighed Whatman glass fiber filters (Buckinghamshire, UK; GF/F, porosity 0.7 µm, combusted 4 h at 450 °C) and kept

frozen (<−80 °C). Bacterial viability samples were obtained by filtration on 0.8 µm Whatman nucleopore filters (24 mm, autoclaved 1 h at 110 °C) and kept frozen (<−80 °C) prior to analysis. Owing to the porosity of the filters, the analyses concerned mainly algae, particles and their attached bacteria. Bacterial abundance and productivity were measured directly onboard the CCGS Amundsen by cytometry (see Bacterial abundance) and ^{3}H-leucine incorporation (see Bacterial productivity).

2.2 Treatment

2.2.1 Chlorophyll a

Concentration of chlorophyll a and phaeopigments retained on the GF/F filters were measured before and after acidification (5% HCl) using a TD-700 Turner Design fluorometer, after 18–24 h extraction in 90% acetone at 4 °C in the dark (Parsons *et al.,* 1984). The fluorometer was calibrated with a commercially available chlorophyll a standard (Anacystis nidulans, Sigma).

2.2.2 Total particulate carbon

At Université Laval, filters were dried for 24 h at 60 °C, weighed again for dry weight determination and then analyzed using a Perkin Elmer carbon-hydrogen-nitrogen-sulfur (CHNS) 2400 Series II instrument to measure TPC. Calibration was done using accurately weighed samples of acetanilide (C_8H_9NO).

2.2.3 Bacterial abundance

Samples were analyzed directly on board the CCGS Amundsen using an Accuri C6 flow cytometer equipped with 488 nm and 633 nm lasers and the standard filter setup. For enumeration of phototrophs, cells were detected on the base of their red fluorescence (FL3) and unfixed samples were analyzed 3 min at a flow rate around 65 µL min^{-1}. Samples were then fixed with 0.25% glutaraldehyde (final concentration) and stained for a minimum of 15 min with SYBR Green I at 1/10,000 of the commercial solution for enumeration of heterotrophic cells (Marie *et al.,* 1999). Trigger was set on the green fluorescence of the SYBR and samples were analyzed 2 min at a flow rate of about 35 µL min^{-1}.

2.2.4. Bacterial productivity

Bacterial production (BP) was measured for 8 to 10 depths per station, distributed in the first 350 m, by [^{3}H]-leucine incorporation (Kirchman *et al.,* 1985) modified for microcentrifugation (Smith and Azam, 1992). Triplicate 1.7 mL aliquots were incubated with a mixture of 50/50 (v/v) [^{3}H]-leucine (Perkin Elmer) and nonradioactive leucine for 4 h at a temperature (1.5 °C) close to that in situ. Samples with 5% trichloroacetic acid added prior to the isotope served as blank. Saturation and time course were performed beforehand to determine the concentration of leucine and minimum incubation time. Leucine incorporation was converted to carbon production using a conservative conversion factor of 1.5 kg C mol^{-1} leucine (Simon and Azam, 1989).

2.2.5. Bacterial viability analysis

The bacterial viability analysis was conducted using a method based on propidium monoazide (PMA). PMA is a photoreactive dye that binds to DNA, inhibiting its replication by PCR. Live cells have intact membranes and are impermeable to PMA, which only influxes into cells with disrupted membranes. The combination of PMA use and PCR provides a rapid and reliable method for discriminating live and dead bacteria. The viability analysis requires two sets of the same sample, one treated with PMA (that gives the quantity of living organisms in the sample) and an untreated one (that gives the total amount of organisms in the sample).

The first step of this method consists of a treatment of the concentrated and filtrated samples with PMA, the filters are then exposed to light, allowing PMA to bind with DNA. For a detailed protocol see Amiraux et al. (2021a).

Nucleic acids were extracted using a chloroform-based method. Filters were placed in 2 mL Eppendorf® tubes and heat-shocked (+80 °C then −80 °C alternately, twice) to improve cell lysis. 100 µL of lysis solution (Tris 20 mM, EDTA 25 mM, lysozyme 1 µg·µL^{-1}) was added and the samples were incubated at 37 °C for 15min. 900 µL of sterile ice-cold water and 900 µL of chloroform were then added and the samples were vortexed five times for 5 s and then centrifuged for 5 min at 10,000 × g. 700 µL of the aqueous phase was collected, transferred to a new tube and any traces of remaining chloroform removed in speed vacuum concentrator (Savant DNA 120, Thermo Scientific ™) for 15 min. Finally, 10 µL of RNase (10 mg·mL^{-1}) was added to the samples, and they were incubated for 30 min at 37 °C or overnight at 4 °C. The DNA obtained was kept frozen at −20 °C for further use.

Absolute quantification of bacterial SSU ribosomal RNA (rRNA) gene was carried out by qPCR with SsoAdvanced™ Sybr Green Supermix on a CFX96 Real-Time System (C1000 Thermal Cycler, Bio-Rad Laboratories, CA, USA) according to the procedure described in Fernandes et al. (2016). For more details about the qPCR program, see Amiraux et al. (2021a).

2.2.6. Lipid extraction

Samples (GF/F filters) were reduced with excess $NaBH_4$ after adding MeOH (25mL, 30 min) to reduce labile hydroperoxides (resulting from photo- or autoxidation) to alcohols, which are more amenable to analysis using gas chromatography–mass spectrometry (GC–MS). Water (25mL) and KOH (2.8 g) were then added and the resulting mixture saponified by refluxing (2 h). After cooling, the mixture was acidified (HCl, 2 N) to pH 1 and extracted with dichloromethane (DCM; 3 × 20 mL). The combined DCM extracts were dried over anhydrous Na_2SO_4, filtered and concentrated by rotary evaporation at 40 °C to give total lipid extracts (TLEs). Aliquots of TLEs were either silylated and analyzed by gas chromatography-electron impact quadrupole time-of-flight mass spectrometry (GC-QTOF) for sterol quantification, or methylated, and then treated with dimethyldisulfide (DMDS) and analyzed by GC–MS/MS for the determination of monounsaturated fatty acid double-bond stereochemistry as previously described by Amiraux et al. (2017). *Cis* and *trans* isomers of monounsaturated fatty acid (MUFA) methyl esters react with DMDS, stereospecifically, to form threo and erythro adducts, which exhibit similar mass spectra but are well-separated by gas chromatography, allowing unambiguous double-bond stereochemistry determination (Buser *et al.,* 1983).

2.2.7. Gas chromatography-tandem mass spectrometry

GC–MS and GC–MS/MS analyses were performed using an Agilent 7890A/7010A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert, 30 m × 0.25 mm, 0.25 µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270 °C. Oven temperature was ramped from 70 °C to 130 °C at 20 °C min^{-1}, then to 250 °C at 5 °C min^{-1} and then to 300 °C at 3 °C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program and then ramped from0.69×10^5 Pa to 1.49×10^5 Pa at 0.04×10^5 Pa min^{-1}. The following mass spectrometer

conditions were used: electron energy 70 eV, source temperature 230 °C, quadrupole 1 temperature 150 °C, quadrupole 2 temperature 150 °C, collision gas (N_2) flow 1.5 mL min^{-1}, quench gas (He) flow 2.25 mL min^{-1}, mass range 50–700 Da, cycle time 313 ms. DMDS derivatives were quantified in multiple reaction monitoring (MRM) mode. Precursor ions were selected from the most intense ions (and specific fragmentations) observed in electron ionization (EI) mass spectra. Trans/cis ratios were obtained directly from peak area measurement of threo and erythron DMDS adducts after analyses, which were carried out three times.

2.2.8. Gas chromatography-EI quadrupole time-of-flight mass spectrometry

Accurate mass measurements were made in full scan mode using an Agilent 7890B/7200 GC/QTOF system (Agilent Technologies, Parc Technopolis – ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Macherey-Nagel; OPTIMA-5MS Accent, 30 m × 0.25 mm, 0.25 µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270 °C. Oven temperature was ramped from 70 °C to 130 °C at 20 °C min^{-1} and then to 300 °C at 5 °C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program. Instrument temperatures were 300 °C for transfer line and 230 °C for the ion source. Nitrogen (1.5 mL min^{-1}) was used as collision gas. Accurate mass spectra were recorded across the range m/z 50–700 at 4 GHz with the collision gas opened. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12,252 from m/z 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was used for daily MS calibration. Compounds were identified by comparing their TOF mass spectra, accurate masses and retention times with those of standards. Quantification of each compound involved extraction of specific accurate fragment ions, peak integration and determination of individual response factors using external standards.

2.2.9. Statistical analysis

The collected data were analyzed using the XLStat version 22.05 software (Adinsoft™). Kruskal-Wallis tests associated with a pairwise multiple comparison (using the Conover-Iman procedure) were performed on trans/cis ratios and on bacterial mortality data, at significance level α =5%.

3. Results

Chlorophyll a concentration in the bottommost layer of ice (0–3 cm) was 0.44, 3.93 and 11.76 µg L^{-1} at St 403, St 409 and St 413, respectively. Chlorophyll a, phaeopigments and particulate organic carbon (POC) concentrations, bacterial abundance (BA) and bacterial production (BP) were measured in the upper 100 m of the water column at each station. Though relatively weak along the whole transect, pelagic chlorophyll a concentrations were found to be highest (up to 0.58 µg L^{-1}) in the upper 30 m of St 409, St 413 and St 418 (Figure 3.2A). The highest phaeopigment concentrations (up to 0.50 µg L^{-1}) were observed between 20 m and 40 m at St 413 (Figure 3.2B). The highest POC concentrations were measured in the topmost waters of St 409, St 413 and St 418 (values reaching 200 mg L^{-1} at the surface of St 418 and at 15 m in the case of St 413) (Figure 3.2C). 10 m for St 413 (Figure 3.3B).

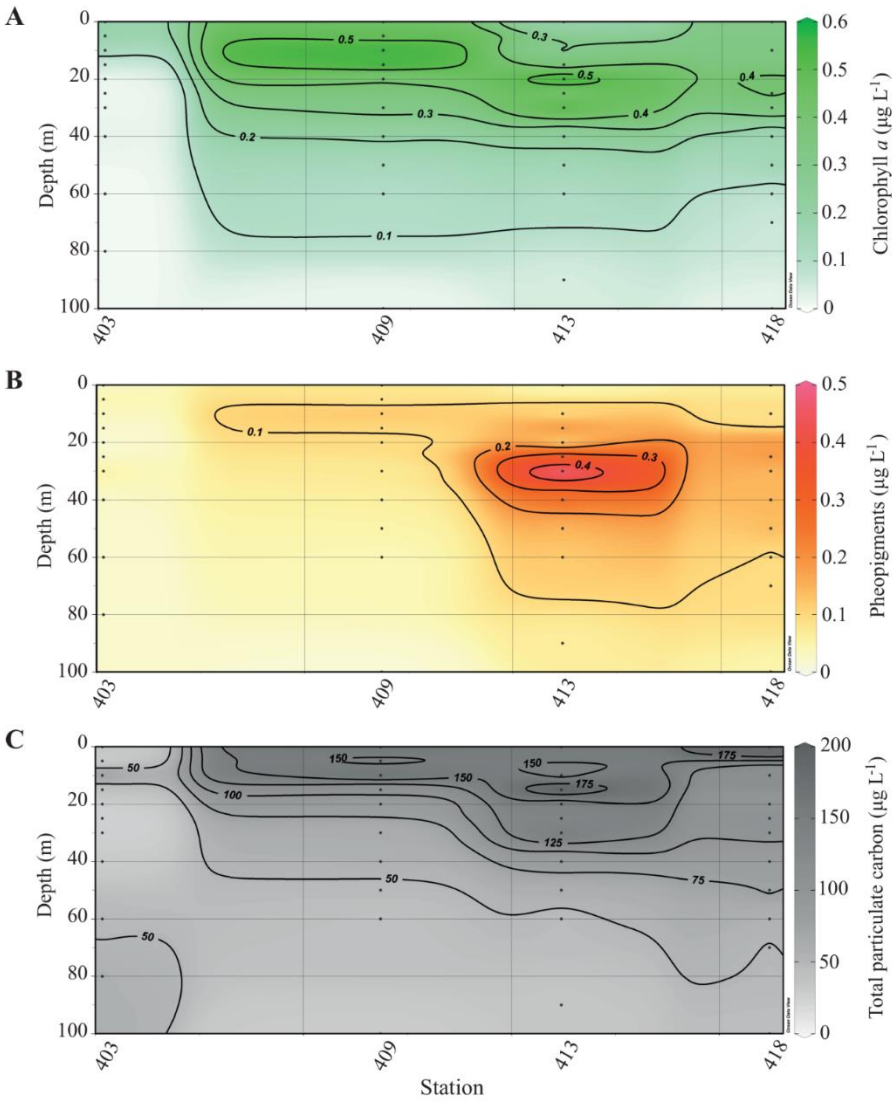

Figure 3.2: Chlorophyll a (A), phaeopigments (B) and total particulate carbon (C) in seawater at the different stations investigated. Data were interpolated and plotted using Ocean Data View v4.7.8 (Schlitzer, 2015).

BA was found to be relatively low at St 403 and St 409, but increased from St 413 to St 418, reaching 1.9 × 10^6 cells mL^{-1} in the upper 30 m of water of St 418 (Figure 3.3A). The highest values of BP (up to 0.33 µgC L^{-1} d^{-1}) were observed at the surface of St 409 and at

To learn more about the nature of the organisms present in the different ice and SPM samples, the relative proportions of the main monounsaturated fatty acids (MUFAs) ($C_{16:1\Delta9}$, $C_{16:1\Delta11}$, $C_{18:1\Delta9}$, $C_{18:1\Delta11}$, $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$), and alcohols ($C_{20:1\Delta11}$ and $C_{22:1\Delta11}$) were measured (Figure 3.4). All the ice samples collected at St 403, St 409 and St 413 were dominated by $C_{16:1\Delta9}$ (palmitoleic) acid. By contrast, $C_{18:1\Delta9}$ (oleic) acid appeared to be the dominant MUFA of most of the SPM samples, except for those collected in the upper 30 m at St. 413 and at the surface and between 25 m and 30 m at St 418 containing a high abundance of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ acids and alcohols (Figure 3.4).

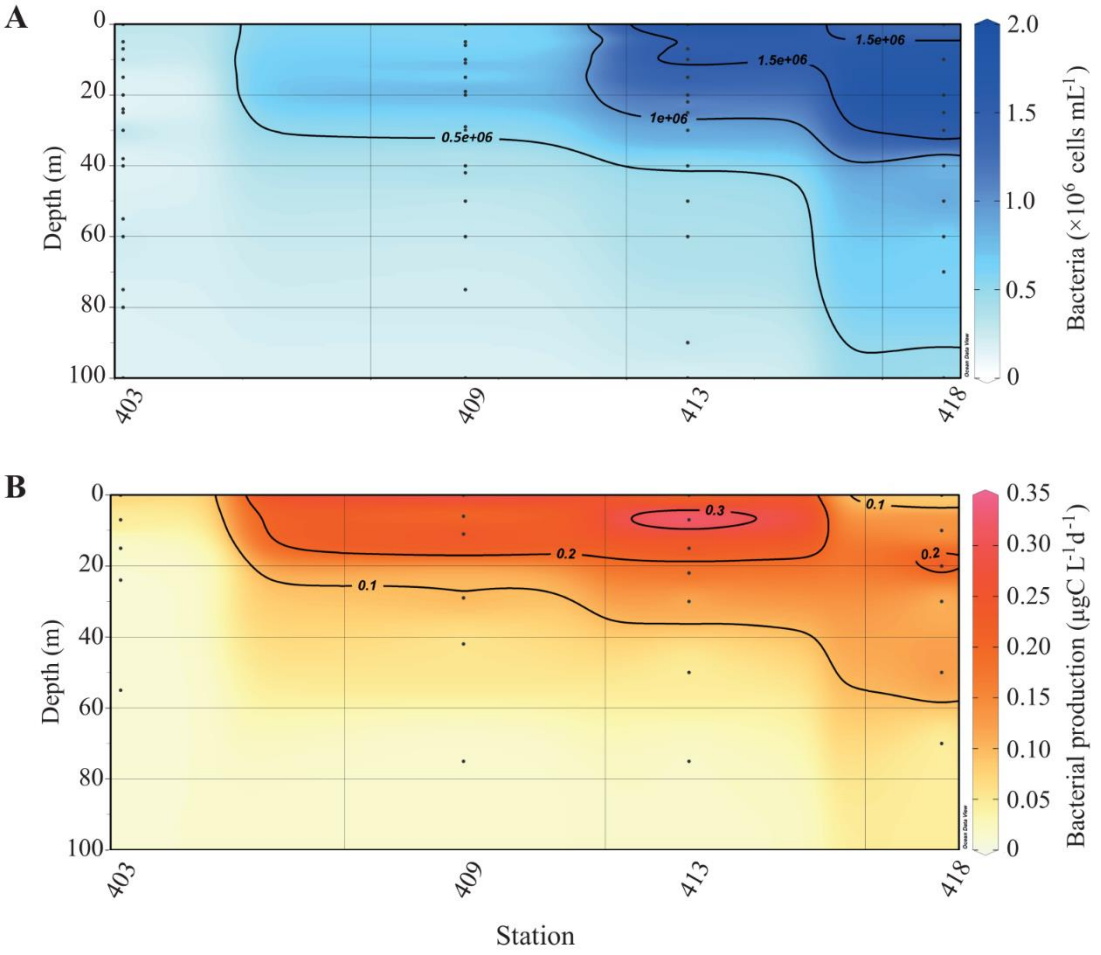

Figure 3.3: Bacterial abundance (BA) (A) and bacterial production (BP) (B) in seawater at the different stations investigated. Data were interpolated and plotted using Ocean Data View v4.7.8 (Schlitzer, 2015).

The main algal sterols – cholest-5,24-dien-3β-ol (desmosterol), 24 methylcholesta-5,22E-dien-3β-ol (brassicasterol), 24- methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol), and 24-ethylcholest-5-en-3β-ol (sitosterol) – were quantified in

ice and water samples at the different stations to confirm the nature of the material present in SPM. Cholesterol, a contaminant often introduced in the samples during their withdrawal and treatment, was excluded from this comparison. Sea ice was found to be dominated by brassicasterol and 24-methylenecholesterol at St 403 and by 24-methylenecholesterol at St 409 and St 413 (Figure 3.5). The deeper SPM samples collected at St 403 showed a dominance of desmosterol, while most of those collected at St 409 and at St 413 were dominated by brassicasterol. Relatively high proportions of desmosterol were also observed in the surface and 20 m samples of St 413 and in the surface and 30 m samples of St 418 (Figure 3.5).

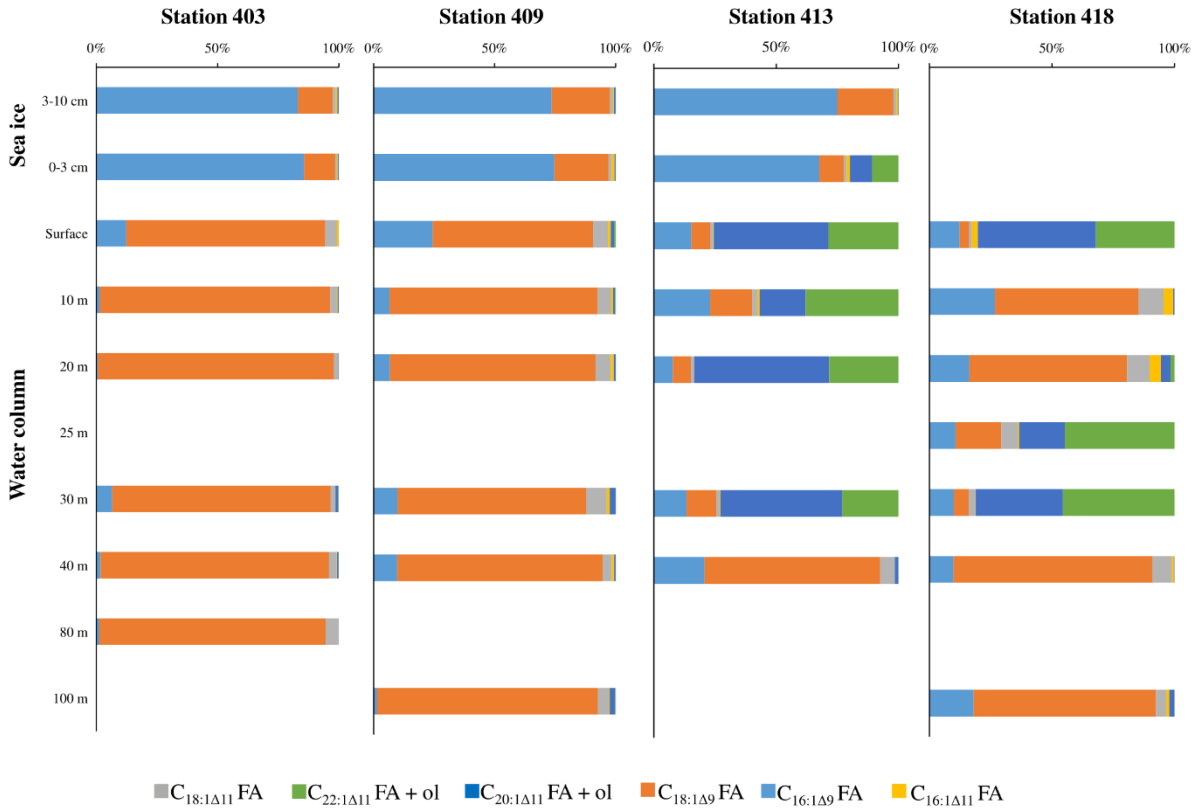

Figure 3.4: Relative proportion of the main monounsaturated fatty acids (FA) and alcohols (ol) in sea ice and underlying seawater at the different stations investigated.

To estimate the stress state of bacteria induced by salinity in brine channels of ice, trans/cis ratios of $C_{16:1\Delta11}$ (hexadec-11-enoic), oleic and $C_{18:1\Delta11}$ (vaccenic) acids were measured in all the samples (Figure 3.6). The results obtained showed a very strong isomerization of hexadec-11-enoic (*trans/cis* ratios reaching 2.1), and vaccenic (*trans/ cis* ratios reaching 0.95) acids, but not of oleic acid in the deeper SPM samples of St 403 and St 409 (Figure 3.7). A strong isomerization of hexadec-11-enoic (*trans/cis* ratio 0.8 and 0.78) and vaccenic acids (*trans/cis* ratios 0.31 and 0.18) was also observed in the 0–3 cm

layer of ice at St 409 and St 413, respectively. At St 418, the three fatty acids were strongly isomerized in the upper SPM samples (*trans/cis* ratio reaching 0.6, 1.0 and 0.45 for hexadec-11-enoic, oleic and vaccenic acids, respectively) (Figure 3.7).

The viability of bacteria was estimated with PMA in sea ice and SPM samples collected at St 409 and in SPM samples collected at St 418. At St 409, a high bacterial mortality was observed in sea ice (88.7 and 84.3% in the 3–10 and 0–3 cm layers, respectively) and in the 10 m SPM sample (62.3%), but not in the 20 m sample (Table 3.1). In the deeper (≥30 m) SPM samples collected at St 418, bacterial mortality was found to be very low, but it was relatively high in the surface and 20 m samples (23.3 and 67.5%, respectively) (Table 3.1).

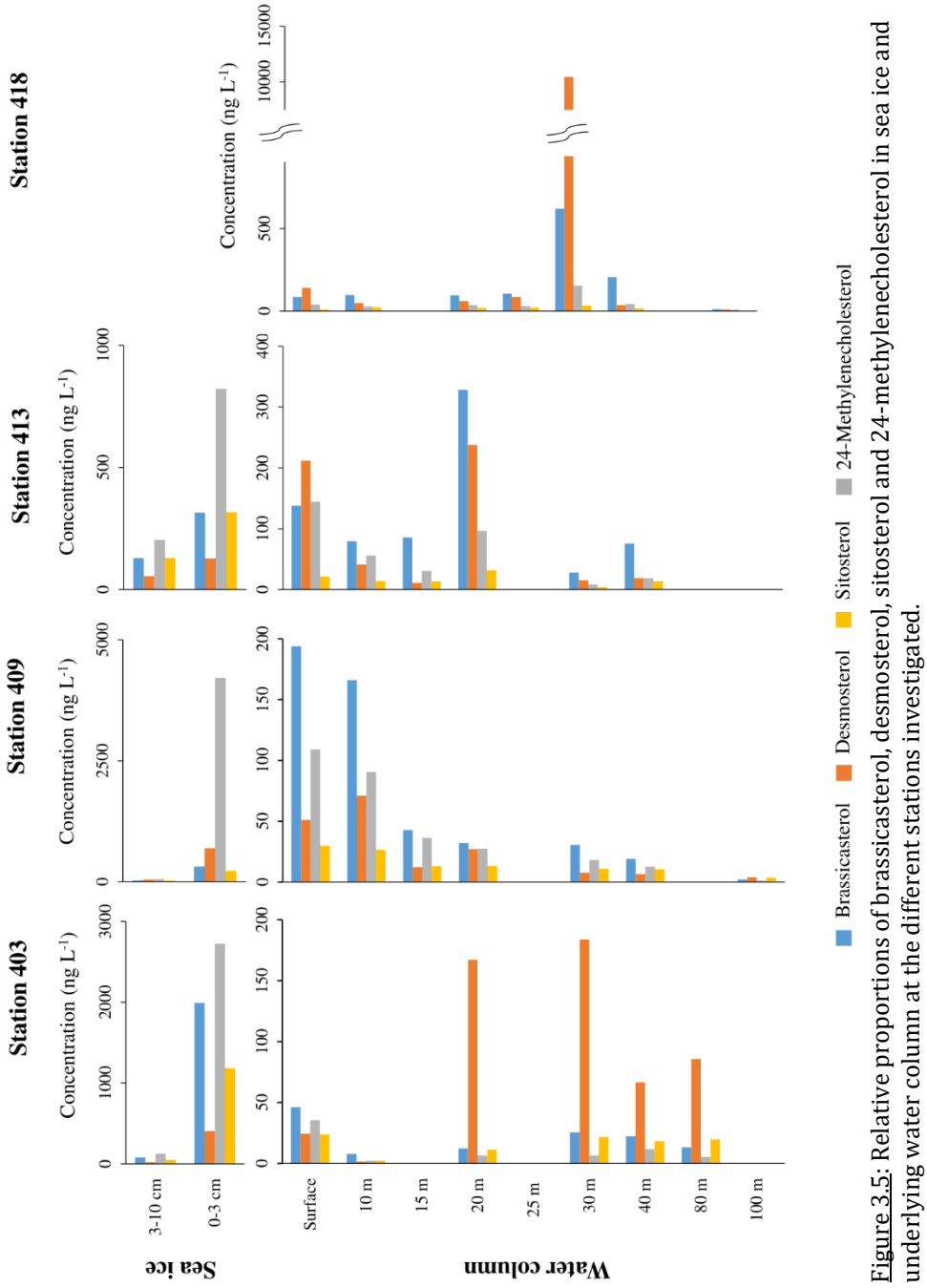

Figure 3.5: Relative proportions of brassicasterol, desmosterol, sitosterol and 24-methylenecholesterol in sea ice and underlying water column at the different stations investigated.

4. Discussion

4.1 Composition of sea ice and SPM samples

The low chlorophyll a concentrations measured in the bottommost ice samples corresponded to only one-tenth of the values previously observed at the time of the sympagic algal bloom at the GreenEdge ice camp located near Broughton Island in Baffin Bay (Figure 3.1) (Amiraux *et al.,* 2019). It thus seems that the bloom of sympagic algae took place before the start of sampling. The increase in chlorophyll a concentration observed in this layer from St 403 to St 413 was thus attributed to the growth of the epiphytic diatom *Melosira arctica*, whose presence was noted during the sampling (Amiraux, unpublished data).

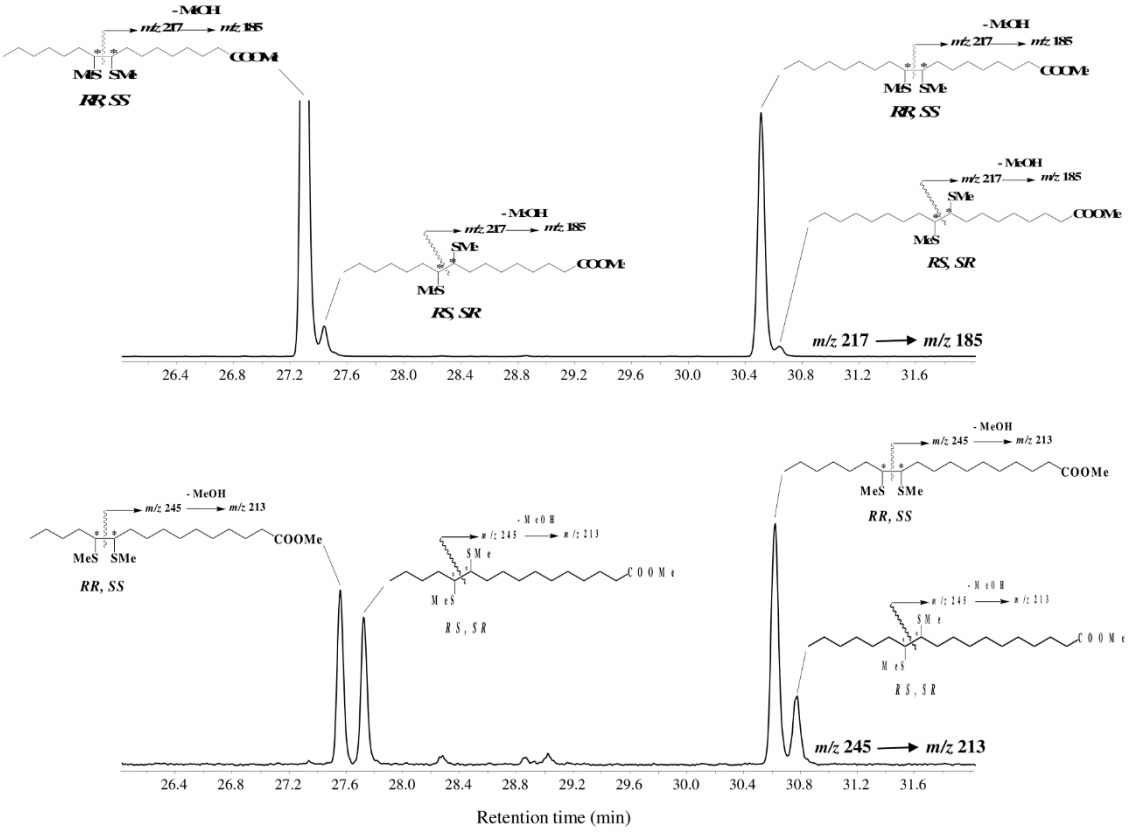

Figure 3.6: MRM chromatograms (m/z 217 → 185 and m/z 245 → 213) of DMDS derivatives of MUFAs in the bottommost layer of ice (0–3 cm) at St 409.

The fatty acid profiles of all the sea ice samples were dominated by $C_{16:1\Delta9}$ (palmitoleic) acid (Figure 3.4) well-known to be the main fatty acid component of sea ice-associated (sympagic or epiphytic) diatoms (Fahl and Kattner, 1993; Falk-Petersen *et al.,* 1998; Leu *et al.,* 2010). The algal community present in the 0–3 cm sample at St 403 seemed to be

mainly composed of pennate and centric (*M. arctica*?) diatoms and Thalassiosirales as suggested by the dominance of brassicasterol and 24-methylenecholesterol (Barrett *et al.,* 1995; Rampen *et al.,* 2010) (Figure 3.5). The increasing proportions of 24-methylenecholesterol observed at St 409 and St 413 were attributed to the presence of increasing amounts of *M. arctica* (containing a significant proportion of this sterol, Smik, unpublished data).

It is well known that sea ice retreat controls the timing of summer plankton blooms in the Arctic Ocean (Janout *et al.,* 2016). From visual observation and knowledge of bloom dynamics, a chlorophyll a threshold of 0.5 μg L^{-1} was defined by Perrette et al. (2011) to identify the blooms in the Arctic. The chlorophyll a concentrations measured in the water column along the transect investigated (up to 0.58 μg L^{-1}) only exceeded this threshold between 0 and 20 m at St 409 and between 15 m and 35 m at St 413 (Figure 3.2A), suggesting the presence of a weak ice-edge bloom at these stations. In the ice-covered water column, chlorophyll may result from: (i) the growth of pelagic phytoplankton or (ii) the release of non-aggregated sympagic or epiphytic algae during melting. The former hypothesis is well supported by the dominance of brassicasterol observed in surface SPM samples (brassicasterol/24- methylenecholesterol ratio 1.0–1.8) (Figure 3.5), which contrasts with the dominance of 24-methylenecholesterol in the bottommost layer of sea ice at these stations (brassicasterol/24-methylenecholesterol ratio 0.07–0.7) (Figure 3.5). This dominance of brassicasterol probably results from the presence of the prymnesiophyte *Phaeocystis pouchetii*, whose sterol fraction is known to consist of almost 100% brassicasterol (Nichols *et al.,* 1991) and which is a main component of under-ice and spring blooms across the Arctic (Riisgaard *et al.,* 2015). Microscopic examination of some SPM samples supported this explanation (e.g. percentage of Phaeocystis to protists at 30 m at St 413 ≈ 50%) (Babin, unpublished data). However, during sea ice melting the species composition of dispersed and aggregated algae may differ significantly (Riebesell *et al.,* 1991). Proportionately more cells of weakly aggregated pennate diatoms containing high proportions of brassicasterol (Rampen *et al.,* 2010) may thus stay suspended, while other more aggregated ice algae (e.g. Thalassiosirales or *M. arctica*) incorporated into sinking particles should rapidly sink out of the euphotic zone (Riebesell *et al.,* 1991).

A high zooplanktonic grazing activity, well supported by the high abundance of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols, was observed in the upper 30 m at St 413 and at the surface and between 25 m and 30 m at St 418 (Figure 3.4).Wax esters are generally the main storage lipids of marine zooplankton in high-latitude species (Lee *et al.,* 2006) and the most

common alkan-1-ols of the wax esters found in herbivorous zooplankton are $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols (Lee and Nevenzel, 1979; Albers *et al.,* 1996). These alcohols are only known to occur in copepods that undergo diapause (Graeve *et al.,* 1994), which are widely distributed in the Arctic.

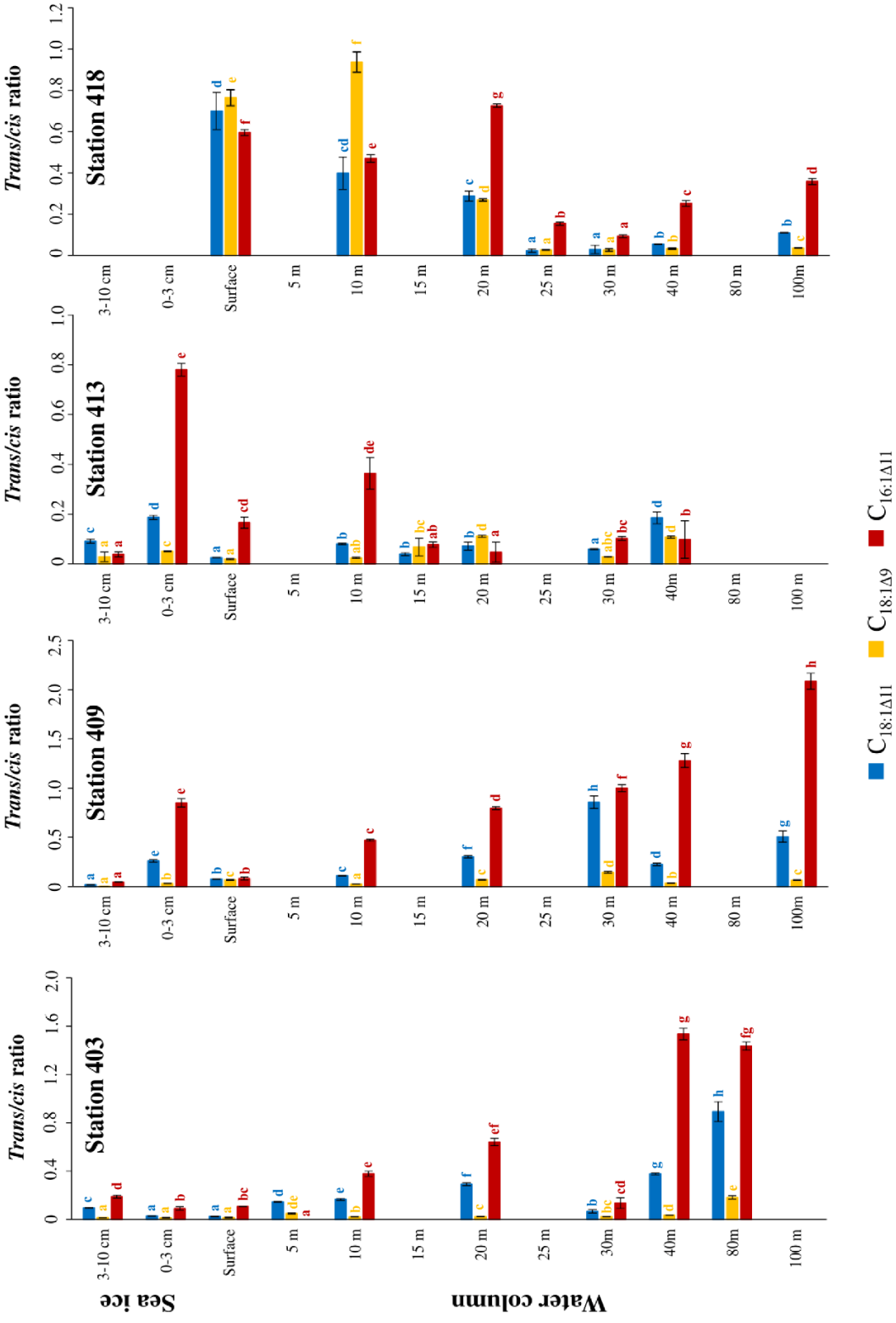

Figure 3.7: Mean Trans/cis ratio of vaccenic ($C_{18:1\Delta11}$), oleic ($C_{18:1\Delta9}$), and hexadec-11-enoic ($C_{16:1\Delta11}$) acids in sea ice and the underlying water column at the different stations investigated (n = 3, analytical triplicates). For each station and each acid, significantly different values are annotated with different letters ($P < 0.05$).

In this area, dominant herbivorous zooplankton are the three large *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* in addition to the smallest *Pseudocalanus* spp. (Sameoto, 1984; Forest *et al.,* 2012). At this period of the year, naupliis and copepodits depend on the bloom to develop into adults, and diapausing adults metabolize primary production into highly rich esters stocks (Conover and Huntley, 1991; Falk-Petersen *et al.,* 2009). A study on the surface copepod community of Baffin Bay from Underwater Vision Profiler (UVP) data revealed that a lot a small copepods were actively feeding in the eastern and ice-free waters (including St 418) during the GreenEdge cruise (Vilgrain *et al.,* submitted). Complementary data from net sampling showed that ice-free stations are dominated by young stages of *C. finmarchicus* and *C. glacialis* (naupliis, and stages CI to CVI) in addition with CII to CIV stages of *C. hyperboreus* (Figure S3.1). All stages of *Pseudocalanus* spp. Were also more abundant in St 413 and 418 with naupliis in particular (Figure S3.1). The large proportion of young herbivorous stages was expected in ice-free stations according to their life cycle strategies (Hirche and Niehoff, 1996; Søreide *et al.,* 2010). Adults of all these species were generally distributed all over the Bay, but diapausing species such as *Calanus* spp., are probably metabolizing esters from phytoplanktonic precursors, which could explain the presence of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols at St 413 and St 418.

Although the degradation of chlorophyll a to phaeopigments occurs in the guts of both large and small macro-zooplankton (Nelson, 1989), these compounds could be detected in significant proportions only at 30 m at St 413 (Figure 3.2B). The low concentrations of phaeopigments observed in the upper SPM samples of St 413 may be attributed to photooxidation processes, well known to degrade such pigments quickly (Welschmeyer and Lorenzen, 1985) and strongly favored at St 413 due to the lack of snow cover and the relatively thin ice (limited to 40 cm). Despite the very high copepod activity present at 30 m at St 418 (Figure 3.5), phaeopigment concentration was found to be very weak (Figure 3.2B), probably owing to a particularly intense photooxidation at this open water station. As expected, in the samples where the presence of high proportions of copepods was indicated by $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols, large proportions of their two main sterols (Harvey *et al.,* 1987) cholesterol (not shown) and desmosterol (Figure 3.5) could be observed.

The deepest SPM samples collected at St 403 were characterized by (i) very low chlorophyll a concentrations (Figure 3.2A), (ii) high proportions of oleic acid (Figure 3.4)

and desmosterol (Figure 3.5) and (iii) lack of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols. Oleic acid is often enriched in secondary producers (Falk-Petersen *et al.,* 1999) and thus commonly interpreted as a marker of heterotrophic feeding (Graeve *et al.,* 1997; Tolosa *et al.,* 2004). Moreover, desmosterol is produced by zooplankton during the conversion of dietary phytosterols to cholesterol (Harvey *et al.,* 1987). Given the absence of alkanols, the presence of copepods in these samples was excluded and that of micro-zooplankton suspected. In Baffin Bay, it is well known that the ice microfauna is dominated by dinoflagellates and ciliates (Michel *et al.,* 2002), rarely observed in the water column, probably because prey are too scarce. These SPM samples thus seem to contain herbivorous micro-zooplankton feeding in ice and then released in the water column during melting. The presence of *trans* MUFAs, typical of stressed sympagic bacteria (see Section 4.2), suggests the simultaneous presence of sympagic herbivorous and bacterivorous micro-zooplankton in these samples. In the deepest samples of St 409 characterized by very low amounts of sterols (Figure 3.5), only bacterivorous micro zooplankton, generally incapable of synthesizing or incorporating sterols (Breteler *et al.,* 2004), seem present.

Table 3.1*:* Mean percentage of dead attached bacteria in sea ice and SPM samples collected at St 409 and St 418 (n=3). For each station, significantly different values are annotated with different letters ($P < 0.05$).

Station	Sample	Dead bacteria (%)
St 409	Ice (3-10 cm)	88.7 ± 4.7 (b)
St 409	Ice (0-3 cm)	84.3 ± 9.7 (b)
St 409	SPM 10 m	62.3 ± 22.06 (ab)
St 409	SPM 20 m	0 ± 11.9 (a)
St 418	SPM surface	23.3 ± 11.3 (b)
St 418	SPM 20 m	67.5 ± 4.1 (c)
St 418	SPM 30 m	3.8 ± 39.0 (ab)
St 418	SPM 40 m	0.6 ± 15.5 (ab)
St 418	SPM 100 m	0 ± 4.6 (a)

SPM material collected between 10 m and 20 m of the open water station St 418 exhibited relatively high proportions of hexadec-11- enoic and vaccenic acids (Figure 3.4), well known to be specific to bacteria (Lambert and Moss, 1983; Sicre *et al.,* 1988). Most of the oleic acid present in these samples also arises from bacteria (see Section 4.2). These samples thus contained a large proportion of bacteria (Figure 3.4). These observations

are consistent with the highest BA measured in these samples (Figure 3.3A), which is similar to those previously measured in spring in sea ice of the Chukchi Sea (0.7–2.5 10^6 cells mL^{-1}; Meiners *et al.,* 2008). Arctic sea ice harbors large amounts of extracellular polymeric substances (EPS) in both the dissolved and particulate fractions (Krembs and Engel, 2001; Krembs *et al.,* 2002; Meiners *et al.,* 2003). Some authors (Riedel *et al.,* 2006; Juhl *et al.,* 2011) previously demonstrated that EPS retained within the melting sea ice in the Arctic could supply a pulse of organic carbon to surface waters after most of the sea-ice algal biomass has been released into the water column. In the pelagic realm, EPS-rich particles, which have been observed to be positively or neutrally buoyant (Azetsu-Scott and Passow, 2004; Meiners *et al.,* 2008), are densely colonized by attached bacteria and are ideal vectors for their horizontal distribution (Meiners *et al.,* 2008). Bacteria use these particles as sites of attachment, possibly to protect them from grazers (Salcher *et al.,* 2005), or as a carbon-rich substrate, which could enhance bacterial production (Riedel *et al.,* 2006). These exopolymers could thus ascend in association with attached bacteria (Azetsu-Scott and Passow, 2004). The highest abundances of bacteria observed at 10 m and 20 m at St 418 (Figure 3.3A) were thus attributed to the release of EPS particles heavily colonized by bacteria during sea ice melting after previous loss of the sympagic algal biomass.

4.2 Stress state of bacteria in sea ice and SPM particles

Cis-trans isomerization of MUFAs has been shown to serve as an adaptive response to chemical or osmotic stress in strains of the widespread genera Pseudomonas and Vibrio (Okuyama *et al.,* 1991; Heipieper *et al.,* 1992; Molina-Santiago *et al.,* 2017). High trans/cis vaccenic acid ratios were previously observed in sinking particles collected during the 2015 and 2016 GreenEdge ice camps (Amiraux *et al.,* 2017; Amiraux *et al.,* 2021a). These high values were attributed to release of non-growing bacteria attached to sympagic algae stressed by salinity in internal brine channels during the early stages of sea ice melting. To determine whether bacteria attached to suspended particles were also stressed by salinity, trans/cis ratios of the main monounsaturated fatty acids present in Pseudomonas sp. and Vibrio sp. (hexadec- 11-enoic, oleic and vaccenic acids) (Lambert *et al.,* 1983; Holmström *et al.,* 1998; Jia *et al.,* 2014) were measured in sea ice and in SPM samples along the transect investigated. Although present in some Vibrio sp. (Lambert *et al.,* 1983; Jia *et al.,* 2014), palmitoleic acid was excluded from these measurements owing to its lack

of specificity (strong dominance in sympagic and pelagic diatoms) (Fahl and Kattner, 1993; Leu *et al.,* 2010).

Very high trans/cis ratios of hexadec-11-enoic and vaccenic acids were observed in the deepest SPM samples of St 403 and St 409 (Figure 3.7), which seemed to be dominated by micro-zooplankton (see Section 4.1). It was previously observed that the lipid composition (fatty acids and neutral lipids) of bacterivorous ciliates resembled that of their prey (Harvey *et al.,* 1987; Boëchat and Adrian, 2005). The high *trans/cis* values observed in these samples were thus attributed to (i) the ingestion of bacteria stressed by salinity in internal brines of sea ice by sympagic ciliates, (ii) the direct incorporation of highly isomerized dietary fatty acids and (iii) the release of these bacterivorous ciliates in the water column during ice melting. The well-known biosynthesis of cis-oleic acid during the metabolism of ciliates (Erwin and Bloch, 1963) is consistent with the relatively weak *trans/cis* ratio of this acid observed in these SPM samples (Figure 3.7). Brine salinity, which could not be measured during the cruise, are expected to be low at the time of sampling (summer). However, high brine salinity values (ranging from 50 to 70) were measured in May 2015 and 2016 in the upper part of the ice at Qikiqtarjuaq (GreenEdge fixed station relatively close to the transect investigated, Figure 3.1) (Amiraux *et al.,* 2019, 2021a). Non-halophilic bacteria strongly affected by these hypersaline conditions in spring could thus have been ingested by sympagic ciliates and trapped in the ice before to be released in the water column during the summer melting period.

It is well known that the uppermost section of the ice experiences the most drastic changes in brine salinity (Ewert and Deming, 2013). As a consequence, bacteria attached to sympagic algae in the bottommost ice are generally not highly affected by osmotic stress (Rontani *et al.,* 2018; Amiraux *et al.,* 2021a). The high trans/cis ratios of hexadec-11-enoic and vaccenic acids measured in the bottommost 3 cm of ice of St 409 and St 413 (Figure 3.6, Figure 3.7) were thus surprising. Given the relative similarity of these ratios with those observed in the deepest samples of St 403 and St 409 (Figure 3.7), this isomerization was attributed to the presence of ciliates fed on salinity-stressed bacteria in internal brine channels and trapped during their discharge at the bottom of ice.

It is generally considered that suspended particles, which constitute most of the standing stock of particulate matter in the ocean (Wakeham and Lee, 1989), sink very slowly through the water column. However, aggregation processes, the extent of which remains to be estimated (Wakeham and Lee, 1989; Hill, 1998), can strongly increase the

settling velocity of these particles and thus their contribution to the seafloor. Sympagic microzooplankton can thus contribute to the transfer of the signature of bacteria stressed by hypersaline conditions in brine channels of sea ice to the sediments.

SPM material collected in the topmost waters of St 418 seems to be composed of EPS particles retained in sea ice and discharged in seawater after the initial release of algal biomass (Riedel *et al.,* 2006; Juhl *et al.,* 2011). Such EPS particles contain high amounts of bacteria (Meiners *et al.,* 2008) that may be of sympagic or pelagic origin. The very high *trans/cis* ratios of hexadec-11-enoic, vaccenic and oleic acids observed in these SPM samples (Figure 3.7) demonstrate that the bacteria attached to EPS particles are strongly stressed by salinity and thus arise from sea ice. The strong isomerization of oleic acid observed also attests to the bacterial origin of this acid. In the presence of osmotic stress, CTI activity is used by bacteria as an urgent response to guarantee survival, before other adaptive mechanisms (Heipieper *et al.,* 2007). Consequently, with no osmotic stress (as is the case in the water column) the *trans/cis* ratio of bacteria stressed by salinity in brine channels of sea ice should decrease to the basic level (Fischer *et al.,* 2010). Since conversion of trans to cis fatty acids is not catalyzed (Eberlein *et al.,* 2018), recovery of the regularly low trans/cis ratio needs de novo synthesis of cis fatty acids and thus depends on bacterial growth rates. The very high values of the trans/cis ratio observed in the topmost waters of St 418 (Figure 3.7) are thus indicative of the non-growing state of bacteria attached to EPS particles. This assumption is well supported by the relatively weak BP measured in these samples (Figure 3.3B) exhibiting the highest BA (Figure 3.3A).

4.3. Viability of attached bacteria in sea ice and SPM particles

PMA treatment showed that most of the bacteria associated with sympagic algae at St 409 had disrupted membranes and so were dead (Table 3.1). These results are consistent with the high mortality of attached bacteria measured in sea ice at the end of the 2016 GreenEdge ice camp (Amiraux *et al.,* 2021a) and attributed to the bactericidal properties of free fatty acids (FFAs) released by sympagic algae under the effect of light stress. The toxicity of FFAs results from their insertion into the bacterial inner membrane, increasing its permeability and letting internal contents leak from the cell, which can result in death (Boyaval *et al.,* 1995; Shin *et al.,* 2007). A high mortality was also observed in the 10 m SPM sample (Table 3.1). This suggests that the algal material present in this sample (Figure 3.5) results from the release of non-aggregated and FFA-producing sympagic diatoms during ice melting rather than from the growth of pelagic algae. By contrast, the

viability of bacteria attached to suspended particles collected at 20 m and dominated by micro-zooplankton was found to be very good (Table 3.1).

Concerning SPM samples collected at St 418, PMA revealed a very low mortality of attached bacteria in the deeper (≥30 m) samples (Table 3.1). This good viability is probably due to the presence of unstressed bacteria associated with copepod or micro-zooplankton material, which dominated these samples (see Section 4.1). By contrast, a lower viability was observed in the samples collected at the surface and at 20 m (23.3 and 67.5% of mortality, respectively) (Table 3.1). The 20 m sample mainly composed of EPS particles thus contained a mixture of dead bacteria (in which the integrity of cell membranes could not be maintained) and non-growing bacteria (where cis-trans isomerization of monounsaturated fatty acids ensured membrane stiffness but not growth). The presence of a significant proportion of zooplanktonic material (potential supports of unstressed bacteria) in the surface sample () could explain the lower mortality (relative to the 20m sample) observed (Table 3.1).

4.4. Considerations about the preservation and transfer of sympagic material to the seafloor

The preservation of sympagic algae during their transfer in the water column depends mostly on grazing by zooplankton and mineralization by their attached bacteria. We previously demonstrated that during the early stages of ice melting, bacteria associated to sinking sympagic material have been strongly stressed in hypersaline brine channels and are thus mainly non-growing in these particles (Amiraux *et al.,* 2017). In contrast, during the advances stages of melting most bacteria associated to sinking ice algae appeared to be stressed by free fatty acids and dead (Amiraux *et al.,* 2021a). Whereas only a small part of the sympagic material is released during the early stages of ice melting, i.e. when bacteria are stressed by hypersaline conditions (Amiraux *et al.,* 2021a, 2021b), a strong cis-trans isomerization of MUFAs was previously observed in Arctic sediments (Rontani *et al.,* 2012; Amiraux *et al.,* 2017). The results obtained during the present study allow to propose an explanation to this paradox. Indeed, during the early stages of ice melting feeding of sympagic microzooplankton on stressed bacteria results to the incorporation and transfer of that stress signal (after aggregation) to the deeper waters, whereas at the advanced stages of melting copepods intensively assimilate the sympagic material (EPS-rich particles and sea ice algae) released in the water column. Due to the

good healthy state of bacteria associated to the resulting copepod fecal pellets, this material should be degraded intensively within the water column contributing only weakly to the sediments. It thus appears that trophic relationships between sea-ice algae, their associated bacteria and zooplanktonic grazers are strongly intricate and need to be more investigated.

5. Conclusions

Lipid analyses and propidium monoazide (PMA) method allowed the monitoring of the stress and viability of attached bacteria in sea ice and SPM samples collected during the GreenEdge 2016 cruise in Baffin Bay, along a transect from sea ice to open water. Our results are summarized in a conceptual trophic network scheme (Figure 3.8).

At the western stations ice covered St 403 and St 409 lipid analyses showed a strong cis-trans isomerization of MUFAs attributed to the presence of sympagic bacterivorous microzooplankton (ciliates) incorporating trans fatty acids after ingestion of bacteria osmotically stressed in hypersaline brine channels of ice (Figure 3.8). At the St 409, the high bacterial mortality measured in sea ice is consistent with that previously observed during the GreenEdge 2016 ice camp (Amiraux *et al.,* 2021a) and is likely due to the release of bactericidal FFAs by sympagic algae under the effect of light stress (Figure 3.8).

At the eastern ice-covered station St 413 and open water station St 418 the lipid analysis showed a high proportion of macro-zooplankton (copepods) (Figure 3.8). SPM material collected in shallower waters at the open water St 418 seems to be mainly composed of EPS-rich particles retained in sea ice and discharged in seawater after the initial release of algal biomass. In those waters, most of the bacteria associated to this material appeared to be either dead or in a non-growing state, while these attached to deeper SPM of St 418 (dominated by zooplanktonic material) were in good healthy state (Figure 3.8).

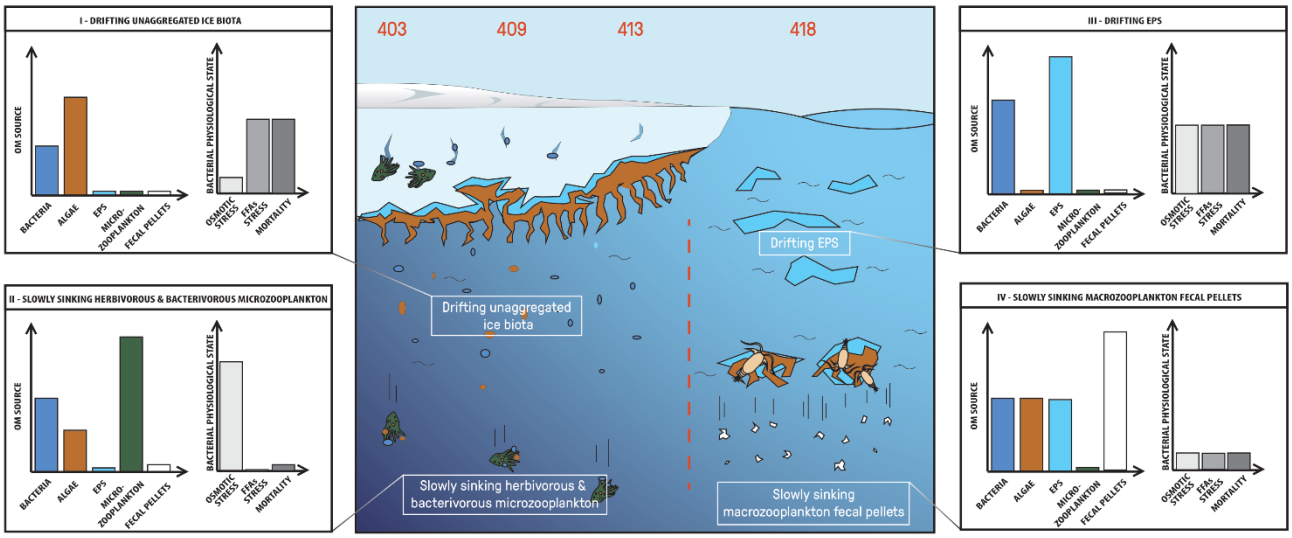

Figure 3.8: Conceptual scheme summarizing the main results obtained.

3.2.3 Informations supplémentaires

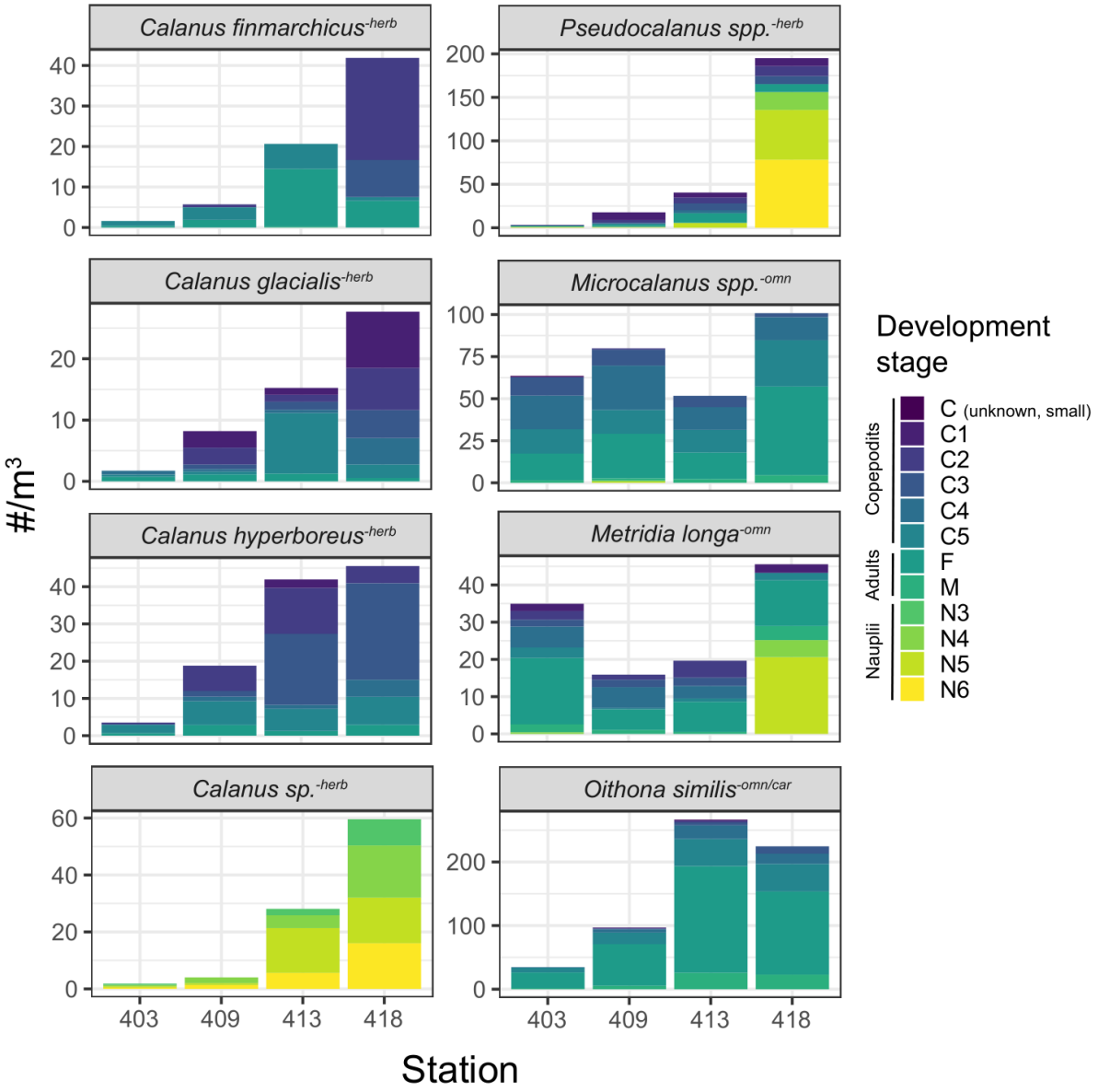

Figure S3.1: Concentrations (number of individuals per m^3) of dominant copepod species at the different stations investigated, according to their development stages. Main feeding modes are indicated -her: herbivorous, -omn: omnivorous, -car: carnivorous. Herbivorous species, and young development stages in particular, show high variations in abundances along sea ice gradients, while it is less clear for omnivorous species.

Bibliographie

Albers, C. S., Kattner, G., & Hagen, W. (1996). The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: Evidence of energetic adaptations. *Marine Chemistry*, *55*(3–4), 347–358. https://doi.org/10.1016/S0304-4203(96)00059-X

Amiraux, R., Belt, S. T., Vaultier, F., Galindo, V., Gosselin, M., Bonin, P., & Rontani, J.-F. (2017). Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers. *Marine Chemistry*, *194*, 89–99. https://doi.org/10.1016/j.marchem.2017.05.006

Amiraux, R., Burot, C., Bonin, P., Guasco, S., Babin, M., & Rontani, J.-F. (2021a). Stress factors resulting from the Arctic vernal sea ice melt: Impact on the viability of the bacterial communities associated to sympagic algae. *Elementa Science of the Anthropocene (under Review)*. https://online.ucpress.edu/elementa/article/8/1/076/114223/Stress-factors-resulting-from-the-Arctic-vernal?searchresult=1

Amiraux, R., Rontani, J.-F., Armougom, F., Frouin, E., Babin, M., Artigue, L., & Bonin, P. (2021b). Bacterial diversity and lipid biomarkers in sea ice and sinking particulate organic material during the melt season in the Canadian Arctic. *Elementa: Science of the Anthropocene*, *9*. https://doi.org/10.1525/elementa.2019.040

Amiraux, R., Smik, L., Köseoğlu, D., Rontani, J.-F., Galindo, V., Grondin, P.-L., Babin, M., & Belt, S. T. (2019). Temporal evolution of IP_{25} and other highly branched isoprenoid lipids in sea ice and the underlying water column during an Arctic melting season. *Elementa Science of the Anthropocene*, *7*(1), 38. https://doi.org/10.1525/elementa.377

Azetsu-Scott, K., & Passow, U. (2004). Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the upper ocean. *Limnology and Oceanography*, *49*(3), 741–748. https://doi.org/10.4319/lo.2004.49.3.0741

Barrett, S. M., Volkman, J. K., Dunstan, G. A., & LeRoi, J.-M. (1995). Sterols of 14 Species of Marine Diatoms (bacillariophyta)1. *Journal of Phycology*, *31*(3), 360–369. https://doi.org/10.1111/j.0022-3646.1995.00360.x

Bates, S. S., & Cota, G. F. (1986). Fluorescence Induction and Photosynthetic Responses of Arctic Ice Algae to Sample Treatment and Salinity1. *Journal of Phycology*, *22*(4), 421–429. https://doi.org/10.1111/j.1529-8817.1986.tb02484.x

Boëchat, I. G., & Adrian, R. (2005). Biochemical composition of algivorous freshwater ciliates: You are not what you eat. *FEMS Microbiology Ecology*, *53*(3), 393–400. https://doi.org/10.1016/j.femsec.2005.01.005

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernandez-Mendez, M., Hendricks, S., Katlein, C., Lalande, C., Krumpen, T., Nicolaus, M., Peeken, I., Rabe, B., Rogacheva, A., Rybakova, E., Somavilla, R., Wenzhofer, F., & RV Polarstern ARK27-3-Shipboard Science Party. (2013). Export of Algal Biomass from the Melting Arctic Sea Ice. *Science*, *339*(6126), 1430–1432. https://doi.org/10.1126/science.1231346

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J. A., & Rapp, J. Z. (2015). Microbial ecology of the cryosphere: Sea ice and glacial habitats. *Nature Reviews Microbiology*, *13*(11), 677–690. https://doi.org/10.1038/nrmicro3522

Boras, J. A., Sala, M. M., Arrieta, J. M., Sà, E. L., Felipe, J., Agustí, S., Duarte, C. M., & Vaqué, D. (2010). Effect of ice melting on bacterial carbon fluxes channelled by viruses and protists in the Arctic Ocean. *Polar Biology*, *33*(12), 1695–1707. https://doi.org/10.1007/s00300-010-0798-8

Boyaval, P., Corre, C., Dupuis, C., & Roussel, E. (1995). Effects of free fatty acids on propionic acid bacteria. *Le Lait*, *75*(1), 17–29. https://doi.org/10.1051/lait:199512

Breteler, W., Koski, M., & Rampen, S. (2004). Role of essential lipids in copepod nutrition: No evidence for trophic upgrading of food quality by a marine ciliate. *Marine Ecology Progress Series*, *274*, 199–208. https://doi.org/10.3354/meps274199

Buser, H. Rudolf., Arn, Heinrich., Guerin, Patrick., & Rauscher, Stefan. (1983). Determination of double bond position in mono-unsaturated acetates by mass spectrometry of dimethyl disulfide adducts. *Analytical Chemistry*, *55*(6), 818–822. https://doi.org/10.1021/ac00257a003

Cho, B. C., & Azam, F. (1988). Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature*, *332*(6163), 441–443. https://doi.org/10.1038/332441a0

Conover, R. J., & Huntley, M. (1991). Copepods in ice-covered seas—Distribution, adaptations to seasonally limited food, metabolism, growth patterns and life cycle strategies in polar seas. *Journal of Marine Systems*, *2*(1–2), 1–41. https://doi.org/10.1016/0924-7963(91)90011-I

Cooksey, K., & Wigglesworth-Cooksey, B. (1995). Adhesion of bacteria and diatoms to surfaces in the sea: A review. *Aquatic Microbial Ecology*, *9*, 87–96. https://doi.org/10.3354/ame009087

Ducklow, H. (2000). Bacterial Production and Biomass in the Oceans. In *Microbial Ecology of the Oceans* (Vol. 1, pp. 85–120). Wiley & Sons.

Dupont, F. (2012). Impact of sea-ice biology on overall primary production in a biophysical model of the pan-Arctic Ocean. *Journal of Geophysical Research: Oceans*, *117*(C8). https://doi.org/10.1029/2011JC006983

Eberlein, C., Baumgarten, T., Starke, S., & Heipieper, H. J. (2018). Immediate response mechanisms of Gram-negative solvent-tolerant bacteria to cope with environmental stress: Cis-trans isomerization of unsaturated fatty acids and outer membrane vesicle secretion. *Applied Microbiology and Biotechnology*, *102*(6), 2583–2593. https://doi.org/10.1007/s00253-018-8832-9

Engel, A., & Passow, U. (2001). Carbon and nitrogen content of transparent exopolymer particles (TEP) in relation to their Alcian Blue adsorption. *Marine Ecology Progress Series*, *219*, 1–10. https://doi.org/10.3354/meps219001

Erwin, J., & Bloch, K. (1963). Lipid metabolism of ciliated protozoa. *Journal of Biological Chemistry*, *238*, 1618–1624.

Ewert, M., & Deming, J. (2013). Sea Ice Microorganisms: Environmental Constraints and Extracellular Responses. *Biology*, *2*(2), 603–628. https://doi.org/10.3390/biology2020603

Fahl, K., & Kattner, G. (1993). Lipid Content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). *Polar Biology*, *13*(6), 405–409. https://doi.org/10.1007/BF01681982

Falk-Petersen, S., Mayzaud, P., Kattner, G., & Sargent, J. R. (2009). Lipids and life strategy of Arctic *Calanus*. *Marine Biology Research*, *5*(1), 18–39. https://doi.org/10.1080/17451000802512267

Falk-Petersen, S., Sargent, J. R., Henderson, J., Hegseth, E. N., Hop, H., & Okolodkov, Y. B. (1998). Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. *Polar Biology*, *20*(1), 41–47. https://doi.org/10.1007/s003000050274

Falk-Petersen, S., Sargent, J. R., Lønne, O. J., & Timofeev, S. (1999). Functional biodiversity of lipids in Antarctic zooplankton: Calanoides acutus, Calanus propinquus, Thysanoessa macrura and Euphausia crystallorophias. *Polar Biology*, *21*(1), 37–47. https://doi.org/10.1007/s003000050330

Fernandes, S. O., Javanaud, C., Michotey, V. D., Guasco, S., Anschutz, P., & Bonin, P. (2016). Coupling of bacterial nitrification with denitrification and anammox supports N removal in intertidal sediments (Arcachon Bay, France). *Estuarine, Coastal and Shelf Science*, *179*, 39–50. https://doi.org/10.1016/j.ecss.2015.10.009

Fernández-Méndez, M., Katlein, C., Rabe, B., Nicolaus, M., Peeken, I., Bakker, K., Flores, H., & Boetius, A. (2015). Photosynthetic production in the central Arctic Ocean during the record sea-ice minimum in 2012. *Biogeosciences*, *12*(11), 3525–3549. https://doi.org/10.5194/bg-12-3525-2015

Fischer, J., Schauer, F., & Heipieper, H. J. (2010). The trans/cis ratio of unsaturated fatty acids is not applicable as biomarker for environmental stress in case of long-term contaminated habitats. *Applied Microbiology and Biotechnology*, *87*(1), 365–371. https://doi.org/10.1007/s00253-010-2544-0

Forest, A., Stemmann, L., Picheral, M., Burdorf, L., Robert, D., Fortier, L., & Babin, M. (2012). Size distribution of particles and zooplankton across the shelf-basin system in southeast Beaufort Sea: Combined results from an Underwater Vision Profiler and vertical net tows. *Biogeosciences*, *9*, 1301–1320.

Forest, A., Tremblay, J.-É., Gratton, Y., Martin, J., Gagnon, J., Darnis, G., Sampei, M., Fortier, L., Ardyna, M., Gosselin, M., Hattori, H., Nguyen, D., Maranger, R., Vaqué, D., Marrasé, C., Pedrós-Alió, C., Sallon, A., Michel, C., Kellogg, C., … Piepenburg, D. (2011). Biogenic carbon flows through the planktonic food web of the Amundsen Gulf (Arctic Ocean): A synthesis of field measurements and inverse modeling analyses. *Progress in Oceanography*, *91*(4), 410–436. https://doi.org/10.1016/j.pocean.2011.05.002

Garrison, D. L., & Buck, K. R. (1986). Organism losses during ice melting: A serious bias in sea ice community studies. *Polar Biology*, *6*(4), 237–239. https://doi.org/10.1007/BF00443401

Glud, R. N., & Rysgaard, S. (2007). *Carbon cycling in Arctic marine ecosystems: Case study Young Sound* (Vol. 58). Museum Tusculanum Press.

Gosselin, M., Legendre, L., Therriault, J.-C., Demers, S., & Rochet, M. (1986). Physical control of the horizontal patchiness of sea-ice microalgae. *Marine Ecology Progress Series*, *29*, 289–298. https://doi.org/10.3354/meps029289

Graeve, M., Kattner, G., & Hagen, W. (1994). Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. *Journal of Experimental Marine Biology and Ecology*, *182*(1), 97–110. https://doi.org/10.1016/0022-0981(94)90213-5

Graeve, M., Kattner, G., & Piepenburg, D. (1997). Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? *Polar Biology*, *18*(1), 53–61. https://doi.org/10.1007/s003000050158

Guckert, J. B. (1986). Phospholipid Ester-Linked Fatty Acid Profile Changes during Nutrient Deprivation of Vibrio cholerae: Increases in the translcis Ratio and Proportions of Cyclopropyl Fatty Acids. *Applied and Environmental Microbiology*, *52*, 794–801.

Harvey, H. R., Eglinton, G., O'Hara, S. C. M., & Corner, E. D. S. (1987). Biotransformation and assimilation of dietary lipids by Calanus feeding on a dinoflagellate. *Geochimica et Cosmochimica Acta*, *51*(11), 3031–3040. https://doi.org/10.1016/0016-7037(87)90376-0

Heipieper, H. J., Diefenbach, R., & Keweloh, H. (1992). Conversion of cis unsaturated fatty acids to trans, a possible mechanism for the protection of phenol-degrading Pseudomonas putida P8 from substrate toxicity. *Applied and Environmental Microbiology*, *58*(6), 1847–1852. https://doi.org/10.1128/AEM.58.6.1847-1852.1992

Heipieper, H. J., Meinhardt, F., & Segura, A. (2003). The cis–trans isomerase of unsaturated fatty acids in Pseudomonas and Vibrio: Biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism. *FEMS Microbiology Letters*, *229*(1), 1–7. https://doi.org/10.1016/S0378-1097(03)00792-4

Heipieper, H. J., Neumann, G., Cornelissen, S., & Meinhardt, F. (2007). Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Applied Microbiology and Biotechnology*, *74*(5), 961–973. https://doi.org/10.1007/s00253-006-0833-4

Hill, P. (1998). Controls on floc size in the sea. *Oceanography*, *11*(2), 13–18. https://doi.org/10.5670/oceanog.1998.03

Hirche, H.-J., & Niehoff, B. (1996). Reproduction of the Arctic copepodCalanus hyperboreus in the Greenland Sea-field and laboratory observations. *Polar Biology*, *16*(3), 209–219. https://doi.org/10.1007/BF02329209

Holmström, C., James, S., Neilan, B. A., White, D. C., & Kjelleberg, S. (1998). Pseudoalteromonastunicata sp. Now, a bacterium that produces antifouling agents. *International Journal of Systematic Bacteriology*, *48*, 1205–1212.

Hoppe, H.-G. (1991). Microbial Extracellular Enzyme Activity: A New Key Parameter in Aquatic Ecology. In R. J. Chróst (Ed.), *Microbial Enzymes in Aquatic Environments* (pp. 60–83). Springer New York. https://doi.org/10.1007/978-1-4612-3090-8_4

Howard-Jones, M. H., Ballard, V. D., Allen, A. E., Frischer, M. E., & Verity, P. G. (2002). Distribution of bacterial biomass and activity in the marginal ice zone of the central Barents Sea during summer. *Journal of Marine Systems*, *38*(1–2), 77–91. https://doi.org/10.1016/S0924-7963(02)00170-7

Janout, M. A., Hölemann, J., Waite, A. M., Krumpen, T., Appen, W.-J. von, & Martynov, F. (2016). Sea-ice retreat controls timing of summer plankton blooms in the Eastern Arctic Ocean.

Geophysical Research Letters, *43*(24), 12,493-12,501. https://doi.org/10.1002/2016GL071232

Jia, J., Chen, Y., Jiang, Y., Tang, J., Yang, L., Liang, C., Jia, Z., & Zhao, L. (2014). Visualized analysis of cellular fatty acid profiles of Vibrio parahaemolyticus strains under cold stress. *FEMS Microbiology Letters*, *357*(1), 92–98. https://doi.org/10.1111/1574-6968.12498

Juhl, A., Krembs, C., & Meiners, K. (2011). Seasonal development and differential retention of ice algae and other organic fractions in first-year Arctic sea ice. *Marine Ecology Progress Series*, *436*, 1–16. https://doi.org/10.3354/meps09277

Junge, K., Eicken, H., & Deming, J. W. (2004). Bacterial Activity at ⁻2 to ⁻20°C in Arctic Wintertime Sea Ice. *Applied and Environmental Microbiology*, *70*, 550–557.

Karner, M., & Herndl, G. J. (1992). Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. *Marine Biology*, *113*, 341–347.

Kim, L. H., & Chong, T. H. (2017). Physiological Responses of Salinity-Stressed *Vibrio* sp. And the Effect on the Biofilm Formation on a Nanofiltration Membrane. *Environmental Science & Technology*, *51*(3), 1249–1258. https://doi.org/10.1021/acs.est.6b02904

Kirchman, D., K'Nees, E., & Hodson, R. (1985). Leucine Incorporation and Its Potential as a Measure of Protein Synthesis by Bacteria in Natural Aquatic Systemst. *Applied and Environmental Microbiology*, *49*(3), 599–607.

Krause-Jensen, D. (2007). Benthic primary production in Young Sound, Northeast Greenland. *Meddelelser Om Grønland*, *58*, 159–174.

Krembs, C., Eicken, H., Junge, K., & Deming, J. W. (2002). High concentrations of exopolymeric substances in Arctic winter sea ice: Implications for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep Sea Research Part I: Oceanographic Research Papers*, *49*(12), 2163–2181. https://doi.org/10.1016/S0967-0637(02)00122-X

Krembs, C., & Engel, A. (2001). Abundance and variability of microorganisms and transparent exopolymer particles across the ice/water interface of melting first-year sea ice in the Laptev Sea (Arctic). *Marine Biology*, *138*, 1370–1377.

Lambert, M. A., Hickman-Brenner, F. W., Hi, J. J. F., & Moss', C. W. (1983). Differentiation of Vibvionaceae Species by Their Cellular Fatty Acid Composition. *International Journal of Systemic Bacteriology*, *33*(4), 777–792.

Lambert, M. A., & Moss, C. W. (1983). Comparison of the effects of acid and base hydrolyses on hydroxy and cyclopropane fatty acids in bacteria. *Journal of Clinical Microbiology*, *18*(6), 1370–1377. https://doi.org/10.1128/JCM.18.6.1370-1377.1983

Lee, R. F., & Nevenzel, J. C. (1979). Wax Esters in the Marine Environment: Origin and Composition of the Wax from Bute Inlet, British Columbia. *Journal of the Fisheries Research Board of Canada*, *36*(12), 1519–1523. https://doi.org/10.1139/f79-222

Lee, R., Hagen, W., & Kattner, G. (2006). Lipid storage in marine zooplankton. *Marine Ecology Progress Series*, *307*, 273–306. https://doi.org/10.3354/meps307273

Leu, E., Wiktor, J., Søreide, J., Berge, J., & Falk-Petersen, S. (2010). Increased irradiance reduces food quality of sea ice algae. *Marine Ecology Progress Series*, *411*, 49–60. https://doi.org/10.3354/meps08647

Loffeld, B., & Keweloh, H. (1996). Cis/trans isomerization of unsaturated fatty acids as possible control mechanism of membrane fluidity inPseudomonas putida P8. *Lipids*, *31*(8), 811–815. https://doi.org/10.1007/BF02522976

Mancuso Nichols, C. A., Garon, S., Bowman, J. P., Raguénès, G., & Guézennec, J. (2004). Production of exopolysaccharides by Antarctic marine bacterial isolates. *Journal of Applied Microbiology*, *96*(5), 1057–1066. https://doi.org/10.1111/j.1365-2672.2004.02216.x

Mari, X. (1999). Carbon content and C:N ratio of transparent exopolymeric particles (TEP) produced by bubbling exudates of diatoms. *Marine Ecology Progress Series*, *183*, 59–71. https://doi.org/10.3354/meps183059

Marie, D., Partensky, F., Vaulot, D., & Brussaard, C. (1999). Enumeration of Phytoplankton, Bacteria, and Viruses in Marine Samples. *Current Protocols in Cytometry*, *10*(1), 11.11.1-11.11.15. https://doi.org/10.1002/0471142956.cy1111s10

Meiners, K., Gradinger, R., Fehling, J., Civitarese, G., & Spindler, M. (2003). Vertical distribution of exopolymer particles in sea ice of the Fram Strait (Arctic) during autumn. *Marine Ecology Progress Series*, *248*, 1–13. https://doi.org/10.3354/meps248001

Meiners, K., Krembs, C., & Gradinger, R. (2008). Exopolymer particles: Microbial hotspots of enhanced bacterial activity in Arctic fast ice (Chukchi Sea). *Aquatic Microbial Ecology*, *52*, 195–207. https://doi.org/10.3354/ame01214

Michel, C., Nielsen, T., Nozais, C., & Gosselin, M. (2002). Significance of sedimentation and grazing by ice micro- and meiofauna for carbon cycling in annual sea ice (northern Baffin Bay). *Aquatic Microbial Ecology*, *30*, 57–68. https://doi.org/10.3354/ame030057

Molina-Santiago, C., Udaondo, Z., Gómez-Lozano, M., Molin, S., & Ramos, J.-L. (2017). Global transcriptional response of solvent-sensitive and solvent-tolerant Pseudomonas putida strains exposed to toluene. *Environmental Microbiology*, *19*(2), 645–658. https://doi.org/10.1111/1462-2920.13585

Nelson, J. R. (1989). Phytoplankton pigments in macrozooplankton feces: Variability in carotenoid alterations. *Marine Ecology Progress Series*, *52*(2), 129–144. JSTOR.

Nichols, P. D., Skerratt, J. H., Davidson, A., Burton, H., & Mcmeekin, T. A. (1991). Lipids of cultured Phaeocystis pouchetii: Signatures for food-web, biogeochemical and environmental studies in Antarctica and the Southern ocean. *Phytochemistry*, *30*(10), 3209–3214. https://doi.org/10.1016/0031-9422(91)83177-M

Okuyama, H., Okajima, N., Sasaki, S., Higashi, S., & Murata, N. (1991). The cis/trans isomerization of the double bond of a fatty acid as a strategy for adaptation to changes in ambient temperature in the psychrophilic bacterium, Vibrio sp. Strain ABE-1. *Biochimica Et Biophysica Acta*, *1084*(1), 13–20. https://doi.org/10.1016/0005-2760(91)90049-n

Parsons, T., Maita, Y., & Lalli, C. (1984). A manual of chemical and biological methods for seawater analysis. *Marine Ecology Progress Series*, *199*, 43–53.

Passow, U. (2002). Transparent exopolymer particles (TEP) in aquatic environments. *Progress in Oceanography*, *55*(3), 287–333. https://doi.org/10.1016/S0079-6611(02)00138-6

Perrette, M., Yool, A., Quartly, G. D., & Popova, E. E. (2011). Near-ubiquity of ice-edge blooms in the Arctic. *Biogeosciences*, *8*(2), 515–524. https://doi.org/10.5194/bg-8-515-2011

Piuri, M., Sanchez-Rivas, C., & Ruzal, S. M. (2003). Adaptation to high salt in Lactobacillus: Role of peptides and proteolytic enzymes. *Journal of Applied Microbiology*, *95*(2), 372–379. https://doi.org/10.1046/j.1365-2672.2003.01971.x

Rampen, S. W., Abbas, B. A., Schouten, S., & Damste, J. S. S. (2010). A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. *Limnology and Oceanography*, *55*(1), 91–105. https://doi.org/10.4319/lo.2010.55.1.0091

Riebesell, U., Schloss, I., & Smetacek, V. (1991). Aggregation of algae released from melting sea ice: Implications for seeding and sedimentation. *Polar Biology*, *11*(4), 239–248. https://doi.org/10.1007/BF00238457

Riedel, A., Michel, C., & Gosselin, M. (2006). Seasonal study of sea-ice exopolymeric substances on the Mackenzie shelf: Implications for transport of sea-ice bacteria and algae. *Aquatic Microbial Ecology*, *45*, 195–206. https://doi.org/10.3354/ame045195

Riisgaard, K., Nielsen, T., & Hansen, P. (2015). Impact of elevated pH on succession in the Arctic spring bloom. *Marine Ecology Progress Series*, *530*, 63–75. https://doi.org/10.3354/meps11296

Rontani, J., Amiraux, R., Lalande, C., Babin, M., Kim, H.-R., & Belt, S. T. (2018). Use of palmitoleic acid and its oxidation products for monitoring the degradation of ice algae in Arctic waters and bottom sediments. *Organic Geochemistry*, *124*, 88–102. https://doi.org/10.1016/j.orggeochem.2018.06.002

Rontani, J., Charriere, B., Petit, M., Vaultier, F., Heipieper, H. J., Link, H., Chaillou, G., & Sempéré, R. (2012). Degradation state of organic matter in surface sediments from the Southern Beaufort Sea: A lipid approach. *Biogeosciences*, *9*(9), 3513–3530. https://doi.org/10.5194/bg-9-3513-2012

Rontani, J.-F., Belt, S. T., Brown, T. A., Amiraux, R., Gosselin, M., Vaultier, F., & Mundy, C. J. (2016). Monitoring abiotic degradation in sinking versus suspended Arctic sea ice algae during a spring ice melt using specific lipid oxidation tracers. *Organic Geochemistry*, *98*, 82–97. https://doi.org/10.1016/j.orggeochem.2016.05.016

Salcher, M., Pernthaler, J., Psenner, R., & Posch, T. (2005). Succession of bacterial grazing defense mechanisms against protistan predators in an experimental microbial community. *Aquatic Microbial Ecology*, *38*, 215–229. https://doi.org/10.3354/ame038215

Sameoto, D. D. (1984). Vertical distribution of zooplankton biomass and species in northeastern Baffin Bay related to temperature and salinity. *Polar Biology*, *2*(4), 213–224. https://doi.org/10.1007/BF00263627

Sazhin, A. F., Romanova, N. D., Kopylov, A. I., & Zabotkina, E. A. (2019). Bacteria and Viruses in Arctic Sea Ice. *Oceanology*, *59*(3), 339–346. https://doi.org/10.1134/S0001437019030196

Schlitzer, R. (2015). *Ocean Data View*.

Shin, S. Y., Bajpai, V. K., Kim, H. R., & Kang, S. C. (2007). Antibacterial activity of bioconverted eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) against foodborne pathogenic bacteria. *International Journal of Food Microbiology*, *113*(2), 233–236. https://doi.org/10.1016/j.ijfoodmicro.2006.05.020

Sicre, M.-A., Paillasseur, J.-L., Marty, J.-C., & Saliot, A. (1988). Characterization of seawater samples using chemometric methods applied to biomarker fatty acids. *Organic Geochemistry*, *12*(3), 281–288. https://doi.org/10.1016/0146-6380(88)90265-3

Simon, M., & Azam, F. (1989). Protein content and protein synthesis rates of planktonic marine bacteria. *Marine Ecology Progress Series*, *51*, 201–213. https://doi.org/10.3354/meps051201

Simon, M., Grossart, H., Schweitzer, B., & Ploug, H. (2002). Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology*, *28*, 175–211. https://doi.org/10.3354/ame028175

Sinensky, M. (1974). Homeoviscous Adaptation—A Homeostatic Process that Regulates the Viscosity of Membrane Lipids in Escherichia coli. *Proceedings of the National Academy of Sciences*, *71*(2), 522–525. https://doi.org/10.1073/pnas.71.2.522

Smith, D., & Azam, F. (1992). A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. *Marine Microbial Food Web*, *6*(2), 107–114.

Smith, S. D., Muench, R. D., & Pease, C. H. (1990). Polynyas and leads: An overview of physical processes and environment. *Journal of Geophysical Research: Oceans*, *95*(C6), 9461–9479. https://doi.org/10.1029/JC095iC06p09461

Søreide, J. E., Leu, E., Berge, J., Graeve, M., & Falk-Petersen, S. (2010). Timing of blooms, algal food quality and Calanus glacialis reproduction and growth in a changing Arctic. *Global Change Biology*, *16*(11), 3154–3163. https://doi.org/10.1111/j.1365-2486.2010.02175.x

Thompson, J., & MacLeod, R. (1971). Functions of Na+ and K+ in the Active Transport of αAminoisobutyric Acid in a Marine Pseudomonad. *The Journal of Biological Chemistry*, *246*(12), 4066–4074.

Thornton, D. C. O. (2002). Diatom aggregation in the sea: Mechanisms and ecological implications. *European Journal of Phycology*, *37*(2), 149–161. https://doi.org/10.1017/S0967026202003657

Tolosa, I., Vescovali, I., LeBlond, N., Marty, J.-C., de Mora, S., & Prieur, L. (2004). Distribution of pigments and fatty acid biomarkers in particulate matter from the frontal structure of the Alboran Sea (SW Mediterranean Sea). *Marine Chemistry*, *88*(3), 103–125. https://doi.org/10.1016/j.marchem.2004.03.005

von Quillfeldt, C. H., Ambrose, W. G., & Clough, L. M. (2003). High number of diatom species in first-year ice from the Chukchi Sea. *Polar Biology*, *26*(12), 806–818. https://doi.org/10.1007/s00300-003-0549-1

Wadhams, P., & Martin, S. (2001). *Ice in the ocean* (2nd ed., Vol. 54). CRC press.

Wakeham, S., & Lee, C. (1989). Organic geochemistry of particulate matter in the ocean: The role of particles in oceanic sedimentary cycles. *Organic Geochemistry - ORG GEOCHEM*, *14*, 83–96. https://doi.org/10.1016/0146-6380(89)90022-3

Welschmeyer, N. A., & Lorenzen, C. J. (1985). Role of herbivory in controlling phytoplankton abundance: Annual pigment budget for a temperate marine fjord. *Marine Biology*, *90*(1), 75–86. https://doi.org/10.1007/BF00428217

Chapitre IV

Utilisation des signaux de stress des bactéries associées aux algues de glace comme marqueur de leur préservation dans les sédiments arctiques Canadiens

Sommaire

4.1 Avant-propos

Dans les deux chapitres précédents, le mauvais état physiologique des bactéries associées au matériel algal sympagique arctique a été démontré. Du fait de leur forte agrégation, de la forte mortalité et de l'inactivité des bactéries qui leur sont associées, il est logique d'imaginer une chute rapide vers les sédiments ainsi qu'une bonne préservation des algues sympagiques tout au long de la colonne d'eau.

Dans ce chapitre, 30 échantillons de sédiments récoltés dans l'Arctique Canadien (mer de Beaufort et baie de Baffin) ont été analysés. Le suivi de différents traceurs de stress bactériens (rapports *trans/cis* des lipides membranaires bactériens indicateur de stress osmotique, activité 10*S*-DOX bactérienne détoxifiant les acides gras libres) et de l'état de dégradation de la matière organique (rapports stanol/Δ^5-stenol) ont donné de nouvelles indications quant à l'état de préservation de la matière algale dans les sédiments.

En effet, en mer de Beaufort et dans la partie ouest de la Baie de Baffin le matériel sympagique relargués lors des premiers stades de fonte de la banquise semble contribuer fortement aux sédiments, contrairement à celui relargué en plus grandes quantités en fin de fonte. Nous avons attribué ce paradoxe à la très forte activité de broutage zooplanctonique (copépodes) souvent associée au relargage des algues de glace en fin de fonte de la banquise. Il est intéressant de noter que le matériel sympagique ne semble pas colonisé fortement par des bactéries pélagiques ou benthiques, cela a été attribué au caractère nocif des acides gras libres produits par les algues de glace qui limite sa colonisation. De son côté, le matériel algal pélagique semble lui plus affecté par le broutage par le zooplancton, qui diminue grandement son export aux sédiments. Ces résultats confirment l'importance du timing du broutage zooplanctonique dans la préservation du matériel algal en Arctique.

Sous l'effet du réchauffement climatique en cours, la contribution sympagique/pélagique à la production primaire arctique va être fortement modifiée. Le rôle de puits de carbone des algues sympagiques devrait donc diminuer progressivement impactant fortement l'intégralité du cycle du carbone dans ces régions.

- Contribution personnelle

Ce chapitre s'appuyant sur l'analyse d'échantillons de sédiments arctiques variés et relativement anciens (de 2005 à 2016), l'échantillonnage n'a évidemment pas été réalisé dans le cadre de cette thèse. Ma contribution à ce chapitre s'est donc limitée à l'analyse en CPG-SM des extraits lipidiques de certains de ces échantillons et à la rédaction et la relecture de l'article, publié dans la revue *Microorganisms*.

4.2 Use of stress signals of their attached bacteria to monitor sympagic algae preservation in Canadian Arctic sediments

4.2.1 Résumé en français

En se basant sur la forte agrégation des algues sympagiques (de glace) et la mortalité ou inactivité élevée des bactéries leur étant associées, il a été précédemment hypothétisé que les algues sympagiques contribuent fortement à l'export du carbone aux sédiments arctiques. Dans cette étude, le contenu lipidique de 30 échantillons de sédiments collectés dans l'Arctique Canadien a été investigué, afin de vérifier cette hypothèse. La détection de de fortes proportions d'acide vaccénique sous forme *trans* (résultant de l'action de l'enzyme bactérienne *cis*-*trans* isomérase (CTI) en conditions hypersalines) et d'acide *10S*-hydroxyhexadec-8(*trans*)-énoique (résultant de l'action de détoxification de la *10S*-DOX bactérienne en présence d'acide palmitoléique libre délétère) ont confirmé : (i) la forte contribution du matériel sympagique à certains sédiments arctiques, et (ii) l'état physiologique altéré des communautés bactériennes associées. Contrairement au matériel d'origine terrestre, les algues sympagiques ayant échappé au broutage du zooplancton semblent bien préservées de toute forme de dégradation biotique dans les sédiments arctiques. La réduction annoncée du couvert de glace de mer, résultant du réchauffement global, devrait entraîner un shift dans la contribution relative des algues sympagique *vs* pélagique aux fonds marins, modifiant ainsi fortement le cycle du carbone.

4.2.2 Article

Use of stress signals of their attached bacteria to monitor sympagic algae preservation in Canadian Arctic sediments

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Article accepté chez *Microorganisms* en décembre 2021

Abstract:

Based on the strong aggregation of sympagic (ice-associated) algae and the high mortality or inactivity of bacteria attached to them, it was previously hypothesized that sympagic algae should be significant contributors to the export of carbon to Arctic sediments. In the present work, the lipid content of 30 sediment samples collected in the Canadian Arctic was investigated to test this hypothesis. The detection of high proportions of *trans* vaccenic fatty acid (resulting from *cis-trans* isomerase (CTI) activity of bacteria under hypersaline conditions) and 10*S*-hydroxyhexadec-8(*trans*)-enoic acid (resulting from 10*S*-DOX bacterial detoxification activity in the presence of deleterious free palmitoleic acid) confirmed: (i) the strong contribution of sympagic material to some Arctic sediments, and (ii) the impaired physiological status of its associated bacterial communities. Unlike terrestrial material, sympagic algae that had escaped zooplanktonic grazing appeared relatively preserved from biotic degradation in Arctic sediments. The expected reduction of sea ice cover resulting from global warming should cause a shift in the relative contributions of ice-associated *vs*. pelagic algae to the seafloor, and thus to a strong modification of the carbon cycle.

1. Introduction

In the Canadian Arctic, primary production is supported by sympagic (ice-associated) algae dominated by pennate diatoms and more specifically by the Naviculaceae, (Poulin *et al.,* 2011) or by the centric diatom *Melosira arctica* (Boetius *et al.,* 2013) during the ice-covered period, and then by phytoplankton in open waters (Gosselin *et al.,* 1997, Wassmann *et al.,* 2011). The contribution of sympagic algae to total primary production varies widely depending on the season and the region (Loose *et al.,* 2011, Fernández-Mendéz *et al.,* 2015). Because of global warming (causing a decrease in sea ice extent and duration), we are currently witnessing a reduced primary production of the sympagic algae and an increase in that of pelagic phytoplankton. Sympagic algae are assumed to be a main source of organic matter reaching the seafloor (Boetius *et al.,* 2013, Amiraux *et al.,* 2017, Rontani *et al.,* 2018, Yunda-Guarin *et al.,* 2020). It is therefore feared that in the future, the biological pump would have a positive feedback effect on global warming. The high contribution of sympagic algae to Arctic sediments was previously attributed to: (i) their strong aggregation resulting from the high concentrations of extracellular polymeric substances (EPSs) produced by these organisms in the ice, which protects biogenic silica of diatom frustules from dissolution (Moriceau *et al.,* 2007) and induces quick settling in the water column (100-500 m d^{-1}; Riebesell *et al.,* 1991), (ii) the fact that aggregated microalgae are not favored prey particles for grazers (Lürling *et al.,* 1996), and (iii) the poor physiological status of their associated bacterial communities (inducing weak mineralization (Amiraux *et al.,* 2017) and biogenic silica dissolution (Bidle *et al.,* 2003) in the water column and surface sediments). These bacterial communities are damaged by: (i) intense osmotic stress induced by changes in salinity in brine channels during the early stage of ice melting (Amiraux *et al.,* 2017), and (ii) the production of bactericidal free fatty acids (FFAs) by sympagic algae latter in the season under the effect of light stress (Amiraux *et al.,* 2020). It is well-known that bacteria colonize nearly all types of particulate organic matter (POM) (e.g. phytodetritus, zooplanktonic fecal pellets, aggregated sympagic algae, and EPS particles, Rieck *et al.,* 2015).

Attachment of bacteria to particles may be favored by the presence of EPSs, which can act as biological glue (Alldredge *et al.,* 1988, Riedel *et al.,* 2006). In sea ice, more than 50% of the bacterial community were found to be associated with particles (Junge *et al.,* 2004), while the contribution of attached bacteria to the total bacterial production was found to

be highly variable in POM (ranging from 0 to 98% in the Beaufort Sea, Garneau *et al.,* 2009, Ortega-Retuerta *et al.,* 2012). Particle attachment is very important for the sinking export of bacteria, since the sinking velocities of attached bacteria are significantly higher than those of free-living bacteria (Lapoussière *et al.,* 2011). This partly explains the overwhelming proportion of bacteria attached to particles in marine sediments (Meyer-Reil, 1984).

To survive under hypersaline conditions, bacteria have developed various strategies such as (i) implementation of active Na+ and K+ ion transport systems (Thompson & MacLeod, 1971), (ii) accumulation of osmocompatible compounds such as glycine betaine or proline (Piuri *et al.,* 2003) or (iii) production of EPSs, which can act as a diffusion barrier (Kim *et al.,* 2017). Another major adaptive response of bacteria of the Pseudomonas and Vibrio genera is to maintain their membrane fluidity by conversion of cis to trans unsaturated fatty acids through the activity of *cis-trans* isomerases (CTIs) (Loffeld *et al.,* 1996, Heipieper *et al.,* 2003). Guckert *et al*. (1986), have proposed a *trans/cis* ratio > 0.1 as an indicator of bacterial stress. A relatively strong CTI activity was previously observed in sea ice and sinking particles collected at the beginning of the melting period in western Baffin Bay (*trans/cis* ratio and trans-vaccenic flux ranging at 25 m from 0.12 to 0.50 and 0 to 4.0 µg d^{-1} m^{-2}, respectively, Amiraux *et al., 2017,* Rontani *et al.,* 2018) and was attributed to the flush of bacteria associated with sympagic algae from internal hypersaline ice brines (Amiraux *et al.,* 2020). The relative stability of trans/cis ratios with depth suggested that bacterial communities associated with sinking sympagic algae were non-growing and thus inactive. Indeed, in the absence of osmotic stress (as is the case in the water column), the trans/cis ratio of bacteria should decrease to a base level (Fischer *et al.,* 2010), requiring de novo synthesis of cis fatty acids (Eberlein *et al.,* 2018) and thus bacterial growth. The similarity of these ratios observed in the water column and in sediments of western Baffin Bay (mean value 0.22 and 0.23, respectively Amiraux *et al.,* 2017) is also indicative of a weak colonization of sinking particles and sediments by unstressed pelagic and benthic bacteria.

FFAs damage bacterial cellular membranes (Greenway *et al.,* 1979, Chamberlain *et al.,* 1991). These compounds can also inhibit some enzyme activity, disrupt electron transport chains, and uncouple oxidative phosphorylation (Desbois & Smith, 2010). Bacteria attached to sympagic diatoms are particularly sensitive to the antibacterial activity of free palmitoleic acid produced by these algae under increasing light intensity (Hu *et al.,* 2008). To detoxify this acid, some bacteria of the Pseudomonas,

Pseudoalteromonas, and *Shewanella* genera (Guerrero *et al.,* 1997, Shoja Chaghervand, 2019) present in Arctic sea ice (Amiraux *et al.,* 2021) use a 10-dioxygenase (10S-DOX) able to convert palmitoleic acid to *10(S)*-hydroperoxyhexadec-8(*trans*)-enoic acid. The flux of this enzymatic oxidation product previously measured in western Baffin Bay at 25 m ranged from 0 to 93 µg d^{-1} m^{-2} (Amiraux *et al.,* 2017). Despite this detoxification strategy, most of the bacteria associated with sinking sympagic algae (up to 70%) at the end of ice melting (when osmotic stress ceased) have disrupted membranes and may thus be considered dead (Amiraux *et al.,* 2020). However, importantly, *10S*-DOX may also be utilized by bacteria to detoxify FFAs released by wounded pelagic diatoms in the presence of copepods (Rontani *et al.,* 2021).

Given that: (i) only bacteria attached to particles (such as aggregated phytodetritus) possess sufficient sinking rates to reach the seafloor and (ii) in the Arctic, sea ice is the largest source of salinity stress (owing to its extent and brine contents), previous observation of high proportions of trans monounsaturated fatty acids in some Arctic sediment samples (Amiraux *et al.,* 2017, Rontani *et al.,* 2018, 2012) was attributed to the presence of sea ice biota in this material.

To confirm this expected strong contribution of sympagic material to the seafloor, we examined the lipid content of 30 sediment samples collected in the Canadian Arctic (Figure 4.1). Despite the recent development of several indices based on the relative proportions of highly branched isoprenoids (HBIs; IP_{25} and tri-unsaturated HBIs) to estimate relative proportions of sympagic vs. pelagic production (Belt *et al.,* 2019, Smik *et al.,* 2016, Brown *et al.,* 2014, 2016), some uncertainties remain about the nature of POM reaching the seafloor. In particular, processes affecting the sinking organic matter and its lipid content through the water column down to the seafloor (such as degradation and remineralization) need further investigation. The aim of this work was thus to use new indicators of the osmotic and chemical stress status of bacteria associated with sympagic material (trans-vaccenic acid and *10S*-hydroxyhexadec-8(*trans*)-enoic acid, respectively) to estimate: (i) the contribution of the sympagic material to the investigated sediments and (ii) the status of the bacteria associated with it. Ratios of stanols (widespread bacterial biohydrogenation products of sterols, Wakeham, 1989, De Leeuw & Baas, 1986) to their parent sterols were also used to confirm the impaired ability of stressed bacteria to degrade sympagic material.

2. Material and methods

2.1. Sediment sampling

Two contrasting regions were selected, namely the Canadian Beaufort Shelf and Baffin Bay. The Beaufort Shelf is a perennially stratified interior shelf influenced by Pacific-derived waters supplied via the Beaufort Gyre and the Alaskan coastal current (Bergeron & Tremblay, 2014). Sediments of this seasonally ice-covered shelf are strongly influenced by the Mackenzie River (Rachold *et al.,* 2004), the largest river draining into the Arctic in terms of sediment and POM (MacDonald *et al.,* 1998). The Mackenzie shelf functions as a vast estuary receiving inputs of both terrestrial and marine sources of organic matter (Magen *et al.,* 2010). By contrast, Baffin Bay is characterized by a strong spatial sea ice variability resulting from the inflow of the West Greenland Current (WGC) composed of the relatively warm Atlantic Irminger Current (IC), which restricts sea ice extent in its eastern part (Myers *et al.,* 2007). The sedimentation rates appeared to be relatively similar in the two zones investigated ranging from 0.04 to 0.20 cm yr^{-1} in the Beaufort Shelf (Richerol *et al.,* 2008, Kuzyk *et al.,* 2013) and from 0.06 to 0.11 cm yr^{-1} in Baffin Bay (Kuzyk *et al.,* 2013). Depths of the different sampling stations (ranging from 7 to 2017 m) are given in Table S4.2.

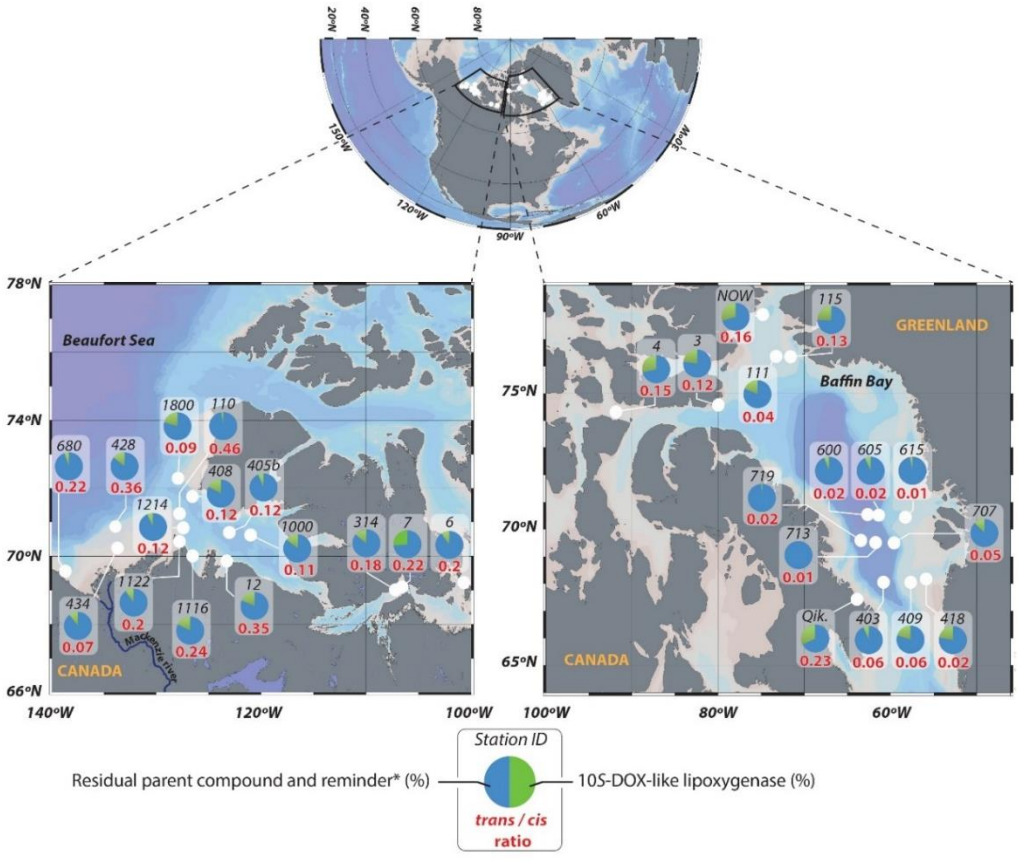

Figure 4.1: Map of the station investigated in the Beaufort Sea and Baffin Bay. The pie charts show the percentage of residual parent compounds (palmitoleic acid) degraded by the 10S-DOX. In red, the trans/cis vaccenic acid ratio. Qik stands for Qikiqtarjuaq.

Sediment samples were collected from a broad range of locations (Figure 4.1) within the Canadian Arctic using an USNEL box corer (50×50×40 cm^3) on board the CCGS Amundsen in 2005 (ArcticNet survey), 2008 (IPY-CFL), 2009 (Malina campaign) and 2015, and 2016 (GreenEdge campaigns). Although a significant decrease in ice concentration under the effect of global warming between 2005 and 2016 was logically to be expected (Comiso & Hall, 2014), vertical particulate organic carbon (POC) exports recorded in the Mackenzie Shelf from 1987 to 2006 (ranging from 1.6 to 1.8 g C m^{-2} yr^{-1} (O'Brien *et al.,* 2006, Forest, 2007, Lalande *et al.,* 2009) seemed relatively unaffected. Moreover, similar values were measured in the Amundsen Gulf (ranging from 2.4 and 6.8 g C m^{-2} yr^{-1}, Lalande *et al.,* 2009, Forest *et al.,* 2010) and in Baffin Bay (ranging from 1.1 to 6.7 g C m^{-2} yr^{-1}, Lalande *et al.,* 2009, Hargrave *et al.,* 2002) justifying a comparative study of the different sediment samples investigated. From each box core, one sample of *ca.* 50 cm^2 was collected from intact sediment surface (0-1 cm) and frozen immediately at -80°C for later analysis.

2.2. Lipid analysis

Samples were reduced with excess $NaBH_4$ after addition of MeOH (25 mL; 30 min) to reduce labile hydroperoxides to alcohols, which are more amenable to analysis using gas chromatography-mass spectrometry (GC-MS). Water (25 mL) and KOH (2.8 g) were then added and the resulting mixture saponified (to break complex lipids down into their consitutent fatty acids) by refluxing (2 h). After cooling, the mixture was acidified (HCl, 2 N) to pH 1 and extracted with dichloromethane (DCM, 3 × 20 mL). The combined DCM extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated by rotary evaporation at 40 °C to give total lipid extracts (TLEs). Aliquots of TLEs were either silylated and analyzed by gas chromatography-electron impact quadrupole time-of-flight mass spectrometry (GC-QTOF) for monounsaturated fatty acid oxidation product quantification, or methylated, treated with dimethyldisulfide (DMDS) and analyzed by GC-MS/MS to determine double bond stereochemistry. *Cis* and *trans* isomers of monounsaturated fatty acid methyl esters react stereospecifically with DMDS to form *threo* and *erythro* adducts, which exhibit similar mass spectra but are well-separated by gas chromatography, allowing unambiguous double bond stereochemistry determination (Buser *et al.,* 1983).

2.3. *Derivatization*

The derivatization method used was silylation allowing the replacement of the active hydrogen atom of carboxylic and alcoholic groups of acids and hydroxyacids by a trimethylsilyl group in one step. TLEs were silylated by dissolving them in 300 µL of a mixture of pyridine and BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide; Supelco; 2:1, v/v) and heating to 50°C (1 h). After evaporation to dryness under a stream of N_2, the derivatized residue was dissolved in a mixture of hexane and BSTFA (to avoid desilylation) and analyzed by GC-MS/MS or GC-QTOF.

2.4. Determination of double bond stereochemistry

TLEs were dissolved in 2 mL of BF_3/methanol (10%) (Sigma-Aldrichn St Louis, MO, USA) and heated at 80 °C (1 h) in a screw cap flask to obtain fatty acid methyl esters (FAMEs). After cooling, an excess of water was added and FAMEs were extracted three times with hexane, dried over anhydrous Na_2SO_4, filtered on Whatman cellulose filters (diameter 90 mm, porosity 11 µm), concentrated using rotary evaporation and transferred to screw cap flasks. After solvent removal (N_2), 200 µL of DMDS (Sigma-Aldrich) and 50 µL of iodine solution (60 µg μL^{-1} in diethyl ether) were added. The mixtures were shaken and heated at 50 °C (48 h), excess iodine was removed by adding 2 ml of a 5% $Na_2S_2O_3$ solution, and lipids were extracted three times with 1 mL of hexane. The extracts were then dried with anhydrous Na_2SO_4, filtered, and concentrated prior to analysis by GC-MS/MS.

2.5. 10S-DOX degradation estimate

Taking into account the production of equal amounts of 9-*trans* and 10-*trans* oxidation products during the photooxidation of the Δ^9 monounsaturated fatty acid (Frankel, 1998, Marchand & Rontani, 2001) and their specific allylic rearrangement to 11-*trans* and 8-*trans* isomers, respectively (Porter *et al.,* 1995), the contribution of the 10*S*-DOX degradation was obtained by the difference between the (10-*trans* + 8-*trans*) and (9-*trans* + 11-*trans*) oxidation products (Galeron *et al.,* 2018).

2.6. Gas chromatography/tandem mass spectrometry

GC-MS and GC-MS/MS analyses were performed using an Agilent 7890A/7000A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis -

ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France 30 m × 0.25 mm, film thickness 0.25 µm) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270 °C and the oven temperature was programmed from 70 °C to 130 °C at 20 °C min^{-1}, then to 250 °C at 5 °C min^{-1} and, finally to 300 °C at 3 °C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program and then programmed from 0.69×10^5 Pa to 1.49×10^5 Pa at 0.04×10^5 Pa min^{-1}. The mass spectrometric conditions were: electron energy, 70 eV; source temperature, 230 °C; quadrupole 1 temperature, 150 °C; quadrupole 2 temperature, 150°C; collision gas (N_2) flow, 1.5 mL min^{-1}; quench gas (He) flow, 2.25 mL min^{-1}; mass range, 50-700 Daltons; cycle time, 313 ms. Quantification was carried out with external standards in multiple reaction monitoring (MRM) mode. Precursor ions were selected from the more intense ions (and specific fragmentations) observed in electron ionization (EI) mass spectra.

2.7. Gas chromatography-EI quadrupole time-of-flight mass spectrometry

Accurate mass measurements were carried out in full scan mode using an Agilent 7890B/7200 GC-QTOF System (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert) (30 m × 0.25 mm, 0.25µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C and the oven temperature was programmed from 70 °C to 130 °C at 20 °C min^{-1} and then to 300 °C at 5 °C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program. Instrument temperatures were 300 °C for the transfer line and 230 °C for the ion source. Nitrogen (1.5 mL min^{-1}) was used as a collision gas. Accurate mass spectra were recorded across the range *m/z* 50–700 at 4 GHz with the collision gas opened. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12.252 from *m/z* 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was used for daily MS calibration. Compounds were identified by comparing their TOF mass spectra, accurate masses and retention times with those of standards. Each compound was quantified by extraction of specific accurate fragment ions, peak integration, and determination of individual response factors using external standards and Mass Hunter software (Agilent Technologies, Parc Technopolis – ZA Courtaboeuf, Les Ulis, France).

2.8. Statistical analysis

The variable investigated being non-parametric, Spearman correlations were performed to determine the correlation between the depth and the *trans/cis* ratio or 10*S*-DOX degradation percentage. Mann-Whitney-Wilcoxon tests were performed to identify any significant differences in (i) *trans/cis* ratio and 10*S*-DOX degradation percentage between Baffin Bay and the Beaufort Sea and (ii) sterol:stanol ratio percentage between sitosterol and brassicasterol.

3. Results and discussion

3.1. Contribution of sympagic material to Arctic sediments

The main fatty acids detected in the TLEs of the different sediments examined were $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1\omega7}$ (palmitoleic), $C_{18:1\omega9}$ (oleic), and $C_{18:1\omega7}$ (vaccenic) acids. These results are in close agreement with previous observations in surface sediments from the Beaufort Sea (Belicka *et al.,* 2004) and the Baffin Bay (Amiraux, unpublished data). Concentrations of palmitoleic acid (well-known to be the main fatty acid component of diatoms, Fahl & Kattner, 1993, Falk-Petersen *et al.,* 1998) and vaccenic acid (typically of bacterial origin, Desbois & Smith, 2012, Hu *et al.,* 2008) were highly variable ranging from 0.2 to 1220 µg g^{-1} and 0.5 to 149 µg g^{-1}, respectively (Table S4.1). As previously observed (Rontani *et al.,* 2018, Belicka *et al.,* 2004), sediments of the Beaufort Shelf, which are under the influence of the Mackenzie River, had high contents of classical tracers of terrestrial higher plants (e.g. betulin, amyrins, dehydroabietic acid and cutin components, Kolattukudy & Cutin, 1980, Otto & Simoneit, 2001).

Trans/cis ratios of vaccenic acid were measured after DMDS treatment in the different surface sediment samples investigated (Figure 4.2). The results obtained (Figure 4.1, Table 4.1 and Table S4.2) showed significantly higher values in the Beaufort Sea than in Baffin Bay (mean ± SE = 0.20 ± 0.03 and 0.06 ± 0.02, respectively; $W = 210$, $p < 0.01$). The efficiency of sympagic-benthic coupling should logically be better in shallower zones. This assumption is well supported by the good anticorrelation observed between *trans/cis* ratio and depth of the different stations (Spearman's rho = -0.67, $p < 0.01$).

Table 4.1: Percentage of 10S-DOX degradation of palmitoleic acid and trans/cis ratio of vaccenic acid observed in sediment samples collected in the Canadian Arctic during the ArcticNet, Malina and GreenEdge expeditions.

	Beaufort Sea Mean ± SE [range]	Baffin Bay Mean ± SE [range]	Overall Mean ± SE [range]
Trans/cis ratio	0.20 ± 0.03[a] [0.46 - 0.07]	0.06 ± 0.02[b] [0.23 - 0.01]	0.14 ± 0.02[c] [0.46 - 0.01]
10*S*-DOX (%)[d]	14.34 ± 1.83 [29.00 - 2.20]	13.56 ± 2.91 [29.70 - 0]	14.01 ± 1.61 [29.70 - 0.00]

[a] n = 17
[b] n = 13
[c] n = 30
[d] Relative to the residual palmitoleic acid and its abiotic degradation products.

A strong contribution sympagic material to the sediments of the Beaufort Sea is well supported by the high ratios previously measured in surface sediments of other stations in this zone (0.65 ± 0.15, n = 6, Rontani *et al.,* 2012). It was previously observed that bacterial osmotic stress resulting from hypersaline conditions in brine channels occurred only during the early stages of ice melting and not at the end of the melting season (Amiraux *et al.,* 2017, 2020). The sympagic material reaching the seafloor of the Beaufort Sea thus seems to be discharged from sea ice at the beginning of melting. The high *trans/cis* ratios observed (Figure 4.1, Table 4.1) ruled out a significant contribution of sympagic algae released in the water column at the advanced stages of ice melting and open water phytoplankton to the sediments.

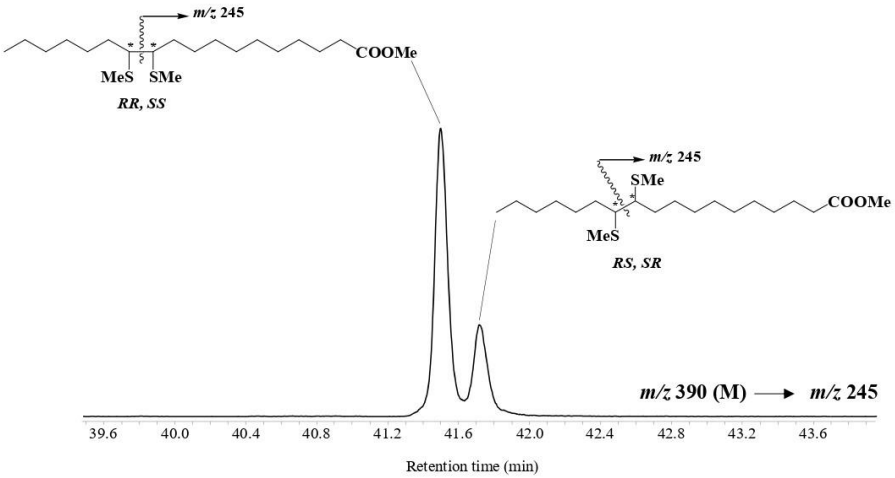

Figure 4.2: MRM chromatograms (m/z 390 → 245) of DMDS derivatives of vaccenic acid in superficial bottom sediment (0–1 cm) collected at St. 428.

A strong contribution of such material (exhibiting very low and no CTI activity, respectively, Amiraux *et al.,* 2020, Rontani *et al.,* 2012) to the sediment should strongly lower the *trans/cis* ratios (by adding bacteria unstressed by salinity with *trans/cis* ratios < 0.1). This assumption is firmly supported by the results of Morata *et al.* (2011), who observed that inputs of sympagic algae to the sediment started to increase from January/February in the Beaufort Sea, while sympagic algal bloom occurred between mid-March and late May (Brown *et al.,* 2011). Juul-Pedersen *et al.* (2008) also observed a sinking export of sympagic algal material in the same zone during winter and early spring (i.e. well before the onset of spring melt). The amount of diatoms exported to the seafloor strongly depends on a match or mismatch between algal production and zooplankton grazing (Nadaï *et al.,* 2021). It is generally considered that copepods do not feed at chlorophyll concentrations < 1 µg L^{-1} (Saunders *et al.,* 2003). The very low chlorophyll concentrations measured in POM between February and March in the Beaufort Sea (0.002-0.003 µg L^{-1}, Morata *et al.,* 2011) should thus not favor zooplankton grazing on sympagic algae during this period. We note that an increase in zooplankton fecal pellet production was observed during this same period (Seuthe *et al.,* 2007), but those authors attributed it to the grazing of non-pigmented sources of food, such as microzooplankton.

Chlorophyll concentration increases strongly in surface waters in late spring and summer at the end of ice melting and during open water diatom bloom (up to 5 µg L^{-1}, Sherr *et al.,* 2009) favoring grazing of sympagic (Michel *et al.,* 1996) and pelagic algae by copepods. Coprophagy can then reduce losses by sinking of the resulting fecal pellets at only a few percent of the pellet production rate (Paffenhöfer & Knowles, 1979). Bacterial decomposition by non-stressed internal bacteria is another mechanism that could prevent pellets sinking to the seafloor (Gowing & Silver, 1983, Smetacek, 1985). Forest *et al.* (2011) previously estimated that 97 % of the primary-produced C was grazed or degraded in the water column of the Amundsen Gulf. The remaining 3% reaching the seafloor thus seem mainly composed of sympagic algae associated with bacteria stressed by salinity and released during the early stages of ice melting. This view is well supported by earlier observations of Morata and Renaud (2008). These authors analyzed sedimentary pigments in the Beaufort Sea and concluded that in the spring, sympagic-algal production largely influenced organic matter inputs to the benthos, while in the summer grazing was responsible for inputs of degraded material.

Whereas a high CTI activity was previously observed in sea ice and sinking particles during the early stages of ice melting and in sediments from a landfast ice station located

at Qikiqtarjuaq near Broughton Island (western Baffin Bay, Amiraux *et al.,* 2017), *trans/cis* ratios were found to be particularly weak in middle and eastern Baffin Bay (Figure 4.1). Interestingly, Yunda-Guarin *et al.* (2020) found more sea ice-derived particulate organic carbon in surface sediments in the western side than in the eastern side of Baffin Bay. These authors attributed this difference to the timing of sea ice retreat. Ice cover is greater in the western part of Baffin Bay than in its eastern side, which is influenced by warmer waters from the western streams of Greenland (Tang *et al.,* 2004). Moreover, recently calculated ecological network analysis indices reveal that the complex eastern Baffin Bay food web favors the classical grazing chain, while the shorter western food web induces a higher carbon export (Saint-Béat *et al.,* 2020). Based on these results, the contrasting high *trans/cis* ratio observed in sediments from Qikiqtarjuaq (Figure 4.1) may be due to a large contribution of sympagic material containing bacteria stressed by salinity. This hypothesis is supported by the similarity of *trans/cis* ratios previously observed at this station in sinking particles and in surface sediments (Amiraux *et al.,* 2017). The very low ratios observed in the other samples from eastern Baffin Bay (Figure 4.1) are in close agreement with the higher contributions of pelagic carbon observed by Yunda-Guarin et *al.* (2020) in these zones.

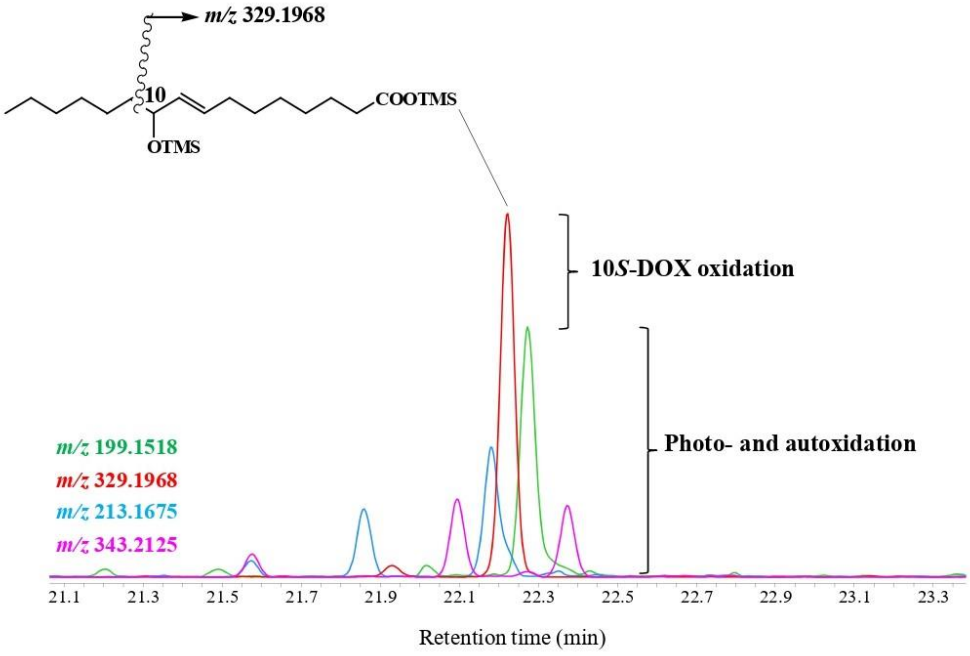

Figure 4.3: Partial ion chromatograms (at m/z 199.1518, 329.1968, 213.1675, and 343.2125) showing the presence of biotic and abiotic palmitoleic oxidation product trimethylsilyl derivatives (including the 10S-DOX degradation product, 10S-hydroxyhexadec-8(trans)-enoic acid) in superficial bottom sediment (0–1 cm) collected at Qikiqtarjuaq.

10-Hydroxyhexadec-8(*trans*)-enoic acid arising from $NaBH_4$ reduction in the corresponding hydroperoxide was detected in most of the sediments investigated (Figure 4.1, Figure 4.3; Table S4.2). The percentages of this hydroxyacid (relative to the parent palmitoleic acid and its degradation products) observed in Baffin Bay and the Beaufort Sea were not significatively different (mean ± SE = 15.20 ± 2.80 and 13.64 ± 1.80%, respectively; $W = 130.5$, $p = 0.69$). The presence of this compound is indicative of bacterial 10*S*-DOX oxidation of palmitoleic acid (Martinez *et al.,* 2010). This enzymatic activity seems to be a detoxification strategy enabling sympagic bacteria to survive the production of deleterious free palmitoleic acid (Desbois & Smith, 2010, Montfort *et al.,* 2000) by sympagic algae (Amiraux *et al.,* 2020). We note that despite this detoxification strategy a high mortality of attached bacteria could previously be observed in sea ice during the sympagic bloom (Amiraux *et al.,* 2020). It was recently observed that 10*S*-DOX could also be employed by some pelagic bacteria to detoxify free palmitoleic acid released by wounded diatoms in the presence of copepods (Rontani *et al.,* 2021). The lack of correlation observed between the percentage of 10*S*-hydroxyhexadec-8(*trans*)-enoic acid and depth (Spearman's rho = -0.22, $p = 0.2465$) suggests a mixed contribution of sympagic and pelagic bacteria to the sedimentary 10*S*-DOX signal. Although 10*S*-hydroxyhexadec-8(*trans*)-enoic acid is thus not sufficiently specific for use as a marker of the contribution of sympagic material to Arctic sediments, it is indicative of the presence of bacteria whose membranes are altered by FFAs. The bacterial communities present in Arctic sediments are thus in part non-growing (i.e. stressed by salinity in brine channels of ice) or dead (i.e. stressed by FFAs in ice or in the water column).

It is generally considered that the deposited algal aggregates are overgrown within a few weeks to months by specific bacterial groups of the surrounding sediment (Rapp *et al.,* 2018), which are likely better adapted to deep-sea environment than surface-derived bacteria (Tamburini *et al.,* 2013). In the Arctic, these benthic bacteria are dominated by members of the *Roseobacter* clade (i.e. the genera *Neptunomonas*, *Arcobacter* and *Sedimentitalea*, Rapp *et al.,* 2018), which all contain large proportions of *cis*-vaccenic acid (Kim *et al.,* 2012, Kim *et al.,* 2016, Yang *et al.,* 2014). The lack of dilution of *trans/cis* ratios of this acid observed in surface sediments from the Beaufort Sea and northern and western Baffin Bay (Figure 4.1, Table 4.1) is thus very surprising. In these zones, sympagic-derived aggregates reaching the seafloor do not appear to be intensively colonized by benthic bacteria. This absence of colonization may be attributed to the

presence of bactericidal FFAs (notably palmitoleic acid) in these aggregates attested by the 10*S*-DOX activity observed in most of them (Figure 4.1, Table S4.2).

3.2. Impact of stress state of bacteria on the degradation of sympagic material in sediments

It is generally considered that the lifetime of organic compounds in marine sediments depends on environmental conditions such as bioturbation, physical mixing, and the presence or absence of oxygen and other electron acceptors (Wakeham & Canuel, 2006). The benthic fauna inhabiting the seafloor is known to: (i) modify the vertical zonation of respiration reactions, (ii) control microbial community assembly, (iii) change the distribution of organic matter (OM), and (iv) influence rates of microbial OM remineralization in surface sediments (Deng *et al.,* 2020). Preservation/degradation of OM also depends on its sources (Keaveney *et al.,* 2020); terrestrial vascular debris generally contain greater proportions of refractory organic carbon than algal material, owing to their geochemical composition (Lacey *et al.,* 2018). The non-growing or dead state of bacteria associated with sympagic algae should strongly impact the degradation conditions of this material in Arctic sediments. Since the conversion of Δ^5-stenol to stanol is generally considered as indicating an intense bacterial degradation (Nishimura, 1978, Gagosian *et al.,* 1980), we compared the stanol/Δ^5-stenol ratios of 24-methylcholesta-5,22-dien-3β-ol (brassicasterol) (common sterol of sympagic diatoms; Belt *et al.,* 2018) and 24-ethylcholest-5-en-3β-ol (sitosterol) (sterol present in some diatoms but also widely distributed in terrestrial vascular plants, Volkman, 2003) in sediment samples from the Beaufort Sea where the highest bacterial stresses were observed (Table 4.1). We note that in these sediments sitosterol is known to result mainly from terrigenous material (Belicka *et al.,* 2004, Gõni *et al.,* 2000). The stanol/Δ^5-stenol ratios obtained for the two sterols were significantly different (mean ± SE = 18.2 ± 1.9% and 52.8 ± 5.9%, for brassicasterol and sitosterol respectively; $W = 36$, $p < 0.01$, Table 4.2). In the case of brassicasterol, the values observed were close to those found in healthy phototrophic organisms (5-20%, Wakeham *et al.,* 1997, Killops & Killops, 2013), attesting to the good biotic preservation of the algal material. By contrast, the measured sitostanol/sitosterol ratio reflects a strong biodegradation of this terrigenous sterol and supports the results of Gõni *et al.* (2005), who estimated that 65% of the old and fossil carbon is respired or buried in the Mackenzie delta. On entering the sea, terrestrial organic matter thus seems

to undergo strong bacterial remineralization. This could be attributed to the involvement of "priming effects" (enhanced remineralization of terrestrial OM in the presence of fresh substrates from pelagic algal sources; Bianchi, 2011, Bianchi *et al.,* 2018, Bonin *et al.,* 2019). By contrast, aggregated sympagic material sinking quickly through the water column with bacteria of poor health status appears well-preserved in surface sediments.

Table 4.2: Stanol/stenol ratio of sitosterol and brassicasterol in sediment samples collected in the Canadian Arctic.

	Sitosterol Mean ± SE [range]	Brassicasterol Mean ± SE [range]
Stanol/stenol ratio (%)	52.8 ± 5.9[a] [84.6 – 14.3]	18.2 ± 1.9[a] [35.8 – 5.5]

[a] n = 17

Under warmer conditions, the Arctic carbon cycle, which is mostly driven by the primary production (sympagic and pelagic algae) and the riverine inputs (including permafrost, Zimov *et al*., 2006), will be impacted. Although the contributions of these sources to the exported carbon have recently been estimated (Boetius *et al.,* 2013, Zimov *et al.,* 2006), their burial efficiency, impacting Arctic CO_2 storage remains uncertain. Here, we monitored their biotic behavior with specific lipid tracers in several surface sediment samples collected in the Canadian Arctic. We show that in the Beaufort Sea and northern and western Baffin Bay, unlike phytoplankton and permafrost, sympagic algae organic matter released during the early stages of ice melt by brine drainage contributes significantly to the exported and buried carbon, owing to: (i) the weak zooplanktonic grazing activity during this period, (ii) its strong aggregation enhancing rates of sinking to the seafloor and (iii) its recalcitrance to demineralizing processes. This particular resistance to biodegradation results from the non-growing (inactive) and dead state of bacteria associated with this sympagic material (Figure 4.4).

We note that Koch *et al.* (2020) previously estimated fluxes of sympagic diatoms in northern Bering and Chukchi Seas with HBI tracers and observed the highest fluxes in July (i.e., at the end and not in the early stages of ice melting). These observations contrasting with ours may be attributed to the particularly strong pelagic-benthic coupling of northern Bering and Chukchi Seas resulting from low grazing pressure due to temporal

mismatch between zooplankton and sympagic algae production (Kitamura *et al.,* 2017) and the shallowness of these zones (Grembier *et al.,* 1988). Match or mismatch of zooplanktonic grazing with sympagic algae fluxes seems to be a true key factor controlling the export of sympagic material to the seafloor (Figure 4.4). In the future, it will be of interest to analyze several Arctic sediments to determine whether the conclusions of this work can be extended to other Arctic zones. During these comparative studies, it will be necessary to use the same research protocol (i.e., the same tracers).

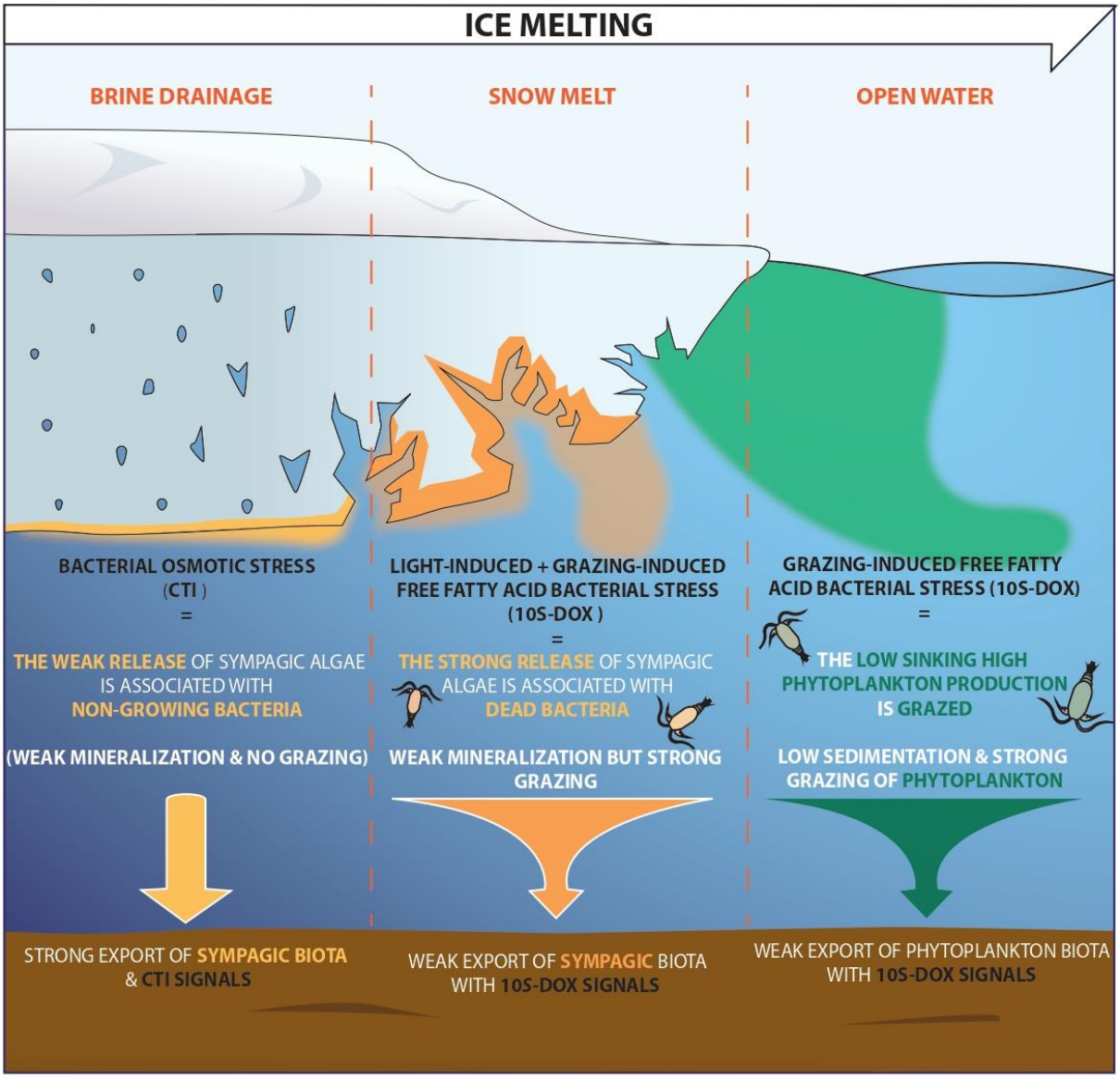

Figure 4.4: Conceptual scheme summarizing bacterial stress and its consequences on sympagic algae export in the Beaufort Sea and northern and western Baffin Bay during the sea ice melting stages: brine drainage, snowmelt and open water.

4.2.3 Informations supplémentaires

Table S4.1: Concentrations (µg g^{-1}) of the main lipids of interest[a] in the different surface sediments investigated.

Station	Brassicasterol	24-Methylene Cholesterol	Sitosterol	Palmitoleic acid	Vaccenic acid[c]
719	0.16	0.11	0.07	3.20	0.68
600	1.09	0.62	0.89	1.82	0.74
605	0.65	0.37	0.44	5.47	0.91
615	1.06	0.43	0.69	3.47	0.91
713	0.09	0.06	0.08	1.93	2.93
707	1.66	0.87	0.89	8.11	1.73
Qik	0.11	0.11	0.08	57.07	26.98
403	0.36	0.28	0.27	0.30	0.18
409	1.07	0.46	0.47	2.39	1.40
418	2.37	0.75	0.38	2.76	0.53
680	0.50	0.30	1.10	1220.85	149.06
110	3.00	1.50	6.20	30.70	80.49
3	0.12	0.03	0.07	6.87	1.89
4	0.28	0.45	0.37	30.36	1.76
NOW	0.02	tr	0.23	6.90	1.14
111	0.76	0.40	0.23	65.87	11.20
115	0.20	0.21	0.08	16.28	5.29
6	1.20	0.86	1.11	18.54	1.11
7	0.03	0.04	0.03	0.32	0.99
314	0.11	0.15	0.13	1.99	3.04
1000	0.02	0.01	0.02	5.88	1.49
405b	tr[b]	tr	0.14	3.37	1.13
408	tr	tr	0.01	2.62	1.72
12	0.04	0.06	0.09	1.65	1.70
1116	0.01	0.01	0.02	6.94	2.33
1122	0.01	0.01	0.10	13.55	1.00
434	0.70	0.33	0.49	426.67	34.89
1214	0.02	0.02	0.03	0.24	0.82
428	0.05	0.03	0.06	65.33	3.83
1800	0.16	0.16	0.17	4.36	6.06

[a] Unspecific lipids (such as saturated fatty acids, oleic acid and cholesterol) were not quantified.

[b] tr = traces (concentrations < 0.01 µg g^{-1}).

[c] Total (*cis* + *trans*)

Table S4.2: Depth, coordinates, trans-vaccenic acid/cis-vaccenic acid ratio and percentage of 10S-DOX product of the different surface sediments investigated.

Station	Depth (m)	Latitude (°N)	Longitude (°E)	*Trans*-vaccenic acid/*cis*-vaccenic acid	10*S*-DOX (%)[a]
719	1948	69.51	-63.23	0.02	1.0
600	2106	70.51	-62.01	0.02	5.6
605	2017	70.49	-61.58	0.02	6.4
615	617	70.50	-58.47	0.01	3.0
713	1892	69.50	-61.58	0.01	0
707	1419	69.51	-59.81	0.05	12.4
Qik	360	67.47	-63.79	0.23	26.8
403	1672	68.03	-60.40	0.06	6.2
409	1404	68.10	-58.00	0.06	20.0
418	380	68.11	-56.23	0.02	22.1
680	115	69.61	-138.21	0.22	5.2
110	385	71.70	-126.48	0.46	2.2
3	811	74.48	-79.75	0.12	22.8
4	347	74.27	-91.77	0.15	29.0
NOW	703	77.84	-74.68	0.16	29.7
111	608	76.30	-73.11	0.04	18.3
115	615	76.30	-71.67	0.13	24.8
6	61	69.18	-100.70	0.20	9.4
7	112	69.00	-106.57	0.22	26.2
314	93	69.07	-106.57	0.18	13.9
1000	362	70.60	-120.92	0.11	15.0
405b	513	70.57	-123.00	0.12	6.3
408	168	71.32	-127.65	0.12	17.0
12	219	69.92	-122.98	0.35	18.3
1116	184	70.05	-126.29	0.24	17.5
1122	15	70.48	-127.59	0.20	10.0
434	7	70.17	-133.59	0.07	10.0
1214	223	70.73	-127.36	0.12	6.6
428	43	70.79	-133.74	0.36	14.0
1800	352	72.20	-127.82	0.09	20.5

[a] Percentage of 10*S*-hydroxyhexadec-8(*trans*)-enoic acid relative to residuel palmitoleic acid and its abiotic oxidation products.

Bibliographie

Alldredge, A. L., & Silver, M. W. (1988). Characteristics, dynamics and significance of marine snow. *Progress in Oceanography*, *20*(1), 41–82. https://doi.org/10.1016/0079-6611(88)90053-5

Amiraux, R., Belt, S. T., Vaultier, F., Galindo, V., Gosselin, M., Bonin, P., & Rontani, J.-F. (2017). Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers. *Marine Chemistry*, *194*, 89–99. https://doi.org/10.1016/j.marchem.2017.05.006

Amiraux, R., Burot, C., Bonin, P., Guasco, S., Babin, M., & Rontani, J.-F. (2020). Stress factors resulting from the Arctic vernal sea ice melt: Impact on the viability of the bacterial communities associated to sympagic algae. *Elementa Science of the Anthropocene*. https://online.ucpress.edu/elementa/article/8/1/076/114223/Stress-factors-resulting-from-the-Arctic-vernal?searchresult=1

Amiraux, R., Rontani, J.-F., Armougom, F., Frouin, E., Babin, M., Artigue, L., & Bonin, P. (2021). Bacterial diversity and lipid biomarkers in sea ice and sinking particulate organic material during the melt season in the Canadian Arctic. *Elementa: Science of the Anthropocene*, *9*(1). https://doi.org/10.1525/elementa.2019.040

Belicka, L. L., Macdonald, R. W., Yunker, M. B., & Harvey, H. R. (2004). The role of depositional regime on carbon transport and preservation in Arctic Ocean sediments. *Marine Chemistry*, *86*(1), 65–88. https://doi.org/10.1016/j.marchem.2003.12.006

Belt, S. T., Brown, T. A., Smik, L., Assmy, P., & Mundy, C. J. (2018). Sterol identification in floating Arctic sea ice algal aggregates and the Antarctic sea ice diatom Berkeleya adeliensis. *Organic Geochemistry*, *118*, 1–3. https://doi.org/10.1016/j.orggeochem.2018.01.008

Belt, S. T., Smik, L., Köseoğlu, D., Knies, J., & Husum, K. (2019). A novel biomarker-based proxy for the spring phytoplankton bloom in Arctic and sub-arctic settings – HBI T25. *Earth and Planetary Science Letters*, *523*, 115703. https://doi.org/10.1016/j.epsl.2019.06.038

Bergeron, M., & Tremblay, J.-É. (2014). Shifts in biological productivity inferred from nutrient drawdown in the southern Beaufort Sea (2003–2011) and northern Baffin Bay (1997–2011), Canadian Arctic. *Geophysical Research Letters*, *41*(11), 3979–3987. https://doi.org/10.1002/2014GL059649

Bianchi, T. S. (2011). The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. *Proceedings of the National Academy of Sciences*, *108*(49), 19473–19481. https://doi.org/10.1073/pnas.1017982108

Bianchi, T. S., Cui, X., Blair, N. E., Burdige, D. J., Eglinton, T. I., & Galy, V. (2018). Centers of organic carbon burial and oxidation at the land-ocean interface. *Organic Geochemistry*, *115*, 138–155. https://doi.org/10.1016/j.orggeochem.2017.09.008

Bidle, K. D., Brzezinski, M. A., Long, R. A., Jones, J. L., & Azam, F. (2003). Diminished efficiency in the oceanic silica pump caused by bacteria-mediated silica dissolution. *Limnology and Oceanography*, *48*(5), 1855–1868. https://doi.org/10.4319/lo.2003.48.5.1855

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernandez-Mendez, M., Hendricks, S., Katlein, C., Lalande, C., Krumpen, T., Nicolaus, M., Peeken, I., Rabe, B., Rogacheva, A., Rybakova, E., Somavilla, R., Wenzhofer, F., & RV Polarstern ARK27-3-Shipboard Science Party. (2013). Export of Algal Biomass from the Melting Arctic Sea Ice. *Science*, *339*(6126), 1430–1432. https://doi.org/10.1126/science.1231346

Bonin, P., Prime, A.-H., Galeron, M.-A., Guasco, S., & Rontani, J.-F. (2019). Enhanced biotic degradation of terrestrial POM in an estuarine salinity gradient: Interactive effects of organic matter pools and changes of bacterial communities. *Aquatic Microbial Ecology*, *83*(2), 147–159. https://doi.org/10.3354/ame01908

Brown, T. A., Belt, S. T., Gosselin, M., Levasseur, M., Poulin, M., & Mundy, C. J. (2016). Quantitative estimates of sinking sea ice particulate organic carbon based on the biomarker IP25. *Marine Ecology Progress Series*, *546*, 17–29. https://doi.org/10.3354/meps11668

Brown, T. A., Belt, S. T., Philippe, B., Mundy, C. J., Massé, G., Poulin, M., & Gosselin, M. (2011). Temporal and vertical variations of lipid biomarkers during a bottom ice diatom bloom in the Canadian Beaufort Sea: Further evidence for the use of the IP25 biomarker as a proxy for spring Arctic sea ice. *Polar Biology*, *34*(12), 1857–1868. https://doi.org/10.1007/s00300-010-0942-5

Brown, T. A., Yurkowski, D. J., Ferguson, S. H., Alexander, C., & Belt, S. T. (2014). H-Print: A new chemical fingerprinting approach for distinguishing primary production sources in Arctic ecosystems. *Environmental Chemistry Letters*, *12*(3), 387–392. https://doi.org/10.1007/s10311-014-0459-1

Buser, H. Rudolf., Arn, Heinrich., Guerin, Patrick., & Rauscher, Stefan. (1983). Determination of double bond position in mono-unsaturated acetates by mass spectrometry of dimethyl

disulfide adducts. *Analytical Chemistry*, *55*(6), 818–822. https://doi.org/10.1021/ac00257a003

Chamberlain, N. R., Mehrtens, B. G., Xiong, Z., Kapral, F. A., Boardman, J. L., & Rearick, J. I. (1991). Correlation of carotenoid production, decreased membrane fluidity, and resistance to oleic acid killing in Staphylococcus aureus 18Z. *Infection and Immunity*, *59*(12), 4332–4337. https://doi.org/10.1128/IAI.59.12.4332-4337.1991

Comiso, J. C., & Hall, D. K. (2014). Climate trends in the Arctic as observed from space. *WIREs Climate Change*, *5*(3), 389–409. https://doi.org/10.1002/wcc.277

De Leeuw, J. W., & Baas, M. (1986). Early-stage diagenesis of steroids. *Methods in Geochemistry and Geophysics*, *24*, 101–123.

Deng, L., Bölsterli, D., Kristensen, E., Meile, C., Su, C.-C., Bernasconi, S. M., Seidenkrantz, M.-S., Glombitza, C., Lagostina, L., Han, X., Jørgensen, B. B., Røy, H., & Lever, M. A. (2020). Macrofaunal control of microbial community structure in continental margin sediments. *Proceedings of the National Academy of Sciences*, *117*(27), 15911–15922. https://doi.org/10.1073/pnas.1917494117

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, *85*(6), 1629–1642. https://doi.org/10.1007/s00253-009-2355-3

Eberlein, C., Baumgarten, T., Starke, S., & Heipieper, H. J. (2018). Immediate response mechanisms of Gram-negative solvent-tolerant bacteria to cope with environmental stress: Cis-trans isomerization of unsaturated fatty acids and outer membrane vesicle secretion. *Applied Microbiology and Biotechnology*, *102*(6), 2583–2593. https://doi.org/10.1007/s00253-018-8832-9

Fahl, K., & Kattner, G. (1993). Lipid Content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). *Polar Biology*, *13*(6), 405–409. https://doi.org/10.1007/BF01681982

Falk-Petersen, S., Sargent, J. R., Henderson, J., Hegseth, E. N., Hop, H., & Okolodkov, Y. B. (1998). Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. *Polar Biology*, *20*(1), 41–47. https://doi.org/10.1007/s003000050274

Fernández-Méndez, M., Katlein, C., Rabe, B., Nicolaus, M., Peeken, I., Bakker, K., Flores, H., & Boetius, A. (2015). Photosynthetic production in the central Arctic Ocean during the record sea-ice minimum in 2012. *Biogeosciences*, *12*(11), 3525–3549. https://doi.org/10.5194/bg-12-3525-2015

Fischer, J., Schauer, F., & Heipieper, H. J. (2010). The trans/cis ratio of unsaturated fatty acids is not applicable as biomarker for environmental stress in case of long-term contaminated habitats. *Applied Microbiology and Biotechnology*, *87*(1), 365–371. https://doi.org/10.1007/s00253-010-2544-0

Forest, A., Bélanger, S., Sampei, M., Sasaki, H., Lalande, C., & Fortier, L. (2010). Three-year assessment of particulate organic carbon fluxes in Amundsen Gulf (Beaufort Sea): Satellite observations and sediment trap measurements. *Deep Sea Research Part I: Oceanographic Research Papers*, *57*(1), 125–142. https://doi.org/10.1016/j.dsr.2009.10.002

Forest, A., Sampei, M., Hattori, H., Makabe, R., Sasaki, H., Fukuchi, M., Wassmann, P., & Fortier, L. (2007). Particulate organic carbon fluxes on the slope of the Mackenzie Shelf (Beaufort Sea): Physical and biological forcing of shelf-basin exchanges. *Journal of Marine Systems*, *68*(1), 39–54. https://doi.org/10.1016/j.jmarsys.2006.10.008

Forest, A., Tremblay, J.-É., Gratton, Y., Martin, J., Gagnon, J., Darnis, G., Sampei, M., Fortier, L., Ardyna, M., Gosselin, M., Hattori, H., Nguyen, D., Maranger, R., Vaqué, D., Marrasé, C., Pedrós-Alió, C., Sallon, A., Michel, C., Kellogg, C., … Piepenburg, D. (2011). Biogenic carbon flows through the planktonic food web of the Amundsen Gulf (Arctic Ocean): A synthesis of field measurements and inverse modeling analyses. *Progress in Oceanography*, *91*(4), 410–436. https://doi.org/10.1016/j.pocean.2011.05.002

Frankel, E. N. (1998). Lipid oxidation. *Progress in Lipid Research*, *19*(1–2), 1–22. https://doi.org/10.1016/0163-7827(80)90006-5

Gagosian, R. B., Smith, S. O., Lee, C., Farrington, J. W., & Frew, N. M. (1980). Steroid transformations in Recent marine sediments. *Physics and Chemistry of the Earth*, *12*, 407–419. https://doi.org/10.1016/0079-1946(79)90122-8

Galeron, M.-A., Radakovitch, O., Charrière, B., Vaultier, F., Volkman, J. K., Bianchi, T. S., Ward, N. D., Medeiros, P. M., Sawakuchi, H. O., Tank, S., Kerhervé, P., & Rontani, J.-F. (2018). Lipoxygenase-induced autoxidative degradation of terrestrial particulate organic matter in estuaries: A widespread process enhanced at high and low latitude. *Organic Geochemistry*, *115*, 78–92. https://doi.org/10.1016/j.orggeochem.2017.10.013

Garneau, M.-È., Vincent, W. F., Terrado, R., & Lovejoy, C. (2009). Importance of particle-associated bacterial heterotrophy in a coastal Arctic ecosystem. *Journal of Marine Systems*, *75*(1), 185–197. https://doi.org/10.1016/j.jmarsys.2008.09.002

Goñi, M. A., Yunker, M. B., Macdonald, R. W., & Eglinton, T. I. (2000). Distribution and sources of organic biomarkers in arctic sediments from the Mackenzie River and Beaufort Shelf. *Marine Chemistry*, *71*(1), 23–51. https://doi.org/10.1016/S0304-4203(00)00037-2

Goñi, M. A., Yunker, M. B., Macdonald, R. W., & Eglinton, T. I. (2005). The supply and preservation of ancient and modern components of organic carbon in the Canadian Beaufort Shelf of the Arctic Ocean. *Marine Chemistry*, *93*(1), 53–73. https://doi.org/10.1016/j.marchem.2004.08.001

Gosselin, M., Levasseur, M., Wheeler, P. A., Horner, R. A., & Booth, B. C. (1997). New measurements of phytoplankton and ice algal production in the Arctic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(8), 1623–1644. https://doi.org/10.1016/S0967-0645(97)00054-4

Gowing, M. M., & Silver, M. W. (1983). Origins and microenvironments of bacteria mediating fecal pellet decomposition in the sea. *Marine Biology*, *73*(1), 7–16. https://doi.org/10.1007/BF00396280

Grebmeier, J., Mcroy, C., & Feder, H. (1987). Pelagic-benthic coupling on the shelf of the northern Bering and Chukchi Seas. I. Food supply source and benthic biomass. *Marine Ecology Progress Series*, *48*. https://doi.org/10.3354/meps048057

Greenway, D. L. A., & Dyke, K. G. H. Y. 1979. (1979). Mechanism of the Inhibitory Action of Linoleic Acid on the Growth of Staphylococcus aureus. *Microbiology*, *115*(1), 233–245. https://doi.org/10.1099/00221287-115-1-233

Guckert, J. B. (1986). Phospholipid Ester-Linked Fatty Acid Profile Changes during Nutrient Deprivation of Vibrio cholerae: Increases in the translcis Ratio and Proportions of Cyclopropyl Fatty Acids. *APPL. ENVIRON. MICROBIOL.*, *52*, 8.

Guerrero, A., Casals, I., Busquets, M., Leon, Y., & Manresa, A. (1997). Oxydation of oleic acid to (E)-10-hydroperoxy-8-octadecenoic and (E)-10-hydroxy-8-octadecenoic acids by Pseudomonas sp. 42A2. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *1347*(1), 75–81. https://doi.org/10.1016/S0005-2760(97)00056-8

Hargrave, B. T., Walsh, I. D., & Murray, D. W. (2002). Seasonal and spatial patterns in mass and organic matter sedimentation in the North Water. *Deep Sea Research Part II: Topical Studies in Oceanography*, *49*(22), 5227–5244. https://doi.org/10.1016/S0967-0645(02)00187-X

Heipieper, H. J., Meinhardt, F., & Segura, A. (2003). The cis–trans isomerase of unsaturated fatty acids in Pseudomonas and Vibrio: Biochemistry, molecular biology and physiological

function of a unique stress adaptive mechanism. *FEMS Microbiology Letters*, *229*(1), 1–7. https://doi.org/10.1016/S0378-1097(03)00792-4

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., & Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. *The Plant Journal*, *54*(4), 621–639. https://doi.org/10.1111/j.1365-313X.2008.03492.x

Junge, K., Eicken, H., & Deming, J. W. (2004). Bacterial Activity at −2 to −20°C in Arctic Wintertime Sea Ice. *Applied and Environmental Microbiology*, *70*, 550–557.

Juul-Pedersen, T., Michel, C., Gosselin, M., & Seuthe, L. (2008). Seasonal changes in the sinking export of particulate material under first-year sea ice on the Mackenzie Shelf (western Canadian Arctic). *Marine Ecology Progress Series*, *353*, 13–25. https://doi.org/10.3354/meps07165

Keaveney, E. M., Radbourne, A. D., McGowan, S., Ryves, D. B., & Reimer, P. J. (2020). Source and quantity of carbon influence its sequestration in Rostherne Mere (UK) sediment: A novel application of stepped combustion radiocarbon analysis. *Journal of Paleolimnology*, *64*(4), 347–363. https://doi.org/10.1007/s10933-020-00141-1

Killops, S. D., & Killops, V. J. (2013). *Introduction to Organic Geochemistry*. John Wiley & Sons.

Kim, H. M., Hwang, C. Y., & Cho, B. C. 2010. (n.d.). Arcobacter marinus sp. Nov. *International Journal of Systematic and Evolutionary Microbiology*, *60*(3), 531–536. https://doi.org/10.1099/ijs.0.007740-0

Kim, H. S., Hyun, D.-W., Lee, J.-Y., Kim, P. S., Whon, T. W., Kang, W., & Bae, J.-W. (2016). Sedimentitalea todarodis sp. Nov., isolated from the intestinal tract of a Japanese flying squid. *International Journal of Systematic and Evolutionary Microbiology*, *66*(9), 3293–3298. https://doi.org/10.1099/ijsem.0.001188

Kim, L. H., & Chong, T. H. (2017). Physiological Responses of Salinity-Stressed *Vibrio* sp. And the Effect on the Biofilm Formation on a Nanofiltration Membrane. *Environmental Science & Technology*, *51*(3), 1249–1258. https://doi.org/10.1021/acs.est.6b02904

Kitamura, M., Amakasu, K., Kikuchi, T., & Nishino, S. (2017). Seasonal dynamics of zooplankton in the southern Chukchi Sea revealed from acoustic backscattering strength. *Continental Shelf Research*, *133*, 47–58. https://doi.org/10.1016/j.csr.2016.12.009

Koch, C. W., Cooper, L. W., Lalande, C., Brown, T. A., Frey, K. E., & Grebmeier, J. M. (2020). Seasonal and latitudinal variations in sea ice algae deposition in the Northern Bering and

Chukchi Seas determined by algal biomarkers. *PLOS ONE*, *15*(4), e0231178. https://doi.org/10.1371/journal.pone.0231178

Kolattukudy, P. E. (1980). Cutin, Suberin, and Waxes. In P. K. Stumpf (Ed.), *Lipids: Structure and Function* (pp. 571–645). Academic Press. https://doi.org/10.1016/B978-0-12-675404-9.50024-2

Kuzyk, Z. Z. A., Gobeil, C., & Macdonald, R. W. (2013). 210Pb and 137Cs in margin sediments of the Arctic Ocean: Controls on boundary scavenging. *Global Biogeochemical Cycles*, *27*(2), 422–439. https://doi.org/10.1002/gbc.20041

Lacey, J. H., Leng, M. J., Vane, C. H., Radbourne, A. D., Yang, H., & Ryves, D. B. (2018). Assessing human impact on Rostherne Mere, UK, using the geochemistry of organic matter. *Anthropocene*, *21*, 52–65. https://doi.org/10.1016/j.ancene.2018.02.002

Lalande, C., Forest, A., Barber, D. G., Gratton, Y., & Fortier, L. (2009). Variability in the annual cycle of vertical particulate organic carbon export on Arctic shelves: Contrasting the Laptev Sea, Northern Baffin Bay and the Beaufort Sea. *Continental Shelf Research*, *29*(17), 2157–2165. https://doi.org/10.1016/j.csr.2009.08.009

Lapoussière, A., Michel, C., Starr, M., Gosselin, M., & Poulin, M. (2011). Role of free-living and particle-attached bacteria in the recycling and export of organic material in the Hudson Bay system. *Journal of Marine Systems*, *88*(3), 434–445. https://doi.org/10.1016/j.jmarsys.2010.12.003

Loffeld, B., & Keweloh, H. (1996). Cis/trans isomerization of unsaturated fatty acids as possible control mechanism of membrane fluidity inPseudomonas putida P8. *Lipids*, *31*(8), 811–815. https://doi.org/10.1007/BF02522976

Loose, B., Miller, L. A., Elliott, S., & Papakyriakou, T. (2011). Sea Ice Biogeochemistry and Material Transport Across the Frozen Interface. *Oceanography*, *24*(3), 202–218.

Lürling, M., & Van Donk, E. (1996). Zooplankton-induced unicell-colony transformation in Scenedesmus acutus and its effect on growth of herbivore Daphnia. *Oecologia*, *108*(3), 432–437. https://doi.org/10.1007/BF00333718

Macdonald, R. W., Solomon, S. M., Cranston, R. E., Welch, H. E., Yunker, M. B., & Gobeil, C. (1998). A sediment and organic carbon budget for the Canadian Beaufort Shelf. *Marine Geology*, *144*(4), 255–273. https://doi.org/10.1016/S0025-3227(97)00106-0

Magen, C., Chaillou, G., Crowe, S. A., Mucci, A., Sundby, B., Gao, A., Makabe, R., & Sasaki, H. (2010). Origin and fate of particulate organic matter in the southern Beaufort Sea – Amundsen Gulf region, Canadian Arctic. *Estuarine, Coastal and Shelf Science*, *86*(1), 31–41. https://doi.org/10.1016/j.ecss.2009.09.009

Marchand, D., & Rontani, J.-F. (2001). Characterisation of photo-oxidation and autoxidation products of phytoplanktonic monounsaturated fatty acids in marine particulate matter and recent sediments. *Organic Geochemistry*, *32*(2), 287–304. https://doi.org/10.1016/S0146-6380(00)00175-3

Martínez, E., Hamberg, M., Busquets, M., Díaz, P., Manresa, A., & Oliw, E. H. (2010). Biochemical Characterization of the Oxygenation of Unsaturated Fatty Acids by the Dioxygenase and Hydroperoxide Isomerase of Pseudomonas aeruginosa 42A2. *Journal of Biological Chemistry*, *285*(13), 9339–9345. https://doi.org/10.1074/jbc.M109.078147

Meyer-Reil, L.-A. (1984). Bacterial Biomass and Heterotrophic Activity in Sediments and Overlying Waters. In J. E. Hobbie & P. J. leB. Williams (Eds.), *Heterotrophic Activity in the Sea* (pp. 523–546). Springer US. https://doi.org/10.1007/978-1-4684-9010-7_23

Michel, C., Legendre, L., Ingram, R. G., Gosselin, M., & Levasseur, M. (1996). Carbon budget of sea-ice algae in spring: Evidence of a significant transfer to zooplankton grazers. *Journal of Geophysical Research: Oceans*, *101*(C8), 18345–18360. https://doi.org/10.1029/96JC00045

Monfort, P., Demers, S., & Levasseur, M. (2000). Bacterial dynamics in first year sea ice and underlying seawater of Saroma-ko Lagoon (Sea of Okhotsk, Japan) and Resolute Passage (High Canadian Arctic): Inhibitory effects of ice algae on bacterial dynamics. *Canadian Journal of Microbiology*, *46*(7), 623–632. https://doi.org/10.1139/w00-024

Morata, N., Poulin, M., & Renaud, P. E. (2011). A multiple biomarker approach to tracking the fate of an ice algal bloom to the sea floor. *Polar Biology*, *34*(1), 101–112. https://doi.org/10.1007/s00300-010-0863-3

Morata, N., & Renaud, P. E. (2008). Sedimentary pigments in the western Barents Sea: A reflection of pelagic–benthic coupling? *Deep Sea Research Part II: Topical Studies in Oceanography*, *55*(20), 2381–2389. https://doi.org/10.1016/j.dsr2.2008.05.004

Moriceau, B., Garvey, M., Ragueneau, O., & Passow, U. (2007). Evidence for reduced biogenic silica dissolution rates in diatom aggregates. *Marine Ecology Progress Series*, *333*, 129–142. https://doi.org/10.3354/meps333129

Myers, P. G., Kulan, N., & Ribergaard, M. H. (2007). Irminger Water variability in the West Greenland Current. *Geophysical Research Letters*, *34*(17). https://doi.org/10.1029/2007GL030419

Nadaï, G., Nöthig, E.-M., Fortier, L., & Lalande, C. (2021). Early snowmelt and sea ice breakup enhance algal export in the Beaufort Sea. *Progress in Oceanography*, *190*, 102479. https://doi.org/10.1016/j.pocean.2020.102479

Nishimura, M. (1978). Geochemical characteristics of the high reduction zone of stenols in Suwa sediments and the environmental factors controlling the conversion of stenols into stanols. *Geochimica et Cosmochimica Acta*, *42*(4), 349–357. https://doi.org/10.1016/0016-7037(78)90265-X

O'Brien, M. C., Macdonald, R. W., Melling, H., & Iseki, K. (2006). Particle fluxes and geochemistry on the Canadian Beaufort Shelf: Implications for sediment transport and deposition. *Continental Shelf Research*, *26*(1), 41–81. https://doi.org/10.1016/j.csr.2005.09.007

Ortega-Retuerta, E., Jeffrey, W. H., Babin, M., Bélanger, S., Benner, R., Marie, D., Matsuoka, A., Raimbault, P., & Joux, F. (2012). Carbon fluxes in the Canadian Arctic: Patterns and drivers of bacterial abundance, production and respiration on the Beaufort Sea margin. *Biogeosciences*, *9*(9), 3679–3692. https://doi.org/10.5194/bg-9-3679-2012

Otto, A., & Simoneit, B. R. T. (2001). Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz formation, Saxony, Germany. *Geochimica et Cosmochimica Acta*, *65*(20), 3505–3527. https://doi.org/10.1016/S0016-7037(01)00693-7

Paffenhöfer, G.-A., & Knowles, S. C. (n.d.). Ecological implications of fecal pellet size, production and consumption by copepods. *Journal of Marine Research*, 16.

Piuri, M., Sanchez-Rivas, C., & Ruzal, S. m. (2003). Adaptation to high salt in Lactobacillus: Role of peptides and proteolytic enzymes. *Journal of Applied Microbiology*, *95*(2), 372–379. https://doi.org/10.1046/j.1365-2672.2003.01971.x

Porter, N. A., Caldwell, S. E., & Mills, K. A. (1995). Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, *30*(4), 277–290. https://doi.org/10.1007/BF02536034

Poulin, M., Daugbjerg, N., Gradinger, R., Ilyash, L., Ratkova, T., & von Quillfeldt, C. (2011). The pan-Arctic biodiversity of marine pelagic and sea-ice unicellular eukaryotes: A first-attempt assessment. *Marine Biodiversity*, *41*(1), 13–28. https://doi.org/10.1007/s12526-010-0058-8

Rachold, V., Eicken, H., Gordeev, V. V., Grigoriev, M. N., Hubberten, H.-W., Lisitzin, A. P., Shevchenko, V. P., & Schirrmeister, L. (2004). Modern Terrigenous Organic Carbon Input to the Arctic Ocean. In R. Stein & R. W. MacDonald (Eds.), *The Organic Carbon Cycle in the Arctic Ocean* (pp. 33–55). Springer. https://doi.org/10.1007/978-3-642-18912-8_2

Rapp, J. Z., Fernández-Méndez, M., Bienhold, C., & Boetius, A. (2018). Effects of Ice-Algal Aggregate Export on the Connectivity of Bacterial Communities in the Central Arctic Ocean. *Frontiers in Microbiology*, *9*, 1035. https://doi.org/10.3389/fmicb.2018.01035

Richerol, T., Rochon, A., Blasco, S., Scott, D. B., Schell, T. M., & Bennett, R. J. (2008). Evolution of paleo sea-surface conditions over the last 600 years in the Mackenzie Trough, Beaufort Sea (Canada). *Marine Micropaleontology*, *68*(1), 6–20. https://doi.org/10.1016/j.marmicro.2008.03.003

Riebesell, U., Schloss, I., & Smetacek, V. (1991). Aggregation of algae released from melting sea ice: Implications for seeding and sedimentation. *Polar Biology*, *11*(4), 239–248. https://doi.org/10.1007/BF00238457

Rieck, A., Herlemann, D. P. R., Jürgens, K., & Grossart, H.-P. (2015). Particle-Associated Differ from Free-Living Bacteria in Surface Waters of the Baltic Sea. *Frontiers in Microbiology*, *6*, 1297. https://doi.org/10.3389/fmicb.2015.01297

Riedel, A., Michel, C., & Gosselin, M. (2006). Seasonal study of sea-ice exopolymeric substances on the Mackenzie shelf: Implications for transport of sea-ice bacteria and algae. *Aquatic Microbial Ecology*, *45*, 195–206. https://doi.org/10.3354/ame045195

Rontani, J., Amiraux, R., Lalande, C., Babin, M., Kim, H.-R., & Belt, S. T. (2018). Use of palmitoleic acid and its oxidation products for monitoring the degradation of ice algae in Arctic waters and bottom sediments. *Organic Geochemistry*, *124*, 88–102. https://doi.org/10.1016/j.orggeochem.2018.06.002

Rontani, J., Charriere, B., Petit, M., Vaultier, F., Heipieper, H. J., Link, H., Chaillou, G., & Sempéré, R. (2012). Degradation state of organic matter in surface sediments from the Southern Beaufort Sea: A lipid approach. *Biogeosciences*, *9*(9), 3513–3530. https://doi.org/10.5194/bg-9-3513-2012

Rontani, J.-F., Charriere, B., Forest, A., Heussner, S., Vaultier, F., Petit, M., Delsaut, N., Fortier, L., & Sempéré, R. (2012). Intense photooxidative degradation of planktonic and bacterial lipids in sinking particles collected with sediment traps across the Canadian Beaufort Shelf (Arctic Ocean). *Biogeosciences*, *9*(11), 4787–4802. https://doi.org/10.5194/bg-9-4787-2012

Rontani, J.-F., Smik, L., Vaultier, F., Widdicombe, C., & Belt, S. T. (2021). Seasonal monitoring of lipid degradation processes in the western English Channel links bacterial 10S-DOX enzyme activity to free fatty acid production by phytoplankton. *Marine Chemistry*, *230*, 103928. https://doi.org/10.1016/j.marchem.2021.103928

Saint-Béat, B., Fath, B. D., Aubry, C., Colombet, J., Dinasquet, J., Fortier, L., Galindo, V., Grondin, P.-L., Joux, F., Lalande, C., LeBlanc, M., Raimbault, P., Sime-Ngando, T., Tremblay, J.-E., Vaulot, D., Maps, F., & Babin, M. (2020). Contrasting pelagic ecosystem functioning in eastern and western Baffin Bay revealed by trophic network modeling. *Elementa: Science of the Anthropocene*, *8*(1). https://doi.org/10.1525/elementa.397

Saunders, P. A., Deibel, D., Stevens, C. J., Rivkin, R. B., Lee, S. H., & Klein, B. (2003). Copepod herbivory rate in a large arctic polynya and its relationship to seasonal and spatial variation in copepod and phytoplankton biomass. *Marine Ecology Progress Series*, *261*, 183–199. https://doi.org/10.3354/meps261183

Seuthe, L., Darnis, G., Riser, C. W., Wassmann, P., & Fortier, L. (2007). Winter–spring feeding and metabolism of Arctic copepods: Insights from faecal pellet production and respiration measurements in the southeastern Beaufort Sea. *Polar Biology*, *30*(4), 427–436. https://doi.org/10.1007/s00300-006-0199-1

Sherr, E. B., Sherr, B. F., & Hartz, A. J. (2009). Microzooplankton grazing impact in the Western Arctic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *56*(17), 1264–1273. https://doi.org/10.1016/j.dsr2.2008.10.036

Shoja Chaghervand, S. (2019). *Characterization of the enzymes involved in the diolsynthase pathway in Pseudomonas aeruginosa* [Universitat de Barcelona]. http://diposit.ub.edu/dspace/handle/2445/139344

Smetacek, V. S. (1985). Role of sinking in diatom life-history cycles: Ecological, evolutionary and geological significance. *Marine Biology*, *84*(3), 239–251. https://doi.org/10.1007/BF00392493

Smik, L., Cabedo-Sanz, P., & Belt, S. T. (2016). Semi-quantitative estimates of paleo Arctic sea ice concentration based on source-specific highly branched isoprenoid alkenes: A further development of the PIP25 index. *Organic Geochemistry*, *92*, 63–69. https://doi.org/10.1016/j.orggeochem.2015.12.007

Tamburini, C., Boutrif, M., Garel, M., Colwell, R. R., & Deming, J. W. (2013). Prokaryotic responses to hydrostatic pressure in the ocean – a review. *Environmental Microbiology*, *15*(5), 1262–1274. https://doi.org/10.1111/1462-2920.12084

Tang, C. C. L., Ross, C. K., Yao, T., Petrie, B., DeTracey, B. M., & Dunlap, E. (2004). The circulation, water masses and sea-ice of Baffin Bay. *Progress in Oceanography*, *63*(4), 183–228. https://doi.org/10.1016/j.pocean.2004.09.005

Thompson, J., & MacLeod, R. A. (1971). Functions of Na+ and K+ in the Active Transport of α-Aminoisobutyric Acid in a Marine Pseudomonad. *Journal of Biological Chemistry*, *246*(12), 4066–4074. https://doi.org/10.1016/S0021-9258(18)62141-6

Volkman, J. (2003). Sterols in microorganisms. *Applied Microbiology and Biotechnology*, *60*(5), 495–506. https://doi.org/10.1007/s00253-002-1172-8

Wakeham, S. G. (1989). Reduction of stenols to stanols in particulate matter at oxic–anoxic boundaries in sea water. *Nature*, *342*(6251), 787–790. https://doi.org/10.1038/342787a0

Wakeham, S. G., & Canuel, E. A. (2006). Degradation and Preservation of Organic Matter in Marine Sediments. In J. K. Volkman (Ed.), *Marine Organic Matter: Biomarkers, Isotopes and DNA* (pp. 295–321). Springer. https://doi.org/10.1007/698_2_009

Wakeham, S. G., Hedges, J. I., Lee, C., Peterson, M. L., & Hernes, P. J. (1997). Compositions and transport of lipid biomarkers through the water column and surficial sediments of the equatorial Pacific Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *44*(9), 2131–2162. https://doi.org/10.1016/S0967-0645(97)00035-0

Wassmann, P., Duarte, C. M., Agustí, S., & Sejr, M. K. (2011). Footprints of climate change in the Arctic marine ecosystem. *Global Change Biology*, *17*(2), 1235–1249. https://doi.org/10.1111/j.1365-2486.2010.02311.x

Yang, S.-H., Seo, H.-S., Lee, J.-H., Kim, S.-J., & Kwon, K. K. (2014). Neptunomonas acidivorans sp. Nov., isolated from sediment, and emended description of the genus Neptunomonas. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_11), 3650–3654. https://doi.org/10.1099/ijs.0.064253-0

Yunda-Guarin, G., Brown, T. A., Michel, L. N., Saint-Béat, B., Amiraux, R., Nozais, C., & Archambault, P. (2020). Reliance of deep-sea benthic macrofauna on ice-derived organic matter highlighted by multiple trophic markers during spring in Baffin Bay, Canadian Arctic. *Elementa: Science of the Anthropocene*, *8*(1). https://doi.org/10.1525/elementa.2020.047

Zimov, S. A., Davydov, S. P., Zimova, G. M., Davydova, A. I., Schuur, E. a. G., Dutta, K., & Chapin III, F. S. (2006). Permafrost carbon: Stock and decomposability of a globally significant carbon pool. *Geophysical Research Letters*, *33*(20). https://doi.org/10.1029/2006GL027484

Chapitre V

Induction d'altération des bactéries associées aux phytodétritus par l'oxygène singulet : effet de l'irradiance et de la pigmentation bactérienne

Sommaire

5.1 Avant-propos

Les chapitres précédents se sont focalisés sur les stress (osmotique et chimique) pouvant affecter les bactéries associées aux algues de glace et expliquer leur faible activité de biodégradation. Il a été démontré précédemment que les bactéries associées à des cellules phytoplanctoniques sénescentes pouvaient également subir des réactions d'oxydation photosensibilisées de Type II (faisant intervenir l'$^1O_2$) (Rontani *et al*., 2003). Dans ce dernier chapitre, nous nous sommes donc intéressés à ce stress photooxydatif en se focalisant sur l'impact de l'intensité de l'irradiance lumineuse et de la présence de pigmentation chez les bactéries sur l'efficacité de ces phénomènes de photooxydation sensibilisés de Type II).

La production d'$^1O_2$ par les organismes photosynthétiques sénescents particulièrement en fin de bloom pourrait être à l'origine de la forte abondance des bactéries du clade *Roseobacter* dans les communautés de bactérioplancton. En effet, on retrouve chez ces dernières de nombreuses espèces pigmentées possédant entre autres des caroténoïdes aux fortes propriétés antioxydantes. Nous avons donc induit la production d'$^1O_2$ à l'aide de cellules sénescentes d'une diatomée (*Thalassiosira*) sous des intensités lumineuses bien contrastées et étudié l'impact de l'$^1O_2$ sur l'oxydation des lipides membranaires et l'activation des gènes de réparation de l'ADN de deux souches bactériennes : *Pseudomonas stutzeri* (bactérie Gram - non-pigmentée) et *Dinoroseobacter shibae* (bactérie Gram – pigmentée). Notre étude s'est appuyée d'une part sur l'analyse de nombreux échantillons de glace et de articules en sédimentation collectés dans l'Arctique canadien (Baie de Baffin et mer de Beaufort) sous des conditions d'irradiance lumineuse contrastées, et d'autre part sur l'analyse des produits d'oxydation photosensibilisée de type II (faisant intervenir l'$^1O_2$) de l'acide vaccénique (acide gras membranaire insaturé typiquement bactérien) ainsi que la mesure de l'expression de gènes impliqués dans la réparation de l'ADN et la régulation des systèmes de défense cellulaires.

L'analyse des échantillons collectés *in situ* a montré une photooxydation très intense des bactéries associées aux algues de glace dans la banquise, que nous avons attribué à l'intensité de l'irradiance solaire dans ce biotope. Cette hypothèse a pu ensuite être confirmée *in vitro*. En effet les résultats obtenus démontrent clairement l'effet de l'intensité de l'irradiance sur le transfert de l'$^1O_2$ chez *P. stutzeri*. Sous de faibles irradiances, l'$^1O_2$ est produit lentement, favorisant l'oxydation des lipides insaturés algaux (effet photodynamique) et limitant son transfert aux bactéries attachées. En revanche, de

fortes irradiances induisent une production rapide et intense d'$^1O_2$, qui diffuse hors des chloroplastes et atteint facilement les bactéries attachées oxydant efficacement leurs composants membranaires insaturés. Si la présence de caroténoïdes chez *D. shibae* lui confére une protection efficace contre le rayonnement UV, il semble inefficace dans le cas de l'$^1O_2$.

Concernant l'impact de l'$^1O_2$ sur l'expression des gènes de réparation de l'ADN et de régulation des mécanismes de défense, il semblerait que les effets observés chez *P. stutzeri* soient dûs à l'action délétère du rayonnent UV. En effet sur-expression des gènes de réparation et de régulation observée dans les contrôles effectués sans algue montrent clairement que la présence de phytodétritus n'influence pas l'activité de ces gènes. Dans le cas de *D. shibae*, nous n'avons pas observé d'effets de l'intensité de l'irradiance sur l'activité des gènes de réparation et de régulation de l'ADN. Toutefois, la présence de caroténoïdes dans cette souche semble lui conférer une résistance particulière au rayonnement UV.

Ces observations ne confortent pas notre hypothèse de départ, à savoir que l'abondance de bactéries du clade *Roseobacter* observée lors de périodes post-bloom doit résulter de la présence de caroténoïdes dans leurs membranes leur permettant de résister aux dommages oxydatifs induits par l'$^1O_2$ produit lors de la sénescence algale. Ces composants leur permettent toutefois de résister efficacement aux dommages oxydatifs induits par le rayonnement UV dans les eaux de surface.

- Contribution personnelle

Ce dernier chapitre fortement pluridisciplinaire, encore en préparation, a fait intervenir de nombreuses techniques. Ma contribution personnelle a tout d'abord consisté en la mise en culture, au suivi de la croissance et au comptage de souches bactériennes (sélectionnées au préalable). Une fois les cinétiques de croissance établies et les cellules algales comptées et lysées, j'ai réalisé de nombreux tests de mise au point (en faisant varier la durée d'incubation, l'intensité de la lumière et le ratio cellules algales : bactéries). J'ai également réalisé le clonage et la mise au point des différentes gammes étalons des gènes d'intérêts (sélectionnés au préalable) pour la quantification des niveaux d'expression de ces derniers. J'ai par la suite réalisé les expérimentations dans les différentes conditions retenues, extrait les lipides et analysé les produits d'oxydation de ces derniers. J'ai également contribué aux extractions d'ARN, à l'obtention des ADNc et à

leur amplification par RTqPCR. Enfin, j'ai fortement participé à la rédaction de ce dernier chapitre.

5.2.2 Article

Singlet oxygen-induced alteration of bacteria associated to phytodetritus: effect of irradiance and bacteria pigmentation

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Article en preparation

1. Introduction

In marine ecosystems, microscopic photosynthetic organisms (phytoplankton) such as diatoms, dinoflagellates or cocolithophores are the main primary producers, capable of producing organic matter through light energy and inorganic nutrients. This organic matter is heavily processed by bacteria, mineralizing approximately 50% of the ocean primary production (PP) (Cole *et al*., 1988, Ducklow *et al*., 1993). The remaining half of the PP enters either the marine food web or is buried into the seafloor sediments through a process called the 'biological pump' (Eppley & Peterson, 1979). Hence, bacterial activity in the oceans is highly correlated with the abundance of primary producers (Cole *et al*., 1988). It has indeed been shown that bacterial activity in the oceans (called secondary production) varies accordingly with phytoplankton blooms in large or small scales (Azam & Malfatti, 2007).

Phytoplankton-bacteria interactions are highly intricate, so that changes in the compositions of the phytoplankton blooms (in terms of abundance and species assemblages) leads to corresponding changes in bacterial community composition (Telling *et al*., 2012; Riemann *et al*., 2000; Pinhassi *et al*., 2004). As this microbial secondary production is a key process to better understand the functioning of marine ecosystem, the phylogenetic composition of bacterioplankton communities (i.e free living planktonic bacteria in marine ecosystems) has been extensively studied. If it is still complicate to generalize bacterioplankton community structures, some important bacterial groups such as *Bacteroidetes* and *Proteobacteria* have been found to be typically abundant during bloom events, regardless of the phytoplankton species (Pinhassi *et al*., 2004; Fandino *et al*., 2001; Rink *et al*., 2007). Amongst them, *Roseobacter*, genus belonging to the *Alphaproteobacterial* class, are often the dominant class among marine phytoplankton-associated bacterial communities (Alavi *et al*., 2001; Buchan *et al*., 2005; González *et al*., 2000; Jasti *et al*., 2005; Rüger & Höfle, 1992). Many studies have investigated this repartition and given some explanations to this apparent domination. Indeed, several well-known physiological characteristics of strains belonging to *Roseobacter* genus such as their high colonization capabilities (Dang & Lovell, 2000), their production of *quorum* sensing molecules (Wagner-Döbler *et al*., 2005) or antimicrobial

compounds (Long & Azam 2001) could explain why they are so efficient at colonizing phytoplankton. Another physiological characteristic of *Roseobacter* species that could explain their high abundance in bacterioplankton assemblages could be the occurrence of the particular Aerobic Anoxygenic Phototrophy (AAP) metabolism among this clade (Allgaier *et al*., 2003). AAP bacteria are capable of photoheterotrophy, a metabolic pathway allowing them to produce energy from light using photosynthetic reaction centers composed of bacteriochlorophyll-a (BChl *a*, Yurkov & Csotonyi, 2009). They are known to contain high amounts of pigments, such as carotenoids (Petit *et al*., 2015), which can protect these organisms against oxidative damage, such as type II photooxidation, a process that may happen during late bloom phases (Rontani *et al*., 2012).

Type II photooxidation processes are light-induced indirect degradation reactions, involving the action of a photosensitizer molecule (typically pigments or aromatic compounds) in its triplet state (Gollnick, 1968). In the oceans and more specifically in photosynthetic cells, chlorophyll, which is an efficient photosensitizer (Foote, 1976) can trigger type II photooxidation processes, especially in late bloom phases, when phytoplankton cells are senescent. Indeed, when senescent algal cells are exposed to light, the energy is no longer used for the photosynthetic pathway, and thus leads to the production of chlorophyll in its triplet state (^{3}Chl, Knox & Dodge, 1985). This produced ^{3}Chl induces the accumulation of deleterious Reactive Oxygen Species (ROS), such as singlet oxygen ($^1O_2$, Nelson, 1993). Given the lifetime and the diffusion capacity of $^1O_2$, it is very likely that its deleterious effects do not only harm algal cells but also their neighboring attached bacteria (Rontani *et* al., 2003; Christodoulou *et al*., 2010, Petit *et al*., 2013, 2015). If it was previously demonstrated that low solar irradiance favors a slow production and diffusion of $^1O_2$ through the membranes, thus increasing photooxidative damage of the unsaturated algal lipids (Amiraux *et al*., 2016), the effects of the intensity of solar irradiance on the photooxidation of the bacteria attached to phytodetritus are totally unknown

$^1O_2$ reacts with a broad range of cellular molecules, mostly unsaturated lipidic compounds such as sterols and unsaturated fatty acids (Rontani & Belt, 2020, membranes, proteins (Morgan *et al*., 2004), or DNA (Agnez-Lima *et al*., 1999; Ravanat *et al*., 2000). When a $^1O_2$ molecule encounters an unsaturated fatty acid, it generates what is called an "ene addition" (Frimer, 1979), which means the addition of the $^1O_2$ on the end of the double bond and the concerted migration of the latter. This leads to the formation of allylic hydroperoxides at each end of the original double bond (Frankel *et al*., 1979). In the case

of bacterial $C_{18:1\omega7}$ (vaccenic) acid, these processes afford 11- and 12- hydroperoxides with an allylic *Trans* double bond, which then undergo stereospecific allylic rearrangement to 13-*Trans* and 10-*Trans* hydroperoxides, respectively (Petit *et al*., 2013). The corresponding hydroxyacids constitute good tracers of type II photooxidation of bacteria in natural samples (Rontani *et* al., 2003). In nucleic acids, guanine reacts with $^1O_2$ to form 8-oxo-7,8dihydro2'-deoxyguanosine (8-oxodG), or ^{0}G (Cadet et al., 2006, 2010), resulting in various mutations (Menck *et al*., 1993). Bacteria respond to adverse conditions by activating gene-expression pathways that increase their chances of survival. They have different strategies to protect themselves against the toxicity of $^1O_2$. Such a pathway — the aptly named SOS response — is induced by genome damage or disruption of DNA replication, and results in the synthesis of DNA repair enzymes and the inhibition of bacterial cell division. Bacterial cells have different strategies to protect themselves against the toxicity of $^1O_2$, these strategies usually involves "preventive" detoxication enzymes such as Super Oxide Dismutase (SOD) or Catalase (CAT) (Glaeser et al., 2011; Kim et al., 2002), an efficient "*Go system"* of three detoxication proteins : MutM, MutT and MutY (Michaels & Miller, 1992) assigned to DNA repair, or the quenching of $^1O_2$ with antioxidant compounds such as carotenoid pigments (Conn *et al*., 1991).

Hence, it has already been hypothesized that bacteria with high amounts of pigments such as carotenoids may tolerate exposition to $^1O_2$ (Dahl *et al*., 1988; Glaeser *et al*., 2010; Petit *et al*., 2015). This resistance to oxidative stress could explain why the *Roseobacter* genus constitute one of the most highly represented bacterial family among bacterioplankton communities during late bloom phases, when phytoplankton cells are senescent. If the effect of pure $^1O_2$ on Gram-negative and Gram-positive bacteria has already been investigated by Dahl *et al*. (1987, 1988), the transfer of $^1O_2$ from senescent phytoplankton to their attached bacteria has been only demonstrated by Rontani *et al.* in 2003. Further studies could demonstrate: (i) the good correlation between the photodegradation state of bacteria attached to phytodetritus and the photooxidation state of phytoplanktonic chlorophyll (Petit *et al*., 2013), and (ii) the inhibition of the transfer of $^1O_2$ by the silica matrix of diatom frustules (Petit *et al*., 2015a). To our knowledge there is no study in the literature where the photooxidation states of pigmented and unpigmented bacteria associated with phytodetritus were compared.

In this study, we thus intended: (i) to determine the effect of solar irradiance intensity on the efficiency of $^1O_2$ transfer from phytodetritus to their attached bacteria, and (ii) to

compare the effect of photo-produced $^1O_2$ on the lipids and DNA of two Gram-negative unpigmented (*Pseudomonas stutzeri*) and pigmented bacteria (*Dinoroseobacter shibae*). The first task involves analyses of several particulate matter and sea ice samples collected in the Arctic under contrasted irradiance regimes and incubation of senescent *Thalassiosira* sp. cells contaminated with *P. stutzeri* and *D. shibae* under two contrasted artificial light conditions. In the second, the damage induced by $^1O_2$ photoproduced from senescent *Thalassiosira* sp. on the lipids and DNA of *D. shibae* and *P. stutzeri* were compared. For the first time, the impact of $^1O_2$ produced during algal senescence on the physiology of associated bacteria at the membrane-lipid and DNA levels was studied.

2. Materials and methods

2.1 Collection of in situ samples

Detailed descriptions (e.g., sampling dates, depths, volumes, etc) of the collection of samples of Arctic marine particulate matter and sea ice were described previously (Rontani et al., 2012, 2016; Amiraux et al., 2020). Briefly, sinking particles were collected in Resolute Passage under ice at 5 and 30 m with Hydro-Bios multi-sediment traps MS12 (Rontani et al., 2016) and at 100 m with Technicap PPS traps in the Amundsen Gulf and on the Mackenzie Shelf (Rontani et al., 2012). Sea-ice samples were collected at a landfast ice station located near Broughton Island in Davis Strait using a Kovacs Mark V 14-cm diameter corer and focusing on the bottommost 10 cm of sea ice.

2.2 Algal material production

The nonaxenic diatom *Thalassiosira* sp. RCC1714 was obtained from the Roscoff Marine station culture collection. Note that the amount of vaccenic acid (which oxidation products were used to monitor photooxidation of attached bacteria) resulting to the weak bacterial contamination of this strain was negligible relative to that present in *P. stutzeri* (1/30) and *D. shibae* (1/15) aliquots. Diatoms were grown in F/2 medium at 17°C in a constant environment-controlled cabinet under an irradiance of 18 $W.m^{-2}$ (Osram, Lumilux 12:12h light/dark cycles). After two months of growth, cell density was measured by counting under microscope, and death of the algal cells was induced by sonication. *Thalassiosira* sp. cells were sonicated on ice using a Branson Sonifier 450 ultrasound generator equipped with a Branson S450A cell disruptor probe, at 240 W for 10 minutes (duty cycle 50%). The sonicated cells were then aliquoted in 50 mL falcon tubes and stored at -20°C for later photooxidation experiments.

2.3 *Bacterial strains and culture conditions*

P. stutzeri ATCC 14405 and *D. shibae* DFL 17 were grown aerobically on a rotary shaker overnight in the dark at 17°C on marine broth medium (US Biologocal ™) diluted to the tenth with sterilized artificial sea water (Kester *et al*., 1967) to prevent the addition of large amounts of easily assimilated organic matter from the inoculum. The media were inoculated with the aerobic pre-culture at a ratio of 1:5 in the early exponential phase (DO_{620nm}, ± 0.020).

At the end of the incubation cells were counted by the technique of epifluorescence in the presence of fluorochrome (4P,6-diamidine-2P-phenylindole dihydrochloride, DAPI) as previously described in Otto (1990).

2.4 *Photooxidation and transfer of $^1O_2$ to the attached bacteria*

In order to test the effect of irradiance on the efficiency of the transfer of $^1O_2$ from phytodetritus to the attached bacteria, these experiments were conducted under two artificial light conditions, one using an Altas Suntest CPS + solar simulator with a Xenon NXE 1500 (500 $W.m^{-2}$) (producing a high solar irradiance in the range 300-800 nm close to this generally observed at the surface of Mediterranean Sea, Sarra *et al*., 2019) and one using a Binder incubator with Osram, Lumilux lamps producing Photosynthetically Available Radiations (PAR) in the range 400-750 nm, with a power of 18 $W.m^{-2}$ (producing a low photosynthetically available radiations (PAR) irradiance in the range 400-750 nm, close to this observed at 50m depth in the Mediterranean Sea, Maraňón et al., 2020).

- *High irradiance experiments*

Experiments were conducted in 100 mL sterilized quartz (exposing the cells to all the solar light spectrum) ad Pyrex (exposing the cells to radiations > 300 nm and thus excluding UV radiations) tubes. Phytodetritus and bacteria were mixed (1:5) in K medium (Keller *et al.,* 1987) (K 25x diluted with sterilized artificial sea water) at a final volume of 100 mL, and immediately exposed to light in the solar simulator. Tubes were maintained at 17°C by submersion in a water bath connected to a cryothermostat. Four sampling times were defined: 0.5, 2, 3.5 and 5 h. At each sampling time, 15 and 10 mL were collected in triplicate, for q-RT PCR and lipids analysis, respectively. The 15 mL samples were filtered (under sterile conditions) on polycarbonate 0,8 µm filters (to collect attached bacteria on particles and not free living) and stored at -80°C for further RNA extraction,

while the 10 mL samples were filtered (under sterile conditions) on 0,8 µm glass fiber filters (GF/F) and stored at -20°C for further lipid extraction. Each experiments was conducted in 4 separate tubes prepared simultaneously in the same conditions. For each sampling time, a single tube was sacrificed.

Control experiments were conducted with no phytodetritus. Bacteria were mixed with sterilized artificial sea water complemented with 1 $g.L^{-1}$ of lactate/acetate (50:50) as a carbon source, placed in 100 mL sterilized quartz tubes and immediately exposed to light in the solar simulator. Three sampling times were defined: 0, 2 and 5 h. At each sampling time, 15 mL were filtered (under sterile conditions) on 0.2 µm polycarbonate filters (to recover bacteria which are free-living in the lack of algal cells) and stored at -80°C for further RNA extraction. For the lipid analysis, 50 mL were sampled (under sterile conditions) and centrifugated with a Beckman & Coulter Allegra X-22 centrifuge at 20,000g for 30 minutes and pelleted. The bacterial cells were then stored at -20°C. Here again, the 3 sampling were conducted in separate control tubes prepared in the same conditions.

- *Low irradiance experiments*

Experiments were conducted in 2 L sterile Erlenmeyer flasks. Phytodetritus and bacteria were mixed (1:5) in K medium (K 25x diluted with sterilized synthetical sea water) in a final volume of 400 mL and immediately exposed to light in the incubator on a VWR analog shaker rotatory stirring plate. Four sampling times were defined: 0.5, 5, 8 and 24h. At each time, 15 mL and 10 mL were collected in triplicate. The 15 mL samples were filtered (under sterile conditions) on polycarbonate 0.8 µm filters and stored at -80°C for further RNA extraction, while the 10 mL samples were filtered (under sterile conditions) on 0.8 µm GF/F and stored à -20°C for further lipid extraction. In this condition, all 4 samplings were conducted in a single Erlenmeyer.

Control experiments were conducted with no phytodetritus. Bacteria were mixed with sterilized artificial sea water complemented with 1 $g.L^{-1}$ of lactate/acetate (50:50) as a carbon source, in a final volume of 200 mL and immediately exposed to light in the incubator on a VWR analog shaker rotatory stirring plate. Two sampling times were defined: 0 and 8 h. At each sampling time, 15 mL were filtered (under sterile conditions) on 0.2 µm polycarbonate filters and stored at -80°C for further RNA extraction. For the lipid analysis, 50 mL were sampled (under sterile conditions) and centrifugated with a

Beckman & Coulter Allegra X-22 centrifuge and pelleted. The bacterial cells were then stored at -20°C. The 2 sampling times were conducted in a single Erlenmeyer flask.

2.5 *Lipid analysis*

Lipid extraction

Samples (GF/F filters) were reduced with excess $NaBH_4$ after adding MeOH (25 mL, 30 min) to reduce labile hydroperoxides (resulting from photo- or autoxidation) to alcohols, which are more amenable to analysis using gas chromatography-mass spectrometry (GC-MS). Water (25 mL) and KOH (2.8 g) were then added and the resulting mixture saponified by refluxing (2 h). After cooling, the mixture was acidified (HCl, 2 N) to pH 1 and extracted with dichloromethane (DCM; 3 × 20 mL). The combined DCM extracts were dried over anhydrous Na_2SO_4, filtered and concentrated by rotary evaporation at 40°C to give total lipid extracts (TLEs).

Silylation

Dry TLEs were derivatized by dissolving them in 300 µL of a mixture of pyridine/bis-(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco; 2:1, v/v) and silylated in a heating block (50°C, 1 h). After evaporation to dryness under a stream of N_2, the derivatized residue was dissolved in ethyl acetate/BSTFA (to avoid desilylation) and analyzed by GC-MS/MS.

Gas chromatography-tandem mass spectrometry (GC-MS/MS)

GC-MS and GC-MS/MS analyses were performed using an Agilent 7890A/7010A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert, 30 m x 0.25 mm, 0.25 µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C. Oven temperature was ramped from 70°C to 130°C at 20°C min^{-1}, then to 250°C at 5°C min^{-1} and then to 300°C at 3°C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69 x 10^5 Pa until the end of the temperature program and then ramped from 0.69 x 10^5 Pa to 1.49 x 10^5 Pa at 0.04 x 10^5 Pa min^{-1}. The following mass spectrometer conditions were used: electron energy 70 eV, source temperature 230°C, quadrupole 1 temperature 150°C, quadrupole 2 temperature 150°C, collision gas (N_2) flow 1.5 $mL.min^{-1}$, quench gas (He) flow 2.25 $mL.min^{-1}$, mass range 50–700 Dalton, cycle time 313 ms. Vaccenic acid photooxidation products were quantified in multiple reaction monitoring (MRM) mode

using the transitions *m/z* 339 → 131 for the parent vaccenic acid and *m/z* 199 → 129, *m/z* 213 → 129, *m/z* 357 → 129 and *m/z* 371 → 129 for its oxidation products (Rontani, 2021). Precursor ions were selected from the most intense ions (and specific fragmentations) observed in electron ionization (EI) mass spectra. Collision-induced dissociation (CID) was optimized by using collision energies at 5, 10, 15 and 20 eV. Quantification involved peak integration and determination of individual response factors using standards and Mass Hunter (Agilent Technologies, Parc Technopolis – ZA Courtaboeuf, Les Ulis, France) software.

Gas chromatography-EI quadrupole time-of-flight mass spectrometry

Accurate mass measurements were made in full scan mode using an Agilent 7890B/7200 GC/QTOF system (Agilent Technologies, Parc Technopolis – ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Macherey-Nagel; OPTIMA-5MS Accent, 30 m x 0.25 mm, 0.25 µm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C. Oven temperature was ramped from 70°C to 130°C at 20°C.min^{-1} and then to 300°C at 5°C.min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69 x 10^{5} Pa until the end of the temperature program. Instrument temperatures were 300°C for transfer line and 230°C for the ion source. Nitrogen (1.5 mL.min^{-1}) was used as collision gas. Accurate mass spectra were recorded across the range *m/z* 50–700 at 4 GHz with the collision gas opened. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12252 from *m/z* 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was used for daily MS calibration. Compounds were identified by comparing their TOF mass spectra, accurate masses and retention times with those of standards. Quantification of each compound involved extraction of specific accurate fragment ions, peak integration and determination of individual response factors using external standards.

Standard compounds

Vaccenic and palmitoleic acids and phytol were obtained from Sigma-Aldrich. Vaccenic acid oxidation was achieved using Fe^{2+}/ascorbate (Loidl-Stahlhofen and Spiteller, 1994). Subsequent reduction of the resulting hydroperoxides in methanol with excess $NaBH_4$ afforded the corresponding hydroxyacids.

2.6 Quantitative RT-PCR

RNA extractions were performed with the High Pure RNA Isolation Kit (Roche Applied Science, United States) according to the supplier's recommendations. After RNA

extraction, quantification and ratios 260/230 and 260/280 were measured on the extract by spectrophotometry (NanoDrop 2000c, Thermo Scientific). The absence of DNA contamination in RNA extracts was verified by qPCR targeting 16s rDNA without reverse transcription step following the protocol of Aigle *et al*. (2017). The cDNAs were synthesized from the total extracted RNAs using random primers and the PrimeScript™ RT reagent Kit (TaKaRa) according to the supplier's recommendations. For each sample, the amount of RNAs, added to the reaction mixture, was adjusted so that the amount of RNAs was identical for each sample. Thus, despite the difference in cell density, the same quantity of RNAs from the different samples was used for the reverse transcription.

The primers were designed according to the bibliography and modified according to the sequence of our strain or were newly designed with the help of a primer-designing tool (Primer3). For standard curve elaboration, the fragments of genes were amplified and cloned into pGEMT-easy vectors (Promega) following the supplier's instructions.

Primer used for the amplification and cloning of the targeted genes for *P. stutzeri* and *D. shibae* are presented in the Table 5.1 and Table 5.2. The PCR reactions (20µl) contained 5 ng of genomic DNA from *P. stutzeri* or *D. shibae*, 1,25 µM of each primer and 10 µl of GoTaq® (Promega). The PCR program consisted in an initial denaturation step of 2 min at 98°C, to activate the polymerase, the cycle (35 repetitions) then started with 10 s of denaturation at 98°C, 20 s of hybridization at the appropriate temperature for the pair of primer used, and an elongation phase of 20 s at 72°C.

The cloned DNA fragments were checked by sequencing. After purification, the plasmid concentrations were determined by spectrophotometry and used for constituting the standard curves for qPCR allowing the quantification of the synthetized cDNAs. The quantity of the various target cDNAs was quantified by qPCR (qPCR Master Mix, Promega) according to the supplier's recommendations. Experiences were made using the CFX Opus Bio-Rad and the associated software. The starting quantity (SQ) was determined using the Bio-Rad CFX manager 2.1 software and transformed into copies per milliliter of culture according to the sample dilution. The quantification of the level of expression of the housekeeping gene (*rpoD*) encoding for a σ70 subunit of the RNA polymerase was used to normalize the gene expression. Each extracted RNAs were analyzed through six technical replicates (two independent reverse transcriptions and three qPCR analyses per cDNAs).

Table 5.1: Sequences and hybridization temperatures (°C) of primers used for the cloning and amplification of targeted genes in *P. stutzeri*

Target Genes	Primer Names	Primer sequence 5' → 3'	Fragment size	PCR hybridization Temperatures
16s	PS_16S-F	AGC-TAG-TTG-GTG-AGG-TAA-AGG	291 pb	49.7°C
	PS_16S-R	GTA-TTA-CCG-CGG-CTG-CTG-GCA		53.1°C
mutT	PS_mutT-F	GGT-ACT-GAT-CCG-CAT-CCC-G	192 pb	62 °C
	PS_mutT-R	TTC-TCC-ATG-AAG-CCT-GCC-G		60°C
mutM	PS_mutM-F	AAG-TCG-AAA-CCA-CCC-GCC	114 pb	58°C
	PS_mutM-R	GCA-CGT-CGA-GAT-CCT-CCG		60°C
mutY	PS_mutY-F	GGC-TAC-TTC-GAC-CGC-TTC-AT	74 pb	62°C
	PS_mutY-R	AGC-ACT-TCA-TCT-TCC-TCG-GC		62°C
rpoH	PS_rpoH-F	GGT-CTG-ATG-AAG-GCG-GTC-AA	209 pb	60°C
	PS_rpoH-R	ACC-TCA-TCG-TTG-TTC-AGC-CA		62°C
rpoE	PS_rpoE-F	TAC-ATG-ACT-CCC-ACG-AAG-CTC	200 pb	49.2°C
	PS_rpoE-R	GTA-GAA-CTC-GGC-ATC-CTC-AGA		
chrR	PS_chrR-F	CGA-GTT-GGA-ATT-ACG-TCG-CG	109 pb	62°C
	PS_chrR-R	CGG-CTC-GAT-CAG-TTC-CTT-GT		62°C
rpoD	PS_rpoD-F	CAG-TCC-CGC-CTC-AAA-GAG-TT	148 pb	62°C
	PS_rpoD-R	CGT-TGA-TGC-CCA-TGT-CGT-TG		62°C

Table 5.2: Sequences and hybridization temperatures (°C) of primers used for cloning and amplification of the target genes in *D. shibae*

Target Genes	Primer Names	Primer sequence 5' → 3'	Fragment size	PCR hybridization Temperatures
16s	Rhodo 240F	GGC-CCG-CGT-TGG-ATF-AGR-TA	276 pb	62°C
	Univ 516R	GTD-TTA-CCG-CGG-CKG-CTGy-RCA		62°C
mutT	DS_mutT-F	GCA-ATT-TCC-CGG-TGG-CAA-G	186 pb	60°C
	DS-mutT-R	ATA-CCC-TGC-CAT-TCC-CGA-CA		60°C
mutM	DS_mutM-F	CGC-AGA-CCC-TCA-TCA-TCC-AT	88 pb	62°C
	DS_mutM-R	GGC-AAA-GCG-TGA-TGA-AAT-GC		60°C
mutY	DS_mutY-F	GGC-TCT-CCG-AGG-TGA-TGC-TG	179 pb	66°C
	DS_mutY-R	CGC-GCA-TTT-CAG-CAG-GTT-G		60°C
rpoH	DS_rpoH-F	ATA-TAC-CAA-TCT-TCC-GGC-CCC-C	106 pb	51.6°C
	DS_rpoH-R	GGC-CAG-CAT-GTA-TTC-CTC-TTC-G		51.6°C
chrR	DS_chrR-F	AGC-TGC-CCA-AAT-CCA-TGA-CA	97 pb	60°C
	DS_chrR-R	TTC-ACA-TCC-TCC-ACA-TCG-CC		62°C
rpoD	DS_rpoD-F	GCA-AGA-CCG-ATG-ACC-AGG-AC	150 pb	64°C
	DS_rpoD-R	TCT-GTT-CCG-AGC-TGA-CCT-GA		62°C

3. Results & discussion

3.1 Effects of irradiation on the efficiency of $^1O_2$ transfer to bacteria

To investigate further the effect of irradiance on the efficiency of the photooxidation processes on bacteria attached to phytoplankton cells, we analyzed a variety (n = 46) of sinking particulate matter and sea ice samples collected in Arctic. Sinking particulate matter samples were collected: (i) at 100 m in summer in open water Beaufort Sea (Rontani *et al*., 2012) and (ii) at 5 and 30 m in spring in ice-covered Resolute Passage (Rontani *et al*., 2016). Sea ice samples (0-10 cm) were collected in spring during the GreenEdge campaign at Qikiqtarjuaq (Baffin Bay, Arctic Ocean, Amiraux *et al*., 2020). To compare the efficiency of Type II photooxidation processes in algal and the bacterial material, we used the ratio vaccenic acid photooxidation % / palmitoleic acid photooxidation %. Indeed, vaccenic acid is generally considered to be specific to bacteria (Lambert & Moss, 1983; Sicre *et al*., 1988), while palmitoleic acid is the main fatty acid component of sympagic diatoms (Fahl & Kattner, 1993; Falk-Petersen *et al.,* 1998; Leu *et al*., 2010). Although palmitoleic acid is also present in several bacteria (e.g., de Carvalho & Caramujo, 2014), the contribution of bacteria to this acid in the samples analyzed (dominated by diatoms) can be considered as negligible relative to this of sympagic algae.

The obtained results (Figure 5.1) show higher vaccenic acid photooxidation %/ palmitoleic photooxidation % ratios in sinking particles collected in open water Beaufort Sean than in these collected in ice-covered Resolute Passage. This difference may be attributed to the mean PAR (Photosynthetically Available Radiations) irradiance in the surface mixed layer, which is considerably higher in open water than in ice-covered zones (365 ± 62 and 10.9 ± 2.7µmol photons $m^{-2}.s^{-1}$, respectively, Alou-Font *et al*., 2016).

The decrease of the ratio observed between 5 and 30 m in the Resolute Passage (Figure 5.1) results likely from the decrease of solar irradiance with depth. These results suggest that photooxidation of bacteria attached to senescent algal cells is enhanced at high irradiances. This assumption is well supported by the very high ratios measured in the bottommost 10 cm of sea ice collected at the GreenEdge ice camp in 2016 (Figure 5.1). Under 75 cm of sea ice, irradiance can reach 105.9 µmol photons $m^{-2}.s^{-1}$ without snow cover and decrease to 51.9 µmol photons $m^{-2}.s^{-1}$ with a snow cover of 1 cm (Lund-Hansen *et al*., 2021). In this solid habitat, the association bacteria-sympagic algae may thus be

maintained at relatively high irradiances during long periods, these conditions strongly favoring the photooxidation of bacteria.

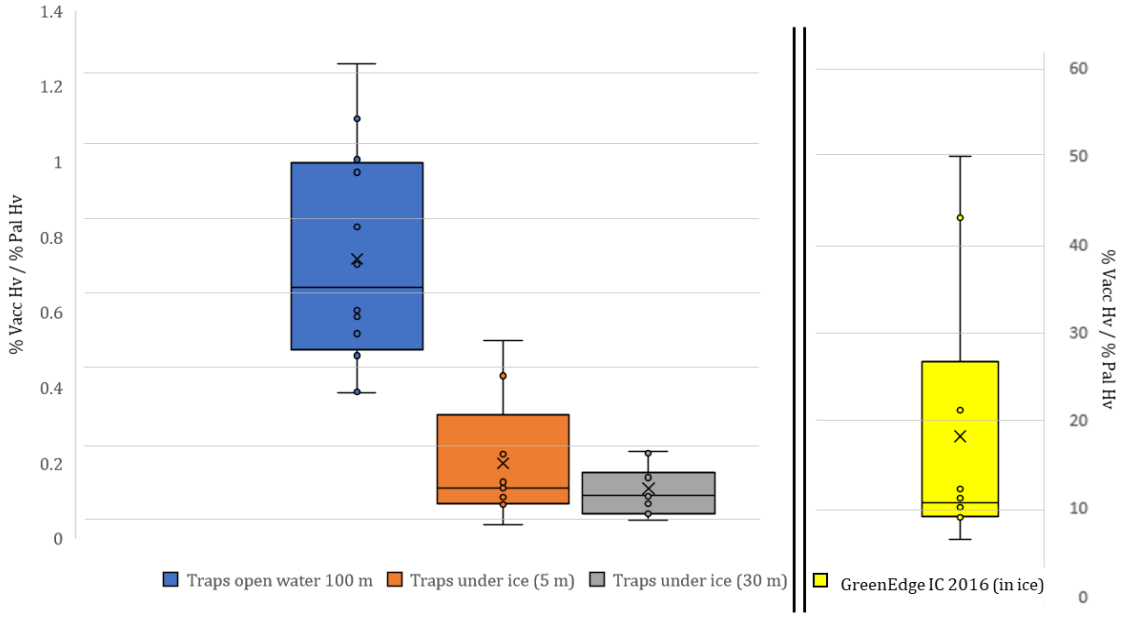

Figure 5.1: Values of the ratio vaccenic acid photooxidation %/palmitoleic acid photooxidation % in particulate matter and sea ice samples collected in Arctic under different irradiance conditions.

It was previously demonstrated that during the early stages of ice melt sympagic bacteria undergo an intense osmotic stress in hypersaline ice brines (Amiraux *et al.*, 2017) and are affected latter in the season by the release of bactericidal free palmitoleic acid by sympagic algae under the effect of light stress (Amiraux *et al.*, 2020). Our results show that these organisms may also undergo a strong photooxidation stress in the ice resulting from a very efficient transfer of $^1O_2$ from senescent sympagic algae.

To confirm this effect of solar irradiance on the efficiency of the transfer of $^1O_2$ from phytodetritus to their attached bacteria, we incubated senescent *Thalassiosira* sp. cells cinoculated with *P. stutzeri* under low PAR, high PAR and UV+PAR irradiances. We used the concentration of photooxidation products of vaccenic acid (nmol L^{-1}) to monitor oxidative damage in attached cells of *P. stutzeri*. Indeed, vaccenic acid is found in high proportions in *P. stutzeri* (Rainey *et al.*, 1994) and is only present in trace amounts in the non-axenic strain of *Thalassiosira* sp. employed.

Interestingly, we failed to detect vaccenic acid photooxidation products in the different controls carried out without algae. As previously observed (Rontani *et* al., 2003) photooxidation of heterotrophic bacterial membranes needs the presence of senescent

phytoplankton cells. Our results showed a strong effect of the intensity of the irradiance on the photooxidation of the bacterial membranes (Figure 5.2). Indeed, under high PAR irradiance the concentration of vaccenic acid photooxidation products reached 2.74 nm/L^{-1} at the first sampling time (0.5 h), whereas at a slightly higher light dose under low irradiance (8 h), the concentration was only of 0.43 nmol.L^{-1} (Figure 5.2).

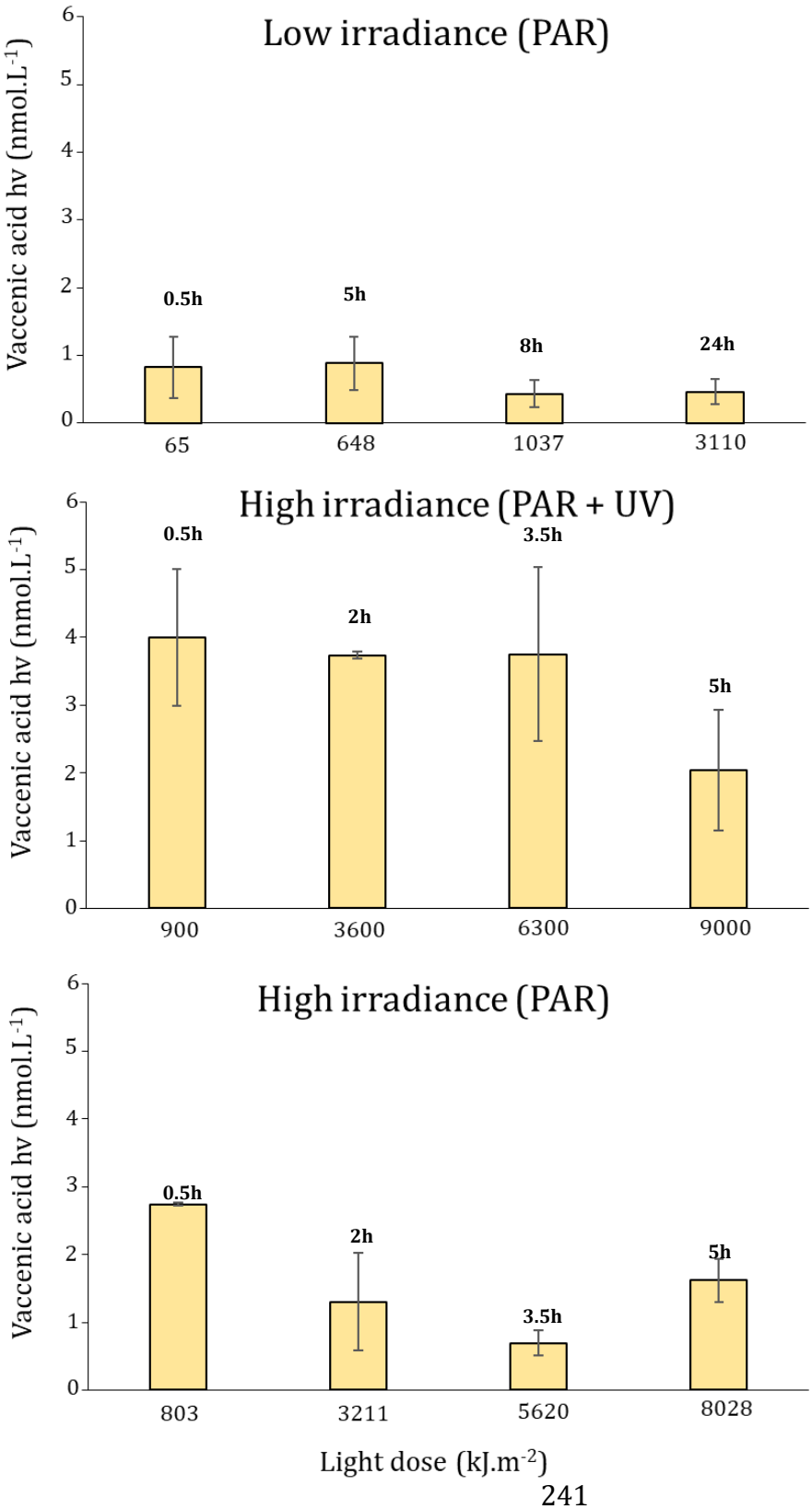

Figure 5.2: Concentrations of vaccenic acid photooxidation products (nmol.L-1) measured in the TLEs of the *Thalassiosira* sp. + *P. stutzeri* experiments under low PAR, high PAR+UV and high PAR irradiances (n = 3).

The highest oxidation of vaccenic acid observed under high UV+PAR irradiance (Figure 5.2) may be attributed to the presence of additional UV radiations. This assumption is well-supported by the profile of photooxidation products obtained under these conditions showing the presence of a high proportion of allylic 11- and 12-hydroxyacids with a *cis* double bond typical of the UV-induced photooxidation of MUFAs (Christodoulou et al., 2010).

It has been shown by Amiraux *et al*, (2016) in senescent diatom cells that low irradiances induce a slow but long production of $^1O_2$ that strongly oxidizes algal unsaturated lipids, through a process called "photodynamic effect" (Knox & Dodge, 1985; Skovsen *et al*., 2005). On the other hand, under high irradiances, the production of $^1O_2$ is stronger but shorter, inducing a strong photooxidation of the sensitizer (Chl a) called "photobleaching" and only a weak photodynamic effect (Amiraux *et al.*, 2016; Rontani *et al*., 2021). This effect of the intensity of solar irradiance on the efficiency of the photodynamic effect in senescent phytoplankton cells was then confirmed *in situ* (Rontani *et al*., 2021).

The effect of irradiance on the efficiency of photooxidation of attached bacteria thus appears to be exactly the opposite of phytoplankton. Under low irradiance, the low flux of $^1O_2$ produced reacts with the unsaturated membrane components of phytodetritus and is thus quenched before to reach attached bacteria. In contrast, under strong irradiance, a higher proportion of $^1O_2$ is efficiently transferred to the attached bacteria, strongly oxidizing their unsaturated lipids (Figure 5.8).

3.2 Effect of pigmentation on the protection of bacteria against $^1O_2$ and UV radiations

One other aim of this work was to demonstrate the role of bacterial pigmentation as a protective agent against the harmful effects of $^1O_2$. To do so, we compared the effects of $^1O_2$ on the photooxidation of the lipids and the activation of the DNA reparation genes in two bacterial strains, *P. stutzeri* (a classical heterotrophic bacteria without carotenoids) and *D. shibae* (an AAP containing a high proportion of vaccenic acid and of a carotenoid, (spheroidenone)) (Biebl *et al*., 2005).

The results obtained in the case of *D. shibae* (Figure 5.3) allow to confirm the enhancement of the efficiency of $^1O_2$ transfer from phytodetritus to their attached bacteria under high irradiance observed above with *P. stutzeri* (Figure 5.3). However, the similarity of the concentrations of vaccenic acid photooxidation products obtained with the two

strains (Figure 5.2 and Figure 5.3) also shows that the presence of spheroidenone in the membranes of *D. shibae* does not protect it against $^1O_2$ damage.

Carotenoids located at the surface of a bacterial membrane can efficiently protect against $^1O_2$-mediated bacterial killing (Tatsuzawa *et al.,* 2000). Those containing two polar hydroxy groups (e.g. zeaxanthin), lie across the monolayer membrane (Ourisson & Nakatani, 1989). In contrast, carotenoids lacking hydroxy groups, such as spheroidenone (dominant in *D. shibae*), may be completely embedded in the lipid layer of the membrane (Strzalka & Gruszecki, 1994). This embedment reduces their likelihood to react with $^1O_2$, which can thus rapidly affect unsaturated molecules in its vicinity. Consequently, in the future, it will be very important to check the effects of $^1O_2$-production by senescent phytoplanktonic cells on bacteria containing more polar carotenoids

In contrasts, the results presented in the Figure 5.3 also show that the presence of additional UV radiations has practically no effect on the unsaturated lipids of D. shibae. If spheroidenone does not seem to protect efficiently the membranes of this strain against the damage of $^1O_2$ transferred from phytodetritus, it seems to play an important role in photoprotection of *D. shibae* against the deleterious effects of UV radiations. Indeed, due to its strong absorption between 300 and 400 nm (Niedzwiedzki *et al.,* 2016), this compound can absorb harmful UV radiations.

3.3 Effect of $^1O_2$ on the activation of the DNA repair system genes

The other aim of this work was to monitor the response of the DNA repair system of pigmented and non-pigmented bacteria to $^1O_2$. Indeed, $^1O_2$ is known to react with a lot of cellular compounds such as lipids, proteins, and DNA (Morgan *et al*., 2004, Agnez-Lima *et al*., 1999; Ravanat *et al*., 2000). Among nucleic acids, $^1O_2$ preferentially reacts with guanine, due to its low redox potential (Cadet & Teoule, 1978, Steenken & Jovanovic, 1997), leading to the formation of 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodG) (Ravanat *et al*., 2004). 8-Oxo-dG has a high mutagenic potential, as it can mispair with adenine, leading to G → T transversion (Shibutani *et al*., 1991), which are the main mutation observed. G → C transversions may also occur in $^1O_2$-damaged bacteria (Agnez-Lima *et al*., 1999, 2001).

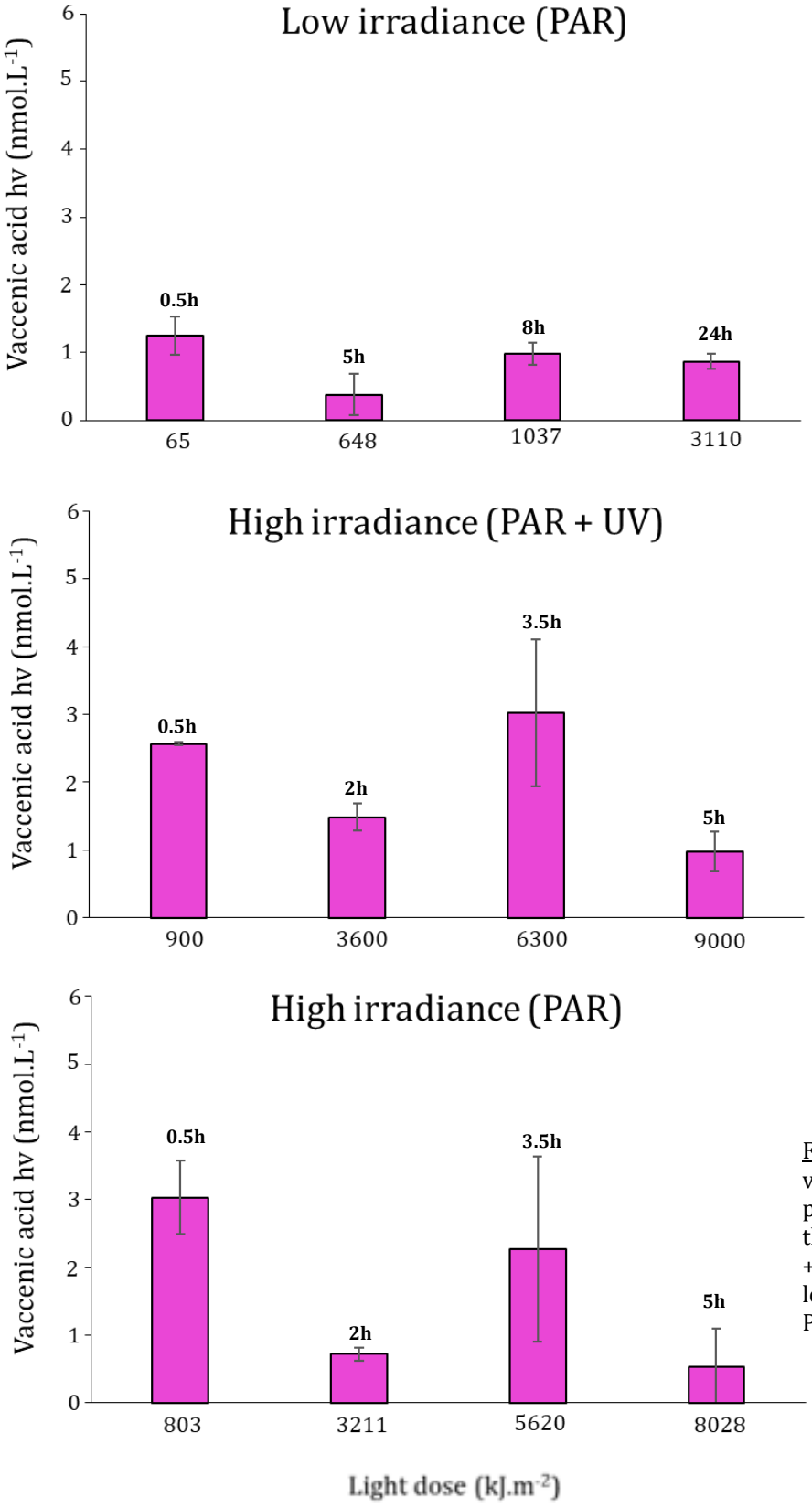

Figure 5.3: Concentrations of vaccenic acid photooxidation products (nmol.L-1) measured in the TLEs of the *Thalassiosira* sp. + *D. shibae* experiments under low PAR, high PAR+UV and high PAR irradiances (n = 3).

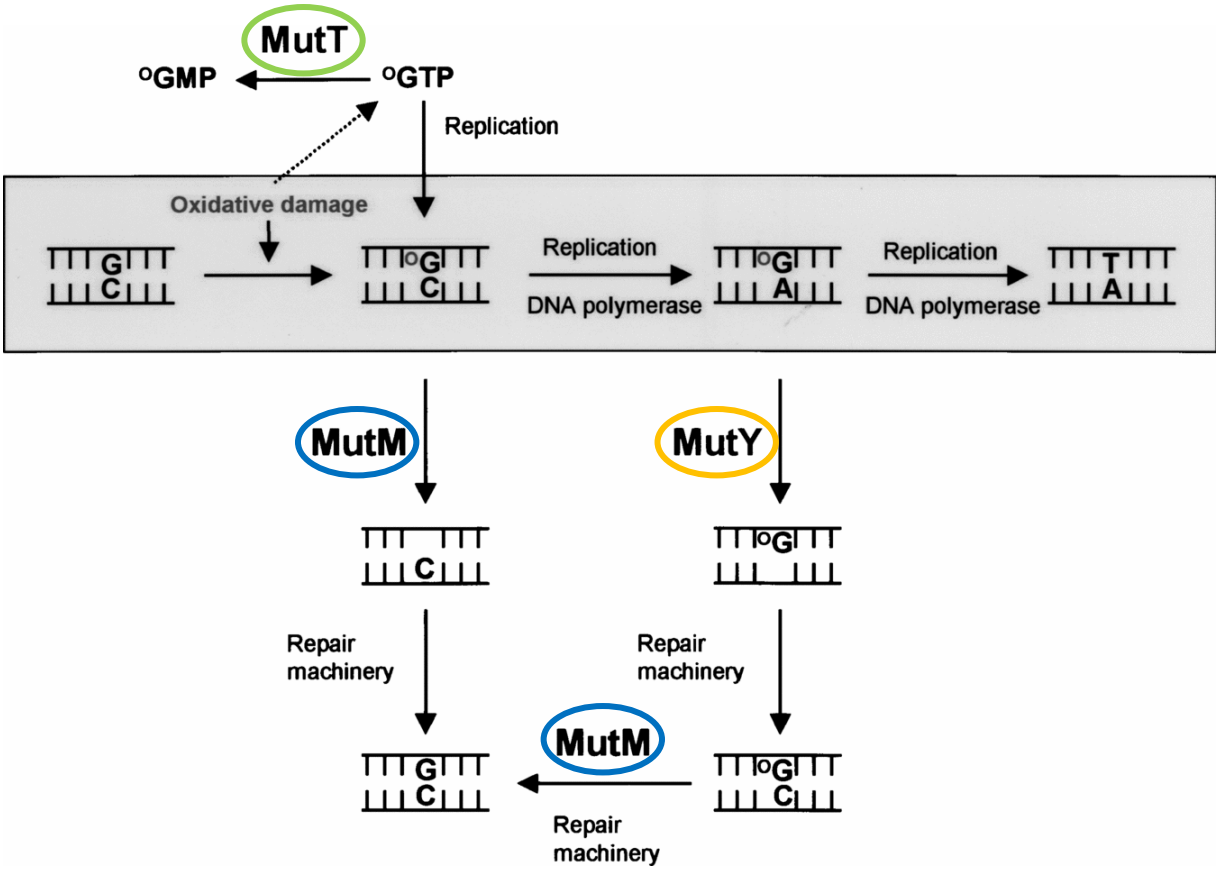

Figure 5.4: Schematized representation of the « GO system". The MutT protein hydrolyzes 8-oxodGTP into 8-oxodGMP, preventing its misincorporation into DNA. If 8-oxodG still incorporates into the DNA matrix, the repair system may follow two pathways: the MutM protein removes 8-oxodG when paired with a cytosine while the MutY protein removes the adenine mispaired with 8-oxodG. The MutM protein then removes the 8-oxodG paired with cytosine.

To monitor the activation of the DNA repair system to $^1O_2$ exposure, we measured the relative expression of several genes related to DNA repair after $^1O_2$-related damages and their respective sigma factors. We studied the relative expression of three genes coding for the three detoxifying proteins of the "Go system": *mutM* (or *fpG*), *mutY* and *mutT*. The MutM and MutY proteins are glycosylases, that respectively removes 8-oxodG paired with cytosine or adenine paired with 8-oxodG (Tchou *et al*., 1991, Boiteux *et al*., 1992, Agnez-Lima *et al*., 2012). MutT is a nucleotide triphosphatase, that detoxifies the nucleotide pool, hydrolyzing 8-oxodGTP into 8-oxodGMP (Costa *et al*., 2003). The functioning of the "GO system" is presented in the Figure 5.4.

$^1O_2$ is also able to induce the expression of genes involved with bacterial SOS response to oxidative stress. In microorganisms, knowledge of the $^1O_2$ specific regulatory mechanisms is based on only few model systems, including the anoxygenic bacterium *Rhodobacter sphaeroides* and several heterotrophic species with *Escherichia coli*. In *R.*

shpaeroides, stress response involves regulation genes by alternative sigma factors RpoS, RpoE and RpoH (Anthony *et al.,* 2004, 2005; Glaeser *et al.,* 2011). The sigma factor RpoE is maintained in an inactive state by forming complex with its cognate anti-sigma factor ChrR (Glaeser *et al*., 2011). Recent studies revealed that under exposure to $^1O_2$, RpoH is activated due to the rapid proteolysis of the RpoE:ChrR complex. RpoH (σ32) is the primary sigma factor controlling the heat shock response but it is also known to be induced by other conditions (for a review see Yura, 2019). See Figure 5.5 for a schematic representation of the regulatory network of the response to photooxidative stress.

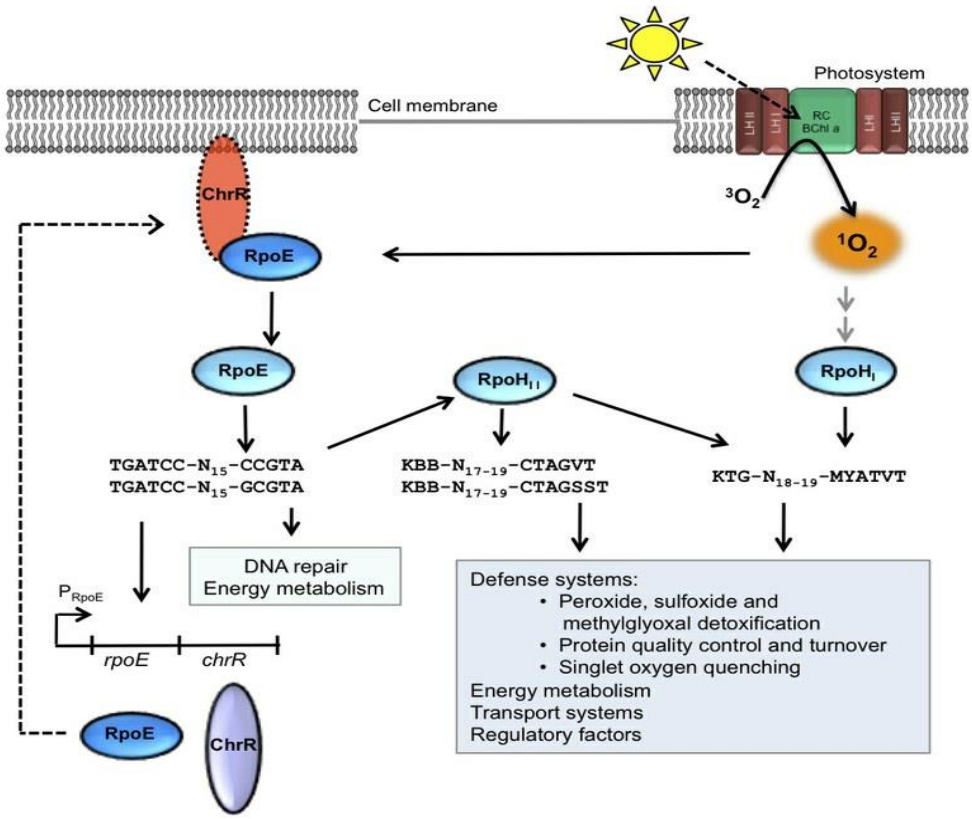

Figure 5.5: Sensing of $^1O_2$ and regulation of the photooxidative stress response. When $^1O_2$ is sensed by cellular sensors, it activates the dissociation of the RpoE:ChrR complex. RpoE then activates the two $RpoH_I$ and $rpoH_{II}$ sigma factors. These two being involved in various cell defense mechanisms. (Glaeser *et al*., 2011)

The results presented in Figure 5.6 and Figure 5.7 show the relative expression of *mutM, mutY, mutT, chrR* and *rpoH* genes in *D. shibae* and *P. stutzeri* after exposure to high or low irradiance in the presence of senescent *Thalassiosira* cells. The expression level of the investigated genes was normalized to that of the *rpoD* gene and expressed as the Log_2 of the relative expression compared to the T0 controls (without algal cells).

Concerning the genes of the "GO system" in *D. shibae*, we observed a significant effect of *Thalassiosira* cells on the expression of *mutY* under high or low irradiances ($P < 0.0001$, $\alpha = 0.05$) with a maximum of 2.83 ± 0.12 and 2.85 ± 0.79-fold increase after 30min under

low irradiance and 5h under high irradiance conditions, respectively (Figure 5.6). There is also a significant over-expression of *mutT* under high and low irradiances ($P = 4.9.\ 10^{-4}$ and $P = 2.6.\ 10^{-4}$, respectively, $\alpha = 0.05$), especially after 5 h under high irradiance and 30 min under low irradiance, with a 1.22 ± 0.6 and 1.45 ± 0.63-fold increase, respectively. Our results also show that there is a significant overall under-expression of *mutM* compared to the controls in *D. shibae* in all conditions ($P < 0.0001$, $\alpha = 0.05$), especially after 30 min under high irradiance conditions with a 2.78 ± 0.68-fold under-expression and after 30 min under low irradiance conditions with a 2.48 ± 0.25-fold under-expression, respectively (Figure 5.6). For *P. stutzeri*, we can see a strong over-expression of *mutY* under high irradiance conditions, with a maximum 4.42 ± 0.89-fold and 4.27 ± 1.11 increase after 2 and 3.5 h respectively (Figure 5.7). There is however no significant effect of the presence of *Thalassiosira* sp. cells on the expression level of *mutY* under high irradiances ($P = 0.072$, $\alpha = 0.05$), as it is also over-expressed in the controls (without algal cells). Indeed, we can see a 3.11 ± 1.39 and 2.5 ± 1.49-fold increase in the 2 and 5 h controls, respectively (Figure 5.7). The other genes of the "GO system" (*mutT* and *mutM*) were not detected in *P. stutzeri*.

Concerning the regulation genes, we observed in *D. shibae* significant effects of senescent *Thalassiosira* sp. cells on the expression level of *chrR* in all conditions ($P < 0.0001$, $\alpha = 0.05$), with a maximum 2.6 ± 0.15-fold increase after 30min under high irradiance conditions (Figure 5.6). It appears that *rpoH* is not impacted by our experiments, as the expression levels of this gene is not highly over or under-expressed in *D. shibae* (Figure 5.6). For *P. Stutzeri*, we observed a strong over-expression of *rpoH* under high irradiance conditions, with an over-expression of 4.6 ± 0.53 and 4.95 ± 0.60-fold increase after 2 and 3.5 h, respectively (Figure 5.7). There is however no significant effect of the presence of *Thalassiosira* sp. cells on the expression level of *rpoH* under high irradiances ($P = 0.167$, $\alpha = 0.05$), as it is also over-expressed in the controls. Indeed, we can see a 2.99 ± 0.76 and 1.65 ± 1.61-fold increase in the 2 and 5 h controls under high irradiances, respectively (Figure 5.7). For *chrR*, we observed a consequent strong under-expression after 5 h under high irradiance (3.17 ± 0.46-fold decrease) and a 3.69 ± 0.16-fold increase in the 24 h control under low irradiance (Figure 5.7).

It has been hypothesized by Dahl *et al*., (1987, 1988, 1989) that $^1O_2$ generated outside the cell could induce cytotoxicity but could not react with double stranded DNA and

trigger any mutations in various bacteria cells, as it first reacts with other cellular compounds such as proteins or membrane's fatty acids.

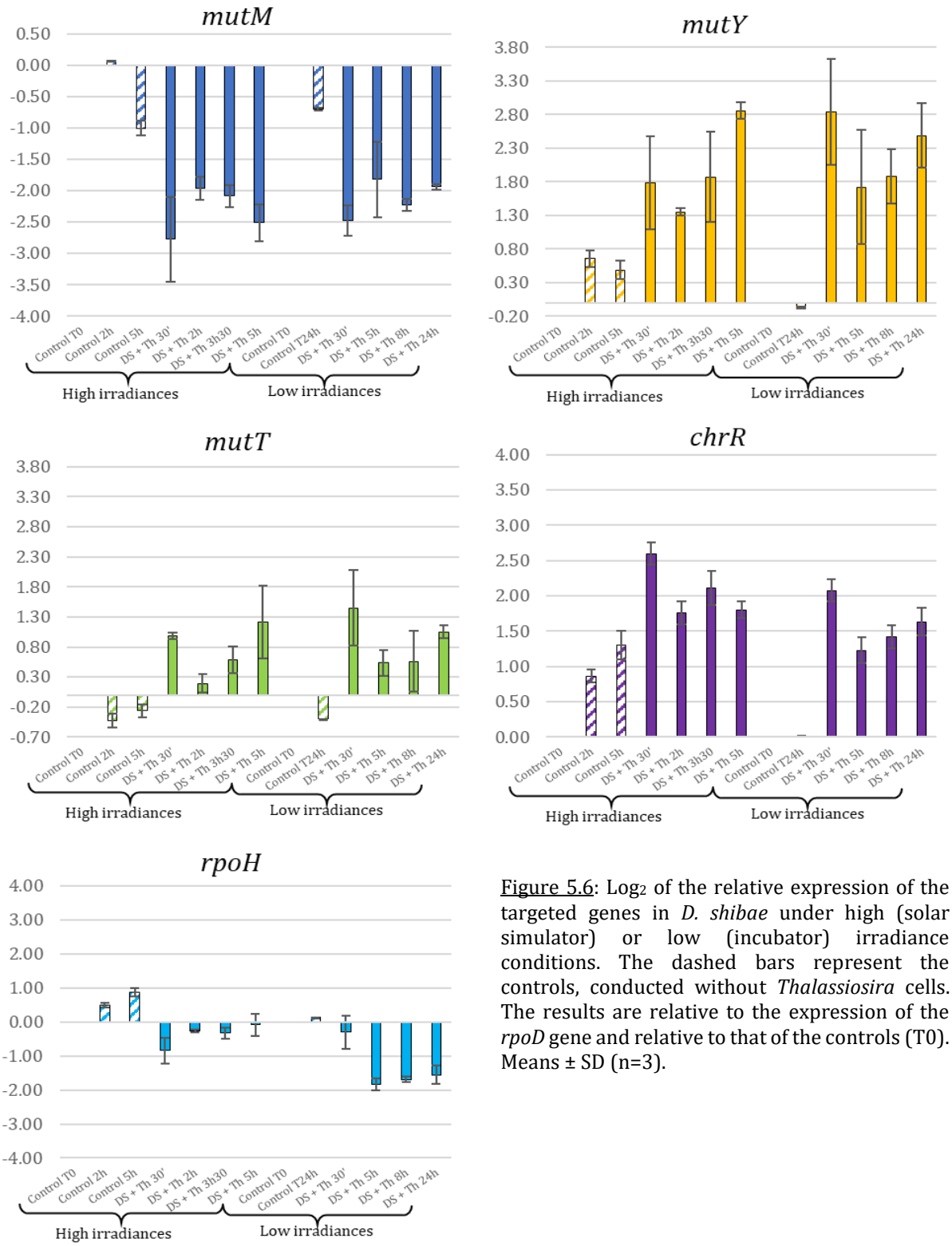

Figure 5.6: Log_2 of the relative expression of the targeted genes in *D. shibae* under high (solar simulator) or low (incubator) irradiance conditions. The dashed bars represent the controls, conducted without *Thalassiosira* cells. The results are relative to the expression of the *rpoD* gene and relative to that of the controls (T0). Means ± SD (n=3).

It happens that the mutagenesis markers that these authors have been monitoring (such as *uvrB, recA, xth* and *nth*) may be bypassed by other DNA repair pathways (Czeczot

et al., 1991, Tudek *et al.*, 1993, Agnez *et al.*, 1996), thus the impact of $^1O_2$ generated outside the cell on DNA is possible.

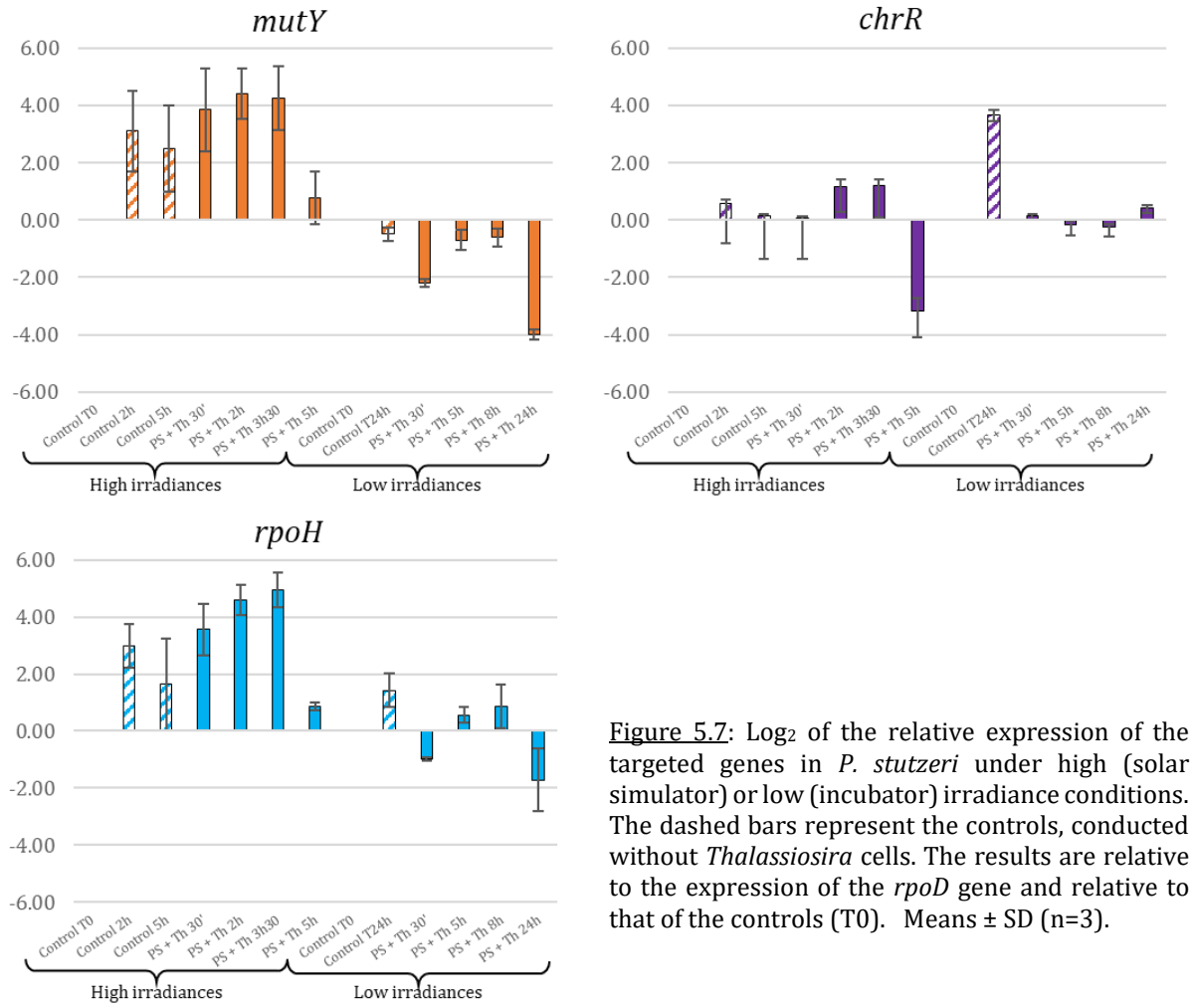

Figure 5.7: Log_2 of the relative expression of the targeted genes in *P. stutzeri* under high (solar simulator) or low (incubator) irradiance conditions. The dashed bars represent the controls, conducted without *Thalassiosira* cells. The results are relative to the expression of the *rpoD* gene and relative to that of the controls (T0). Means ± SD (n=3).

It appears that the activity of the "GO system" in *D. shibae* is sufficient to prevent any strong DNA damage. Indeed, the activity of *mutT* in *D. shibae* actively detoxifies the nucleotide pool, while *mutY* removes the adenine mispaired with 8-oxodG. The apparent under-expression of *mutM* may be surprising at first, as this gene is a part of the "GO system" and reacts to the exposure to $^1O_2$ (Tchou *et al.*, 1991, Boiteux *et al.*, 1992), at least in heterotrophic bacteria. It appeared to Cavalcante *et al*, (2002), that *mutM* deficient *E. coli* cells mutants were less sensitive to cytotoxicity induced by $^1O_2$ than *mutY* deficient mutants, this could be indicating that *mutM* is less important than *mutY* in the "GO system", or that all the mutations happening in the cells of *D. shibae* were G → T transversions (which is the main mutation observed). Due to the reaction of $^1O_2$ with

unsaturated membranes' lipids of *D. shibae* (Figure 5.3) strongly limiting its transfer inside the cells and the spheroidenone photoprotection, it appears that the impact of $^1O_2$ and UV on the DNA of *D. shibae* is limited. Indeed, the absence of overall over expression of the *rpoH* gene indicates that there is no activation of the $^1O_2$-related stress response. It has been previously observed in *R. sphaeroides* (a model organism for AAP bacteria) and in *D. shibae*, that exposure to $^1O_2$ rapidly triggers the activation of *rpoH* and the subsequent stress response genes (Nam *et al*., 2013, Nuss *et al.,* 2009, Tomasch *et al.,* 2011). In *R. sphaeroides*, the activation of the *rpoH* (especially $RpoH_{II}$) gene leads to the activation of a variety of genes, involved mainly in establishing and maintaining the respiratory chain (Nuss *et al*., 2010) or in maintaining a quinone pool, limiting the accumulation of toxic $^1O_2$ byproducts (Søballe & Poole, 2000). It is important to note that the effect observed on *mutY* in *D. shibae* may also be due to the indirect action of $^1O_2$ on cellular components. Indeed, $^1O_2$ can reacts with cellular compounds to form other oxidative agents, which may damage DNA and induce G → T and G → C transversions in the same way as $^1O_2$ does (Cadet *et al*., 1997, Devasagayam *et al*., 1991, Kasai *et al*., 1986, Retèl *et al*., 1993). Moreover, regardless of the gene and the over or under expression, there no to little effect of the intensity of the irradiance on the expression of *mutM, mutY* and *mutT* in *D. shibae* ($P = 0.23$, 0.37 and 0.21, respectively, $\alpha = 0.05$). under both irradiance regimes, it seems that the very weak damage induced by $^1O_2$ on the DNA are repaired.

In *P. stutzeri,* the effects observed on the expression of "GO system" and stress response regulation genes under high irradiances cannot be attributed to $^1O_2$, as we see an over expression of *rpoH* and *mutY* also in the controls without algal cells (Figure 5.7). The effects thus observed are likely due to UV radiations. Wich can explain the absence of expression of the *mutT* and *mutM*. Indeed, as *P. stutzeri* cells are already in a poor physiological state under high irradiances (i.e. damaged membranes), the defense mechanism induced by the expression of *rpoH* may lead to other stress response pathways (for a review see Goosen & Moolenaar, 2008). Moreover, it has already been shown that UV-light may also activate the *mutY* gene (Tajiri *et al*., 1995), as UV can induce DNA mutation the same way $^1O_2$ does (Douki *et al*., 1999).

4. Conclusion

In this work, we studied the impact of type II photooxidation processes (involving a triplet state sensitizer and the production of $^1O_2$) on bacteria attached to senescent phytoplanktonic cells, with a focus on the photooxidation of the membrane lipids and the activation of the DNA repair system. We intended to: (i) test the effect of irradiance on the efficiency of the transfer of $^1O_2$ from the senescent algal cells to their attached bacteria, and (ii) determine if the presence of carotenoids may actively protect bacteria against the harmful effects of $^1O_2$

High irradiances strongly favor the photooxidation of the unsaturated components of bacterial membranes. Under these conditions, $^1O_2$ produced in high quantities reacts weakly with membranes components of algal chloroplasts and is thus efficiently transferred to their attached bacteria inducing strong oxidative damage (Figure 5.8). Note that high irradiances induce a strong but short production of $^1O_2$, as the latter also damages the triplet state sensitizer in the algal cell (here ^{3}Chl).

On the other hand, low irradiances favor the photooxidation of the unsaturated algal lipids. Indeed, in these conditions, the weak amounts of $^1O_2$ generated react with unsaturated lipids of the algal cells and are thus quenched before to reach bacterial membranes (Figure 5.8). These results were also confirmed *in situ*, where we observed the same tendencies in a variety of environmental samples. The transfer of $^1O_2$ from senescent algae to their attached bacteria appeared to be particularly efficient in sea ice where the association sympagic algae-bacteria is maintained at relatively high irradiances during long periods.

The other aim of this work was to test if the presence of carotenoids in the membranes of bacteria could act as a protective agent against $^1O_2$. Comparison of the results obtained with the bacteria *D. shibae* and *P. stutzeri* showed that under high irradiance conditions (where the transfer of $^1O_2$ to the bacterial cells is highly favored), the presence of the carotenoid spheroidenone in *D. shibae* does not prevent $^1O_2$-induced oxidative alterations of its unsaturated membrane lipids. However, these conclusions cannot be extended to the whole of carotenoids since the antioxidant properties of these compounds are structurally-depend. In contrast, spheroidenone appeared to protect efficiently *D. shibae* against UV damage.

Finally, in this work we studied the activation of the DNA repair system and its regulation after exposure to $^1O_2$. Our results showed first that DNA damage induced by

$^1O_2$ generated outside the cell is possible. In the case of *D. shibae* the efficient detoxifying activity of *mutT* and *mutY* is indicative of a limited impact of $^1O_2$ on the DNA of this strain (Figure 5.9). This limited impact (resulting likely from the scavenging of $^1O_2$ in the membranes and the protection against UV radiations, Figure 5.9) is supported by the apparent inactivity of *rpoH* gene, responsible of oxidative stress response in photoheterotrophic bacteria. In *P. stutzeri*, $^1O_2$ reacts first with unsaturated lipids, while the effects we observed on DNA are most likely due to UV radiations. As *P. stutzeri* cells are in a poor physiological state (unsaturated membrane's lipids damaged), it appears that the activity of the *rpoH* gene may trigger other critical stress response mechanisms.

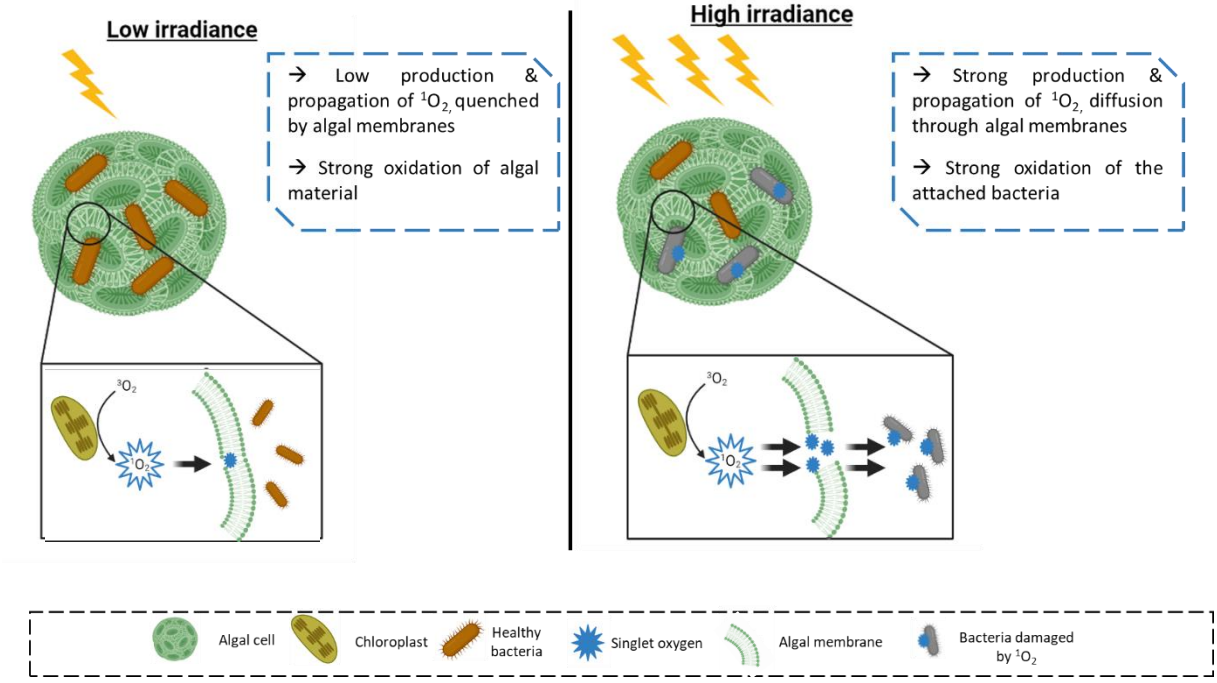

Figure 5.8: Conceptual scheme of the effect of $^1O_2$ on bacteria attached to senescent phytoplankton cells under low or high irradiance

On the basis of these results, the dominance of *Roseobacter* clade among bacterioplankton communities associated to algal blooms cannot be attributed to the protective action of their carotenoids against $^1O_2$-damages.

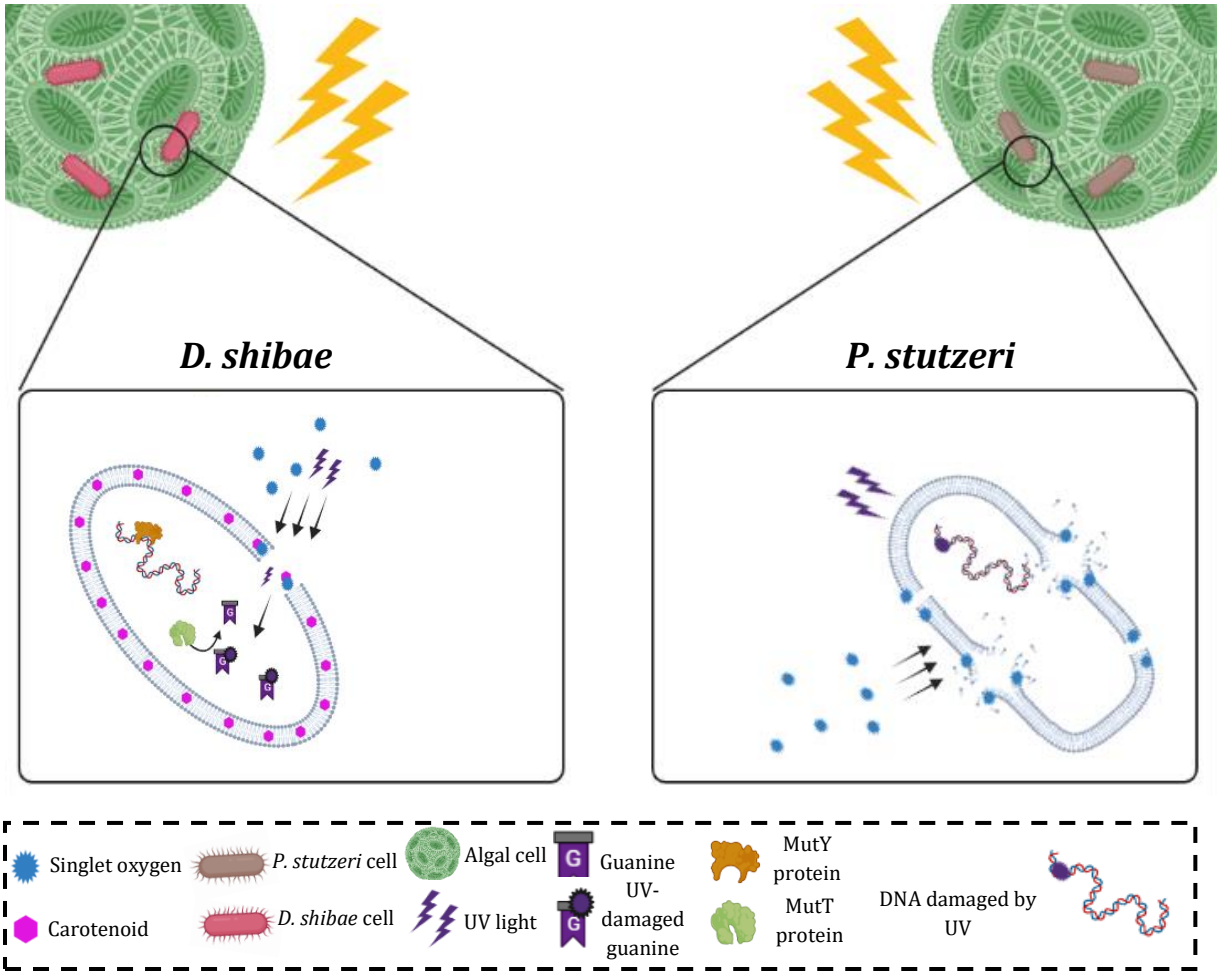

Figure 5.9*:* Conceptual scheme representing the effect of $^1O_2$ on DNA of *P. stutzeri* and *D. shibae* under high irradiances. In *P. stutzeri*, $^1O_2$ is quenched by unsaturated membrane lipids. The damage observed on DNA is thus attributed to UV. In *D. shibae*, the carotenoids found in its membrane protect its unsaturated lipids from $^1O_2$ but not from UV radiations, that induces DNA damage. It appears that *mutY* and *mutT* in *D. shibae* actively detoxify the nucleotide pool and remove the adenine mispaired with UV-damaged guanine and thus protects DNA.

Bibliographie

Agnez, L. F., de Oliveira, R. L. C., Di Mascio, P., & Menck, C. F. M. (1996). Involvement of *Escherichia coli* exonuclease III and endonuclease IV in the repair of singlet oxygen-induced DNA damage. *Carcinogenesis*, *17*(5), 1183–1185. https://doi.org/10.1093/carcin/17.5.1183

Agnez-Lima, L. F., Mascio, P. D., Napolitano, R. L., Fuchs, R. P., & Menck, C. F. M. (1999). Mutation Spectrum Induced by Singlet Oxygen in Escherichia coli Deficient in Exonuclease III. *Photochemistry and Photobiology*, *70*(4), 505–511. https://doi.org/10.1111/j.1751-1097.1999.tb08245.x

Agnez-Lima, L. F., Melo, J. T. A., Silva, A. E., Oliveira, A. H. S., Timoteo, A. R. S., Lima-Bessa, K. M., Martinez, G. R., Medeiros, M. H. G., Di Mascio, P., Galhardo, R. S., & Menck, C. F. M. (2012). DNA damage by singlet oxygen and cellular protective mechanisms. *Mutation Research/Reviews in Mutation Research*, *751*(1), 15–28. https://doi.org/10.1016/j.mrrev.2011.12.005

Agnez-Lima, L. F., Napolitano, R. L., Fuchs, R. P. P., Mascio, P. D., Muotri, A. R., & Menck, C. F. M. (2001). DNA repair and sequence context affect 1O2-induced mutagenesis in bacteria. *Nucleic Acids Research*, *29*(13), 2899–2903. https://doi.org/10.1093/nar/29.13.2899

Aigle, A., Bonin, P., Iobbi-Nivol, C., Méjean, V., & Michotey, V. (2017). Physiological and transcriptional approaches reveal connection between nitrogen and manganese cycles in Shewanella algae C6G3. *Scientific Reports*, *7*(1), 44725. https://doi.org/10.1038/srep44725

Alavi, M., Miller, T., Erlandson, K., Schneider, R., & Belas, R. (2001). Bacterial community associated with Pfiesteria-like dinoflagellate cultures. *Environmental Microbiology*, *3*(6), 380–396. https://doi.org/10.1046/j.1462-2920.2001.00207.x

Allgaier, M., Uphoff, H., Felske, A., & Wagner-Döbler, I. (2003). Aerobic Anoxygenic Photosynthesis in Roseobacter Clade Bacteria from Diverse Marine Habitats. *Applied and Environmental Microbiology*, *69*(9), 5051–5059. https://doi.org/10.1128/AEM.69.9.5051-5059.2003

Alou-Font, E., Roy, S., Agustí, S., & Gosselin, M. (2016). Cell viability, pigments and photosynthetic performance of Arctic phytoplankton in contrasting ice-covered and open-water conditions during the spring–summer transition. *Marine Ecology Progress Series*, *543*, 89–106. https://doi.org/10.3354/meps11562

Amiraux, R., Belt, S. T., Vaultier, F., Galindo, V., Gosselin, M., Bonin, P., & Rontani, J.-F. (2017). Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers. *Marine Chemistry*, *194*, 89–99. https://doi.org/10.1016/j.marchem.2017.05.006

Amiraux, R., Burot, C., Bonin, P., Massé, G., Guasco, S., Babin, M., Vaultier, F., & Rontani, J.-F. (2020). Stress factors resulting from the Arctic vernal sea-ice melt: Impact on the viability of bacterial communities associated with sympagic algae. *Elementa: Science of the Anthropocene*, *8*(1), 076. https://doi.org/10.1525/elementa.076

Amiraux, R., Jeanthon, C., Vaultier, F., & Rontani, J.-F. (2016). Paradoxical effects of temperature and solar irradiance on the photodegradation state of killed phytoplankton. *Journal of Phycology*, *52*(3), 475–485. https://doi.org/10.1111/jpy.12410

Anthony, J. R., Newman, J. D., & Donohue, T. J. (2004). Interactions Between the Rhodobacter sphaeroides ECF Sigma Factor, σE, and its Anti-sigma Factor, ChrR. *Journal of Molecular Biology*, *341*(2), 345–360. https://doi.org/10.1016/j.jmb.2004.06.018

Anthony, J. R., Warczak, K. L., & Donohue, T. J. (2005). A transcriptional response to singlet oxygen, a toxic byproduct of photosynthesis. *Proceedings of the National Academy of Sciences*, *102*(18), 6502–6507. https://doi.org/10.1073/pnas.0502225102

Azam, F., & Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nature Reviews Microbiology*, *5*(10), 782–791. https://doi.org/10.1038/nrmicro1747

Biebl, H., Allgaier, M., Tindall, B., Koblizek, M., Lünsdorf, H., Pukall, R., & Wagner-Döbler, I. (2005). Dinoroseobacter shibae gen. Nov., sp nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. *International Journal of Systematic and Evolutionary Microbiology*, *55*, 1089–1096. https://doi.org/10.1099/ijs.0.63511-0

Boiteux, S., Gajewski, E., Laval, J., & Dizdaroglu, M. (1992). Substrate specificity of the Escherichia coli Fpg protein formamidopyrimidine-DNA glycosylase: Excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry*, *31*(1), 106–110. https://doi.org/10.1021/bi00116a016

Buchan, A., González, J. M., & Moran, M. A. (2005). Overview of the Marine Roseobacter Lineage. *Applied and Environmental Microbiology*, *71*(10), 5665–5677. https://doi.org/10.1128/AEM.71.10.5665-5677.2005

Cadet, J., Berger, M., Douki, T., Morin, B., Raoul, S., Ravanat, J. L., & Spinelli, S. (1997). Effects of UV and visible radiation on DNA-final base damage. *Biological Chemistry*, *378*(11), 1275–1286.

Cadet, J., Douki, T., & Ravanat, J.-L. (2010). Oxidatively generated base damage to cellular DNA. *Free Radical Biology and Medicine*, *49*(1), 9–21. https://doi.org/10.1016/j.freeradbiomed.2010.03.025

Cadet, J., Ravanat, J.-L., Martinez, G. R., Medeiros, M. H. G., & Mascio, P. D. (2006). Singlet Oxygen Oxidation of Isolated and Cellular DNA: Product Formation and Mechanistic Insights. *Photochemistry and Photobiology*, *82*(5), 1219–1225. https://doi.org/10.1562/2006-06-09-IR-914

Cadet, J., & Teoule, R. (1978). Comparative Study of Oxidation of Nucleic Acid Components by Hydroxyl Radicals, Singlet Oxygen and Superoxide Anion Radicals. *Photochemistry and Photobiology*, *28*(4–5), 661–665. https://doi.org/10.1111/j.1751-1097.1978.tb06991.x

Cavalcante, A. K. D., Martinez, G. R., Di Mascio, P., Menck, C. F. M., & Agnez-Lima, L. F. (2002). Cytotoxicity and mutagenesis induced by singlet oxygen in wild type and DNA repair deficient Escherichia coli strains. *DNA Repair*, *1*(12), 1051–1056. https://doi.org/10.1016/S1568-7864(02)00164-7

Christodoulou, S., Joux, F., Marty, J.-C., Sempéré, R., & Rontani, J.-F. (2010). Comparative study of UV and visible light induced degradation of lipids in non-axenic senescent cells of Emiliania huxleyi. *Marine Chemistry*, *119*(1), 139–152. https://doi.org/10.1016/j.marchem.2010.01.007

Cole, J., Findlay, S., & Pace, M. (1988). Bacterial production in fresh and saltwater ecosystems: A cross-system overview. *Marine Ecology Progress Series*, *43*, 1–10. https://doi.org/10.3354/meps043001

Conn, P. F., Schalch, W., & Truscott, T. G. (1991). The singlet oxygen and carotenoid interaction. *Journal of Photochemistry and Photobiology B: Biology*, *11*(1), 41–47. https://doi.org/10.1016/1011-1344(91)80266-K

Costa, R. M. A., Chiganças, V., da Silva Galhardo, R., Carvalho, H., & Menck, C. F. M. (2003). The eukaryotic nucleotide excision repair pathway. *Biochimie*, *85*(11), 1083–1099. https://doi.org/10.1016/j.biochi.2003.10.017

Czeczot, H., Tudek, B., Lambert, B., Laval, J., & Boiteux, S. (1991). Escherichia coli Fpg protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus visible light in vivo and in vitro. *Journal of Bacteriology*, *173*(11), 3419–3424.

Dahl, T. A., Midden, W. R., & Hartman, P. E. (1989). Comparison of killing of gram-negative and gram-positive bacteria by pure singlet oxygen. *Journal of Bacteriology*, *171*(4), 2188–2194.

Dahl, T. A., Robert Midden, W., & Hartman, P. E. (1988). Pure exogenous singlet oxygen: Nonmutagenicity in bacteria. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *201*(1), 127–136. https://doi.org/10.1016/0027-5107(88)90119-4

Dahl, ThomasA., RobertMiddenand, W., & Hartman, PhilipE. (1987). Pure Singlet Oxygen Cytotoxicity for Bacteria. *Photochemistry and Photobiology*, *46*(3), 345–352. https://doi.org/10.1111/j.1751-1097.1987.tb04779.x

Dang, H., & Lovell, C. R. (2000). Bacterial Primary Colonization and Early Succession on Surfaces in Marine Waters as Determined by Amplified rRNA Gene Restriction Analysis and Sequence Analysis of 16S rRNA Genes. *Applied and Environmental Microbiology*, *66*(2), 467–475. https://doi.org/10.1128/AEM.66.2.467-475.2000

de Carvalho, C., & Caramujo, M.-J. (2014). Fatty Acids as a Tool to Understand Microbial Diversity and Their Role in Food Webs of Mediterranean Temporary Ponds. *Molecules*, *19*(5), 5570–5598. https://doi.org/10.3390/molecules19055570

Devasagayam, T. P. A., Steenken, S., Obendorf, M. S. W., Schulz, W. A., & Sies, H. (1991). Formation of 8-hydroxy(deoxy)guanosine and generation of strand breaks at guanine residues in DNA by singlet oxygen. *Biochemistry*, *30*(25), 6283–6289. https://doi.org/10.1021/bi00239a029

Douki, T., Perdiz, D., Grof, P., Kuluncsics, Z., Moustacchi, E., Cadet, J., & Sage, E. (1999). Oxidation of Guanine in Cellular DNA by Solar UV Radiation: Biological Role†. *Photochemistry and Photobiology*, *70*(2), 184–190. https://doi.org/10.1111/j.1751-1097.1999.tb07988.x

Ducklow, H. W., Kirchman, D. L., Quinby, H. L., Carlson, C. A., & Dam, H. G. (1993). Stocks and dynamics of bacterioplankton carbon during the spring bloom in the eastern North Atlantic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *40*(1), 245–263. https://doi.org/10.1016/0967-0645(93)90016-G

Eppley, R. W., & Peterson, B. J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, *282*(5740), 677–680. https://doi.org/10.1038/282677a0

Fahl, K., & Kattner, G. (1993). Lipid Content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). *Polar Biology*, *13*(6), 405–409. https://doi.org/10.1007/BF01681982

Falk-Petersen, S., Sargent, J. R., Henderson, J., Hegseth, E. N., Hop, H., & Okolodkov, Y. B. (1998). Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. *Polar Biology*, *20*(1), 41–47. https://doi.org/10.1007/s003000050274

Fandino, L., Riemann, L., Steward, G., Long, R., & Azam, F. (2001). Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. *Aquatic Microbial Ecology*, *23*, 119–130. https://doi.org/10.3354/ame023119

Foote, C. (1976). Photosensitized oxidation and singlet oxygen: Consequences in biological systems. In *Free radicals in biology* (p. 133). Academic Press.

Frankel, E. n, Neff, W., & Bessler, T. (1979). Analysis of autoxidized fats by gas chromatography-mass spectrometry: V. Photosensitized oxidation. *Lipids*, *14*, 961–967. https://doi.org/10.1007/BF02533431

Frimer, A. A. (1979). The reaction of singlet oxygen with olefins: The question of mechanism. *Chemical Reviews*, *79*(5), 359–387. https://doi.org/10.1021/cr60321a001

Glaeser, J., Nuss, A. M., Berghoff, B. A., & Klug, G. (2011). Singlet Oxygen Stress in Microorganisms. In *Advances in Microbial Physiology* (Vol. 58, pp. 141–173). Elsevier. https://doi.org/10.1016/B978-0-12-381043-4.00004-0

Glaeser, S. P., Grossart, H.-P., & Glaeser, J. (2010). Singlet oxygen, a neglected but important environmental factor: Short-term and long-term effects on bacterioplankton composition in a humic lake. *Environmental Microbiology*, *12*(12), 3124–3136. https://doi.org/10.1111/j.1462-2920.2010.02285.x

Gollnick, K. (1968). Type II Photooxygenation Reactions in Solution. In W. A. Noyes, G. S. Hammond, & J. N. Pitts (Eds.), *Advances in Photochemistry* (pp. 1–122). John Wiley & Sons, Inc. https://doi.org/10.1002/9780470133361.ch1

González, J. M., Simó, R., Massana, R., Covert, J. S., Casamayor, E. O., Pedrós-Alió, C., & Moran, M. A. (2000). Bacterial Community Structure Associated with a Dimethylsulfoniopropionate-Producing North Atlantic Algal Bloom. *Applied and Environmental Microbiology*, *66*(10), 4237–4246.

Goosen, N., & Moolenaar, G. F. (2008). Repair of UV damage in bacteria. *DNA Repair*, *7*(3), 353–379. https://doi.org/10.1016/j.dnarep.2007.09.002

Jasti, S., Sieracki, M. E., Poulton, N. J., Giewat, M. W., & Rooney-Varga, J. N. (2005). Phylogenetic Diversity and Specificity of Bacteria Closely Associated with Alexandrium spp. And Other Phytoplankton. *Applied and Environmental Microbiology*, *71*(7), 3483–3494. https://doi.org/10.1128/AEM.71.7.3483-3494.2005

Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A., & Tanooka, H. (1986). Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen

radicals and evidence for its repair. *Carcinogenesis*, *7*(11), 1849–1851. https://doi.org/10.1093/carcin/7.11.1849

Keller, M. D., Selvin, R. C., Claus, W., & Guillard, R. R. L. (1987). Media for the Culture of Oceanic Ultraphytoplankton1,2. *Journal of Phycology*, *23*(4), 633–638. https://doi.org/10.1111/j.1529-8817.1987.tb04217.x

Kester, D. R., Duedall, I. W., Connors, D. N., & Pytkowicz, R. M. (1967). Preparation of Artificial Seawater. *Limnology and Oceanography*, *12*(1), 176–179. https://doi.org/10.4319/lo.1967.12.1.0176

Kim, S.-Y., Kim, E.-J., & Park, J.-W. (2002). Control of Singlet Oxygen-induced Oxidative Damage in Escherichia coli. *BMB Reports*, *35*(4), 353–357. https://doi.org/10.5483/BMBRep.2002.35.4.353

Knox, J. P., & Dodge, A. D. (1985). Singlet oxygen and plants. *Phytochemistry*, *24*(5), 889–896. https://doi.org/10.1016/S0031-9422(00)83147-7

Lambert, M. A., & Moss, C. W. (1983). Comparison of the effects of acid and base hydrolyses on hydroxy and cyclopropane fatty acids in bacteria. *Journal of Clinical Microbiology*, *18*(6), 1370–1377.

Leu, E., Wiktor, J., Søreide, J., Berge, J., & Falk-Petersen, S. (2010). Increased irradiance reduces food quality of sea ice algae. *Marine Ecology Progress Series*, *411*, 49–60. https://doi.org/10.3354/meps08647

Loidl-Stahlhofen, A., Hannemann, K., & Spiteller, G. (1994). Generation of α-hydroxyaldehydic compounds in the course of lipid peroxidation. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, *1213*(2), 140–148. https://doi.org/10.1016/0005-2760(94)90020-5

Long, R. A., & Azam, F. (2001). Antagonistic Interactions among Marine Pelagic Bacteria. *Applied and Environmental Microbiology*, *67*(11), 4975–4983. https://doi.org/10.1128/AEM.67.11.4975-4983.2001

Lund-Hansen, L. C., Bjerg-Nielsen, M., Stratmann, T., Hawes, I., & Sorrell, B. K. (2021). Upwelling Irradiance below Sea Ice—PAR Intensities and Spectral Distributions. *Journal of Marine Science and Engineering*, *9*(8), 830. https://doi.org/10.3390/jmse9080830

Marañón, E., Van Wambeke, F., Uitz, J., Boss, E. S., Pérez-Lorenzo, M., Dinasquet, J., Haëntjens, N., Dimier, C., & Taillandier, V. (2020). *Deep maxima of phytoplankton biomass, primary production and bacterialproduction in the Mediterranean Sea during late spring* [Preprint]. Biodiversity and Ecosystem Function: Microbial Ecology & Geomicrobiology. https://doi.org/10.5194/bg-2020-261

Menck, C. F. M., Di Mascio, P., Agnez-Lima, L. F., Ribeiro, D. T., & Costa de Oliveira, R. (1993). *Genetic deleterious effects of singlet oxygen*. *16*, 328–336.

Michaels, M. L., & Miller, J. H. (1992). The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *Journal of Bacteriology*, *174*(20), 6321–6325.

Morgan, P. E., Dean, R. T., & Davies, M. J. (2004). Protective mechanisms against peptide and protein peroxides generated by singlet oxygen. *Free Radical Biology and Medicine*, *36*(4), 484–496. https://doi.org/10.1016/j.freeradbiomed.2003.11.021

Nam, T.-W., Ziegelhoffer, E. C., Lemke, R. A. S., & Donohue, T. J. (2013). Proteins Needed to Activate a Transcriptional Response to the Reactive Oxygen Species Singlet Oxygen. *MBio*, *4*(1), Article 1. https://doi.org/10.1128/mBio.00541-12

Nelson, J. R. (1993). Rates and possible mechanism of light-dependent degradation of pigments in detritus derived from phytoplankton. *Journal of Marine Research*, *51*(1), 155–179. https://doi.org/10.1357/0022240933223837

Niedzwiedzki, D. M., Tronina, T., Liu, H., Staleva, H., Komenda, J., Sobotka, R., Blankenship, R. E., & Polívka, T. (2016). Carotenoid-induced non-photochemical quenching in the cyanobacterial chlorophyll synthase–HliC/D complex. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1857*(9), 1430–1439. https://doi.org/10.1016/j.bbabio.2016.04.280

Nuss, A. M., Glaeser, J., Berghoff, B. A., & Klug, G. (2010). Overlapping Alternative Sigma Factor Regulons in the Response to Singlet Oxygen in Rhodobacter sphaeroides. *Journal of Bacteriology*, *192*(10), 2613–2623. https://doi.org/10.1128/JB.01605-09

Nuss, A. M., Glaeser, J., & Klug, G. (2009). RpoHII Activates Oxidative-Stress Defense Systems and Is Controlled by RpoE in the Singlet Oxygen-Dependent Response in Rhodobacter sphaeroides. *Journal of Bacteriology*, *191*(1), 220–230. https://doi.org/10.1128/JB.00925-08

Otto, F. (1990). Chapter 11 DAPI Staining of Fixed Cells for High-Resolution Flow Cytometry of Nuclear DNA. In Z. Darzynkiewicz & H. A. Crissman (Eds.), *Methods in Cell Biology* (Vol. 33, pp. 105–110). Academic Press. https://doi.org/10.1016/S0091-679X(08)60516-6

Ourisson, G., & Nakatani, Y. (1989). Bacterial Carotenoids as Membrane Reinforcers: A General Role for Polyterpenoids: Membrane Stabilization. In N. I. Krinsky, M. M. Mathews-Roth, & R. F. Taylor (Eds.), *Carotenoids: Chemistry and Biology* (pp. 237–245). Springer US. https://doi.org/10.1007/978-1-4613-0849-2_16

Petit, M., Bonin, P., Amiraux, R., Michotey, V., Guasco, S., Armitano, J., Jourlin-Castelli, C., Vaultier, F., Méjean, V., & Rontani, J.-F. (2015). Dynamic of bacterial communities attached to lightened phytodetritus. *Environmental Science and Pollution Research*, *22*(18), 13681–13692. https://doi.org/10.1007/s11356-015-4209-0

Petit, M., Sempéré, R., Vaultier, F., & Rontani, J.-F. (2013). Photochemical Production and Behavior of Hydroperoxyacids in Heterotrophic Bacteria Attached to Senescent Phytoplanktonic Cells. *International Journal of Molecular Sciences*, *14*(6), 11795–11815. https://doi.org/10.3390/ijms140611795

Petit, M., Suroy, M., Sempere, R., Vaultier, F., Volkman, J. K., Goutx, M., & Rontani, J. (2015a). Transfer of singlet oxygen from senescent irradiated phytoplankton cells to attached heterotrophic bacteria: Effect of silica and carbonaceous matrices. *Marine Chemistry*, *171*, 87–95. https://doi.org/10.1016/j.marchem.2015.02.007

Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., & Marrasé, C. (2004). Changes in Bacterioplankton Composition under Different Phytoplankton Regimens. *Applied and Environmental Microbiology*, *70*(11), 6753–6766. https://doi.org/10.1128/AEM.70.11.6753-6766.2004

Rainey, P. B., Thompson, L. I. P., Norbert, & Palleron, J. (1994). *Genome and fatty acid analysis of Pseudomonas stutzeri*.

Ravanat, J.-L., Di Mascio, P., Martinez, G. R., Medeiros, M. H. G., & Cadet, J. (2000). Singlet Oxygen Induces Oxidation of Cellular DNA. *Journal of Biological Chemistry*, *275*(51), 40601–40604. https://doi.org/10.1074/jbc.M006681200

Ravanat, J.-L., Martinez, G. R., Medeiros, M. H. G., Di Mascio, P., & Cadet, J. (2004). Mechanistic aspects of the oxidation of DNA constituents mediated by singlet molecular oxygen. *Archives of Biochemistry and Biophysics*, *423*(1), 23–30. https://doi.org/10.1016/j.abb.2003.10.018

Retèl, J., Hoebee, B., Braun, J. E. F., Lutgerink, J. T., van den Akker, E., Handayani Wanamarta, A., Joenje, H., & Lafleur, M. V. M. (1993). Mutational specificity of oxidative DNA damage. *Mutation Research/Genetic Toxicology*, *299*(3), 165–182. https://doi.org/10.1016/0165-1218(93)90094-T

Rink, B., Seeberger, S., Martens, T., Duerselen C, D., Simon, M., & Brinkhoff, T. (2007). Effects of phytoplankton bloom in a coastal ecosystem on the composition of bacterial communities. *Aquatic Microbial Ecology*, *48*, 47–60. https://doi.org/10.3354/ame048047

Rontani, J., & Belt, S. T. (2020). Photo- and autoxidation of unsaturated algal lipids in the marine environment: An overview of processes, their potential tracers, and limitations. *Organic Geochemistry*, *139*, 103941. https://doi.org/10.1016/j.orggeochem.2019.103941

Rontani, J.-F. (2021). *Lipid Oxidation Products: Useful Tools for Monitoring Photo- and Autoxidation in Phototrophs*. Cambridge Scholars Publishing.

Rontani, J.-F., Amiraux, R., Smik, L., Wakeham, S. G., Paulmier, A., Vaultier, F., Sun-Yong, H., Jun-oh, M., & Belt, S. T. (2021). Type II photosensitized oxidation in senescent microalgal cells at different latitudes: Does low under-ice irradiance in polar regions enhance efficiency? *Science of The Total Environment*, *779*, 146363. https://doi.org/10.1016/j.scitotenv.2021.146363

Rontani, J.-F., Belt, S. T., Brown, T. A., Amiraux, R., Gosselin, M., Vaultier, F., & Mundy, C. J. (2016). Monitoring abiotic degradation in sinking versus suspended Arctic sea ice algae during a spring ice melt using specific lipid oxidation tracers. *Organic Geochemistry*, *98*, 82–97. https://doi.org/10.1016/j.orggeochem.2016.05.016

Rontani, J.-F., Charriere, B., Forest, A., Heussner, S., Vaultier, F., Petit, M., Delsaut, N., Fortier, L., & Sempéré, R. (2012). Intense photooxidative degradation of planktonic and bacterial lipids in sinking particles collected with sediment traps across the Canadian Beaufort Shelf (Arctic Ocean). *Biogeosciences*, *9*(11), 4787–4802. https://doi.org/10.5194/bg-9-4787-2012

Rontani, J.-F., Koblížek, M., Beker, B., Bonin, P., & Kolber, Z. S. (2003). On the origin of cis-vaccenic acid photodegradation products in the marine environment. *Lipids*, *38*(10), 1085–1092. https://doi.org/10.1007/s11745-006-1164-z

Rüger, H.-J., & Höfle, M. G. Y. 1992. (1992). Marine Star-Shaped-Aggregate-Forming Bacteria: Agrobacterium atlanticum sp. nov.; Agrobacterium meteori sp. nov.; Agrobacterium ferrugineum sp. nov., nom. rev.; Agrobacterium gelatinovorum sp. nov., nom. rev.; and Agrobacterium stellulatum sp. nov., nom. rev.†. *International Journal of Systematic and Evolutionary Microbiology*, *42*(1), 133–143. https://doi.org/10.1099/00207713-42-1-133

Sarra, A. di, Bommarito, C., Anello, F., Iorio, T. D., Meloni, D., Monteleone, F., Pace, G., Piacentino, S., & Sferlazzo, D. (2019). Assessing the Quality of Shortwave and Longwave Irradiance Observations over the Ocean: One Year of High-Time-Resolution Measurements at the Lampedusa Oceanographic Observatory. *Journal of Atmospheric and Oceanic Technology*, *36*(12), 2383–2400. https://doi.org/10.1175/JTECH-D-19-0018.1

Shibutani, S., Takeshita, M., & Grollman, A. P. (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, *349*(6308), 431–434. https://doi.org/10.1038/349431a0

Sicre, M.-A., Paillasseur, J.-L., Marty, J.-C., & Saliot, A. (1988). Characterization of seawater samples using chemometric methods applied to biomarker fatty acids. *Organic Geochemistry*, *12*(3), 281–288. https://doi.org/10.1016/0146-6380(88)90265-3

Skovsen, E., Snyder, J. W., Lambert, J. D. C., & Ogilby, P. R. (2005). Lifetime and Diffusion of Singlet Oxygen in a Cell. *The Journal of Physical Chemistry B*, *109*(18), 8570–8573. https://doi.org/10.1021/jp051163i

Søballe, B., & Poole, R. K. Y. 2000. (2000). Ubiquinone limits oxidative stress in Escherichia coli. *Microbiology*, *146*(4), 787–796. https://doi.org/10.1099/00221287-146-4-787

Steenken, S., & Jovanovic, S. V. (1997). How Easily Oxidizable Is DNA? One-Electron Reduction Potentials of Adenosine and Guanosine Radicals in Aqueous Solution. *Journal of the American Chemical Society*, *119*(3), 617–618. https://doi.org/10.1021/ja962255b

Strzałka, K., & Gruszecki, W. I. (1994). Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. I. An EPR spin label study. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1194*(1), 138–142. https://doi.org/10.1016/0005-2736(94)90212-7

Tajiri, T., Maki, H., & Sekiguchi, M. (1995). Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in Escherichia coli. *Mutation Research/DNA Repair*, *336*(3), 257–267. https://doi.org/10.1016/0921-8777(94)00062-B

Tatsuzawa, H., Maruyama, T., Misawa, N., Fujimori, K., & Nakano, M. (2000). Quenching of singlet oxygen by carotenoids produced in Escherichia coli – attenuation of singlet oxygen-mediated bacterial killing by carotenoids. *FEBS Letters*, *484*(3), 280–284. https://doi.org/10.1016/S0014-5793(00)02149-9

Tchou, J., Kasai, H., Shibutani, S., Chung, M. H., Laval, J., Grollman, A. P., & Nishimura, S. (1991). 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proceedings of the National Academy of Sciences*, *88*(11), 4690–4694. https://doi.org/10.1073/pnas.88.11.4690

Teeling, H., Fuchs, B., Becher, D., Klockow, C., Kabisch, A., Bennke, C., Kassabgy, M., Huang, S., Mann, A., Waldmann, J., Weber, M., Kopf, A., Otto, A., Lange, J., Bernhardt, J., Reinsch, C., Hecker, M., Peplies, J., Bockelmann, F.-D., & Amann, R. (2012). Substrate-Controlled

Succession of Marine Bacterioplankton Populations Induced by a Phytoplankton Bloom. *Science (New York, N.Y.)*, *336*, 608–611. https://doi.org/10.1126/science.1218344

Tomasch, J., Gohl, R., Bunk, B., Diez, M. S., & Wagner-Döbler, I. (2011). Transcriptional response of the photoheterotrophic marine bacterium Dinoroseobacter shibae to changing light regimes. *The ISME Journal*, *5*(12), 1957–1968. https://doi.org/10.1038/ismej.2011.68

Tudek, B., Laval, J., & Boiteux, S. (1993). SOS-independent mutagenesis in lacZ induced by methylene blue plus visible light. *Molecular and General Genetics MGG*, *236*(2), 433–439. https://doi.org/10.1007/BF00277144

Wagner-Döbler, I., Thiel, V., Eberl, L., Allgaier, M., Bodor, A., Meyer, S., Ebner, S., Hennig, A., Pukall, R., & Schulz, S. (2005). Discovery of Complex Mixtures of Novel Long-Chain Quorum Sensing Signals in Free-Living and Host-Associated Marine Alphaproteobacteria. *ChemBioChem*, *6*(12), 2195–2206. https://doi.org/10.1002/cbic.200500189

Yura, T. (2019). Regulation of the heat shock response in *Escherichia coli*: History and perspectives. *Genes & Genetic Systems*, *94*(3), 103–108. https://doi.org/10.1266/ggs.19-00005

Yurkov, V., & Csotonyi, J. T. (2009). New Light on Aerobic Anoxygenic Phototrophs. In *The purple phototrophic bacteria* (pp. 31–55). Springer Berlin Heidelberg. https://doi.org/10.1007/978-1-4020-8815-5_3

Chapitre VI

Conclusions et perspectives

Sommaire

6.1 Conclusions

Ces travaux de thèse portent sur l'étude des interactions entre algues de glace et bactéries associées, et plus particulièrement sur l'impact de l'état physiologique de ces dernières sur leur activité de biodégradation du matériel algal. Les organismes photosynthétiques sympagiques et d'eau libre jouent un rôle primordial dans le fonctionnement des écosystèmes arctiques, tant par leur qualité de producteur primaire que par leur habilité à exporter le carbone atmosphérique vers les fonds océaniques. Si des études ont souligné la faible activité et le mauvais état physiologique apparent des bactéries dans ces milieux, il est capital de comprendre comment les différents stress subis par ces dernières influencent la préservation des algues tout au long de leur chute dans la colonne d'eau. Afin de mieux appréhender les différents processus à l'œuvre dans ces conditions particulières, cette thèse a fait intervenir : (i) une approche *In situ,* basée sur l'analyse de nombreux échantillons de glace, de particules en suspension ou en sédimentation et de sédiments, collectés lors de diverses campagnes dans l'Arctique Canadien ; (ii) ainsi qu'une approche *In vitro*, visant à comprendre en détail et investiguer l'impact du stress photo-oxydatif subit par les bactéries.

Les premiers résultats de cette thèse, reportés dans le chapitre II, ont montré l'importance relative des stress halin et chimique, grâce à l'analyse d'échantillons de glace et particules en sédimentation collectés lors de la saison de fonte de 2016 au camp de glace du projet GreenEdge. Si la salinité élevée dans les canaux de saumure de la glace est un facteur de stress important pour les bactéries sympagiques, nos résultats n'ont indiqué qu'un stress halin limité (rapport *trans/cis* de l'acide vaccénique faible) dans les échantillons analysés. Ces résultats sont toutefois cohérents avec la salinité relativement faible mesurée lors de la période d'échantillonnage, comparé aux valeurs extrêmes qu'il est possible de mesurer dans la glace. Des valeurs de ce rapport *trans/cis* indiquant un véritable état de stress des bactéries (> 0.1) n'ont été observées qu'au début de la période de fonte.

L'utilisation du PMA sur les échantillons d'ADN récoltés lors de cette campagne a permis d'estimer la mortalité des bactéries associées aux algues de glace. Les résultats, indiquant jusqu'à 75% de mortalité des bactéries associées en fin de fonte, suggèrent donc l'importance d'un facteur de stress bactérien autre que la salinité. Ce stress a été attribué à la présence de FFAs (acides gras libres) bactéricides. En effet, les algues de glace sont connues pour produire ces molécules hautement bactéricides, notamment en cas de stress

lumineux ou en fin de bloom. Ainsi, ce premier chapitre montre que le stress halin n'a un faible impact sur la préservation du matériel algal qu'en début de fonte, alors que la production de FFAs bactéricide par les algues sympagiques en fin de bloom induit un stress chimique important. Ainsi, en raison de la propension des algues de glace à former des agrégats (augmentant alors leur vitesse de chute dans la colonne d'eau) et du mauvais état physiologique des bactéries qui leur sont associées, les algues sympagiques devraient fortement contribuer à l'export de carbone atmosphérique vers les sédiments arctiques.

Le chapitre III de cette thèse, complémentaire au second, s'est intéressé à des échantillons de particules en suspension collectés en baie de Baffin lors de la saison de fonte de 2016, sur un transect allant de la glace à l'eau libre. Cet échantillonnage a ainsi permis de suivre les interactions algues de glace/bactéries associées lors de la transition du mode de vie sympagique à pélagique.

Un fort signal de stress halin a été observé dans les échantillons de particules en suspension collectées sous la glace, même à des profondeurs importantes (rapport *trans/cis* de l'acide vaccénique de 0.95 à 80m), ce qui a mis en évidence des interactions trophiques plus complexes dans la glace. En effet, ces forts signaux de stress halin ont été attribués à la présence de microzooplancton bactérivore (ciliés) ayant consommé des bactéries stressées par la salinité dans la glace en incorporant sans les altérer les acides gras de leurs proies avant d'être relargués dans la colonne d'eau.

Ici encore, le pourcentage de mortalité des bactéries associées a été estimé *via* l'utilisation du PMA. Les résultats ont indiqué une forte mortalité des bactéries dans la glace (jusqu'à 88.7%) ainsi qu'en eau libre (67.5%). Dans la glace, la mortalité observée est encore une fois attribuée aux FFAs bactéricides, secrétés par les algues de glace en fin de bloom, confirmant l'importance de ce stress sur l'activité des bactéries associées. Dans les échantillons de particules récoltés en eaux libres, un fort signal de stress halin subit par les bactéries a été observé (rapport *trans/cis* de l'acide vaccénique de 0.45 dans les échantillons de surface). Etant donné la capacité des bactéries à synthétiser *de novo* des acides gras *cis* lorsque le stress halin cesse, les ratios *trans/cis* élevés mesurés en eau libre révèlent donc la présence de bactéries en mauvais état physiologique incapables de synthétises de nouveaux acides gras. Il s'est avéré que ce signal de stress halin provient ici de bactéries associées à des particules d'EPS flottantes issues de la glace, donc fortement stressées par la salinité. Le contenu lipidique de ces échantillons a également

démontré la forte présence de grandes quantités de copépodes, broutant efficacement le matériel sympagique. Si l'importance des stress halin et chimique (lié aux FFAs) sur les bactéries sympagiques a été confirmée lors de cette étude, l'intégrité du matériel algal reste étroitement liée à l'activité de broutage du du micro- et macrozooplancton. Les interactions trophiques au sein de la glace restent donc complexes et méritent d'être étudiées plus en détail.

Ces deux premiers chapitres ont mis en évidence le rôle joué par les stress osmotiques et chimiques sur l'état physiologique des bactéries associées aux algues de glace en Arctique. Dans le chapitre suivant, nous avons voulu déterminer quel était le réel impact de ces stress sur la préservation du matériel algal sympagique dans les sédiments. Pour ce faire de nombreux échantillons de sédiments de surface collectés en mer de Beaufort et en baie de Baffin ont été analysés, et un focus sur les traceurs de stress halin (rapport *trans/cis* des acides gras bactériens), de stress chimique (activité bactérienne 10*S*-DOX détoxifiant les FFAs) et de dégradation de la matière organique (rapport stanol/ Δ^5-stenol) a été réalisé.

Les résultats obtenus confirmé la présence de bactéries stressées par la salinité, avec des valeurs de ratio *trans/cis* élevées, plus particulièrement dans les sédiments de surface de la mer de Beaufort et la partie ouest de la Baie de Baffin. Dans ces zones, le matériel algal relargué en début de fonte (quand le stress halin est le plus important) associé à des bactéries fortement stressées contribue donc à l'export de matériel sympagique vers les sédiments. Cet export est favorisé par la faible activité de broutage zooplanctonique observée à ce stade de la fonte. Cependant, ce broutage est généralement beaucoup plus intense au moment de relargage massif des algues sympagiques en fin de fonte limitant ainsi fortement l'export de matériel sympagique.

La présence de rapports *trans/cis* élevés dans les sédiments indique que les bactéries présentes sont originaires de la glace (où la salinité est élevée), et que les particules ne sont pas colonisées par des bactéries en bon état physiologique (avec un rapport *trans*/*cis* < 0,1) qui viendraient alors diluer ce signal. La présence d'acide 10-hydroxyhexadec-8(*trans*)-énoïque, résultant de la détoxification de l'acide palmitoléique libre par la 10*S*-DOX bactérienne atteste de la présence de quantités elevées de FFAs dans certains échantillons analysés. L'absence de colonisation des phytodétritus sympagiques par es bactéries pélagiques et benthiques pourraient donc être attribuée à la présence de FFAs bactéricides. En effet, les particules originaires de la surface sont généralement

rapidement colonisées par les bactéries des milieux profonds lorsqu'elles atteignent les sédiments, ces dernières étant mieux adaptées aux conditions des fonds marins.

La récalcitrance du matériel sympagique aux processus de biodégradation a été confirmée en analysant les rapports stanol/Δ^5-stenol de deux stérols typiques : le sitostérol (marqueur de M.O d'origine terrestre) et le brassicastérol (stérol commun retrouvé dans les algues sympagiques). Les résultats obtenus ont montré que si le matériel d'origine terrestre est intensément biodégradé par les bactéries benthiques, la M.O d'origine sympagique est bien préservée dans les sédiments de surface.

Ces résultats confirment le rôle clef joué par l'état de stress des bactéries associées aux algues sympagiques et le timing du broutage zooplanctonique dans la préservation de ce matériel. Au début du cycle de fonte, contrairement au matériel d'origine terrestre, les algues sympagiques relarguées dans la colonne d'eau contribuent grandement à l'export de carbone vers les sédiments, en raison : (i) du mauvais état physiologique des bactéries qui leur sont associées (stress halin et chimique), (ii) de la faible activité de broutage zooplanctonique au début de la fonte, et (iii) de la récalcitrance de ce matériel à la biodégradation.

En raison des perturbations liées au changement climatique, le cycle du carbone arctique sera inévitablement perturbé, notamment par le changement de la contribution sympagique/pélagique à la production primaire. Si les contributions relatives de ces deux compartiments à la P.P ont déjà été investiguées, de grandes incertitudes demeurent en ce qui concerne la préservation de ce matériel le long de sa chute vers les sédiments. L'analyse de matériel particulaire en sédimentation et de sédiments de surface de différentes zones arctiques lors de séries à long terme serait un bon moyen de gommer ces incertitudes.

Lors du dernier chapitre nous nous sommes intéressés à l'impact du stress photochimique, et plus particulièrement les réactions photo-sensibilisées de type II, faisant intervenir une molécule photo-sensibilisatrice (ici la chlorophylle) et des espèces réactives de l'oxygène ($^1O_2$) sur les bactéries attachées aux cellules algales.

En effet, en fin de bloom, les cellules photosynthétiques sénescentes sont l'objet de réactions de photo-oxydation de type II résultant en la production d'$^1O_2$. Cette espèce très réactive de l'oxygène ayant de grandes affinités avec les composés cellulaires insaturés comme les lipides ou l'ADN (plus particulièrement la guanine). L'objectif de ce chapitre était d'induire la production d'$^1O_2$ dans des cellules de *Thalassiosira* sp. sénescentes en

présence de deux souches bactériennes spécifiques : *Pseudomonas stutzeri* (bactérie ubiquiste non-pigmentée) et *Dinoroseobacter shibae* (bactérie AAP pigmentée), afin d'attester ou non de l'avantage physiologique des bactéries pigmentées face à ce stress. Des conditions d'irradiance contrastées ont également été testée (forte et faible), représentant respectivement l'intensité lumineuse reçue par des cellules en surface et à 50 m en mer Méditerranée.

Nous avons pu observer que sous de fortes irradiances, l'$^1O_2$ est produit en grandes quantités et est transmis efficacement aux bactéries associées, alors que sous de faibles irradiances favorisant l'oxydation des lipides algaux, (effet photodynamique) il est désactivé avant d'atteindre ces dernières. En utilisant les produits d'oxydation spécifiques de l'acide vaccénique et en étudiant l'expression des gènes de réparation de l'ADN liés à l'impact de l'$^1O_2$, nous avons pu mettre en évidence l'avantage des bactéries pigmentées. En effet les analyses lipidiques ont montré une oxydation deux fois plus importante chez *P. stutzeri* que chez *D. shibae* sous forte irradiance. Toutefois, les caroténoïdes présents dans les membranes de cette dernière ne la protègent pas contre les effets délétères de l'$^1O_2$ mais contre ceux des UV. Si les résultats produits dans ce chapitre ne démontrent pas d'effet de l'$^1O_2$ sur l'ADN mais celui des rayonnements UV, ces derniers ne sont pas moins intéressants, et mettent en avant le réel avantage écologique des bactéries pigmentées vis-à-vis de ce stress dans le milieu naturel. En effet, en raison de ses membranes fortement détériorées par la photooxydation, *P. stutzeri* (et les bactéries non-pigmentées) subit un stress photochimique bien plus important que *D. shibae* (et les bactéries pigmentées). Ces résultats permettent d'un peu mieux comprend les dynamiques des communautés de bactérioplancton lors d'un bloom.

6.2 Perspectives

Les résultats obtenus tout au long de cette thèse ont permi de mieux comprendre les interactions complexes entre algues de glace, bactéries associées et l'environnement si particulier qu'est celui de l'Océan Arctique. Ces résultats donnent ainsi une meilleure vue de l'activité de dégradation du matériel sympagique par les bactéries arctiques, et permettront d'intégrer les facteurs mis en avant lors de ce travail (stress halin, chimique, photochimique) dans les différents modèles des flux de carbone dans ces régions. Ces travaux de thèse ont également ouvert la voie à de nouvelles études plus poussées sur les facteurs de stress influençant l'activité de dégradation du bacterioplancton arctique. En effet, l'utilisation des différents marqueurs de stress utilisés dans cette thèse (rapport

trans/cis, présence de FFAs, activité de la 10*S*-DOX bactérienne, rapport de biodégradation des stérols, PMA…) pourrait être étendue et proposée lors des futures campagnes d'échantillonnage en zone arctique. L'utilisation de ces traceurs de stress bactériens donnerait alors une vision plus générale et permettrait potentiellement de vérifier si ces phénomènes sont récurrents au sein de la glace. Comme il a été suggéré dans le chapitre IV, l'analyse poussée des traceurs exposés ci-dessus dans des sédiments arctiques pourrait être une source importante concernant l'état de préservation et l'origine du matériel alimentant les fonds marins.

L'évolution des nouvelles technologies de bio-informatique associée aux nombreuses données collectées lors de campagnes d'échantillonnage internationales serait également un outil puissant, permettant d'avoir des informations précises et rapides sur la présence de certains gènes et protéines associées en Arctique. Initialement prévue dans le cadre de cette thèse, mais n'ayant pu être réalisée faute de temps, une des tâches du projet BACSTRESS (CNRS-EC2CO microbien) visait à suivre l'expression de différents gènes marqueurs de stress halin, chimique et photochimique dans de nombreux échantillons issus des chapitres II et III en utilisant des techniques novatrices de RTqPCR à haut débit. Pour mener à bien ce projet, l'outil Ocean Gene Atlas (OGA), mis au point récemment par Villard *et al*, (2018), permettrait, à partir d'une séquence nucléotidique ou du numéro d'identification PFAM d'une protéine, d'obtenir son abondance, sa localisation et sa distribution taxonomique. Ceci permettrait de designer les meilleurs couples d'amorces spécifiques pour des échantillons environnementaux. Cet outil est encore plus intéressant dans le contexte de cette thèse, depuis que les nombreuses données collectées lors de la mission Tara Arctic ont été implémentées à OGA, ce qui permettrait alors de rechercher l'abondance et la distribution taxonomique des gènes liés à la réponse bactérienne aux stress halin, chimique ou photochimique.

L'étude de l'Arctique, quelle que soit la discipline, doit tenir compte des conséquences du réchauffement global sur son fonctionnement. Ainsi, comme il a été abordé lors de cette thèse, les interactions entre les algues de glace et leurs bactéries associées sont inévitablement destinées à être perturbées. L'augmentation de température, la diminution de la quantité de glace en mer et sa fonte accélérée vont grandement perturber les milieux arctiques, plus particulièrement la production primaire, secondaire et à terme tout le cycle du carbone. Il est donc capital d'étudier l'évolution de l'impact des stress

halin, chimique et photochimique sur l'activité de dégradation du bactérioplancton arctique dans le contexte du réchauffement climatique. La diminution de la quantité de glace va également perturber la contribution sympagique / pélagique de la production primaire, ainsi que le timing de broutage zooplanctonique, le tout impactant inévitablement sa migration et la préservation de ce matériel lors de sa chute vers les sédiments.