2017-08-20

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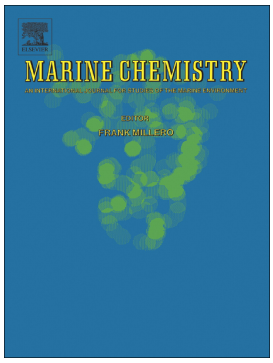
Amiraux, R

10.1016/j.marchem.2017.05.006
Marine Chemistry
Elsevier BV

Accepted Manuscript



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PII: S0304-4203(17)30034-8
DOI: doi: 10.1016/j.marchem.2017.05.006
Reference: MARCHE 3455

To appear in: *Marine Chemistry*

Received date: 20 January 2017
Revised date: 19 April 2017
Accepted date: 24 May 2017

Please cite this article as: Rémi Amiraux, Simon T. Belt, Frédéric Vaultier, Virginie Galindo, Michel Gosselin, Patricia Bonin, Jean-François Rontani , Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers, *Marine Chemistry* (2017), doi: 10.1016/j.marchem.2017.05.006

Monitoring photo-oxidative and salinity-induced bacterial stress in the Canadian Arctic using specific lipid tracers

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Abbreviations: Extracellular polymeric substances (EPS); Photosynthetically active radiation (PAR); Dioxygenase (DOX); Lipoxygenase (LOX); *Cis-trans* isomerase (CTI); Monounsaturated fatty acids (MUFAs); Polyunsaturated fatty acids (PUFAs); Singlet oxygen ($^1O_2$); Total lipid extracts (TLEs); Organic matter (OM); Particulate organic matter (POM); Multiple reaction monitoring (MRM); Electron ionization (EI); Dimethyldisulfide (DMDS); Gas chromatography–mass spectrometry (GC–MS);

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Abstract

We determined, and compared, the abiotic stress state of algae and their attached bacterial communities following their release into the water column during the Arctic sea ice melt season using specific lipid markers that are characteristic of type II photo-oxidation processes, together with those associated with *cis-trans* isomerase and 10*S*-DOX-like lipoxygenase activity, which are indicative of salinity stress. More specifically, parent lipids and some of their oxidation products were quantified in sinking particles (from sediment traps) collected from the Beaufort Sea, Resolute Passage and the Davis Strait (Canadian Arctic) in 2009, 2012 and 2015, respectively. Our data show that salinity- and light-induced bacterial stress processes are temporally decoupled, with the former occurring at the beginning of ice melting and the latter observed during the final stages of ice melt and in subsequent open waters. The salinity-induced bacterial stress seen during the early stages of ice melting contrasts the healthy state of sea ice algae under the same hypersaline conditions. As sea ice melt progresses, brine channels become hyposaline, thus reducing the bacterial salinity stress, while ice algae become susceptible to photodegradation by singlet oxygen ($^1O_2$), despite relatively low temperatures and irradiance. However, the presence of high amounts of extracellular polymeric substances (EPS) encapsulating the algae likely prevents the diffusion of $^1O_2$ to the attached bacteria. Photo-oxidative stress is enhanced further in open waters due to the low amounts of EPS. The lower amounts of active bacteria in the Arctic compared to temperate or tropical regions is thus attributed to the combined action of efficient salinity stress in spring and photo-oxidative stress in summer. Outcomes from this study also imply that surface sediments from the Canadian Arctic contain relatively high quantities of sea ice-derived organic matter that has undergone a strong degree of salinity-induced stress.

Keywords: Light stress; Salinity stress; Lipid biomarkers; *Cis-trans* isomerase; 10*S*-

DOX-like lipoxygenase; Bacteria; Ice algae; Phytoplankton; EPS; Arctic environment

1. Introduction

It is well recognized that bacteria play a key role in the transformation of organic matter (OM) in aquatic ecosystems (Kirchman et al., 2000; Arrigo et al., 2005). Marine microbial communities can be characterized in three categories of cells: (i) dead cells that play no active or potential role in the recycling of elements, (ii) living and active cells that play a functional role and participate in the production of biomass at the time of sampling and (iii) living but inactive cells that might play a role in the future (Howard-Jones et al., 2002).

Howard-Jones et al. (2002) previously determined bacterial activity in the marginal ice zone of the Barents Sea and suggested that a significant fraction (25-80%) of Arctic bacterioplankton is dormant or inactive. The high inactivity of bacteria in the Arctic could potentially be the result of stress processes occurring in sea ice. Indeed, changes in physical and chemical factors can be extreme within the semi-enclosed sea ice system, which contains an intricate network of brine channels characterized by the presence of highly variable salinity, pH, dissolved gas concentrations and dissolved inorganic nutrients (Thomas and Papadimitriou, 2003). Bacterial assemblages in sea ice are exposed to extreme salinity fluctuations (e.g. sea ice salinity can range from 230 to 30 between -30°C and -2°C) (Evert and Deming, 2013) and these changes in brine salinity and temperature can occur over relatively short timescales (e.g. days). Increased salinity exposes organisms to high salinity pressure, which drives water outside of the cell and results in potential dehydration, loss of turgor pressure and reduction of cell volume. The major adaptive response of many microorganisms, including bacteria, is to retain membrane fluidity through 'homeoviscous

adaptation' (Sinensky, 1974). This process is facilitated by changes in fatty acid composition of membrane lipids and, most notably, by conversion of *cis* to *trans* unsaturated fatty acids (Loffeld and Keweloh, 1996; Heipieper et al., 2003). Based on the value of the *trans/cis* fatty acid ratio generally observed in environmental samples (close to 0.1), Guckert et al. (1986) suggested that *trans/cis* ratios > 0.1 may be an indicator of bacterial starvation or stress.

Peroxidation of membrane lipids by lipoxygenases (LOXs) constitutes another mechanism by which microorganisms may change their membrane fluidity (Bhattacharjee, 2014) and such enzymatic activity can increase in plants at higher salinity (Tirajoh et al., 2005). However, the activity of LOXs may also play an important role in promoting oxidative damage during periods of environmental stress (Thompson et al., 1987). For many years, LOXs were believed to be specific to eukaryotes (animals, plants and fungi) (Kühn and Thiele, 1999; Oliw, 2002; Liavonchanka and Feussner, 2006), although recently, LOXs have also been detected in some environmental Gram-negative bacteria (for a review see Hansen et al., 2013). These enzymes contain non-heme iron and catalyze the introduction of one dioxygen molecule into polyunsaturated fatty acids (PUFA), such as $C_{18:2}$, forming a conjugated hydroperoxy diene. Although LOX-induced peroxidation of monounsaturated fatty acids (MUFAs) normally occurs only very slowly (Martínez et al., 2010), a new LOX-like enzyme (10*S*-DOX) has been found in *Pseudomonas aeruginosa* and shown to be an effective oxidant of MUFAs (Guerrero et al., 1997; Busquets et al., 2004; Martínez et al., 2010).

Bacteria attached to phytodetritus may also be affected by Type II (i.e. involving singlet oxygen ($^1O_2$)) photosensitized oxidation processes, which appear to be particularly prevalent in senescent Arctic phytoplankton (Rontani et al., 2012; 2016), despite low temperatures and solar irradiances (Amiraux et al., 2016). $^1O_2$ is generated by the photosensitization of ground state oxygen ($^3O_2$) by chlorophyll (Foote, 1976) during the

senescence of phytoplanktonic cells (Nelson, 1993) and is well known to react with the unsaturated lipid components of phytoplanktonic membranes to produce allylic hydroperoxides (see review by Rontani, 2012). $^1O_2$ can also induce significant damage to the attached bacteria (Rontani et al., 2003; Christodoulou et al., 2010; Petit et al., 2015), largely due to the hydrophobic microenvironments membranes of phytodetritus. In fact, despite the generally short lifetime of $^1O_2$, its maximum diffusive distance is around 300 nm in cell membranes (Wilkinson et al., 1995; Skovsen et al., 2005; Ogilby, 2010), which is ca. twice the thickness of the phytoplankton-bacteria membrane assembly (140-150 nm). Cellular damage resulting from the transfer of substantial amounts of $^1O_2$ from phytoplanktonic cells to their attached bacteria may also be significant due to the lack of efficient photo-protective and antioxidant systems in most of the latter (Garcia-Pichel, 1994). $^1O_2$ can thus react with monounsaturated fatty acids (Rontani et al. 2003; Christodoulou et al. 2010), DNA (Agnez-Lima et al. 1999; Ravanat et al. 2000) and bacterial proteins (Morgan et al. 2004), inducing growth limitation or senescence. To date, however, a more detailed investigation of the role that bacterial stress plays in the preservation (or otherwise) of attached sympagic/pelagic algae between their source environments and underlying sediments has not been carried out. To address this, in the current study, we measured specific lipid tracers of photo-oxidative and salinity stress to investigate the potential importance of these processes in bacterial constituents of sinking particles in the following three environments in the Canadian Arctic: (i) summer open waters (Beaufort Sea, 2009), (ii) spring sea ice with relatively high transmitted light (Resolute Passage, 2012) and (iii) spring sea ice with relatively low transmitted light (Davis Strait, 2015).

2. Materials and Methods

2.1. Sampling

The first set of sinking particles was collected in the Beaufort Sea during summer 2009. Sample recovery ranged from 7 to 16 days between August 2nd and 31st. Time series sediment traps (Technicap PPS 4/3; 24 cups; cylindrical-conical shape; collecting area: 0.125 m^2) were deployed at 100 m on 4 mooring lines located in the Amundsen Gulf (CA16, CA05) and on the Mackenzie Shelf (CA10, G09) (Fig. 1). For these two times series, long-term sediment traps were rinsed thoroughly before deployment with freshwater and seawater following the JGOFS protocol (Knap et al., 1996). Sample cups were filled with filtered seawater (Whatman glass fiber filters, nominal porosity of 0.7 μm) and adjusted to a salinity of 35 with NaCl. Formalin was added to preserve the material collected (5% v/v, sodium borate buffered). For this study, each trap was fitted with a plastic baffle mounted in the opening, to prevent the entrance of larger organisms.

The second sampling of sinking particles was conducted every three days from May 18th to June 23rd in spring 2012 at a landfast ice station (74°43.613'N, 95°33.496'W; water column depth: 90 m) located between Griffith Island and Sheringham Point (Cornwallis Island) in Resolute Passage (Fig. 1). During the sampling period, snow cover ranged from 5 to 16 cm and sea ice thickness from 127 to 93 cm. Hydro-Bios multi-sediment traps MS12 were deployed at 5 m and 30 m from the bottom ice. The interceptor traps, fixed to a tripod on the sea ice, were made of polyvinyl chloride (PVC) with an internal diameter of 13 cm and an aspect ratio (height:diameter) of 4.

The third set of sinking particle samples was collected every 48 hours from April 30th to June 10th during spring 2015. Short-term sediments traps were deployed at 2 and 25 m with two mooring lines at a landfast ice station located near Broughton Island (67°28.766'N;

63°47.579'W; water column depth: 350 m) in Davis Strait (Fig. 1). Sediment traps were made of polyvinyl chloride (PVC) and had an aperture diameter of 15 cm. During this period, snow and ice thicknesses ranged from 6 to 52 cm and from 144 to 101 cm, respectively. Surface sediment samples (ca. 0–1 cm) were also collected from box cores at a very close sampling location (67°28.531'N, 63°47.356'W; water column depth 379 m) on board the CCGS Amundsen in 2015, as part of the Green Edge project.

Sub-samples from each sediment trap were filtered through pre-weighed Whatman glass fiber filters (porosity 0.7 µm, diameter 25 or 47 mm, combusted 4 h at 450°C) and kept frozen (<-20°C) prior to lipid analysis. Due to the porosity of the filters, the lipid analysis concerns mainly algae and their attached bacteria, with exclusion of free bacteria. Surface sediment samples (ca. 0–1 cm) were directly frozen at <-20°C and stored until analysis.

2.2. Lipids and their degradation products employed for photo-oxidation and salinity stress estimation

Type II photosensitized oxidation of unicellular algae could be monitored in all samples through the identification of 24-methylcholesta-5,24/28-dien-3β-ol (24-methylenecholesterol) (a sterol typical of phytoplankton, Volkman, 1986; 2003) and its specific $^1O_2$-mediated photo-oxidation products (6α/β-hydroperoxy-24-methylcholesta-4,24/28-dien-3β-ols). These hydroperoxides were quantified (after $NaBH_4$ reduction to the corresponding diols) and the sterol photo-oxidation percentage was estimated using the following equation: sterol photo-oxidation % = (Δ^4-3β,6α/β-dihydroxysterol %) × (1 + 0.3) / 0.3 (Christodoulou et al. 2009; Rontani et al. 2009). Furthermore, monitoring of the photo-oxidation of bacteria was based on quantification of *cis*-vaccenic acid (a typical biomarker for Gram-negative bacteria, Sicre et al., 1988; Keweloh and Heipieper, 1996) and a

group of four isomeric hydroxyacids obtained from $NaBH_4$ reduction of the corresponding hydroperoxyacids (i.e. 11-hydroxyoctadec-*trans*-12-enoic, 12-hydroxyoctadec-*trans*-10-enoic, 13-hydroxyoctadec-*trans*-11-enoic, and 10-hydroxyoctadec-*trans*-11-enoic acids) produced during $^1O_2$-mediated oxidation (Marchand and Rontani, 2001) and their allylic rearrangement (Porter et al., 1995). In order to monitor salinity stress in bacteria, we measured the *trans/cis* ratio of the same fatty acid. After DMDS treatment of *cis* and *trans* vaccenic acid methyl esters, quantification of the *threo* and *erythro* adducts permitted calculation of the *trans*/*cis* ratio. We also examined (or re-examined) the profiles of oxidation products of MUFAs, focusing on the proportion of 10-hydroxyhexadec-8(*trans*)-enoic acid, the main 10*S*-DOX-like lipoxygenase oxidation product of palmitoleic acid ($C_{16:1\omega9}$) (Guerrero et al., 1997; Busquets et al., 2004; Martínez et al., 2010).

2.3. Lipid Analysis

Samples were reduced with excess $NaBH_4$ after addition of 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 x 20 ml). The combined DCM extracts were dried over anhydrous Na_2SO_4, filtered and concentrated via 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, then treated with dimethyldisulfide (DMDS) and analyzed by GC–MS/MS for the determination of double bond stereochemistry. *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 well-separated by gas chromatography, allowing unambiguous double bond stereochemistry determination (Buser et al., 1983).

2.3.1. Monounsaturated fatty acid oxidation product quantification

TLEs were silylated by dissolving them in 300 µl of a mixture of pyridine and BSTFA (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. Quantification of monounsaturated fatty acids and their oxidation photoproducts was achieved by comparison of individual GC–MS responses with those obtained from an external standard of oleic acid and its photoproducts of known concentration. Some of the palmitoleic acid (and its oxidation products) concentration data have been reported previously by Rontani et al. (2016).

2.3.2. Determination of double bond stereochemistry

TLEs were dissolved in 2 mL of BF_3/methanol (10%) (Sigma-Aldrich) 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 of 11 µm), and concentrated using rotary evaporation before being 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. Following shaking, the mixtures were heated at 50°C (48 h), excess iodine was removed by addition of 2 ml of a 5% $Na_2S_2O_3$ solution, and lipids were extracted three times with 1 ml hexane. The extracts were subsequently dried with anhydrous Na_2SO_4, filtered and concentrated prior to analysis by GC–MS/MS.

2.3.3. 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) (30 m × 0.25 mm, 0.25 μm film thickness) capillary column was employed. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C and the oven temperature programmed 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 programmed 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 employed: 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. 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.

2.3.4. 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, 0.25 μm film thickness) capillary column was employed. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C and the oven

temperature 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 transfer line and 230°C for the ion source. Accurate mass spectra were recorded across the range *m/z* 50-700 at 4 GHz. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12252 from *m/z* 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was utilized for daily MS calibration.

3. Results and discussion

3.1. Sampling site characteristics

The main aim of this study was to monitor the influence of abiotic stress factors on primary producers and their attached bacteria at different stages of spring sea ice melting season. Since the summer 2009 sampling took place in ice-free waters following the end of the melt season, the collected material likely comprised mainly pelagic phytoplankton which grew under relatively high irradiance (Pidwirny, 2006) and consistent salinity. In contrast, the 2012 and 2015 sampling reflected the release of sympagic (ice–associated) material during the spring sea ice melt, although the conditions between these two years were somewhat different. For example, during spring 2012, sea ice conditions were characterized by a thinner snow cover (mean 10 cm, range 5–16 cm) compared to 2015 (mean 28 cm, range 6–52 cm), which resulted in an increase in transmitted light, as expected (Mundy et al., 2005). Thus, across the sampling periods, the under-ice transmitted PAR in 2012 was 17.2 ± 13.8 µmol photons m^{-2} s^{-1}, while in 2015, it was only 2.7 ± 2.3 µmol photons m^{-2} s^{-1} (Mundy C.J. and Verin G., pers. comm.). Further, the mean pack ice temperature was -2.0 ± 1.4°C and -3.5 ± 1.4°C in 2012 and 2015, respectively, which was reflected in differences in sea ice brine volume and salinity. Thus, mean brine volume was lower in 2012 (0.2%) than in 2015 (10.5%) and mean

brine salinity was also reduced in 2012 (37.8) compared to 2015 (58.6). These data suggest that the majority of the interstitial brine channels had already been flushed by percolation of downward flowing meltwater prior to the main sampling interval in 2012, while, in contrast, only modest changes within the sea ice brine network occurred during the sampling period in 2015. As such, the sea ice was in a more advanced melt state in 2012 compared to 2015.

Finally, to ensure that our lipid analyses of sediment trap material collected during 2012 and 2015 could be attributed to ice algae and their attached bacteria, we also compiled some taxonomic information on the majority biota. In 2012, the main algal constituents were pennate diatoms such as *Nitzschia frigida* (25%), *Navicula pelagica* (6.5%) and *Navicula sp. 6* (3.5%) (Lessard S. and Poulin M., pers. comm.), while in 2015, *N. frigida* (1-20%) and *Fragilariopsis oceanica* (2-24%) were the dominant species (Lalande C., pers. comm.), thus confirming a sea ice origin for the majority of the biota in the sediment traps for both sample sets (Poulin et al., 2011).

3.2. Photo-oxidative stress

Due to the filtering method adopted during sampling, this study focuses on the abiotic stress experienced by the algae and their attached bacteria rather than bacteria in their free form. Identification of oxidation products of palmitoleic acid and vaccenic acid in the sinking particles collected from the Beaufort Sea in 2009 and Resolute Passage in 2012 provided evidence for photo-oxidation of algae and their attached bacteria, respectively (Fig. 2). In contrast, none of these photo-oxidation products were detected in the sinking particles collected from Davis Strait in 2015.

The photo-oxidation of algae observed in the samples collected in 2009 and 2012 might, intuitively, be attributed to higher transmitted light during the respective sampling

periods (see section 3.1). In practice, however, an enhancement in photosensitized oxidation processes in senescent phototrophic cells is associated with lower irradiance levels (Amiraux et al., 2016). To explain this apparent paradox, Amiraux et al. (2016) suggested that high irradiance conditions result in both production of $^1O_2$ and photodestruction of sensitizers, such that $^1O_2$ formation is intense but relatively short. In contrast, lower irradiance induces longer $^1O_2$ production rates that result in increased membrane photodamage (Amiraux et al., 2016). However, photo-oxidative damage is also strongly dependent on the physiological state of phototrophic cells (Nelson, 1993; Merzlyak and Hendry, 1994). Thus, since the highest photo-oxidative damage was observed in samples from 2009 and 2012, which had the highest associated transmitted PAR values, we suggest that the algae in these samples were in a poorer physiological state compared to those collected in 2015 and we provide further justification for this conclusion as follows.

In a previous study, a higher proportion of palmitoleic acid oxidation products were observed at 5 m compared to 30 m in the 2012 sediment trap samples, indicative of increased photo-oxidative damage of ice algae in the upper euphotic zone (Rontani et al., 2016). One characteristic of Arctic sea ice algae is their ability to produce high amounts of extracellular polymeric substances (EPS), which play multiple roles in the entrapment and survival of these micro-organisms within the sea ice matrix (Krembs et al., 2001; Riedel et al., 2006, 2007; Ewert and Deming, 2013). The production of EPS facilitates the attachment of algae to their substrate (i.e. sea ice) but also allows the formation of micro-aggregates of algal cells that can remain intact even after ice melt (Riebesell et al., 1991). The aggregation state of ice algae thus determines their sedimentation rate and their residence time within the euphotic zone. Therefore, Rontani et al. (2016) previously attributed the greater algal preservation in the 30 m sediment traps to an enhanced contribution of highly aggregated senescent sea ice algae that settled rapidly out of the euphotic zone (Lalande et al., 2016). Here, the relative

aggregation states of the ice algae in 2012 and 2015 were estimated by determination of the concentration ratio of palmitoleic acid at two sediment trap depths (i.e. $[C_{16:1\omega9}]_{\text{deep sediment trap}}$ /$[C_{16:1\omega9}]_{\text{shallow sediment trap}}$). The substantially higher ratio in 2012 (mean = 678) compared to 2015 (mean = 0.7) confirms the higher aggregation state of ice algae in 2012 and, by implication, a poorer physiological state. This conclusion is also supported by the observations of Rontani et al. (2012), who showed an increase in dead phytoplankton cells for particles having a long residence time in the euphotic zone of Arctic open waters, and by a decrease of ice algal growth and survival in hypo- versus hypersaline environments (salinities of 16 and 65, respectively; Ralph et al., 2007).

A cross-plot of % photo-oxidation of *cis*-vaccenic acid versus % photo-oxidation of 24-methylenecholesterol illustrates the variation of photo-oxidation of attached bacteria relative to that of the algae (Fig. 2). Interestingly, in spring 2012, the % photo-oxidation of *cis*-vaccenic acid relative to that of 24-methylenecholesterol was generally greater at 5 than 30 m suggesting a weaker efficiency of $^1O_2$ transfer from phytodetritus to attached bacteria in the deeper sinking particles, possibly resulting from the higher aggregated state of ice algae in these samples. Indeed, highly aggregated sea ice algal cells are normally enclosed by increased amounts of EPS, which provide a physical and/or chemical barrier through which $^1O_2$ must pass prior to interacting with attached bacteria. This hypothesis is supported by data obtained from the Beaufort Sea samples (2009), which reveal a much higher photo-oxidation state of bacteria relative to phytoplankton (Fig. 2). In this setting, primary productivity over the Mackenzie Delta/Beaufort Shelf (3.3×10^{12} g y^{-1} of particulate organic carbon) is due mainly to phytoplanktonic blooms dominated by diatoms and haptophytes (Hill et al., 2005; Coupel et al., 2015) that occur during late spring and summer (Macdonald et al., 1998) such that EPS content would be expected to be relatively low. Indeed, Krembs et al. (2002) showed previously that EPS concentrations in Arctic sea ice are typically an order of magnitude

higher than for under-ice and open water environments (Krembs and Engel, 2001; Meiners et al., 2003). The lower production of EPS by open water phytoplankton should thus favor $^1O_2$ transfer from phytodetritus to attached bacteria and, as a result, increase the susceptibility of the latter towards photo-oxidative damage. Overall, therefore, the low temperatures and low solar irradiances associated with high latitude environments appear to promote Type II photosensitized oxidation processes in both phytodetritus (Amiraux et al., 2016) and their attached bacteria. This is potentially explained by a reduced $^1O_2$ diffusion outside of cell membranes at lower temperatures, together with longer production times of $^1O_2$ under low irradiance conditions, as described earlier, and by Amiraux et al. (2016). Finally, an exponential increase in the photo-oxidation state of bacteria compared to the attached phytoplankton for Arctic open waters (Beaufort Sea samples) has not been observed previously in temperate (Christodoulou et al., 2009) or tropical (Rontani et al., 2011) regions.

3.3. Salinity stress

A major adaptive response of microorganisms to rapid increases in salinity is the conversion of *cis* to *trans* unsaturated fatty acids in order to retain their membrane fluidity (Loffeld and Keweloh, 1996; Heipieper et al., 2003). Previously, Guckert et al. (1986) suggested that *trans/cis* ratios > 0.1 in environmental samples may be indicative of bacterial stress, with isomerization induced by the action of the *cis-trans* isomerase (CTI) enzyme (Heipieper et al., 2003).

The *trans/cis* ratio of vaccenic acid in the sinking particles collected from the Beaufort Sea (2009) was zero (i.e. no *trans* isomer could be detected) and was mostly < 0.1 at both 5 and 30 m (Fig. 3a, b) in the samples from Resolute Bay (2012), suggesting that bacteria in these samples were in a healthy state. In contrast, *trans/cis* ratios for material collected from

Davis Strait (2015) were mostly above the bacterial stress threshold of 0.1, with values ranging from 0.04 to 0.28 (mean 0.16 ± 0.08) (Fig. 3c) and 0.12 to 0.50 (mean 0.27 ± 0.12) (Fig. 3d) at 2 and 25 m, respectively. The occurrence of highest bacterial stress at 25 m reflects the observations of Riebesell et al. (1991) and Rontani et al. (2016), who showed that less metabolically active algal particles tend to be concentrated in aggregates with higher sinking rates.

The absence of notable CTI activity in sediment trap samples collected from the Beaufort Sea in 2009 (Rontani et al., 2012) implies that bacteria associated with phytoplankton in ocean settings are not susceptible to salinity-induced stress, presumably reflecting the near uniformity of open water salinity. On the other hand, the difference in the *trans/cis* ratio (and thus the CTI activity) between the samples taken in 2012 and 2015 could potentially be attributed to the contrasting brine characteristics of the respective sea ice matrices owing to their different stages of sea ice melt (see Section 3.1).

Some previous studies have suggested that the *trans/cis* ratio may not represent an entirely reliable proxy for durable stress in the environment (Fischer et al., 2010) since bacteria may substitute the action of the CTI (which is only used as an acute response to permit survival) by other mechanisms, thus reducing the measured values (Fischer et al., 2010). However, this recovery mechanism is mostly dependent on bacterial growth rates, so any stability of the *trans/cis* ratio observed within the water column should thus indicate the senescence of bacteria or, at least, their poor physiological state. During 2015 sampling, the similarity of the *trans/cis* ratios measured in the 25 m sediment traps (mean value weighted by the sinking flux = 0.22) and in the underlying sediment (0.23) (Fig. 3d), combined with the absence of salinity-induced stress for pelagic sinking particles (see Beaufort Sea samples) indicates: (i) a poor physiological state of bacteria in sinking particles; (ii) a low dilution of the stress signal between the water column and the surface sediment; (iii) that the sedimentary

organic material is composed mainly of sea-ice particulate organic matter (POM) released during brine drainage. In this instance, the sea ice algae are relatively aggregated (with moderate sinking rates) and experience only a relatively small degree of biotic (due to the poor physiological state of bacteria) and photo-oxidative (due to the healthy state of ice algae) degradation. In addition, we suggest that the occurrence of relatively high *trans/cis* ratios in sediments collected from other Arctic regions (Rontani et al., 2012) may not be due to the presence of thiyl radicals (produced during the reaction of thiols with hydroperoxides) as proposed previously, but to the presence of sea ice bacteria affected by salinity stress.

Turning our attention to enzymatic responses, and following a previous report of a bacterial 10*S*-DOX-like lipoxygenase enzyme capable of oxidizing MUFAs (Guerrero et al., 1997; Busquets et al., 2004; Martínez et al., 2010), we examined, or re-examined, the profiles of oxidation products of these lipids in our various sediment trap samples. As background, $^1O_2$-mediated photo-oxidation of Δ^9 monounsaturated fatty acids (e.g. palmitoleic acid) produces equal amounts of 10-*trans* and 9-*trans* hydroperoxides, which can undergo stereoselective allylic rearrangement (Porter et al., 1995) to their 8-*trans* and 11-*trans* regioisomers, respectively. In contrast, autoxidation (i.e. free radical-induced oxidation) affords mixtures of 9-*trans*, 10-*trans*, 11-*trans*, 11-*cis*, 8-*trans*, and 8-*cis* allylic hydroperoxides, which also contain equal proportions of the 9-*trans* and 10-*trans* isomers as the major products (Frankel, 1998).

In the sinking particles collected from Davis Strait in 2015, we identified a predominance of the 10-hydroperoxyoctadec-8(*trans*)-enoic acid (relative to the 9-*trans* isomer) (Fig. 4), which we attribute to the involvement of a specific enzymatic degradation process and, more specifically, a bacterial 10*S*-DOX-like lipoxygenase enzyme. Consistent with this suggestion, the selective conversion of oleic acid to 10(*S*)-hydroperoxyoctadec-8(*trans*)-enoic acid by *Pseudomonas aeruginosa* 42A2 was observed previously (Guerrero et

al., 1997; Busquets et al., 2004; Vidal-Mas et al., 2005) and attributed to a lipoxygenase-like enzyme, subsequently called 10*S*-DOX (Martínez et al., 2010; Estupiñàn et al., 2014; 2015). Such an enzyme, which is particularly active in the case of MUFAs containing double bonds in position Δ^9 (Brash et al., 2014), can convert palmitoleic acid to 10(*S*)-hydroperoxyhexadec-8(*trans*)-enoic acid (Brash et al., 2014). The involvement of 10*S*-DOX-like lipoxygenase enzymatic activity in our samples collected from Davis Strait (2015) is also supported by the detection (in some cases) of trace quantities of 7,10-dihydroxyhexadec-8(*trans*)-enoic acid (Fig. 5) formed from the specific action of 7*S*,10*S*-hydroperoxide diol synthase (linked to the 10*S*-DOX-like lipoxygenase enzymatic activity) (Estupiñàn et al., 2014) on 10(*S*)-hydroperoxyhexadec-8(*trans*)-enoic acid. 10*S*-DOX-like lipoxygenase enzymatic activity was only detected in samples collected in 2015, however, with percentages of oxidation products reaching 29.5% and 43.5% of the sum of residual palmitoleic acid and its biotic and abiotic oxidation products at 2 and 25 m, respectively (Fig. 6a, b). The parallel response of both bacterial salinity stress tracers (10*S*-DOX-like lipoxygenase oxidation and *cis-trans* isomerization products) in our three samples sets may be attributed to similarities concerning the catalytic mechanism of these two enzymes (Okuyama et al., 1998) and the organisms exhibiting such enzymatic activities. Indeed, CTI was found previously in the bacteria belonging to *Vibrio* (Heipieper et al., 2003) or *Pseudomonas* genus (Heipieper et al., 2003; Mrozik et al., 2005; Fischer et al., 2010), whereas 10*S*-DOX-like lipoxygenase was only reported for *Pseudomonas aeruginosa* (Martínez et al., 2010).

On the other hand, although palmitoleic acid is a major constituent of numerous bacteria (Oliver and Colwell, 1973; Viso and Marty 1993), it is also a principal component of sea ice diatoms (Fahl et al., 1993). Since bacterial 10*S*-DOX-like lipoxygenase may also be expressed extracellularly (Kim et al., 2000), the presence of 10*S*-DOX-like lipoxygenase oxidation products of palmitoleic acid in the 2015 samples may thus result not only from the

oxidation of this membrane component of bacteria, but also from the degradation of sea ice algae by their attached bacteria.

Finally, the iron present in lipoxygenases is usually present in the inactive ferrous (Fe^{+2}) state, such that an activation step is required for these enzymes to enter the catalytic cycle. This activation involves the reaction of the native ferrous enzyme with hydroperoxides, thus affording an active ferric (Fe^{+3}) enzyme and an alkoxyl radical (Garreta et al., 2013). As such, it is interesting to note that the generation of radicals during the iron-based catalytic cycle may also serve as a catalyst for autoxidation processes (Fuchs and Spiteller, 2014; Spiteller and Afzal, 2014). The simultaneous presence of high proportions of 10*S*-DOX-like lipoxygenase enzymatic (15%) and autoxidative (35%) palmitoleic acid oxidation products observed in surface sediments collected in spring 2015 (Fig. 6b) therefore supports the potential role played by bacterial 10*S*-DOX-like lipoxygenase enzymes in the induction of autoxidative degradation processes of algal material in the underlying sediments.

3.3. Biogeochemical implications

In the Arctic, the main constituents of sedimentary organic matter may be derived from sea ice, open water and terrestrial sources (Subba Rao and Platt 1984; McCleeland et al., 2014), and may be subject to alteration both within their native environments and within sediments, which can modify their burial efficiency. Understanding the significance and the timelines of these alterations is therefore important for evaluating biogeochemical cycling of organic matter at high latitudes.

Our analysis of degradation pathway-specific lipid tracers in settling particulate matter

obtained from different regions of the Canadian Arctic provides new information about a potential timeline associated with bacterial stress during sea ice melt. We summarize our findings in the form of a conceptual scheme (Fig. 7) that can be described as follows. During the early stages of sea ice melt (Fig 7a), brine pockets get interconnected and slightly released in channels, hypersaline conditions arise, and ice algae are generally in a healthy state (Ralph et al., 2007). Consequently, photodegradation processes in ice algae and in their attached bacteria are negligible and cell aggregation is only moderate (Riesebell et al., 1991). In contrast, these hypersaline conditions strongly impact the bacterial community associated with the sympagic algae, and its resulting poor physiological state likely contributes to the preservation of the algal material prior to deposition in the underlying surface sediments. This conclusion is supported by relatively high values of *trans/cis* fatty acid ratios measured previously in Arctic surface sediments (Rontani et al., 2012), which indirectly attests to a strong contribution of sea ice material to high latitude sedimentary OM. However, due to the likely presence of 10*S*-DOX-like lipoxygenase enzymatic activity in seafloor sediments, and its ability to induce free radical oxidation reactions (Spiteller and Afzal, 2014), OM in sediments could also be strongly autoxidized.

With continued warming, sea ice melt progresses (Fig. 7b), snow depth decreases, high salinity brines are released, and the leaching of brine by downward percolation of melt water strongly reduces the overall salinity in brine channels. Under these hyposaline conditions, the physiological state of ice algae decreases favoring the photo-oxidative damage, despite the relatively low transmitted light and temperature (Amiraux et al., 2016). However, the presence of EPS surrounding this senescent material (Riesebell et al., 1991) likely limits the transfer of $^1O_2$ to the attached bacteria, thus protecting them from photo-oxidation. During this phase, although a large amount of ice algal material experiences high sinking rates (enhanced by its poor physiological and high aggregation state), its contribution

to the seafloor is relatively weak due to the increased efficiency of biotic degradation that results from reduced salinity and photo-oxidative stress on sea ice bacterial communities.

Finally, following ice melt and during open water conditions in summer (Fig. 7c), salinity stress is absent and phytoplankton aggregation is negligible compared to under sea ice (Fig. 7a,b), resulting in longer residence times of senescent algal cells in the euphotic zone, and thus to an enhancement of photo-oxidative degradation in algae and their associated bacteria. Due to the very high photo-oxidation state of these bacteria, good biotic preservation of algal material might be expected. However, due to the very low sedimentation rate of this POM, with further colonization and degradation by active mesopelagic bacteria, this does not seem to be the case.

4. Conclusion

By measuring various lipids and their characteristic oxidation products in sinking particles released from Arctic sea ice during the vernal melting period and in summer, we have shown that Arctic bacteria, when attached to certain algae, undergo a strong salinity stress in spring and photo-oxidative stress in summer. Salinity stress (monitored via the induction of CTI and 10*S*-DOX-like lipoxygenase enzyme) occurs mainly during the early stages of sea ice melt, when the salinity conditions in the brine channel network become hypersaline. Under such conditions, the good physiological state of ice algae prevents significant photo-oxidation. In contrast, photo-oxidative stress processes become active in subsequent stages of ice melt, or in open waters. The relatively small amounts of active bacteria in Arctic compared to temperate or tropical zones may be thus attributed to the combined action of (i) salinity stress induced by the release of brines during the melting period in spring and (ii) the enhancement of photodegradation processes in summer due to the long residence time of particles in the euphotic layer, as observed previously for Arctic algae (Rontani et al., 2012). This study also

suggests that the organic matter content of Arctic surface sediments may be comprised almost exclusively of sea ice-derived material, which has undergone a strong salinity stress and which is only weakly biotically degraded. Moreover, this study confirmed the use of products of 10*S*-DOX-like lipoxygenase enzymatic oxidation and *cis-trans* isomerase activity as tracers of bacterial salinity stress in the Arctic.

The estimation of organic matter sources supplying surface sediments is a crucial carbon cycling issue. Although the high *trans/cis* ratios measured in some surface sediments from the Arctic (Rontani et al., 2012) suggests a strong contribution of ice algal material to underlying sediments, it would, nonetheless, be interesting to confirm this conclusion by measuring 10*S*-DOX-like lipoxygenase enzymatic activity and bacterial diversity in both sea ice and sediments from other regions.

Acknowledgements

This work was carried out in the framework of the transverse axis DEBAT (DEgradation Biotique et Abiotique de la maTière organique en milieu marin: processus et interactions) of the MIO. It was supported by the MICROMAR (INSU - EC2CO-Microbien) and GREENEDGE projects. The GREENEDGE project is funded by the following French and Canadian programs and agencies: ANR (Contract #111112), CNES (project #131425), IPEV (project #1164), CSA, Fondation Total, ArcticNet, LEFE and the French Arctic Initiative (GREENEDGE project). This project would not have been possible without the support of the Hamlet of Qikiqtarjuaq and the members of the community as well as the Inuksuit School and its Principal Jacqueline Arsenault. The project is conducted under the scientific coordination of the Canada Excellence Research Chair on Remote sensing of Canada's new Arctic frontier and the CNRS & Université Laval Takuvik Joint International laboratory (UMI3376). The field campaign was successful thanks to the contribution of J. Ferland, G. Bécu, C. Marec, J.

Lagunas, F. Bruyant, J. Larivière, E. Rehm, S. Lambert-Girard, C. Aubry, C. Lalande, A. LeBaron, C. Marty, J. Sansoulet, D. Christiansen-Stowe, A. Wells, M. Benoît-Gagné, E. Devred and M.-H. Forget from the Takuvik laboratory, C.J. Mundy from the University of Manitoba as well as F. Pinczon du Sel and E. Brossier from Vagabond. We also thank Québec-Océan, the CCGS *Amundsen* and the Polar Continental Shelf Program for their in-kind contribution in polar logistic and scientific equipment. We especially thank C. Nozais from the Université du Québec à Rimouski for providing the sediment traps, C. Lalande (project leader), C. Aubry, and T. Dezutter from the Université Laval Joint International Laboratory, Takuvik, for allowing the 2015 short term sediment trap deployment and G. Massé for the seafloor surface sediment material. We are also grateful to G. Verin and Dr. C.J. Mundy for providing transmittance values, S. Lessard M. Poulin for taxonomic data and Dr. H.J. Heipieper for helpful discussions. We also thank the OCEANOMED project (FEDER n°1166-39417) for the funding of the apparatus employed during this work. Finally, we gratefully acknowledge two anonymous reviewers for their useful and constructive comments.

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Figure Captions

Fig. 1. Map of the study area with locations of the different stations investigated in the Beaufort Sea, Resolute Passage (Canadian Arctic Archipelago) and Davis Strait.

Fig. 2. Relationships between the percentage of photo-oxidation of vaccenic acid and the one of photo-oxidation of 24-methylenecholesterol in sinking particles collected at 100 m (■) during summer 2009 in the Beaufort Sea and at 5 m (○) and 30 m (♦) during spring 2012 in Resolute Passage.

Fig. 3. *Trans/cis* ratios and salinity stress threshold ratio of 0.1 (---) in sinking particles collected at (A) 5 m and (B) 30 m in Resolute Passage during spring 2012 and at (C) 2 m and (D) 25 m in Davis Strait (near Broughton Island) during spring 2015. The *trans/cis* ratio found for the seafloor surface sediment (0-1 cm) from Davis Strait is also shown in (B).

Fig. 4. (A) Partial ion chromatograms (at *m/z* 199.1518, 213.1675, 329.1968, and 343.2125) showing the predominance of 10-*trans* hydroperoxide in the profile of palmitoleic oxidation products and (B) main oxidation products of palmitoleic acid by 10*S*-DOX-like lipoxygenase.

Fig. 5. Partial TOF-MS of 7,10-dihydroxyhexadec-8(*trans*)-enoic acid TMS derivative.

Fig. 6. Percentage of residual palmitoleic acid and autoxidation, photo-oxidation and 10*S*-DOX-like lipoxygenase degradation products in sinking particles collected in Davis Strait during spring 2015 at (A) 2 m and (B) 25 m and at the seafloor surface sediment (0-1 cm).

Fig. 7. Conceptual scheme summarizing bacterial stress and its consequences upon the behavior of particulate organic material during (A) the brine drainage, (B) the downward percolation of meltwater into the brine channels and (C) open water conditions in the Canadian Arctic.

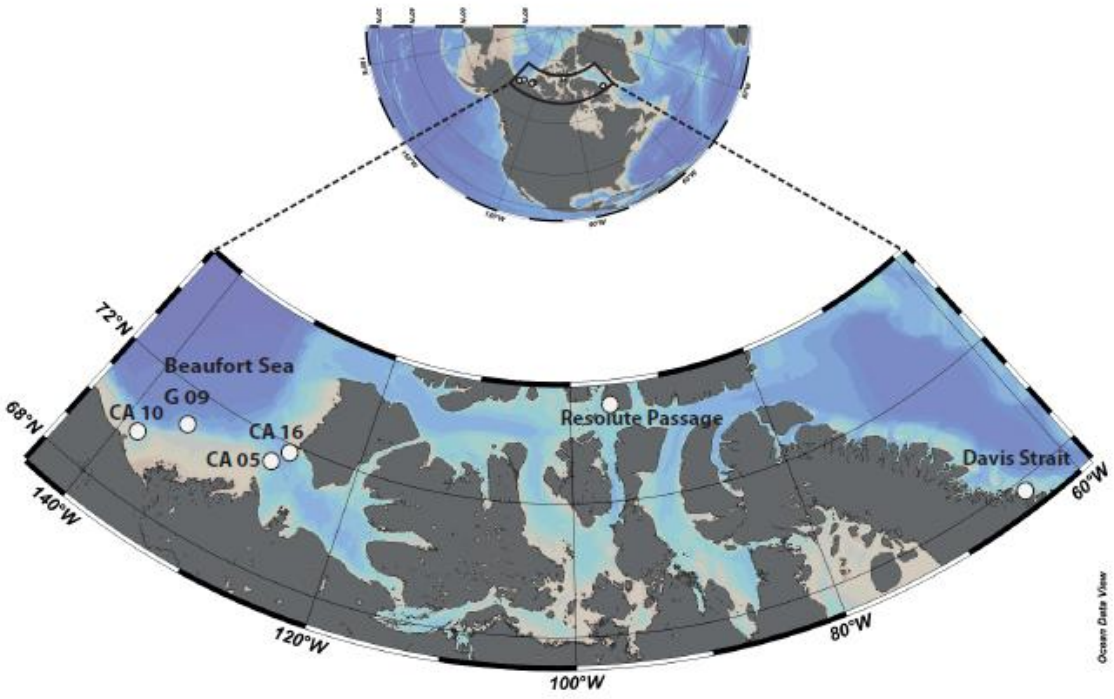


Fig. 1

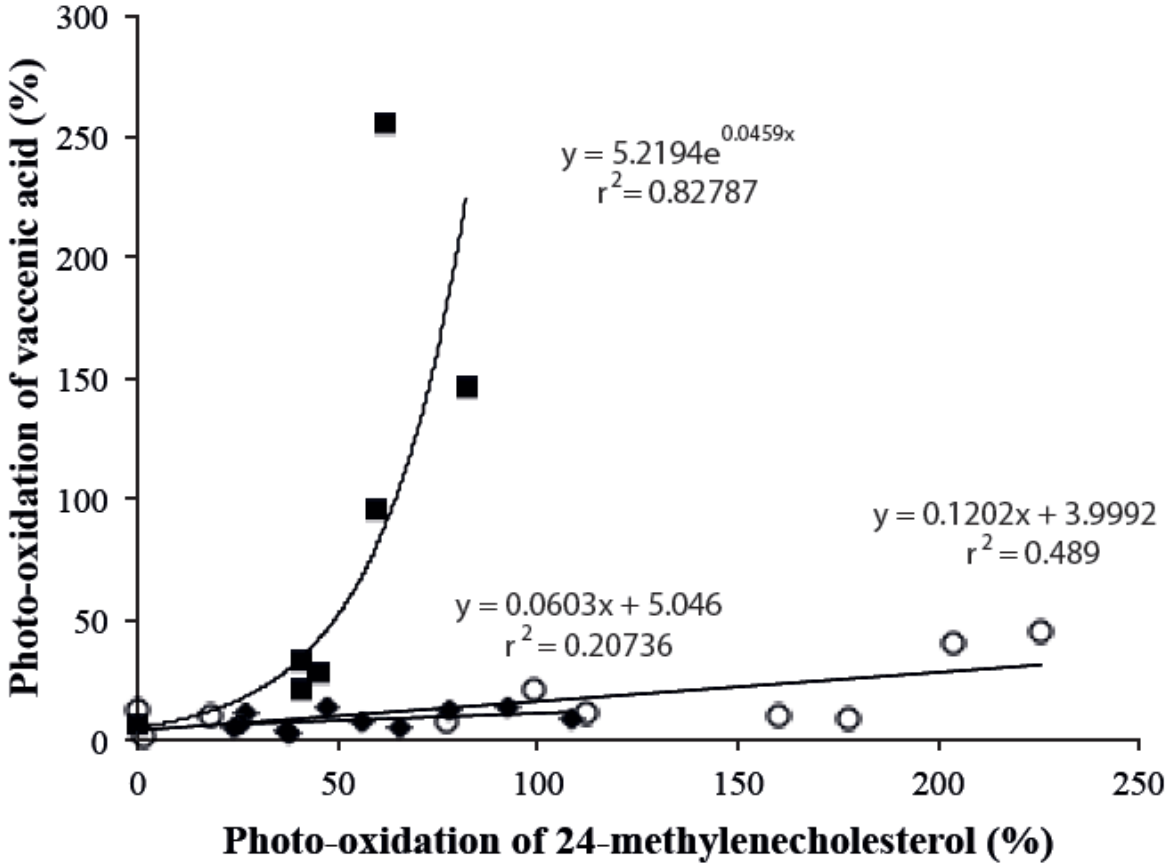


Fig. 2

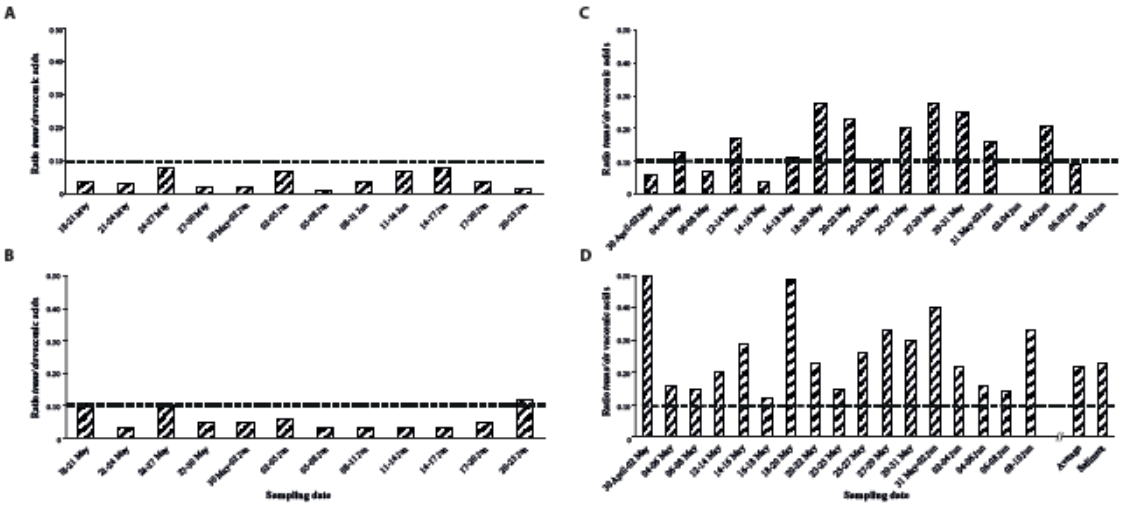
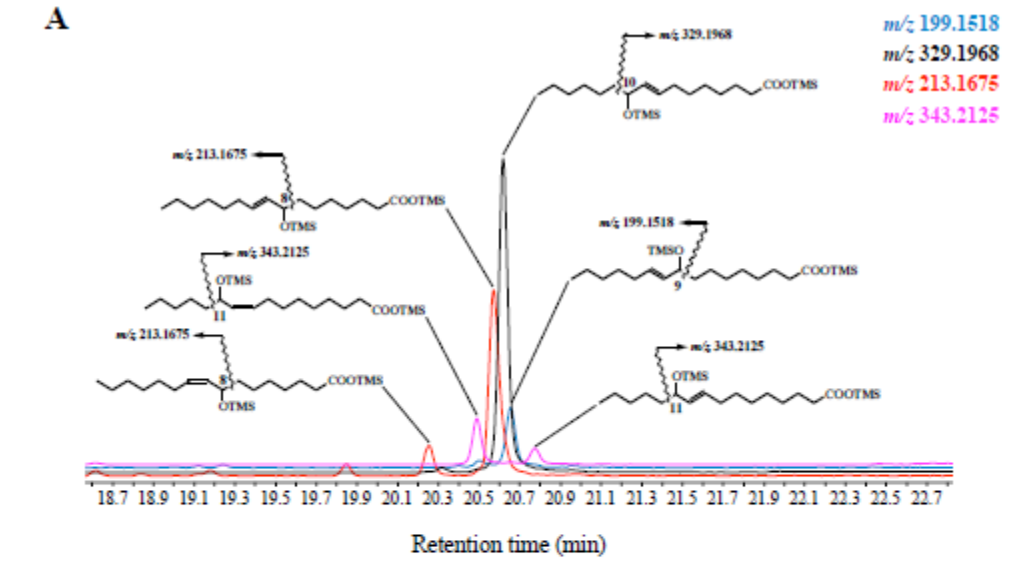


Fig. 3



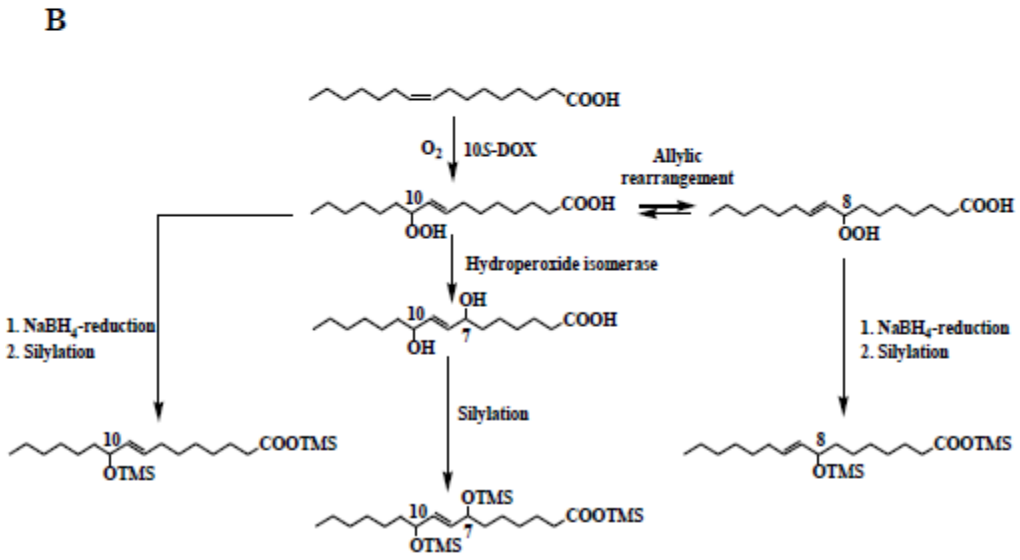


Fig. 4

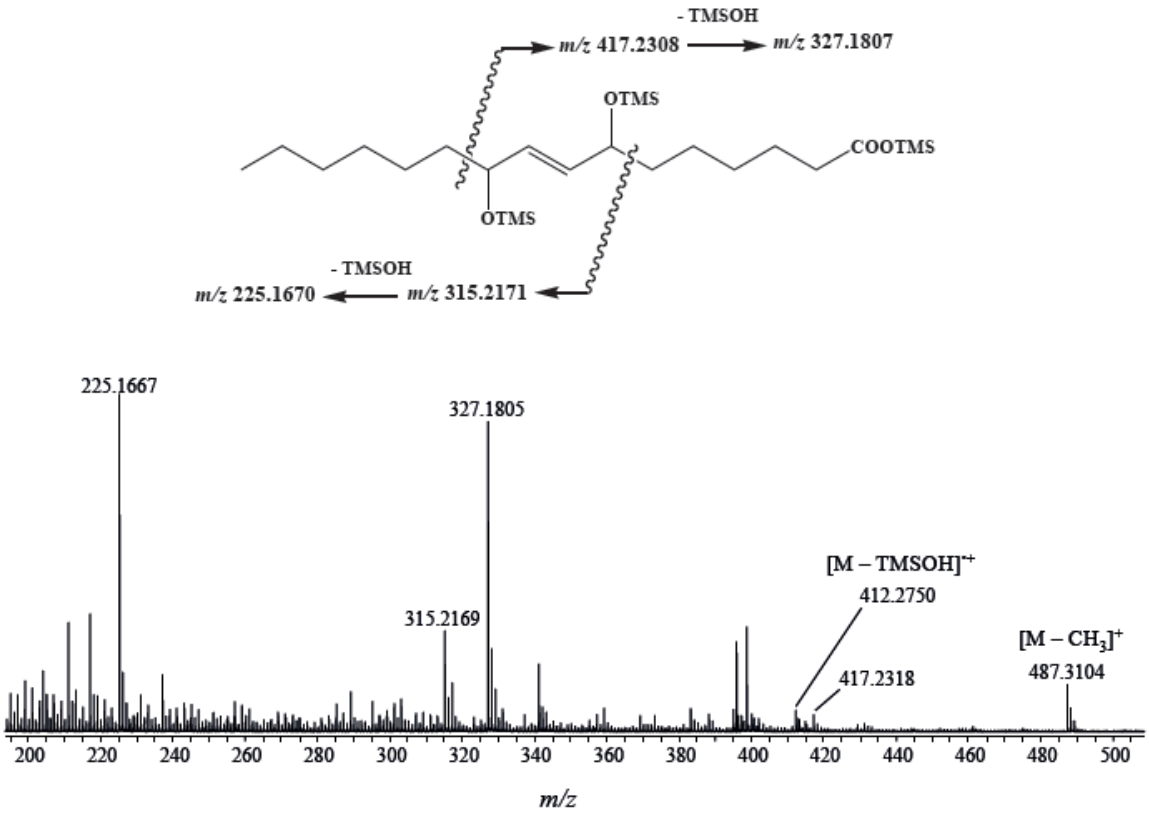


Fig. 5

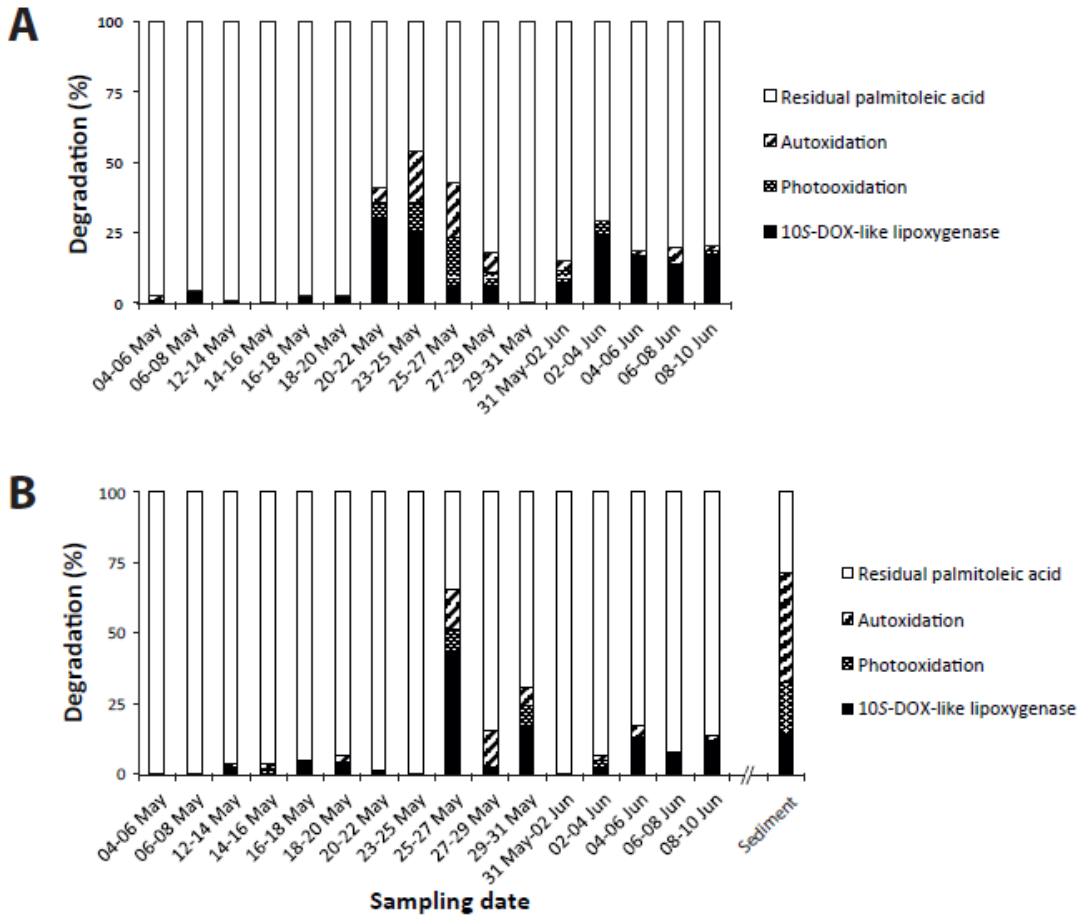


Fig. 6

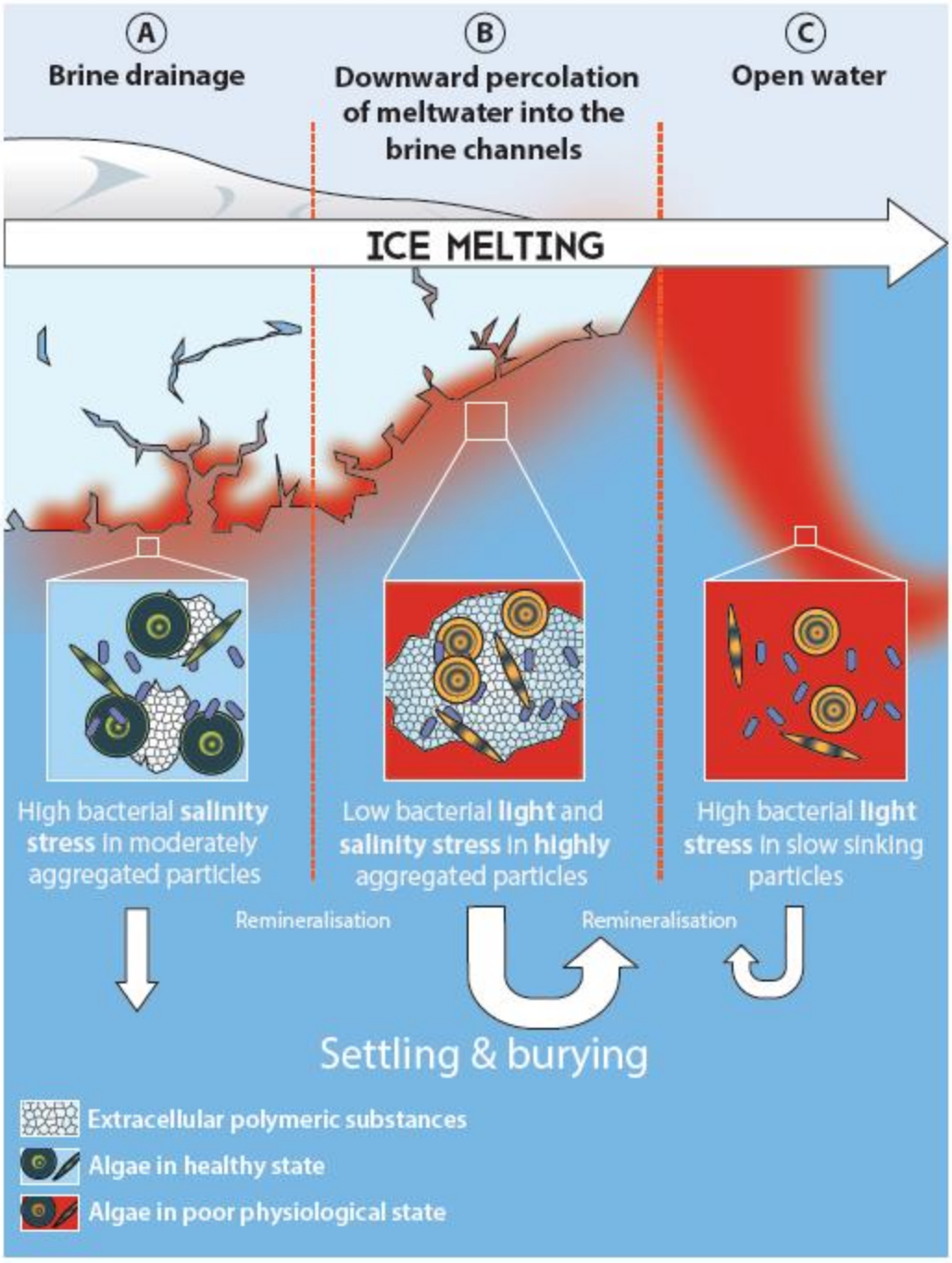


Fig. 7

Highlights

- Salinity and light bacterial stresses are temporally decoupled during ice melting
- 10*S*-DOX and CTI activity, two relevant biomarkers of bacterial salinity stress
- Seafloor sediment organic matter seems almost exclusively composed of sea ice biota