# Response of dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) cell quotas to salinity and temperature shifts in the sea-ice diatom *Fragilariopsis cylindrus*

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**Abstract**

Sea ice is an extreme environment known to host microbial communities which produce high concentrations of the metabolites dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO). These two compounds are involved in the cycling of the climate cooling gas dimethylsulfide (DMS). Despite decades of research, the drivers of these large concentrations in sea ice remain largely unknown. In this study, we use a cell culture approach to quantify for the first time the DMSP and DMSO cell quotas for the diatom *Fragilariopsis cylindrus* under combined shifts of temperature and salinity typically encountered in the sea ice brine habitat. In doing so, we investigate two suggested potential metabolic functions for DMSP and DMSO: osmoregulation and cryoprotection. We observed an increase of both DMSP:Chl-*a* and DMSO:Chl-*a* in multiple experiments with salinities of 75 and 100 at constant temperature, which suggest osmoregulation for both compounds in the diatom cell. Stronger salinity shifts to 150 induced lethal osmotic shock resulting in massive cell death. Interestingly, combining salinity shifts with temperature shifts (as low as -7.4°C) did not modify drastically the DMSP and DMSO cell quotas, which may indicate that the cryoprotectant function of DMSP and DMSO in our diatom cultures was not-relevant. Also, decreasing the salinity to 20 at constant temperature suggested no cellular adaptation in terms of DMSP and DMSO cell quotas.

**Keywords**

Sea ice – DMSP – DMSO – *Fragilariopsis cylindrus* – Salinity - Temperature

**Introduction**

The biogenic sulfur compounds dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) are key components in the cycling of the climate-active sulfur gas dimethylsulfide (DMS) in the marine environment (Liss et al. 1997; Stefels et al. 2007). DMS is an important precursor of sulfate aerosols which modify the atmosphere and cloud's albedo. A potential climate-cooling feedback loop involving oceanic DMS production, sulfate aerosols, and albedo was presented by Charlson et al. (1987). This hypothesis has since been heavily discussed in the scientific community (see Quinn and Bates (2011) and Green and Hatton (2014) for an overview). DMS could nevertheless be an essential component in the atmosphere of polar regions which are far from the anthropogenic aerosols emissions (Lana et al. 2012; Levasseur 2013; McCoy et al. 2015).

DMSP and DMSO are produced by a large diversity of aquatic micro- and macroalgae (Keller 1989; Stefels 2000; Simó and Vila-Costa 2006; Hatton and Wilson 2007; Borges and Champenois 2015, 2017). Intracellular functions of DMSP and DMSO are not well understood but both molecules have been suggested to act e.g. as osmoregulators (Dickson and Kirst 1986), cryoprotectants (Karsten et al. 1996) or antioxidants (Sunda et al. 2002). Intracellular DMSP and DMSO are released in the aquatic environment by multiple processes such as senescence, grazing, viral lysis or cell-controlled exudation

(all these processes are reviewed in Stefels et al. (2007)). After their release in the environment, the DMSP and DMSO pools are controlled by both algal and bacterial processes. The main pathways of degradation of DMSP by bacteria are first a combination of demethylation and demethiolation and second and enzymatic cleavage to DMS and acrylate (Kiene and Linn 2000; Yoch 2002). Cleavage of DMSP to DMS can also be carried out by algal DMSP-lyase (Stefels 2000; Niki et al. 2000; Steinke et al. 2002). Additionally, DMS can result from the algal and bacterial reduction of DMSO (Stefels et al. 2007; Spiese et al. 2009). Despite the volatility of DMS, its emissions to the atmosphere are largely reduced by bacterial consumption and photochemical oxidation occurring in the water environment (see Stefels et al. (2007) for an overview). Recently, a new intermediate sulfur compound, dimethylsulfoxonium propionate (DMSOP) was added to the DMS cycle by Thume et al. (2018). DMSOP appears to be produced by algae and bacteria and could have similar cellular functions as DMSP and DMSO.

In the polar ocean, the dynamic of dimethyled sulfur compounds is strongly influenced by the development of seasonal sea ice. Sea ice is a periodically permeable physical layer that influences the transfers of many gases ($CO_2$, $O_2$, $CH_4$, DMS, …) between the ocean and the atmosphere (Loose et al. 2011; Zhou et al. 2013, 2014a, b; Crabeck et al. 2014). Also, sea ice is considered as a biogeochemical reactor producing large quantities of molecules among which DMSP, DMSO and DMS (Tison et al. 2010). The transposition of the oceanic DMS cycle to sea ice is a real challenge considering the high disparity of living conditions between both environments. This is why, despite twenty years of field observations, the factors driving the DMS cycle in sea ice remain poorly understood (Kirst et al. 1991; Levasseur et al. 1994; Trevena and Jones 2006; Tison et al. 2010; Carnat et al. 2014, 2016). In particular, there is a lack of knowledge about the response of microalgal cells to the large seasonal environmental variations in their brine habitat. Given the metabolic functions suggested for DMSP and DMSO, it is very likely that the variations of brine abiotic conditions (e.g. temperature, salinity, light) greatly impact the intracellular concentration of DMSP and DMSO. Hence, experimental approaches based on microalgal cell cultures in controlled conditions are highly needed to improve our understanding of the DMS cycle in sea ice.

Lyon et al. (2016) were the first to measure the intracellular concentration of DMSP in the sea ice diatom *Fragilariopsis cylindrus* experimenting shifts of salinity. *F.cylindrus* is a pennate diatom living in both polar regions, and one of the key diatoms of the Southern Ocean growing both in the water column and the sea ice (Kang and Fryxell 1992). Diatoms as *F.cylindrus* are generally considered as low individual producers of DMSP compared to haptophytes and dinoflagellates (Keller 1989; Kirst et al. 1991). However, in sea-ice, diatoms dominate the algal assemblages and therefore still significantly contribute to the pool of DMSP by their large biomass accumulations (Trevena et al. 2000, 2003). Lyon et al. (2016) tested four shifts of salinity from 10 to 70 without temperature changes. In their study,

*F.cylindrus* was growing in environmental conditions relatively far from the most extreme variations observed in the sea ice brine habitat (in isolated winter brine pockets, salinity can exceed 200 and temperature, in equilibrium with salinity, can drop below -20°C (Thomas and Dieckmann 2002)). Curson et al. (2018) reproduced the same experimental approach as Lyon et al. (2016). They observed that both the DMSP production by *F.cylindrus* and a gene coding for its production (*DSYB)* were similarly regulated under an increase of salinity and nitrogen depletion.

DMSP and DMSO are part of a large pool of molecules that help the microalgae to deal with the consequences of environmental variations in brine channels. Indeed, those variations strongly impact many processes in the cells such as photoinhibition (Palmisano et al. 1987), decrease of photosynthetic activity (Ralph et al. 2005), modification of the photosystem II (Misra et al. 2001; Ralph et al. 2007; Krell et al. 2007; Petrou et al. 2011), formation of toxic ions in the cell and also disruptions of respiration, enzymatic activity and membrane permeability (Sudhir and Murthy 2004). DMSP and DMSO, as well as glycine betaine or homarine (Kirst 1996), are considered as high osmotic tolerance molecules. Other molecules like amino acids, polyols or disaccharides also contribute to preserve the cell from osmotic stress. Changes of the environmental conditions surrounding the cell also lead to the formation of polysaccharide-rich extracellular polymeric substances (EPS) (Krembs et al. 2002; Krembs and Deming 2008; Marx et al. 2009; Mishra and Jha 2009; Underwood et al. 2010; Krembs et al. 2011). EPS are produced by microorganisms living in various habitat (water, soils and biofilms) (Donot et al. 2012) and are typically observed in many diatoms (Hoagland et al. 1993). Genomic studies revealed the complexity of the system of production of EPS by *F.cylindrus* under simulation of ice growth (Aslam et al. 2012, 2018). This complexity appeared to be necessary to challenge the extreme and variable conditions in sea ice. The same studies also showed that increase of salinity and decrease of temperature induced a reduced growth but an increased EPS production suggesting the importance of EPS in the acclimation system in sea ice. As part of this acclimation system, DMSP and DMSO pathways of production could also be influenced by the combination of salinity and temperature changes (Carnat et al. 2018). DMSP and/or DMSO response to temperature changes could also suggest a cryoprotectant function in the cell as mentioned by Karsten et al. (1996). Antifreeze proteins (AFPs), sometimes referenced as ice-binding proteins (Janech et al. 2006; Krell et al. 2008), are other biogenic molecules produced by phytoplankton in the brine habitat (Bayer-Giraldi et al. 2010). AFPs have an impact on the thermal hysteresis (TH), the capacity of lowering the freezing point, and on the inhibition of the recrystallisation (RI). Hyperactive AFPs are able to modify the freezing point by 3 to 6 °C (Duman 2001). Nevertheless, the most hyperactive AFP recorded in *F.cylindrus* can only decrease the freezing point by 0.25°C, suggesting that this protein is not strongly involved in the TH (Uhlig et al. 2011). To influence the freezing point, cryoprotecting molecules have to accumulate in the cell or in EPS structures around the cell (Bayer-Giraldi et al. 2011). Hence, given that DMSP and DMSO accumulate in the cells, they

could impact the freezing point if they are synthesized as cryoprotectants. Based on mathematical estimations, Lee et al. (2001) showed however that intracellular concentrations of DMSO are largely inferior to the concentration necessary to decrease the freezing point by 2°C.

In this context, we tested the hypothesis that DMSP and DMSO could have a role of cryoprotectant and osmolyte in the sea-ice diatom *F.cylindrus*. The effect of temperature and salinity on DMSP and DMSO cellular content were investigated in laboratory batch cultures of *F.cylindrus.* The range of salinity (20 to 150) and temperature (4°C to -7.4°C) chosen for the experiment covers a large part of the natural variability measured in the brine along the seasons.

**Materials and methods**

Culture conditions

Axenic cultures of *F.cylindrus* were maintained in exponential growth at 4°C under a salinity of 34 (S34) and a 16:8 light:dark cycle (75 µE $m^{-2}$ $s^{-1}$) in a refrigerated incubator (RUMED® Rubarth Apparate GmbH). *F.cylindrus* was growing in filtered (0.2 µm Whatman® cellulose acetate filter) and sterilized Antarctic sea water from the McMurdo Sound enriched with sea salts (Instant Ocean®) and diluted with milliQ water to obtain S34. A F/2 medium (Guillard and Ryther 1962), vitamin and antibiotics (Penicillin-G and Streptomycin) were also added to the water to respectively create an excess in nutrients and prevent bacterial growth. The final concentrations of the main nutrients in the culture were 883 µM, 107 µM and 36.3 µM for nitrogen, silica and phosphorus respectively.

The protocol of Lyon et al. (2016) was adapted to include experiments with changes of temperature. Four salinities were studied in our experiments: salinity 20 (S20), 75 (S75), 100 (S100) and 150 (S150). The effect of salinity increase (compared to the control culture at S34) was tested with and without a decrease of temperature (-2.3°C, -3.9°C and -7.4°C respectively for S75, S100 and S150). Note that the decrease of salinity to 20 was tested without change of temperature. Salinities studied were obtained by dilution of cultures at S34 and 4°C with milliQ water or saltier solution respectively for decreasing or increasing salinity. For each salinity studied, a 1L-culture of *F.cylindrus* in exponential growth at S34 and 4°C was divided into three sub-cultures. The first sub-culture was dedicated to a shift of salinity alone. The second underwent the same shift of salinity combined with a shift of temperature while the third remained at 4°C and S34 and was considered as a control culture.

To obtain S20, we diluted three times a fraction of a 1L-culture at S34 and 4°C with milliQ water over 8 hours, shifting the culture by 5 salinity units per dilution. To increase the salinity to 75, 100 or 150, we mixed a fraction of the 1L-culture with a saltier solution (100, 120 or 210 respectively) over 8 hours. These increases of salinity were performed in three steps shifting the salinity by +14 (S75), +22 (S100) and +39 (S150). In addition, the subculture also dedicated to a change of temperature was put in a cooling alcohol bath (PolyScience®) designed to test negative temperatures. In parallel, the associated

control culture was mixed over 8 hours with a quantity of sea water at 4°C and at S34 similar to the volume of milliQ water or salty solution used to reach the salinity studied, in order to simulate the same dilution of biomass in the same period. After dilution, all cultures were enriched with fresh F/2 medium to ensure non-limiting nutrients conditions during the tests.

This experimental setup resulted in 11 cultures including four replicates of the control culture (S34 and 4°C) and 7 experiments of changes of salinity and/or temperature (only one replicate by change). These 11 cultures were sampled at $T_0$ and after 24 h, 48 h, 72 h according to Lyon et al. (2016). We also decided to sample after 9 days to get an overview of the longer-term impact of tested conditions. Sampling of the cultures targeted the cell density, the carbon biomass and the concentration in Chlorophyll-*a* (Chl-*a*), DMSP and DMSO. Chl-*a*, DMSP and DMSO were sampled as triplicates in each culture (i.e. technical replicates).

Analyses

Biological properties

Samples of algal cultures were kept in Lugol-glutaraldehyde solution (1% v:v) for cell measurements. Cell density was determined by inverted microscopy following the Uthermöhl method (Hasle 1978). The inverted microscope was also used to measure cell size and determine specific biovolumes (Hillebrand et al. 1999). The biovolume was then converted in carbon biomass using the equations of Menden-Deuer et Lessard (2000). A constant carbon biomass of 3.2 ± 2.2 pgC $cell^{-1}$ was assumed for *F.cylindrus* based on multiple cell measurements (n = 1040 cells).

A volume between 15 and 25 mL (depending on the growth stage of the algae) was filtered (glass microfibers GF/F filters 25mm, Whatman®) to determine the Chl-*a* concentration. Chl-*a* was extracted with 90% acetone and measured with a Kontrom SFM25 fluorimeter (Holm-Hansen et al. 1965). A solution of spinach chlorophyll (1000 µg $L^{-1}$) was used to create the calibration standards.

Sulfur compounds quantification

Each algae culture was sampled to obtain three technical replicates of particulate DMSP and DMSO (referred as $DMSP_p$ and $DMSO_p$ in this study). Both were obtained by filtration of 5 mL of culture through a grilled glass microfibers GF/F filter 25 mm (Whatman®). After sampling, filters were stored in grilled vials and immersed in 3 mL of milliQ water. Samples were then bubbled in a microwave oven for 9 seconds directly after the sampling to inhibit the DMSP-lyase activity that converts DMSP in DMS (Kinsey and Kieber 2016). Before storage at 4°C, the samples were also acidified with $H_2SO_4$ 50% to stop all further biological activities. All the samples were stored in previously grilled glass vials and closed with a cap with a butyl/PTFE septum.

All the samples were initially purged for 20 min before analysis to eliminate residual DMS in the vial. DMSP was then measured after transformation in DMS by an alkaline hydrolysis with NaOH pellets at

4°C in the dark during 24h (Dacey and Blough 1987). DMSO was measured after conversion to DMS by $TiCl_3$ in another replicate (Kiene and Gerard 1994; Deschaseaux et al. 2014). Resulting DMS from both chemical reactions was analyzed by a gas chromatograph associated with a purge-and-trap system (Carnat et al. 2014). The sample was bubbled with pure helium (99.999%) to purge the DMS (flow rate = 25 mL $min^{-1}$). DMS then flew in a -30°C cold bath to trap residual water vapor (Andreae and Barnard 1984). Finally, DMS was trapped in a PTFE loop (1/8" OD) immersed in liquid nitrogen (-196°C). At the end of the purge step, the PTFE loop was transferred in boiling water and DMS was injected in the gas chromatograph (GC). The GC used was a Trace GC Interscience® equipped with a flame photometric detector (FPD) and Micropacked RTX Sulfur column (1m x 0.75 mm ID). The temperature of the FPD was fixed at 250°C and $H_2$, dry air and makeup gas $N_2$ flows at 50 mL $min^{-1}$, 60 mL $min^{-1}$ and 60 mL $min^{-1}$ respectively. The cycle of temperature of the GC oven started at 60°C to reach 150°C at a rate of 30°C $min^{-1}$. Then the oven was kept at 150°C for 3 min and then cooled down to 60°C. We also used a second GC (Agilent®7890A) equipped with a dual FPD (sulfur and phosphorus filter) and a sulfur-specific capillary column (Agilent J&W®DB-A, 30m x 0.32 mm ID) parametrized as the first GC. Both GC were calibrated using DMS standards (pure DMS >99%, Merck) from 0.015 to 3 nmol in 3 mL. From those standards, we obtained linear regressions ($R^2 > 0.99$) between the square root of peak areas and the number of nanomoles of DMS in 3 mL.

Statistical analysis and data treatment

Data representing our control (S34 and 4°C) is presented as a mean of four biological replicates. All salinity and temperature treatment data were non-replicated and therefore are presented as a mean of technical replicate measurements.

No statistical methods were conducted to compare responses to changes in salinity and temperature due to lack of biological replication. Instead, all treatment responses are reported as trends relative to the control.

**Results**

Changes observed in the control cultures (constant salinity : S34, constant temperature : T = 4°C)

The values show in this section correspond to the mean of the values observed for the four replicates of the control culture. These mean values are further used as the control values for our salinity and temperature experiments in the following section and are shown as green lines in **Fig.*1*** and **Fig.*2***.

Chl-*a* in the control culture increased over 9 days, from 19.2 ± 8.2 to 59.5 ± 19.1 µg $L^{-1}$ (**Fig.*1*a** to **c**). $DMSP_p$ and $DMSO_p$ also increased during the same period, from 24.1 ± 8.9 to 82.4 ± 36.6 nM and from 23.5 ± 7.0 to 78.3 ± 28.1 nM respectively (**Fig.*1*d** to **i**). $DMSP_p$:Chl-*a* (1.2-1.5 mmolS gChl-$a^{-1}$) and $DMSO_p$:Chl-*a* (1.3-1.5 mmolS gChl-$a^{-1}$) ratios in the control culture were relatively similar and showed very little changes over time (**Fig.*2*a** to **f**).

In like fashion, intracellular concentrations ($DMSP_p$ and $DMSO_p$ concentration with respect to the cell volume) remained relatively constant during the 9-days experiment (in average 13.8 mmol $L_{cell}^{-1}$ and 12.9 mmol $L_{cell}^{-1}$ for $DMSP_p$ and $DMSO_p$ respectively) (based on **Fig.*1*** and Online resource #1). Finally, the $DMSP_p$:$DMSO_p$ ratio in the control culture was relatively close to 1 during the whole experiment (between 0.9 and 1.1, **Fig.2g** to **i**).

Changes observed following an increase in salinity (at constant temperature: T = 4°C)

At S75, the Chl-*a* concentration was lower than the control (S34) but remained constant over the duration of the experiment (11.7-13.8 µg $L^{-1}$, **Fig.1a**). $DMSP_p$ and $DMSO_p$ concentrations increased at S75 to reach, on day 9, concentrations (66.5 ± 5.3 nM and 53.7 ± 12.2 nM respectively), that were not much different from the control culture values on day 9 (**Fig.1d** and **g**).

At higher salinities, the Chl-*a* concentration was lower than at S34, and decreased to near-0 after 9 days at S100 and S150 (**Fig.*1*a**). This suggested an increase in mortality when the salinity increased. At S100, $DMSP_p$ increased similarly to the control during the 3 first days of the experiment before decreasing between day 3 and day 9 (**Fig.*1*d**), while $DMSO_p$ remained constant during the 9-day experiment (**Fig.1g**). At S150, $DMSP_p$ and $DMSO_p$ were lower than the control values, and decreased to very low concentrations (3.1 and 6.0 nM respectively) after 9 days (**Fig.1d** and **g**).

As a result, the $DMSP_p$:Chl-*a* ratio increased and was higher than the control value at both S75 and S100. Furthermore, this increase was higher at S75 (5.7 mmolS gChl-$a^{-1}$) than at S100 (4.5 mmolS gChl-$a^{-1}$) (**Fig.*2*a**). Given the rapid decrease of both Chl-*a*, $DMSP_p$ and $DMSO_p$ to extremely low values at S150, we considered that the ratios in this condition were not reliable. They are therefore not shown in **Fig.*2***. We observed a higher increase of $DMSO_p$:Chl-*a* at S100 than at S75 with a value of 8.8 mmolS gChl-$a^{-1}$ recorded at S100 after 9 days (**Fig.*2*d**). Finally, the $DMSP_p$:$DMSO_p$ ratio was higher than the control value at S75 (0.9-1.4) and lower than the control value at S100 (0.5-1.0) (**Fig.*2*g**).

Changes observed following an increase in salinity associated with a decrease in temperature

The observations made were relatively similar with and without a decrease of temperature associated to the increase of salinity. Chl-*a* remained constant (9.5-15.3 µg $L^{-1}$) at S75 and a temperature of -2.3°C (**Fig.*1*b**), but decreased with time for the two other tested conditions, with the strongest decrease observed at S150 and a temperature of -7.4°C (**Fig.1b**).

$DMSP_p$ increased after 9 days at S75, showing a similar trend as in the control culture (**Fig.*1*e**). At S100 and S150, $DMSP_p$ remained constant during the 3 first days before decreasing. After 9 days at S150 and -7.4°C, $DMSP_p$ strongly decreased down to 4.5 nM (**Fig.*1*e**). $DMSO_p$ increased at S75, reachingvalues of 48.5 nM after 9 days (**Fig.*1*h**). At S100, $DMSO_p$ did not changed from day 0 to day 9 (**Fig.*1*h**) and, at S150, after a maximum of 32 nM at day 2, $DMSO_p$ decreased to 8.4 nM after 9 days (**Fig.*1*h**).

The $DMSP_p$:Chl-*a* ratio tripled in 9 days at S75 and a temperature of -2.3°C, reaching 6.2 mmolS gChl-$a^{-1}$ (**Fig.2b**). At S100 and a temperature of -3.9°C, this ratio reached a constant values (around 3 mmolS gChl-$a^{-1}$) after 2 days which is three times higher than the control value (**Fig.2b**). For the same reason as in the experiments where only salinity was modified, we decided not to show the ratio for the experiment at S150 and a temperature of -7.4°C. The $DMSO_p$:Chl-*a* ratio at S75 and S100 increased in a similar way to reach 3.2 and 3.5 mmolS gChl-$a^{-1}$ after 9 days respectively (**Fig.2e**). The $DMSP_p$:$DMSO_p$ ratio was up to 2-times the control value at S75 (1.0-2.0) and approximates the control at S100 (0.8-1.2) (**Fig.*2*h**).

Changes observed following a decrease in salinity (at constant temperature, T = 4°C)

At S20, the Chl-*a* concentration increased in a similar way to the control culture, reaching 53.5 µg $L^{-1}$ after 9 days (**Fig.*1*c**). Both $DMSP_p$ and $DMSO_p$ increased faster than in the control culture to reach 64.6 nM and 50.7 nM after 3 days. Then, $DMSP_p$ and $DMSO_p$ decreased from day 3 to day 9, but their values were not very different from the control (**Fig.*1*f** and **i**).

The $DMSP_p$:Chl-*a*, $DMSO_p$:Chl-*a* and $DMSP_p$:$DMSO_p$ showed similar trends than the control culture (**Fig.2c**, **f** and **i**).

## Discussion

DMSP and DMSO in polar oceanic conditions (control cultures)

The polar diatom *F. cylindrus* should be physiologically adapted to the control conditions tested in this study, which are close to the polar oceanic conditions in terms of temperature and salinity. Our observations are consistent with previous experiments which showed no alteration of the *F.cylindrus* growth in those conditions which are used as stock conditions (Krell et al. 2008; Bayer-Giraldi et al. 2011; Lyon et al. 2016; Paajanen et al. 2017). The growth rate at S34 and 4°C in this study is similar to Lyon et al. (2016) (0.22 doubling $day^{-1}$ in our 9-day study versus 0.25 doubling $day^{-1}$ over 7 days, data not shown).

The production of DMSP and DMSO is species-specific and inside the diatom community, some are higher producers of DMSP than *F.cylindrus* (Keller et al. 1999; Spielmeyer and Pohnert 2012) and others lower producers (Hatton and Wilson 2007; Bucciarelli et al. 2013), with community intracellular concentrations ranging from 0.1 to 39.3 mmol $L_{cell}^{-1}$. Intracellular $DMSP_p$ measured for *F.cylindrus* in our experiments (13.8 mmol $L_{cell}^{-1}$) was therefore in the lower part of the range measured for diatoms. The $DMSP_p$:Chl-*a* ratio of 1.4 mmolS gChl-$a^{-1}$ obtained in this study was in the range of the $DMSP_p$:Chl-*a* ratio of 4 ± 6 mmolS gChl-$a^{-1}$ recorded for diatoms in polar oceanic conditions (Keller 1989, Stefels et al. 2007). Similar $DMSP_p$:Chl-*a* (1.9 ± 2.9 mmolS gChl-$a^{-1}$, Lee et al. (2001)) but lower $DMSO_p$:Chl-*a* ratio (0.2 ± 0.5 mmolS gChl-$a^{-1}$, Lee et al. (2001)) were recorded in bottom ice dominated by by *F.cylindrus* and other diatoms. Our experiments conducted at S34 showed similar trends as Lyon et al. (2016) with

an increase of both Chl-*a* and $DMSP_p$ and a constant $DMSP_p$:Chl-*a* ratio over the 9 days (**Fig.3**). Constant $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* ratios measured at S34 and 4°C suggest that the increase of $DMSP_p$ and $DMSO_p$ could be exclusively correlated to the growth of the algal population. Also, the same proportion of $DMSP_p$ and $DMSO_p$ ($DMSP_p$:$DMSO_p$ close to 1) observed for this study suggests equal importance for both compounds in the studied diatom. This is somewhat surprising because, except for some measurements in coastal areas or in the Antarctic Peninsula (Simó and Vila-Costa 2006), higher $DMSP_p$ than $DMSO_p$ have generally been reported in sea ice and waters worldwide (Lee et al. 2001; Simó and Vila-Costa 2006; Carnat et al. 2016), resulting in $DMSP_p$:$DMSO_p$ ratio exceeding 10. This large difference in $DMSP_p$:$DMSO_p$ ratio could partially be explained by differences in methodology between the studies (e.g. overestimation or underestimation of DMSO depending on the applied reducing protocol, see Deschaseaux et al. (2014)). Also, ratios measured in the natural environment are very likely influenced by a wide range of processes that are not present in controlled experiments.

DMSP and DMSO in response to salinity shifts

At S75, measured Chl-*a* concentration was lower than the control but remained at a constant level during the 9-days experiment (also shown by cell density evolution, Online resource #1) . This means that cultures of *F. cylindrus* are not able to grow at S75 but are able to maintain. Diatoms, and in particular *F.cylindrus*, appear to be more tolerant to changes of salinity than other species like chlorophytes or dinoflagellates (Ryan et al. 2004; Yang et al. 2011; Søgaard et al. 2011; Petrou et al. 2011). This tolerance suggests adaptations to extreme conditions, including synthesis of molecules to avoid salinity stress. Measurements of $DMSP_p$ and $DMSO_p$ at S75 revealed that both compounds increased with time and that the $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* ratios showed an increase of respectively 4 and 3 times compared to the control culture. Despite quantitatively lower ratios, highlighting the need of additional biological replicates to refine the DSC cell quotas, the increasing trend observed in our DMSP cell quotas is similar to the results obtained by Lyon et al. (2016) (**Fig.3**) supporting the osmoprotectant function assumed for DMSP and DMSO.

Quite different results are observed for the higher salinities. At S100, the Chl-*a* decreased over the 9 days and the $DMSP_p$ concentration was lower than the one measured at S75. However, we recorded a $DMSP_p$:Chl-*a* ratio still 3 times higher than the control culture. At S100, *F.cylindrus* thus appears to be more affected by salinity condition than at S75 although it still manages to maintain. This is consistent with the results of Søgaard et al. (2011) who considered that this diatom was at its limit of resistance to salinity at S100. This resistance up to S100 could be attributed to the synthesis of DMSP and DMSO by the diatom. The observed positive trends of the $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* ratios must nevertheless be taken with caution, given the fact that the ratio increase mainly results from the decrease of Chl-*a* and not from an increase of $DMSP_p$ or $DMSO_p$. However, the $DMSP_p$:$DMSO_p$ ratio of

0.5 observed at S100 could suggest that DMSO is more useful for the cell than DMSP as an osmolyte or in another cellular function. An alternative explanation could be the emergence of important oxidative stress at S100 in addition to osmotic stress. Indeed, salinity stress appears to induce the production of reactive oxygen species (ROS) in the chloroplast, which are responsible of oxidative damages in the cell (reviewed in Mittler 2002). An antioxidant function has been proposed for DMSP (Sunda et al. 2002). DMSP is known to react with ROS to produce DMSO which can accumulate in the cell (Simó et al. 1998, 2000; Lee and De Mora 1999). This could explain the higher concentration of $DMSO_p$ observed at S100 relative to $DMSP_p$ concentration, showed by the decrease of the $DMSP_p$:$DMSO_p$ ratio. Furthermore, DMSP was recently localized in high quantities in the chloroplast of a dinoflagellate (Raina et al. 2017), which could also be the case with diatoms. Similarly, the gene *DSYB*, coding for an enzyme involved in the production of DMSP, showed high transcription levels in chloroplast and mitochondria of the Haptophyta *Prymnesium parvum* (Curson et al. 2018).

At salinities higher than S100, the mechanisms of protection against an osmotic shock might become ineffective. Indeed, we observed a decrease of Chl-*a,* carbon and cell density (Online resource #1) that all converged to negligible amounts at the end of the 9-days experiment. $DMSP_p$ and $DMSO_p$ concentrations also drop to very low values. This could mean that the osmoregulatory function of DMSP and DMSO in *F.cylindrus* is inefficient at those high salinities and/or that the algal metabolism is damaged by the environmental conditions.

In the present study, the growth of *F.cylindrus* is similar at S20 and S34 as shown by the Chl-*a* concentration. This suggests that the growth of the diatom is not impacted at S20 which is consistent with studies that reported a higher tolerance from sea ice algae to a decrease of salinity than to an increase of salinity (Bates and Cota 1986; Søgaard et al. 2011). When the salinity decreases, the osmolytes are in excess and should be evacuated from the cells (Kirst 1996). If DMSP and DMSO are supposed to act as osmolytes in the diatom, it is possible, therefore, that at S20, $DMSP_p$ and $DMSO_p$ decrease. This decrease could be due to a decrease of production, an excretion through the cellular membrane and/or a recycling by the metabolism as proposed by Lyon et al. (2016). These authors observed a gradual decrease of $DMSP_p$ at S20 which had no effects on the growth and was not correlated to an increase of dissolved DMSP. They suggested that this intracellular DMSP was recycled by the cell in other compounds, without revealing the identity of those compounds. They also suggested that when the salinity decrease is higher, the recycling is too slow and the only fast way to eliminate DMSP is the excretion in the environment which is supported by a large increase of dissolved DMSP and a high percentage of permeable cells at S10. Results obtained at S20 in the present experiment were close to the control culture regarding DMSP and DMSO cell quotas. Therefore, unlike Lyon et al. (2016), we suppose that the conditions experienced by *F.cylindrus* at S20 were not strong enough to require the excretion and/or the recycling of $DMSP_p$ and $DMSO_p$.

DMSP and DMSO in response to covariation of salinity and temperature

Previous experiments have shown that polar diatoms can grow exponentially at temperatures as cold as -4 or -6°C (Aletsee and Jahnke 1992). In our study, cultures of *F. cylindrus* showed maintenance at S100 and temperatures as low as -3.9°C in the laboratory. At S150 (corresponding to a colder temperature of -7.4°C), *F. cylindrus* showed more difficulties to survive. We hypothesize that DMSP and DMSO could be involved, as osmolyte and cryoprotectant, in the surviving of the diatom until S100 and a temperature of -3.9°C. Covariation of salinity and temperature changes showed surprising results in terms of DMSP and DMSO cell quotas. Indeed, we observed similar trends for $DMSP_p$ and $DMSO_p$ concentrations with or without changes of temperature suggesting no additional effect of temperature on $DMSP_p$ and $DMSO_p$. Lee et al. (2001) already concluded about a non-cryoprotectant function of DMSO. Note, however, that the temperatures tested were warmer than the temperature of the freezing point for each salinity tested. Thus, lower temperatures closer to the freezing point might have a stronger impact on the DMSP and DMSO cell quotas.

This study aimed to assess the impact of salinity and temperature shifts, as experienced by polar diatoms in sea ice brines, on the DMSP and DMSO cell quotas. Controlled experiments conducted with the sea ice diatom *F.cylindrus* showed an increase of $DMSP_p$ and $DMSO_p$ concentrations with increasing salinity up to 100. These results bring observational support to the osmoregulation function proposed for both DMSP and DMSO, with both compounds helping polar diatoms to resist to high salinity increases. Additionally, the covariation test with both increase of salinity and decrease of temperature leads us to minimize the potential cryoprotectant function of DMSP and DMSO in *F.cylindrus*. Indeed, the covariation of salinity and temperature and the variation of salinity only showed globally similar trends along the experiments. The experiment with the decrease of salinity to 20 showed no difference with the control culture in terms of DMSP and DMSO cell quotas suggesting no specific acclimation of *F. cylindrus* to this decrease of salinity.

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**Compliance with Ethical Standards**

The authors state that there is no conflict of interest

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**Figure captions**

**Fig.1** Changes of Chlorophyll-a (Chl-*a*) and particulate DMSP ($DMSP_p$) and DMSO ($DMSO_p$) concentrations for three sets of 9-days experiments conducted on *F.cylindrus*: increase of salinity (S) at constant temperature (T) (a, d, g), increase of salinity associated with a decrease of temperature (b, e, h) and decrease of salinity at constant temperature (c, f, i). For each, the control culture is the green line at S = 34, T = 4°C. Shifts of salinity to S20, S75, S100 and S150 are represented by purple triangles red squares, yellow diamonds and blue dots respectively. The control culture is the mean of 4 biological replicates, and the global standard deviation is based on the standard deviation calculated in each replicate. Note that the standard deviation can be inferior to the symbol thickness. DMSP and DMSO concentrations are presented as the number of moles of sulfur by liter of culture.

**Fig.2** Changes of ratios $DMSP_p$:Chl-a, $DMSO_p$:Chl-a and $DMSP_p$:$DMSO_p$ during three sets of 9-days experiments conducted on *F.cylindrus*: increase of salinity (S) at constant temperature (T) (a, d, g), increase of salinity associated with a decrease of temperature (b, e, h) and decrease of salinity at constant temperature (c, f, i). For each, the control culture is the green line at S = 34, T = 4°C. Shifts of salinity to S20, S75, S100 and S150 are represented by purple triangles, red squares, yellow diamonds and blue dots respectively. Control culture is the means of 4 experiments.

**Fig.3** Chlorophyll-*a* (Chl-*a*), particulate DMSP ($DMSP_p$) and the ratio $DMSP_p$:Chl-*a* obtained from this study (S34 (green dots) and S75 (red dots)) and from Lyon et al. (2016) (S34 (half green dots) and S70 (half orange dots)).