Dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) cell quotas variations due to sea ice shifts of salinity and temperature in the Prymnesiophyceae *Phaeocystis antarctica*

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Abstract

The Southern Ocean, including the seasonal ice zone (SIZ), is a source of large sea-air fluxes of dimethylsulfide (DMS), a climate active gas involved in Earth cooling processes. In this area, the prymnesiophyte *Phaeocystis antarctica (P.antarctica)* is one of the main producers of dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO), two metabolites that are precursors of DMS. These algae are also present in sea ice and could contribute substantially to the high DMSP and DMSO concentrations observed in this habitat. DMSP and DMSO production in sea ice by *P.antarctica* could be promoted by it living in extreme environmental conditions. We designed cell culture experiments to test that hypothesis, focusing on the impact of shifts of temperature and salinity on the DMSP and DMSO cell quotas. Our experiments show an increase in DMSP,O cell quotas following shifts in salinity (34 to 75, at 4°C), suggesting a potential osmoregulator function for both DMSP and DMSO. Stronger salinity shifts (up to 100) directly impact cell growth and induce a crash of the cultures. Combining salinity (34 to 75) and temperature (4°C to -2.3°C) shifts induces higher increases of DMSP and DMSO cell quotas also suggesting an implication of both metabolites in a cryoprotectant system. Experimental cell quotas (including diatom *Fragilariopsis cylindrus* quotas from a previous study) are then used to reconstruct DMSP and DMSO profiles in sea ice based on the biomass and taxonomy. Finally, the complexity of the transposition of rates obtained in the experimental domain to the real world is discussed.

Keywords

Sea ice – DMSP – DMSO – Phaeocystis antarctica – Salinity – Temperature

Introduction

The prymnesiophyceae *Phaeocystis antarctica* is considered a major contributor (36-45%) to annual primary production in the coastal Antarctic waters (Schoemann et al. 2005; Smith et al. 2006). *P.antarctica* is particularly dominant during phytoplankton blooms occurring during and after the sea-ice seasonal melt in the late-spring and summer (Rousseau et al. 2007; Smith et al. 2003). *P.antarctica* influences the biochemical cycles in the Southern Ocean (Verity et al. 2007), in particular, the carbon (DiTullio et al. 2000; Schoemann et al. 2005) and sulfur (Stefels 2000; Stefels et al. 2007) cycles. In the sulfur cycle, *P.antarctica* is involved in the production of two dimethylated sulfur compounds (DSC), dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) (Kinsey and Kieber 2016), which are the biogenic precursors of the climate active gas dimethylsulfide (DMS) (Liss et al. 1997; Stefels et al. 2007). In the mid-1980s, it was determined that high concentrations of DMSP and DMS measured in and over the cold and temperate ocean waters could be linked to blooms of *Phaeocystis* sp. (Barnard et al. 1984; Baumann et al. 1994; Stefels et al. 1995; Crocker et al. 1995 and references therein). The importance of DMS was put forward by Charlson et al. (1987), who suggested a key role for DMS in climate-cooling feedback (the CLAW hypothesis). In the atmosphere, DMS is the biogenic precursor of sulfur aerosols which could counteract the effect of anthropic greenhouse gases on the Earth radiative balance. Their observations have since been challenged by modelling studies (see Quinn and Bates (2011) for an overview) which suggest a minor impact of DMS fluxes in the global loop proposed by the CLAW hypothesis. However, the atmospheric role of DMS (i.e. precursor of sulphate aerosols) remains relevant, especially over the polar regions (Levasseur 2013) which are far from anthropogenic aerosol emissions.

There is no consensus about the intracellular function of DMSP and DMSO in organisms producing or assimilating these demethylated sulfur compounds. DMSP was assumed to act as an osmoregulator (Dickson and Kirst 1986), a cryoprotectant (Karsten et al. 1996), an antioxidant (Sunda et al. 2002), grazers deterrent (Strom et al. 2003) and a ‘‘trash- can’’ for reduced compounds and excess energy (Stefels 2000). Similarly, Lee and De Mora (1999) highlighted the role of cryoprotectant and osmoregulator for DMSO but also the role of intracellular electrolyte modifier and antioxidant. Further, DMSO appears to be involved in an antioxidant cascade in the cell (Sunda et al. 2002). DMSP ends up in the surrounding aquatic environment after cell grazing, viral lysis, senescence or exudation episodes (Stefels et al. 2007). DMSO, for its part, easily diffuses through the cell membranes (Jacob and Wood 1967).

If a large part of the DMSP pool is degraded by bacterial demethylation and demethiolation processes, a small part (at most 17%) is converted into DMS by algal and bacterial processes (Archer et al. 2002; Kiene and Linn 2000; Yoch 2002). In particular, *P.antarctica* synthesizes enzymes DMSP-lyases that cleave the DMSP into DMS and acrylate (Del Valle et al. 2011). These lyases are linked to the cell

membrane and could be excreted from the cell through secretory vesicles as suggested by Orellana et al. (2011) and accumulate in the gelatinous matrix of *P.antarctica* colonies. The DMSO pool is also controlled by algal and bacterial processes and, through these, can be reduced to DMS (Stefels et al. 2007; Spiese et al. 2009). The fate of the DMS in the marine environment varied. A large portion is involved in bacterial processes while photooxidation and emissions to the atmosphere also occur (see Stefels et al. (2007) for a review).

Sea ice is a permeable layer that plays a role in the exchanges of many gases (CO_2, O_2, CH_4, DMS, …) between the ocean and the atmosphere (Loose et al. 2011; Zhou et al. 2013, 2014 a, b; Crabeck et al. 2014). Measurements of DSC were conducted in Antarctic sea ice for at least three decades (Carnat et al. 2014; 2016 for an overview). In the beginning, only DMS and DMSP were recorded but with the improvement of sulfur analytical techniques, DMSO has also been measured in sea ice samples (Hatton et al. 1994; Simó et al. 1996; Simó et al. 1998; Lee et al. 2001). These measurements revealed that DSC can be much higher than the concentration measured in oceanic waters and vary with time and space. Maximum concentrations of a few thousand nanomoles of DMSP (up to 5349 nM) and DMSO (up to 2097 nM) were recorded both in pack and fast ice (Carnat et al. 2016, 2014; Kirst et al. 1991; Tison et al. 2010). In polar oceanic regions, the DMS cycle is highly influenced by the presence of this annual sea ice cover which impacts the concentration, the production and the exchanges of DSC with the ocean and the atmosphere (Tison et al. 2010).

Also, sea ice is the host of high biomass which produces a large number of molecules including DMSP, DMSO and DMS (Tison et al. 2010). As part of this biomass, *P.antarctica* has been regularly observed in sea ice and could initiate the oceanic spring bloom when sea ice melt (Gibson et al. 1990; Garrison et al. 2003; Kennedy et al. 2012). In addition, sea ice is characterized by brine salinities that can exceed 200, temperatures that can drop below -18°C, light levels that can be extremely low (<5 µmol photon m^{-2} s^{-1}) and by the occurrence of nutrient gradients which are extreme for living organisms (Thomas and Dieckmann 2010). These extreme conditions strongly impact vital cellular processes such as photosynthesis, respiration, enzymatic activity or membrane permeability (Sudhir and Murthy 2004; Ralph et al. 2005, 2007; Petrou et al. 2011). Nevertheless, polar microalgae (such as *P.antarctica* or *F.cylindrus*, among others) are able to react to these stress conditions by producing numerous molecules which help to maintain the integrity of the cell. Among these, osmolytes such as glycine betaine, DMSP, DMSO; thermo-tolerants (antifreeze proteins, extracellular polymeric substances (EPS), DMSP, DMSO) or antioxidants (carotenoids, ascorbates, tocopherols, reduced glutathione, DMSP, DMSO and various antioxidant enzymes) were all detected in sea ice brine conditions (Kirst 1996; Zhang et al. 2005; Janech et al. 2006; Janknegt et al. 2008; Krell et al. 2008). For these reasons, the cycle of DSC and sea ice growth and decay are closely linked.

After decades of research, processes driving the DMS cycle are still not well constrained (Carnat et al. 2016, 2014; Kirst et al. 1991; Levasseur et al. 1994; Tison et al. 2010; Trevena and Jones 2006). Also, although the extreme environmental conditions in sea ice and the related metabolic functions of both DMSP and DMSO are established, the pathways of DSC production are only partly understood.

In the polar area, the cycle of DSC is strongly influenced by the diversity in physiology and biomass of the different phytoplankton groups. As an example, diatoms are known to be lower DSC producers than prymnesiophyceae (Keller 1989). Hence, it is important to test how variations of abiotic factors (salinity, temperature, light) impact the DSC pool for a large panel of species. Up to now, most of the research focusing on the impact of salinity on the DSC were conducted on diatoms. Yang et al. (2011), Kettles et al. (2014) and Lyon et al. (2016) have all observed a positive impact of an increase of salinity on the intracellular DMSP, respectively on the benthic diatom *Skeletonema costatum*, *Thalasiosiria pseudonana* and the polar diatom *F.cylindrus*. Diatoms are more easily studied in laboratory than prymnesiophyceae which present a more complex life cycle including free-living single cells and a colonial stage (Rousseau et al. 2007). Some experimental studies were however conducted on the prymnesiophyceae (Vairavamurthy et al. 1985; Stefels and Dijkhuizen 1996; Van Rijssel and Gieskes 2002) but were not focused on the extreme temperature and salinity occurring in the polar area.

In this study, we propose a cell culture approach based on *P.antarctica*. This approach is relatively new for the prymnesiophyceae. In controlled laboratory conditions, we address the impact of variations of temperature and salinity on the DMSP and DMSO cell quotas to test their supposed cryoprotectant and osmoregulator functions. The used range of temperature (4°C to -7.4°C) and salinity (20 to 150) is chosen to cover a large part of the seasonal variations experimented by the microalgae in their real environment (brines in sea ice).

Experimental

Culture conditions

Cultures of *P.antarctica* (CCMP1374) from the Roscoff Culture Collection (France) were maintained in exponential growth at 4°C under a salinity of 34 (S34) and a 16:8 light:dark cycle (100 µE m^{-2} s^{-1}) in a cooling incubator (FITOCLIMA S600, ARALAB®). The growth medium for algae was prepared using filtered (0.2µm Whatman® cellulose acetate filter) and sterilized Antarctic seawater at S34 from the Ross Sea enriched with F/2 medium and vitamins (B1, B12 and H) (Guillard and Ryther 1962) to create an excess in nutrients. The total concentrations of nitrogen, silica and phosphorus in the growth medium before algal inoculation were 883 µM, 107 µM and 36 µM, respectively. These were calculated from the F/2 medium protocol. A complex of antibiotics (Penicillin-G and Streptomycin) was also added to prevent bacterial development. Considering that F/2 medium contains iron (10 µM before algal inoculation), our experiments are not iron-depleted.

We tested four salinity levels on *P.antarctica* at constant temperature (4°C): 20 (S20), 75 (S75), 100 (S100) and 150 (S150). The three increases of salinity were also tested with a decrease of temperature (-2.3°C, -3.9°C and -7.4°C respectively for S75, S100 and S150). For each salinity experimented, we started by dividing a 1L culture of *P.antarctica* at exponential growth (S34 and 4°C) in three sub-cultures (**Supplementary material 1**). The first one, called control culture, remained at 4°C and S34. The second was diluted stepwise at 4°C with ultrapure water or with a high salinity solution to decrease (S20) or increase (S75, S100 and S150) the salinity. The third underwent the same shift of salinity (S75, S100 and S150) and was placed in a cooling alcohol bath to change the temperature (to -2.3°C, -3.9°C or -7.4°C).

Changes of salinity were obtained by means of three successive dilutions over 8 hours. The salinity was incrementally decreased by addition of ultrapure water (three salinity increments of 4.6). Conversely, a subculture was mixed three times with a salty solution (100, 120 or 210) to achieve S75, S100 and S150, respectively (salinity increment of +14, +22 and +39, respectively) (**Supplementary material 2**). The S34 control solution was also diluted with seawater of salinity S34 to mimic the dilution of the other treatments and related decrease of cell concentration. Thereby, we simulated an identic dilution of biomass during the same period between the three sub-cultures. Note also that fresh F/2 medium was added in all cultures to avoid a limitation of nutrients during the tests.

The 11 cultures obtained by this protocol (i.e. four replicates at S34 and 4°C and seven experiments of environmental variations with only one replicate for each) were studied over 9 days with sampling at T_0 and after 24h, 48h, 72h and 9 days. Cultures were sampled to follow up the concentration in Chlorophyll-*a*, DMSP and DMSO.

Analyses

Chlorophyll-a

Chl-*a* concentrations were obtained by filtering a volume between 15 mL and 25 mL of algal culture (glass microfibers GF/F filters 25 mm, Whatman®). Extraction of Chl-*a* was performed with acetone (90%) and Chl-*a* was measured with a Kontrom® SFM25 fluorimeter (Holm-Hansen et al. 1965). Standards used for the calibration were prepared from a solution of spinach Chlorophyll (1000 µg L^{-1}).

DMSP and DMSO analysis

The intracellular fractions of DMSP and DMSO, referred as particulate DMSP and DMSO ($DMSP_p$ and $DMSO_p$ respectively), were analysed for all the studied conditions. Preliminary experiments showed that *P.antarctica* produced an amount of $DMSP_p$ and $DMSO_p$ largely superior to the upper limit of detection (0.5 – 0.6 nmol per mL) of our gas chromatograph (GC, Agilent®7890A). Sampling consisted to filtrate 0.15 mL of culture mixed with 4.85 mL of water at the same salinity (factor 1:20), through a muffled filter (Glass microfibers GF/F filter 25 mm, Whatman®). This step was replicated three times for both $DMSP_p$ and $DMSO_p$ (i.e. technical replicates). Then, filters were stored in muffled vials in 3 mL

of ultrapure water. Directly after the sampling, the samples were bubbled in a microwave oven until boiling to prevent the activity of DMSP-lyases who are able to convert DMSP in DMS, especially in *Phaeocystis sp.* (Kinsey and Kieber 2016). After a last step of acidification with H_2SO_4 50% to prevent biological development, the samples were closed with a cap with a butyl/PTFE septum and stored at 4°C in the dark. Before analysis, samples were purged for 20 min to remove residual DMS in vials.

Analysis of $DMSP_p$ and $DMSO_p$ were performed by gas chromatography after respectively an alkaline hydrolysis to DMS (addition of NaOH pellets at 4°C in the dark for 24h (Dacey and Blough 1987)) and conversion to DMS with $TiCl_3$ (Deschaseaux et al. 2014; Kiene and Gerard 1994). After chemical reaction, each sample was connected to a purge-and-trap system (P&T) coupled with a GC (Carnat et al. 2014). The P&T consisted, first, in bubbling the sample with pure helium (99.999%) to purge the DMS (flow rate = 25 mL min^{-1}). Second, the purged DMS was going through a water vapour trap and was finally trapped in a PTFE loop (1/8" OD) immersed in liquid nitrogen (-196°C). After a purge of 20 minutes, the PTFE loop was transferred in boiling water and desorbed DMS was injected in the GC. We used an Agilent®7890A GC 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). The temperature of the FPD was maintained at 250°C and the flows of H_2, dry air and makeup gas (N_2) were at 50 mL min^{-1}, 60 mL min^{-1} and 60 mL min^{-1} respectively. Carrier gas was He. In the GC oven, the applied cycle of temperature started at 60°C and increased to 150°C with a rate of 30°C min^{-1}. The temperature was maintained at 150°C for 3 min before returned to 60°C. GC calibration was performed with DMS standards (pure DMS >99%, Merck) from 0.015 to 3 nmol in 3 mL. Number of nanomols of DMS in our samples were determined from the linear regression created from the square root of areas of standards peaks.

Statistical analysis and data treatment

The four sub-cultures conducted at S34 and at 4°C were grouped to obtain only one data set in these conditions called control culture. In this case, we averaged the biological parameters (Chl-*a*, $DMSP_p$ and $DMSO_p$) at each day of the sampling period. In each sub-culture, these parameters were themselves issued from an averaging of 2 to 5 measurements. Therefore, we use a weighed relation to calculate the standard deviation of the control culture:

$$\text{Standard deviation} = \sqrt{\frac{\sum_{i=1}^{4} n_i . STDV_i^2}{n_{tot}}},$$

where n_i and $STDV_i$ are respectively the number of observations (Chl-*a*, $DMSP_p$ or $DMSO_p$) for each sub-culture and the standard deviation of these observations for each sub-culture. n_{tot} is the total number of observations from the four sub-cultures.

No statistical analysis was conducted to compare the response of *P.antarctica* to salinity and/or temperature treatments due to a lack of biological replicates.

Estimation of natural DSC brines contents

In order to link experimental data to field observations, we attempted estimation reconstruction of the brines DSC concentrations using the specific DMSP and DMSO cell rates measured in this laboratory study (*P.antarctica*) and in Wittek et al. (2020) (*F.cylindrus*). We chose field locations where the biotic (taxonomy, biomass) and physico-chemical (temperature, salinity) data sets were available from sea ice layers where DSC were also recorded: the YROSIAE and ISPOL field campaigns. YROSIAE stations (3) sourced from Antarctic fast ice (McMurdo Sound) sampled in late spring 2011 (YRS1) and in early spring 2012 (YRS5 & 7) (Carnat et al. 2014). ISPOL stations (7) were sampled in Antarctic pack ice (western Weddell Sea) in summer 2004 (Tison et al. 2008, 2010).

In this simplistic approach, we postulated that the taxonomic composition in sea ice was restricted to two major groups: diatoms and flagellates (including *Phaeocystis* sp., dinoflagellates and other flagellates). Hence, for our calculation, we considered that the production of DMSP and DMSO by the group of diatoms could be estimated through the empirical relations from our experiment on *F.cylindrus* (Wittek et al. 2020) and the DSC production of flagellates by the *P.antarctica* empirical relations (this study).

In practice, Chl-*a* and taxonomic composition were first used to reconstruct the cell abundance in each group. Then brine salinity was used with our empirical DSC cell quotas to calculate DMSP and DMSO concentration that could be attributed to each group. Finally, calculated DMSP and DMSO concentrations from each group were combined and compared to measured DMSP and DMSO in sea ice. It should be noted that calculated DMSP and DMSO were particulate DSC ($DMSP_p$ and $DMSO_p$) while measured DSC were total DMSP and total DMSO ($DMSP_t$ and $DMSO_t$).

Results

P.antarctica cultures

Control culture (S34 at T = 4°C)

Chl-*a*, $DMSP_p$ and $DMSO_p$ measurements made on the four biological replicates at S34 and 4°C are shown in **Fig. *1*a** (colored symbols). Globally, the mean of these parameters increased over the 9 days experiment, but we noted some contrast between the four replicates. In particular, the 4th replicate showed the higher increases in Chl-*a*, $DMSP_p$ and $DMSO_p$ concentration which mainly occurred between day 3 and day 9. For this replicate, we measured $DMSP_p$ and $DMSO_p$ concentrations up to 12073 nM and 12192 nM, respectively.

We also computed the $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* ratios for these four biological replicates (**Fig. 1b**). These ratios showed no increase over the 9 days except for the $DMSP_p$:Chl-*a* ratio of the 3rd replicate which increases from 74.0 to 169.2 mmolS gChl-a^{-1}.

A mean evolution of Chl-*a*, $DMSP_p$, $DMSO_p$ and of the two ratios was then calculated (solid black lines in **Fig. *1***) and used as the control S34 and 4°C curves for our experiments shown in **Fig.2** and **Fig.3** (green lines)**Erreur ! Source du renvoi introuvable.**. Over the 9 days experiment, Chl-*a* (**Fig.2Erreur ! Source du renvoi introuvable.a** to **c**), $DMSP_p$ (**Fig.2Erreur ! Source du renvoi introuvable.d** to **f**) and $DMSO_p$ (**Fig.2Erreur ! Source du renvoi introuvable.g** to **i**) increased near-linearly. During the experiment, the Chl-*a*, DMSP and DMSO concentration increased by 3 to 4-fold (from 21.0 ± 10.2 to 68.6 ± 36.9 µg L^{-1} for Chl-*a*, from 1498.4 ± 746.9 to 6446.0 ± 2793.1 nM for DMSP and from 1697.1 ± 823.3 to 5303.4 ± 3348.4 nM for DMSO).

$DMSP_p$:Chl-*a* increased from day 0 to day 9 (from 75.7 ± 33.3 to 106.7 ± 45.0 mmolS gChl-a^{-1}, **Fig.3Erreur ! Source du renvoi introuvable.a** to **c**) while $DMSO_p$:Chl-*a* remained constant (mean = 80.6 mmolS gChl-a^{-1}, **Fig.3Erreur ! Source du renvoi introuvable.d** to **f**) during the whole experiment. The $DMSP_p$:$DMSO_p$ ratio (not shown in **Fig. *1***) slightly increased during 9 days and reached 1.4 (**Fig.3Erreur ! Source du renvoi introuvable.g** to **i**)

Salinity increase (S75, S100 and S150 at T = 4°C)

Results from the experiments conducted at S75, S100 and S150 at constant temperature are also presented in **Fig.2Erreur ! Source du renvoi introuvable.** and **Fig.3Erreur ! Source du renvoi introuvable.** (**a**, **d** and **g** for both graphs). Note that we have not plotted the $DMSP_p$:Chl-*a*, $DMSO_p$:Chl-*a* and $DMSP_p$:$DMSO_p$ ratios for the experiment at S150 (**Fig.3Erreur ! Source du renvoi introuvable.a**, **d** and **g**) due to the crash of the culture (i.e. Chl-*a* dropped to 0 after 9 days, **Fig.2Erreur ! Source du renvoi introuvable.a**).

At S75, Chl-*a* decreased with days to reach a value 7-fold lower than the control after 9 days (10.0 µg L^{-1}, **Fig.2Erreur ! Source du renvoi introuvable.a**). During the same period, $DMSP_p$ and $DMSO_p$ quickly reached a plateau around 1550 nM and 1150 nM respectively (**Fig.2Erreur ! Source du renvoi introuvable.d** and **g**). $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* ratios both increased up to 3-fold over the study period (from 47.3 mmolS gChl-a^{-1} to 162.3 mmolS gChl-a^{-1} and from 34.7 mmolS gChl-a^{-1} to 112.9 mmolS gChl-a^{-1}, **Fig.3Erreur ! Source du renvoi introuvable.a** and **d**). The $DMSP_p$:$DMSO_p$ ratio tended to stabilize around 1.4 after 9 days (**Fig.3Erreur ! Source du renvoi introuvable.g**).

When salinity increased to S100, Chl-*a* directly decreased after the shift of salinity to reach 0 at day 9 (**Fig.2Erreur ! Source du renvoi introuvable.a**). As Chl-*a*, $DMSP_p$ and $DMSO_p$ concentrations decreased over days. At day 9, measured values for $DMSP_p$ and $DMSO_p$ were under the limit of detection of the GC (**Fig.2Erreur ! Source du renvoi introuvable.d** and **g**). The $DMSP_p$:Chl-*a* ratio was lower than S34 and S75 for 3 days and decreased down to 0 at day 9 (**Fig.3Erreur ! Source du renvoi introuvable.a**). For the $DMSO_p$:Chl-*a* ratio, values were similar than S34 and S75 during the first 3 days and then fell

to 0 at day 9 (**Fig.3Erreur ! Source du renvoi introuvable.d**). The $DMSP_p$:$DMSO_p$ ratio decreased during 3 days and no data was available on day 9 (**Fig.3Erreur ! Source du renvoi introuvable.g**).

Lower values were observed at S150. The Chl-*a* also directly decreased after the salinity shift and already reached 0 after 3 days (**Fig.2Erreur ! Source du renvoi introuvable.a**). $DMSP_p$ concentrations measured were much lower than the control values and the other experiments values. Two values were available for $DMSP_p$, the others were under the limit of detection (0.03 nM) of the GC and were considered as 0 nM (**Fig.2Erreur ! Source du renvoi introuvable.d**). $DMSO_p$ concentrations were not plotted because they were at the limit of detection where the uncertainty is high.

Salinity increase and temperature decrease (S75, S100 and S150 at T = -2.3°C, -3.8°C and -7.4°C, respectively)

Data from experiments combining an increase of salinity with a decrease of temperature are shown in **Fig.2Erreur ! Source du renvoi introuvable.** and **Fig.3Erreur ! Source du renvoi introuvable.** (**b**, **e** and **h**). As in the previous section, we have not plotted the ratios for the experiment at S150 and a temperature of -7.4°C due to the collapse of the culture (**Fig.3Erreur ! Source du renvoi introuvable.b**, **e** and **h**).

At S75 and a temperature of -2.3°C, Chl-*a* slightly decreased after the shift of conditions and tended to stabilize between day 2 and day 9 with a Chl-*a* value 7-fold lower than the control at the end of the experiment (13.4 µg L^{-1}, **Fig.2Erreur ! Source du renvoi introuvable.b**). The $DMSP_p$ concentration increased less than the control experiment but nevertheless tripled over the 9-days experiment to reach 3140.6 nM at day 9 (**Fig.2Erreur ! Source du renvoi introuvable.e**). During the same period, the $DMSO_p$ concentration increased the first 2 days and, as observed for Chl-*a* concentration, reached a plateau around 1800 nM lower than the control (**Fig.2Erreur ! Source du renvoi introuvable.h**). Consequently, we observed an increase by 5-fold of the $DMSP_p$:Chl-*a* ratio which reached 234.1 mmolS gChl-a^{-1} at day 9 (**Fig.3Erreur ! Source du renvoi introuvable.b**) and an increase by 5-fold of the $DMSO_p$:Chl-*a* ratio the first 3 days followed by a decrease to 138.1 mmolS gChl-a^{-1} at day 9 (**Fig.3Erreur ! Source du renvoi introuvable.e**). Both ratios were higher than the control value after 9 days. The $DMSP_p$:$DMSO_p$ ratio showed a minimum after 2 days (0.4), due to a small decrease of $DMSP_p$ while the value was around 1.5 during the rest of the experiment, which was not far from the control at day 9 (**Fig.3Erreur ! Source du renvoi introuvable.h**).

Stronger conditions such as S100 and a temperature of -3.9°C showed a quick decrease of Chl-*a* to 3.3 µg L^{-1} which was lower than the control after 9 days (**Fig.2Erreur ! Source du renvoi introuvable.b**). In these conditions, $DMSP_p$ and $DMSO_p$ concentrations stayed constant during the 9 days experiment at lower values than the control (averages = 456.1 nM and 545.3 nM respectively, **Fig.2Erreur ! Source du renvoi introuvable.e** and **h**). The $DMSP_p$:Chl-*a* ratio increased the first 2 days and then maintains around 120 mmolS gChl-a^{-1} for the rest of the experiment (**Fig.3Erreur ! Source du renvoi**

introuvable.b). The $DMSO_p$:Chl-*a* ratio showed similar evolution and values than observed at S75 and a temperature of -2.3°C (**Fig.3Erreur ! Source du renvoi introuvable.e**). The $DMSP_p$:$DMSO_p$ ratio slowly decreased over the 9 days (**Fig.3Erreur ! Source du renvoi introuvable.h**).

Observations made for the experiment conducted at S150 were similar at a temperature of 4°C and -7.4°C. Indeed, the Chl-*a* concentration quickly fell down to low value (1 µg L^{-1}, **Fig.2Erreur ! Source du renvoi introuvable.b**). Also, besides the measure at T0, the $DMSP_p$ data were all lower than the limit of detection of the GC (**Fig.2Erreur ! Source du renvoi introuvable.e**). As previously mentioned, $DMSO_p$ measured at S150 were at the limit of detection and therefore not shown (see above).

Salinity decrease (S20 at 4°C)

At S20, Chl-*a* slightly decreased after the shift of salinity, and the concentration was almost 3-fold lower than the control after 9 days (**Fig.2Erreur ! Source du renvoi introuvable.c**). $DMSP_p$ and $DMSO_p$ slowly varied over the 9 days experiment and both stabilized around 3000 nM which was 2-fold lower than the control (**Fig.2Erreur ! Source du renvoi introuvable.f** and **i**). The $DMSP_p$:Chl-*a* ratio at S20 was similar to the control (average = 90.6 mmolS gChl-a^{-1}) while the $DMSO_p$:Chl-*a* ratio increased higher but remains in the standard deviation of the control with a value of 127.8 mmolS gChl-a^{-1} after 9 days (**Fig.3Erreur ! Source du renvoi introuvable.c** and **f**). The $DMSP_p$:$DMSO_p$ ratio stayed around 1 (value inferior to the control at 1.4) along the experiment (**Fig.3Erreur ! Source du renvoi introuvable.i**).

Estimation of natural DSC brines contents

Empirical relations

From this study (**Fig.3**) and (Wittek et al. 2020), we computed empiric relations between $DMSP_p$ and $DMSO_p$ cell quotas and brine salinity for *P.antarctica* and *F.cylindrus* (**Fig.4**) using data from experiments conducted at S20 and 4°C, S34 and 4°C and S75 and -2.4°C. Experiments at S100 and S150 are not considered because of the growth limitation observed for both algae under these conditions. Also, we preferred the experiment at S75 and -2.4°C to the one at S75 and 4°C to obtain the conditions closest to those encountered by algae *in situ*. Whatever the temperature, results obtained at S75 were similar for both algae (**Fig.3** and Wittek et al. (2020)).

The DMSP and DMSO cell contents were much higher in *P.antarctica* than in *F.cylindrus* (**Fig.4**). Typically, for the same concentration of Chl-*a* in our experiments, $DMSP_p$ and $DMSO_p$ were 1 to 2 order of magnitude higher in the prymnesiophyceae. However, when salinity increased from S34 to S75, both DMSP and DMSO cell quotas showed a higher increase for *F.cylindrus* (multiplied by 4.0 and 2.3, respectively) than for *P.antarctica* (multiplied by 2.2 and 1.7, respectively). This could support the idea that a higher concentration in DSC provides an advantage to *P.antarctica* when surrounding conditions suddenly vary, while in order to deal with the stress, *F.cylindrus* needs to quickly increase its DSC content. Impact of the decrease of salinity to S20 is less clear for both species and both DSCs.

Reconstructed profiles

DMSP and DMSO profiles reconstructed as described in the previous section are plotted in **Fig. *5*** (YROSIAE)**Erreur ! Source du renvoi introuvable.** and **Fig. 6** (ISPOL). Two alternative calculations are presented: one only based on the diatom fraction (squares) and the other combining diatoms and flagellates (inverted triangles). Field data (i.e. brine salinity, Chl-*a*, taxonomic fraction and measured DMSP and DMSO) are also shown in these figures. Considering that field data were previously described (Tison et al. 2010; Carnat et al. 2014), we will only highlight their major trends and focus on the comparison of the reconstructed versus measured DSC concentrations.

Summer stations from ISPOL suggested a transition from potentially active gravity drainage (with brine salinities higher than underlying water value) to brine stratification with snow melt contribution at the later stages (Tison et al. 2008). Spring YROSIAE stations showed potentially active gravity drainage throughout the depth, with a clear slow down for station YRS1 (end of November) (Carnat et al. 2014). Chl-*a* from both campaigns was mainly recorded at the bottom and rarely exceeded 1 µg L^{-1} in interior and surface ice. Diatoms dominated the bottom ice in YROSIAE and ISPOL stations and surface of YRS1. Flagellates were developed at the surface ice of YRS5 and YRS7 and at all ISPOL stations as well in interior ice for the whole data set. DMSP and DMSO profiles were largely dominated by bottom layers. Carnat et al. (2014) observed a local DMSP maximum in interior ice correlated to a shift of texture between columnar and platelet ice (**Fig. 5**).

In the following, unless mentioned otherwise, calculated DMSP and DMSO are described with both diatoms and flagellates considered. Clear contrast exists between the two sampling campaigns. In YROSIAE, calculated DSC were higher than measured DSC in all bottom layers, but also for the whole profile of YRS5 and the surface layer of YRS7 (**Fig. 5Erreur ! Source du renvoi introuvable.**). If only diatoms were considered, bottom calculated DSC concentrations from YROSIAE were of the same order of magnitude than measured DSC. By contrast, calculated bottom DSC were always lower than measured DSC in ISPOL stations, whichever calculation was considered (**Fig. 6**). For ISPOL stations, calculated and measured DMSP were close together at surface layers, especially from ISP3 to ISP7. Calculated DMSO was similar to measured DMSO at the surface of ISP1 and ISP2 but was higher from ISP3 to ISP7.

Discussion

Growth and DSC cell quotas in polar oceanic conditions (control culture)

P.antarctica appears to be well adapted to the polar oceanic conditions tested in this study (S34, 4°C). These conditions are also observed in the Southern Ocean where *P.antarctica* dominates the spring and early summer blooms (Smith et al. 1998; Garcia et al. 2009). Despite a predominance of the colonial stage of *P.antarctica* in polar blooms, this stage is not observed in our controlled cultures. This

might suggest that all the conditions required for colonial development are not satisfied. These conditions are not clear for *P.antarctica* although, following some authors, the presence of grazers could have induced the switch to the colonial form in the field (Verschoor et al. 2004; Van Donk et al. 2011). Nevertheless, the single cells successfully grew in our culture bottles with a quasi-linear increase of Chl-*a* over the 9-days and a final cell density reaching almost 10^9 cells L^{-1} (**Supplementary material 3**). The growth rate of *P.antarctica* in this study is 0.18 d^{-1} which is lower than the maximum growth rate of 0.35 d^{-1} at 4°C recorded by Wang et al. (2010). We also observe a quasi-linear increase with time of $DMSP_p$ and $DMSO_p$ to reach concentrations around 6000 nM. Measured concentrations in $DMSP_p$ are of the same order of magnitude than previous measurements obtained on *Phaeocystis* sp. with similar cell density (Stefels and van Boekel 1993; Stefels and van Leeuwe 1998; Tang et al. 2009). In terms of cell quotas, the DMSP cell quota reaches a constant value after two days while the DMSO cell quota remains constant from day 0 to day 9. These constant values suggest that the evolution of DMSP and DMSO cell contents in *P.antarctica* are mainly linked to the increase of biomass in non-stressed conditions.

Impact of salinity on growth and DSC cell quotas at a constant temperature

Increasing salinity decreases the *P.antarctica* growth in our experiments. At S75, Chl-*a* show a 2-time decrease but the population still maintains over the 9 days, while above S100 the population crashes. Despite it survives, we make the assumption that the growth of *P.antarctica* is already challenged at S75. Indeed, we observe that both DMSP and DMSO cell quotas increase up to 3-fold over the experiment. Thus, it appears that the surviving part of the algal population increases its intracellular DSC which could improve its abilities to resist to the increased osmotic constraint at S75. This supports the potential role of osmoregulator attributed to DMSP and DMSO when phytoplankton cells are exposed to osmotic stress. The osmotic function in the cell is handled by ions and organics molecules. The latter, such as proline, betaine or DMSP, also act as compatible solutes for proteins under osmotic shock and, contrary to ions, they do not impact the enzyme activities at high concentration (Kirst 1990). It was also assumed that algal species could accumulate various osmolytes (Dickson and Kirst 1986; Hellebust 1985; Karsten and Kirst 1989). Therefore, we could consider that DMSP and DMSO are synthesized together since they are chemically related. Also, the solubility of the compatible molecules is essential in case of high osmotic stress (Hellebust 1985). DMSO could, therefore, be an excellent osmoregulator candidate because the molecule is dipolar and thus soluble in water (Zumdahl and DeCoste 2013). However, the synthesis of both DMSP and DMSO is highly “energy-consuming” which could be an obstacle to their production. Nevertheless, under stress such as an increase of salinity, we might assume that the energy is fully dedicated to the prevention of damages. A longer experiment might have helped us to capture a long-term beneficial effect of DMSP and DMSO production.

At S100 and S150, Chl-*a*, $DMSP_p$ and $DMSO_p$ decrease to negligible values. Despite DMSP and DMSO cell quotas similar to the control for two days at S100, the presence of DMSP and DMSO as osmoregulator products in *P.antarctica* appears not to be efficient enough to counteract the damage from the extreme salinities and to prevent the death of the algal population.

In this study, the growth of *P.antarctica* is radically different when the salinity decreases to 20 compared to the growth at S34. Indeed, we observe a decrease in Chl-*a* over the experiment. In comparison to the control culture, the DMSO cell content slightly increases (1.6 times at day 9) while the DMSP cell content is similar. This suggests that *P.antarctica* and its growth are impacted by the decrease of salinity. In reaction to the stress generated by the salinity, *P.antarctica* could have increased its DSC cell contents. These results are similar to those observed for *P.globosa* by Speeckaert et al. (2019) and could suggest oxidative stress in the cell. Indeed, as suggested by Liu et al. (2012) in the halophile green algae *Dunaliella salina*, hypo-osmotic stress at S20 could induce the accumulation in the chloroplast of reactive oxygen species (ROS) such as H_2O_2 or the hydroxyl radical $^{\bullet}OH$. ROS are naturally produced as by-products of the respiration and the photosynthesis in chloroplasts and mitochondria (Lesser 2006). ROS act in the cell as signalling molecules but can cause cell damages, in particular at the molecular level, on proteins, lipids and DNA (Lesser 2006; Mittler et al. 2011). Organisms are able to eliminate these ROS but in case of an unbalance between production and elimination, oxidative stress occurs (Van Alstyne 2008). In this context, the ROS production could be enhanced during osmotic stress (Tanou et al. 2009). It is, indeed, assumed in various organisms that the main metabolic processes (Calvin cycle, CO_2 assimilation, respiration…) can be impacted by stress and induce production of ROS (Ahmad 2014; Apel and Hirt 2004; Lesser 2006). DSC are known to be involved in an antioxidant system to scavenge the ROS in the cell with DMSO as final product (Sunda et al. 2002). Since DMSP has been located in the chloroplast (Raina et al. 2017), we could hypothesize from our experiment at S20 that DSC variations sustain an antioxidant system when the salinity decreases. The decrease of the $DMSP_p$:$DMSO_p$ ratio also supports this antioxidant hypothesis where $DMSP_p$ could be oxidised in $DMSO_p$.

Impact of covariation of temperature and salinity on growth and DSC cell quotas

Kennedy et al. (2012) show that *P.antarctica* can tolerate and grow down to a temperature of -3°C. Therefore, the conditions tested in this study (-2.3°C to -7.4°C) are quite extreme for the growth of *P.antarctica*. At S75 and a temperature of -2.3°C, Chl-*a* decreases for two days before slightly increases until day 9. A similar increase is also observed in the density measurements (**Supplementary material 3**). At the same time, we observe an increase of the DMSP cellular content with a 5-fold increase in 9 days while the DMSO cellular content increases by 5-fold for 3 days followed by a slight decrease on day 9. The ratio reached on day 9 is higher than the one observed for the experiment at S75 only. This

demonstrates that the covariation of salinity and temperature has a higher impact on the specific production of DMSP by *P.antarctica* than salinity alone, and therefore suggests an intracellular function of osmoregulator as well as cryoprotectant. This increased production of DMSP could efficiently help the algae to resist to the extreme conditions, resulting in the observed increase of Chl-*a* on day 9. The evolution of the DMSO cell content is quite different in timing and intensity. We observe that after 3 days the $DMSO_p$:Chl-*a* reaches a plateau which could suggest that the amount of intracellular DSC required to handle the stress is achieved. Also, the $DMSO_p$:Chl-*a* ratio increases faster and reaches higher levels than measured at S75 with a constant temperature of +4°C. It appears that $DMSO_p$ also plays a role of osmoregulator and/or cryoprotectant supporting the survival of the algal population during the experiment. Note that the temperature values used here are higher than the minimal temperature potentially observable in sea ice at brine salinities S75 (-4°C). At even lower temperatures, closer to the in-situ temperature, a higher impact on algal growth and DSC cell quotas could be observed or, alternatively, induce higher mortality rates.

The two others experiments of covariations (S100/temperature of -3.9°C and S150/temperature of -7.4°C) show extremely low Chl-*a* values after 9 days meaning that the algal population collapses in those conditions, as it was already suggested for other polar microalgae (Søgaard et al. 2011). The evolution of the DMSP and DMSO cell contents from the experiment at S100 and a temperature of -3.9°C could suggest that production of both DSC still occurs in *P.antarctica*. At S150 and a temperature of -7.4°C, measurements of DSC were extremely low or even non-existent due to the fast decline of the algal population in these conditions.

Estimation of natural DSC brines contents from laboratory culture experiments

In the Southern Ocean, the autumn and winter surface waters are often dominated by dinoflagellates and nanoflagellates such as *P.antarctica* (Krell et al. 2005; Niemi et al. 2011). At that moment, surface waters algal communities can be embedded in the sea ice matrix during its formation through various processes such as the scavenging by frazil, wave pumping, or the growth of the skeletal layer at the ice-water interface (reviewed in Horner et al. (1992) and Arrigo (2016)). Incorporation in sea ice tends to mainly select species who develop mechanisms to survive in the sea ice extreme living conditions. As the ice grows, the structure of algal communities in sea ice becomes contrasted between the bottom and the upper sea ice layers. It appears that diatoms such as *F.cylindrus* survive at the bottom part of sea ice by producing, among other molecules, extracellular polymeric substances (EPS) (Aslam et al. 2018, 2012; Günther and Dieckmann 2001; Horner et al. 1992; Niemi et al. 2011). In the upper ice layer, the extreme evolution of environmental conditions (S increases to over 200 and temperature decreases as low as -18°C) cause the decline of diatoms, and it has been assumed that some flagellates could survive by developing a cyst stage (Günther and Dieckmann 1999; Stoecker et al. 2002). In spring,

improvement of the light conditions initiates the algal development in the ice matrix. The bottom diatoms assemblage increases to reach up to 97% of the autotroph (Garrison et al. 2005) and sea ice becomes colonized by diatoms blooms often dominated by *F.cylindrus* (Gleitz et al. 1998; Günther and Dieckmann 2001; Krell et al. 2005). Flagellates as *P.antarctica* appear to grow in conditions similar to the water column (i.e. a salinity around 34 and a temperature higher than the freezing point (-1.8 °C)) such as the surface slush layer (Garrison et al. 2005), the late spring melt ponds (Horner et al. 1992), the open polynyas (Arrigo et al. 1999; DiTullio and Smith 1996) or when the sea ice surface is flooded by sea water (Lizotte 2001). Also, blooms of *P.antarctica* have been observed in surface water diluted by meltwater. This leads to a stratification of the surface waters which promotes the development of *P.antarctica* at the expense of diatoms, especially when the mixed layer is deep because this species is adapted to low irradiance (Alderkamp et al. 2012; Arrigo et al. 2010; Fonda Umani et al. 2005).

In this study and in Wittek et al. (2020) we observed a link between DMSP and DMSO cell quotas and temperature and salinity variation for the two sympagic algae. This link also appears to be taxonomic dependent. Therefore, DMSP and DMSO profiles measured in sea ice could be driven by the environmental conditions in the brine habitat and by the taxonomic diversity which is also controlled by the living conditions in sea ice. This leads us to compare our experimental data set to field observations through the reconstruction of brines DSC profiles.

Our primary hypothesis assumed that the flagellates observed in sea ice have all the same DSC cell quotas as *P.antarctica*. However, inside this group, there is a broad diversity of DMSP and DMSO production from no-DSC producers to “high” DSC producers as the dinoflagellates (Stefels et al. 2007). Hence, this disparity of production, the lack of detailed taxonomy based on DSC and our approach could explain the difference between calculated and measured DSC.

At the YROSIAE bottom layers, larger calculated DSC concentrations compared to measured DSC could be due to a flagellate population which produces less DSC than *P.antarctica*. If we consider only the fraction of diatoms, we observe that calculated and measured DSC are closer and that calculated is now lower than measured, suggesting poor DSC-producers in the flagellate fraction. Similarly, overestimations observed in interior and surface ice (YRS5 and YRS7) occur in layers highly dominated by flagellates where the part of lower producers than *P.antarctica* could be important. DMSO calculated at the surface from ISP3 to ISP7 is also larger than the measured DMSO **Erreur ! Source du renvoi introuvable.**but not observed for DMSP. These stations are subject to a decrease of salinity by flooding and snow meltwater percolation which could challenge our approach (Tison et al. 2010, 2008) and explain part of observed differences between measured and estimated values. Some layers (YROSIAE) in the data set experimented higher salinities than the range of salinity chosen to establish the empiric relation (S20 to S75)**Erreur ! Source du renvoi introuvable.**. Therefore, overestimation

could be due to the assumption that DSC cell quotas increase over S75 while mortality is already observed at S100 and decreases the DSC cell quotas.

In contrast, lower calculated than measured DSC in YROSIAE and ISPOL could be explained by higher DSC producers such as the dinoflagellates. However, at the ISPOL bottom layers, flagellates are almost absent**Erreur ! Source du renvoi introuvable.** and the variability in diatom DSC production cannot explain the difference (Stefels et al. 2007). We suggest that these layers show a high concentration of dissolved DSC because we calculate a particulate DSC production while total DSC were measured on the field. This is nevertheless challenged by the high DSC turnover controlled by bacteria in sea ice which could quickly transform dissolved DSC (Asher et al. 2011). Movements of DMSP and DMSO in the brine channels could also explain higher measured concentrations in those bottom layers. Convective movements and diffusion in the largely porous bottom ice might indeed bring DSC from the upper sea ice layers.

These results show that the reconstruction of DSC profiles is challenging in high taxonomic diversity ecosystems such as sea ice. Indeed, DSC cell quotas vary between microalgae groups and species (Stefels et al. 2007). In particular, estimating the DSC from the fraction of flagellates is complex. Therefore, our hypothesis based on *F.cylindrus* and *P.antarctica* is too restrictive to correctly estimates the DSC profiles. Also, the empiric relations between salinity and DSC cell quotas are only based on temperature and salinity experiments while these quotas could also be influenced by others external factors such as light conditions, nutrient concentration or the presence of grazers (Lee and De Mora 1999; Strom et al. 2003; Sunda et al. 2002). Hence, a more complex experimental setup and the study of DSC cell quotas for a larger taxonomic diversity could improve the estimation of DSC profiles in sea ice.

Conclusion

In this study, we propose a cell approach to study the DMSP and DMSO cell quotas from *P.antarctica.* Even if the applied temperatures do not represent the real thermal conditions in sea ice, we observe that both DMSP and DMSO could play the function of osmoregulator as well as cryoprotectant in the cell. We put forward that the DMSP and DMSO cell quotas allow to resist to variations of salinity and temperature to S75 and -2.3°C as suggested by the survival of the Chl-*a* under exposition to these conditions for 9 days. We also report a maximal DMSO cell quotas reaching 175.9 mmolS gChl-a^{-1}. We observe that *P.antarctica* is more impacted by salinity over 75 compared to diatoms which could explain its lower occurrence in winter sea ice and its ability to form cysts.

In addition, we suppose that DMSP and DMSO could be involved in an antioxidant system induced by a decrease of salinity to 20. This assumes that some reactive oxygen species could be produced at S20 and react with DMSP to produce DMSO as suggested by the increase of the DMSO cell quotas in our experiment.

Also, we try to estimate the DMSP and DMSO profiles in real sea ice using the DSC cell quotas measured in laboratory for *P.antarctica* but also for another species previously studied in a similar way, *F.cylindrus*. This approach is based on the hypothesis that diatoms and flagellates are only represented in sea ice by *F.cylindrus* and *P.antarctica*. The exercise remains challenging in reproducing DMSP and DMSO production in flagellate dominated layers. DMSP and DMSO cell quotas from other emblematic species of the sea ice habitat would clearly improve this approach. It is also clear that other factors than salinity and temperature will impact the DSC cell content such as light, nutrient composition or oxygen concentration. A similar approach to this study, modulating these other factors would improve our understanding of the DSC cycle for the sea ice phytoplanktonic groups. A longer experiment would also be considered to cover the entire life cycle of *P.antarctica* in a DSC-cycle perspective.

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Supplementary material

The supplementary material shows useful details to reproduce our experimental setup. We present also the cell density measurements.

Conflicts of Interest

The authors declare no conflicts of interest

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Figure captions

Fig. 1 Biological replicates conducted at S34 and 4°C used as the control run. **a.** Chl-*a*, $DMSP_p$ and $DMSO_p$ measured in each biological replicate at S34 and 4°C. Technical replicates of Chl-a, DMSPp and DMSOp are shown by symbols (red dots, green squares, yellow triangles and blue stars, respectively for the biological replicate n°1, 2, 3 and 4). Means of the technical replicates for each biological replicate are shown by dashed lines (red, green, yellow and blue dashed lines for the biological replicate n°1, 2, 3 and 4 respectively). The global mean with its standard deviation is shown as a solid black line. **b.** $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* obtained in each biological replicates (red dots, green squares, yellow triangles and blue stars, respectively for the biological replicates n°1, 2, 3 and 4). The global mean with its standard deviation is shown as a solid black line

Fig.2 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 *P.antarctica*: 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 all group of experiments, 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 diamond and blue dots respectively. The control culture is the mean of 4 biological replicates, and the global standard deviation is based on the standard deviations calculated in each replicate. Note that the standard deviation can be smaller than the symbol thickness

Fig.3 Changes of ratios $DMSP_p$:Chl-*a*, $DMSO_p$:Chl-*a* and $DMSP_p$:$DMSO_p$ for three sets of 9-days experiments conducted on *P.antarctica*: 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 experiment, the control culture is the green line at S = 34, T = 4°C and is the mean of four biological replicates. Shifts of salinity to S20, S75 and S100 are represented by purple triangles, red squares and yellow diamonds respectively.

Fig.4 Evolution of $DMSP_p$:Chl-*a* and $DMSO_p$:Chl-*a* ratio as a function of salinity (20 to 75) from experiments of covariation conducted on *F.cylindrus* (**a**, Wittek et al. (2020)) and *P.antarctica* (**b**, this study). Dashed lines show the polynomial regressions which are used as empirical relation. Standard deviations are obtained from the standard deviation on measurements of DMSP,O and Chl-*a* and using the appropriate error propagation for a ratio.

Fig. 5 Measured field profiles of DMSP (blue dots), DMSO (yellow dots), brine salinity (orange dots) and Chl-*a* (green dots) for three YROSIAE stations. Taxonomic fractions are also plotted with diatoms (yellow) and flagellates (dark green). Reconstructed DMSP and DMSO using combined diatoms and flagellates (D+F, blue and yellow inverse triangle) are compared to the measured DMSP and DMSO profiles (dots). Reconstructed DMSP and DMSO using diatoms only (D, blue and yellow squares) are also shown.

Fig. 6 Measured field profiles of DMSP (blue dots), DMSO (yellow dots), brine salinity (orange dots) and Chl-*a* (green dots) for six ISPOL stations. Taxonomic fractions are also plotted with diatoms (yellow) and flagellates (dark). Reconstructed DMSP and DMSO using combined diatoms and flagellates (D+F, blue and yellow inverse triangle) are compared to the measured DMSP and DMSO profiles (dots). reconstructed DMSP and DMSO using diatoms only (D, blue and yellow squares) are also shown.