University of Groningen

Effect of osmotic stress on the uptake, production and exudation of climate-active compound dimethylsulphoniopropionate by sea-ice algae

Flóra Murakeözy-Kis (s5194326)
Course: Honours - Research
Project II (HCSE21405)
Supervisor: Dr. Jacqueline Stefels

August 31, 2025

Contents

Abstract

Dimethylsulfoniopropionate (DMSP) links algal stress physiology to climate via its conversion to dimethyl sulfide (DMS). The 24-hour responses of the haptophyte *Chrysochromulina sp.* and the sea-ice dinoflagellate *Polarella glacialis* were quantified to hyper- and hypo-osmotic shock (25 to 45 ppt) after a 7-day acclimation period to one of the given salinities. Using two tracers ($NaH^{13}CO_3$, D_3-DMSP) with the proton-transfer-reaction mass spectrometry (PTR-MS) method, *de novo* DMSP synthesis, uptake of dissolved DMSP, production of dissolved DMSP, and tested salinity treatment effects were analyzed. Photosynthetic efficiency (F_v/F_m) and mean cell size showed no clear changes over the 24-hour experiment. DMSP cycling was strongly species-specific: *Chrysochromulina sp.* increased intracellular DMSP via both synthesis and uptake and invested more biomass carbon in DMSP at high exposure salinity, whereas *P. glacialis* produced DMSP at a relatively higher rate in response to higher salinity, while allocating a smaller DMSP-C:POC fraction, and showing higher dissolved DMSP production (exudation) under hyperosmotic conditions. Across species, exposure salinity exerted much stronger effects than acclimation salinity: upshock generally enhanced particulate and dissolved DMSP production, downshock reduced them, while volatile DMS release was unchanged. Both species assimilated DMSP from the medium. The simultaneous production and release of DMSP could be a sign of compensation for a short-term DMSP-overproduction strategy that rapidly raises intracellular osmolarity to prevent plasmolysis, followed by controlled exudation to avoid hypo-osmotic swelling. The lower reliance on external DMSP, as well as the higher production rates of both particulate and dissolved DMSP in *P. glacialis* may be a feature of adaptation to rapid salinity fluctuations and low biomass in sea-ice brine pockets. These species-specific homeostatic strategies refine expectations for DMSP–DMS fluxes and climate feedbacks in polar seas undergoing rapid climate-related change and salinity variability.

1 Introduction

Dimethyl sulfide (DMS) is a volatile sulfur compound produced through both anthropogenic and natural processes. While human-derived DMS emmissions occur in varying concentrations worldwide, the dominant natural source of DMS originates from marine algae, having widespread production across taxa. DMS is formed through the enzymatic cleavage of dimethylsulfoniopropionate (DMSP), a molecule filfilling multiple physiological roles in algae, including functions as an osmolyte, antioxidant, cryoprotectant, and grazing deterrent (Bullock, Luo, and Whitman, 2017; Stefels, 2000). Due to its high atmospheric lifetime, DMS forms a great fraction of the global sulfur budget (Khan et al., 2016). DMS thus provides a direct mechanistic link between marine microbiology and planetary climate through its atmospheric oxidation products, which impacts cloud properties (Charlson et al., 1987).

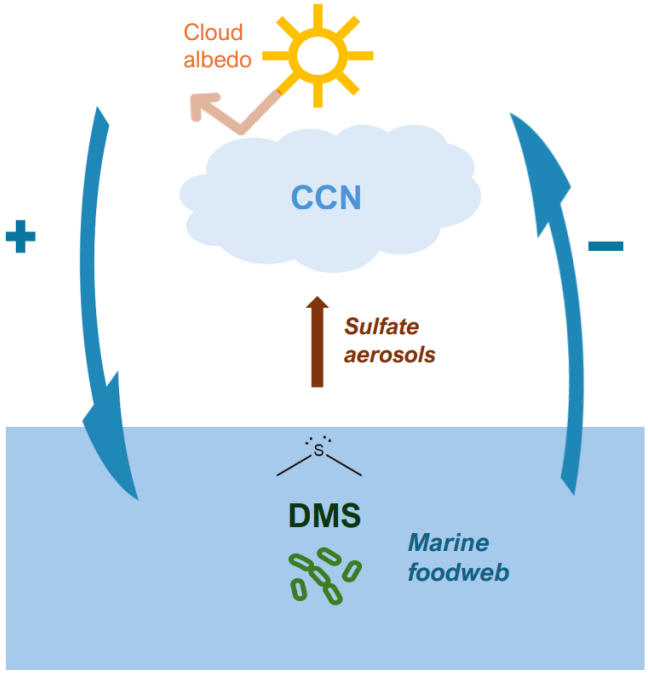

Figure 1 – Visual summary of the CLAW hypothesis. [Figure design based on Caruana et al., 2009].

The role of DMSP in global climate has been formalized in the CLAW hypothesis, proposed by Robert Jay Charlson, James Lovelock, Meinrat Andreae and Stephen G. Warren (Charlson et al., 1987). This hypothesis posits that marine algae-derived DMS creates a negative feedback loop through which it helps regulate global climate and temperature. This is possible due to their ability to act as cloud condensation nuclei, similarly to anthropogenic sulphur compounds (van Rijssel & Gieskes, 2002). The mechanism behind this process is the following: as DMSP is cleaved into the volatile DMS, either through bacterial consumption, sloppy grazing, or other processes, it is released into the atmophere. Once airborne, DMS oxidizes to sulfur dioxide (SO_2) and in turn sulfate aerosols (SO_4^{-2}), which serve a principal role in cloud formation and enhancing surface albedo. Due to its relatively high atmospheric lifetime, DMS is a major contributor to the global sulfur budget, and greater concentrations are associated with surface cooling via increased cloud formation, subsequently forming a negative feedback on DMSP production by marine algae (Charlson et al., 1987; Stefels et al., 2007; Simó, 2001). Empirical evidence supports this hypothesis in both industrial regions and during phytoplankton blooms (Kloster et al., 2007). However, more recent climate models reveal that this negative feedback effect is not globally uniform. The impact of the temperature shifts on DMSP-producing algae appears to be latitude-dependent, with opposite effects of DMS-related temperature drops in lower latitude and higher latitude ecosystems. (Lana et al., 2011)

The ongoing effects of climate change, such as ocean warming, salinity shifts, acidification and sea ice melt, call for a mechanistic understanding of the regulation of DMSP production in polar habitats. It has been established that sea-ice melt has increased local surface albedo

by 25% during the last 30 years (Pistone, Eisenman, & Ramanathan, 2014), leading to an acceleration in further climate warming and sea level rise. Furthermore, satellite-based studies have reported a 20% increase in net primary productivity due to a higher light availability, the main limiting factor, in the Arctic Ocean between 1998 and 2009 (Arrigo, 2014). These changes, coupled with accelerated sea-ice melting and its impact on albedo and associated DMSP cycling, underscore significant potential for biogeochemical and climate feedbacks in the polar regions (Stefels et al., 2007). These differences could have effects felt globally; based on current estimates of oceanic DMS output, a 50% decline in DMS production could lead to a 1.6 °C increase in global temperature, whereas a doubling could induce a 0.9 °C decrease Kettle et al., 1999). These uncertainties are most consequential in rapidly changing polar seas, where warming, salinity shifts, and sea-ice loss are reshaping primary productivity and albedo .

Sea-ice microalgae form a great part of the marine food web. For instance, ice algae may contribute up to 25% of the annual primary production of ice-covered waters and provide an important food source for krill populations, especially during seasons when phytoplankton biomass is scarce in the water column (Torstensson et al., 2015). One of the most distinctive polar dinoflagellates, *Polarella glacialis*, is notable for its high DMSP synthesis rate (Mallia, 2025, unpbl. data). Moreover, a great body of literature supports that DMSP production in many microalgae is regulated in response to salinity, often being upregulated under hypersaline and downregulated under hyposaline conditions (Shaw, Sekar, & Ramalingam, 2022). Similar regulation is observed for glycine betaine (GBT), another compatible solute, which is not only synthesized but also absorbed from the environment by Antarctic sea-ice diatoms such a*s Nitzschia lecointei, Navicula cf. perminuta,* and *Fragilariopsis cylindrus* (Wittek et al., 2020). Microalgae accumulate various compatible solutes including DMSP, proline, and glycerol to regulate osmotic stress, particularly within extreme environments such as sea-ice brine channels where salinity can greatly exceed that of seawater (Wittek et al., 2020).

Brine channel habitats in sea ice present acute and rapid salinity shifts, resulting from freeze-thaw cycles and changes in ice matrix volume. Survival in these environments depends on rapid physiological adaptation—regulation and recycling of osmolytes (DMSP, GBT, proline, glycerol) (Stefels, 2000). These mechanisms may also shape the microbial communities within brine systems, affecting local chemical gradients and facilitating algal-bacterial interactions (Arrigo, 2014). While bacterial cycling and uptake of DMSP via specific transporters are well characterized, evidence of algal DMSP uptake and its regulatory mechanisms remains sparse, though emerging studies indicate opportunistic use and uptake of DMSP by some microalgal species (Gao et al., 2020).

Despite the established importance of DMSP in sulfur cycling and osmoprotection (Bullock, Luo, & Whitman, 2017), detailed and mechanistic understanding of how DMSP production, release and uptake are regulated in polar algae, especially in the variable environmental pressures experienced in brine channels and considering climate change-related variability, remain incomplete. The current study aims to investigate the contrast in DMSP production and utilization between the cosmopolitan *Chrysochromulina sp.* and the polar-adapted *Polarella glacialis* under controlled laboratory conditions, subjecting both species to long- and short-term salinity

shifts while tracing the fate of DMSP and carbon using *in vivo* stable isotope labelling and mass spectrometry, according to the proton-transfer-reaction mass spectrometry (PTR-MS) method proposed by Stefels, Dacey, and Elzenga (2009). Given the established role of DMSP as a compatible solute in many dinoflagellates and haptophytes (Stefels, 2000), and the high concentrations of the compound found in both *Chrysochromulina sp.* and *P. glacialis* (Scarratt et al., 2002; Thomson, 2000), it is expected that chemical cycling of the compound will be in reaction to salt-stress in these species. Moreover, uptake of the compound is expected, as this would reduce metabolic energy expenditure on the synthesis of DMSP during increased osmotic stress. The dissolved fraction of the produced DMSP is expected to increase when experiencing osmotic downshock, and decrease during upshock conditions, as this would correspond with the hypothesis of stabilising DMSP levels under osmotic stress. All in all, this study aims to shed light on both short- and long-term adaptation to salinity in *Chrysochromulina* and *P. glacialis*, possibly representing cosmopolitan and highly specialized polar algae more generally, and how this is coupled to their DMSP homeostasis.

2 Materials and methods

The physiological repsonses of *Chrysochromulina sp.* *P. glacialis* were investigated using the PTR-MS method. After acclimation to the light, temperature and nutrient and salinity ('high' or 'low') regimes, cultures were exposed to either a hyper- or hypo-osmotic shock. Stable isotope tracers (^{13}C and D_3-DMSP) were added to track *de novo* synthesis of DMSP and particulate organic carbon (POC). Over a 24-hour incubation period, cell density and size, photosynthetic efficiency, carbon pools, DMSP production, uptake and release were tracked by taking samples both at the start (t_0) and end (t_{24}) of the shock period. Production rates were derived from isotope incorporation using established models, and treatment effects were evaluated through linear mixed-effects models with post hoc contracts.

2.1 Culturing

Experiments were performed with two nonaxenic microalgal cultures: the haptophyte *Chrysochromulina sp.* (Prymnesiaceae) and dinoflagellate *Polarella glacialis* (Suessiaceae). Cultures were grown in 250-mL Erlenmeyer conical flasks (glass) in natural seawater enriched with trace elements, vitamins, and nutrients according to Guillard (1975). Cultures were kept at 4 °C, under sterile conditions in a Bronson Valoya BXNS1 culture cabinet. The light intensity was 75 µmol m^{-2} s^{-1} PAR (Valoya BXNS1 LED lights).

2.2 Salt-stress experiments

Salt-stress experiments were carried out to determine the response of DMSP synthesis relative to exposure to hyper- and hypo-osmotic shock, through either increasing or decreasing the salt-content of the culture medium. The experimental setup included sterile glass serum bottles with final salinities of 25 ('low') and 45 ('high') parts per thousand (ppt). The high salinities were

reached through the addition of sea salts to filtered sea water. The low salinities were reached through the addition of filtered sea water to non-saline medium, consisting of MiliQ, nutrients and natrium carbonate. The cultures were manually agitated regularly to avoid settling.

Each experiment was started at the beginning of the day, at the same time after the start of the photoperiod. Triplicate stock cultures were placed in 250 mL erlenmeyer flasks and let acclimate to the given salinity (25 or 45 ppt.) for the duration of 7 days. Each stock culture was then split into (three times) two 100 mL fractions: one fraction would be placed in a 45 ppt medium, and the other in a 25 ppt medium , which was created using the same method as for the medium of the stock cultures. All fractions were incubated for 24 hours under the same light conditions and in the same temperature as for the stock cultures. To determine the synthesis of *de novo* DMSP and POC, samples were taken for DMSP and POC analyses and for determining photosynthetic parameters at the start and end of the experiment. Each 100 mL serum bottle received 50 µL $NaH_{13}CO_3$ (0.03M) and 200µL D_3DMSP (73 µM) after the t_0 measurements, yielding final concentrations of 15.0 µM and 146 nM, respectively. See Fig. 2 for a schematic representation of the experiment setup, and Table 1 for an overview of the parameters measured throughout the study.

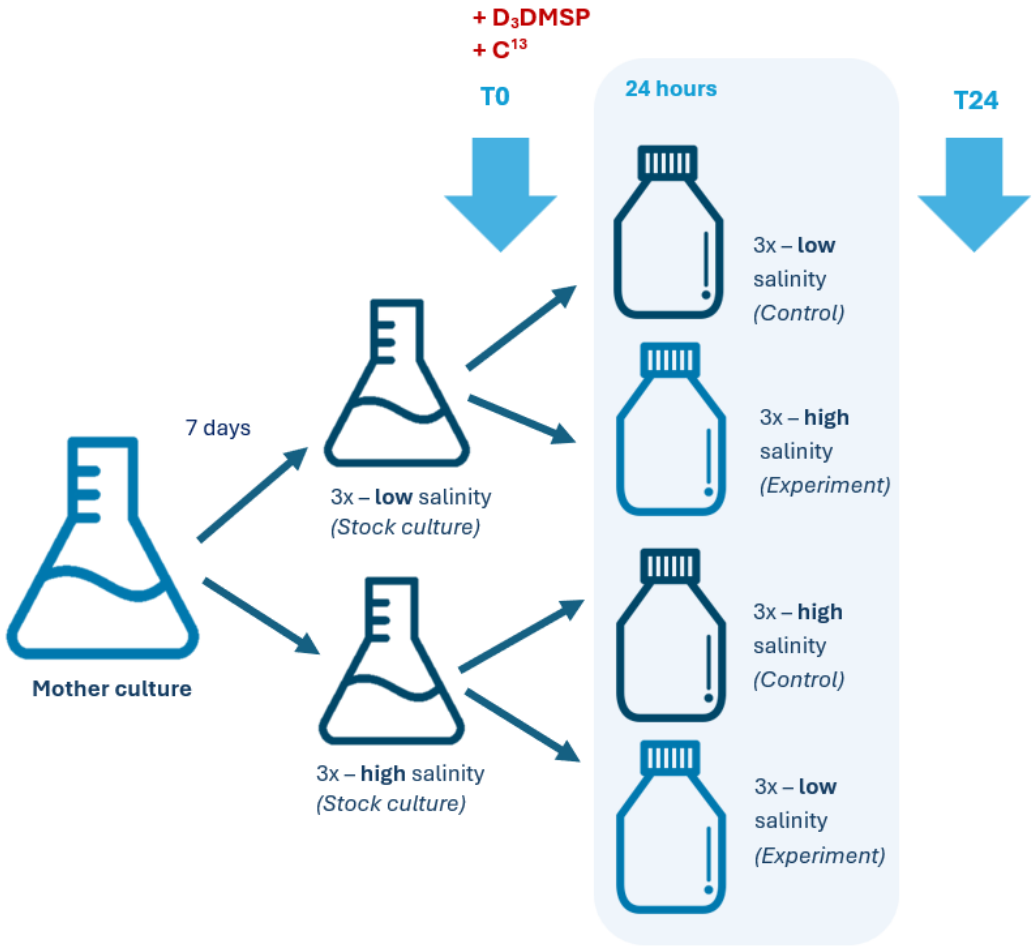

Figure 2 – Schematic representation of the salt-stress experiments: The mother culture (*Chrysochromulina sp.* or *P. glacialis*) was transferred to Erlenmeyer flasks containing seawater of either high (45 ppt.) or low (25 ppt.) osmolarity after 7 and 14 days, respectively, to account for differences in growth rate. Both species were acclimated for 7 days. After acclimation, the stock cultures were transferred to serum bottles for the experimental phase. Cells acclimated to low osmolarity were exposed to high salinity, and vice versa, while control groups were maintained at the same salinity as during acclimation. Following the transfer, D_3-DMSP and ^{13}C were added to all cultures. Measurements were taken at the start (T_0) and after 24 hours of exposure to the new conditions (T_{24}). Each condition was carried out in triplicate for both species.

Table 1 – Comparison of parameters measured at T0 and T24. PAM refers to Pulse-Amplitude Modulation fluorometry, POC to Particulate Organic Carbon, and DiC to Dissoved inorganic Carbon.

T0	**T24**
Cell count	Cell count
PAM	PAM
POC	POC
DMSP (dissolved + total)	DMSP (dissolved + total + particulate)
DiC	–

2.3 DMSP measurements

DMSPp was sampled at the start of the experiment and after 24 h of incubation. From each serum bottle, approximately 15 mL of culture was filtered onto a 2.5 cm Whatman GF/F filter (0.10–0.20 bar). Ten milliliters of the filtrate was transferred to a crimp-sealed vial and immediately analyzed for DMS. To quantify dissolved DMS(P), half a NaOH pellet was added to the vial before sealing, and samples were left at room temperature for at least 24 h to ensure complete hydrolysis of DMSP to DMS. For total DMS, DMSPp, and DMSPd, 10 mL of unfiltered culture was mixed with 10 mL of Milli-Q in a crimp-sealed vial, treated with NaOH in the same way, and stored until analysis. Incorporation of ^{13}C into the DMS moiety of the particulate pool was measured with proton transfer reaction mass spectrometry (PTR-MS, Ionicon GmbH, Innsbruck, Austria), following the method described by Stefels, Dacey, and Elzenga (2009).

The enrichment of $NaH^{13}CO_3$ in the cultures was determined by measuring the dissolved inorganic carbon (DIC) pool. For this, 0.5 mL of culture was acidified with 10% orthophosphoric acid (H_3PO_4), releasing CO_2 which was stripped with ultrapure N_2 (100 mL min^{-1}) and analyzed directly using a cavity ring-down spectroscopy isotopic gas analyzer (G2201-i, Picarro). Particulate organic carbon (POC) was sampled both at the start and after 5 h of incubation. For this, 30 mL of culture was filtered onto pre-combusted (5 h at 525 °C) 2.5 cm Whatman GF/F filters (0.10–0.20 bar). Filters were stored at –20 °C until analysis, then exposed to 37% fuming HCl in a desiccator for 4 h to remove inorganic carbon, and dried overnight at 60 °C. POC content and isotopic enrichment were analyzed using a combustion module coupled to the same isotopic analyzer.

2.4 Cell counts and size

Cell counts and size were measured using the Invitrogen Countess 3 Automated Cell Counter (Thermo Fisher Scientific; Waltham, Massachusetts, U.S), with every sample being measured 6 times. To improve cell visibility, *P. glacialis* samples were stained with 10 µL lugol stain, while *Chrysochromulina sp.*chromulina cells were measured unstained.

2.5 Carbon-isotope measurements

The enrichment of $NaH^{13}CO_3$ in the experimental cultures was quantified within the dissolved inorganic carbon (DIC) pool using a custom-built purging system connected to a cavity ring-down spectroscopy isotopic gas analyzer (G2201-i Isotopic Analyzer, Picarro). For this, 0.5 mL of culture was added to 10% orthophosphoric acid (H_3PO_4), converting all DIC into CO_2. The released CO_2 was stripped from the sample with ultra-pure N_2 at a flow rate of 100 mL min^{-1} and analyzed directly by the instrument.

Particulate organic carbon (POC) samples were collected both before isotope addition and after 5 hours of incubation by filtering 30 mL of culture onto pre-combusted (5 h at 525 °C) 2.5-cm Whatman GF/F filters (filtration pressure: 0.10–0.2 bar). Filters were stored at –20 °C until analysis. Prior to measurement, filters were exposed to 37% fuming HCl in a desiccator for 4 hours to remove inorganic carbon, then dried overnight at 60 °C. POC content and isotopic enrichment were determined with a combustion module linked to the same isotopic analyzer (G2201-i, Picarro).

2.6 Calculation of DMSP and POC production rates

2.6.1 DMSP-Production

De novo DMSP production rates were estimated from the incorporation of ^{13}C into the particulate DMSP pool (DMSPp), using the MRP model described by Stefels, Dacey, and Elzenga (2009). DMSP was measured with proton transfer reaction mass spectrometry (PTR-MS) after alkaline hydrolysis to protonated DMS. The ^{13}C signal was taken from the relative increase at mass 64, compared to the total signal of DMS isotopes (m63, m64, m65). The specific production rate of DMSP (μ_{DMSP}) was calculated as:

$$\mu_{DMSP} = \frac{\ln\left(\frac{^{64}MP_{eq} - {}^{64}MP_0}{^{64}MP_{eq} - {}^{64}MP_t}\right)}{\Delta t \cdot \alpha_{kin}} \tag{1}$$

Here, $^{64}MP_t$ is the fraction of singly ^{13}C-labelled DMSP at time t, given as $\frac{m64}{m63+m64+m65} \cdot 100$. $^{64}MP_0$ is the natural background level of mass 64 (∼3%), caused by deuterium, ^{13}C, and ^{33}S isotopes. $^{64}MP_{eq}$ is the equilibrium percentage of mass 64 in newly made DMSP (single ^{13}C label), and α_{kin} is a correction factor for fractionation, equal to 1.06 (Stefels, Dacey, & Elzenga, 2009).

2.6.2 Particulate Organic Carbon

POC production rates were calculated from the isotopic enrichment of bulk POC during the incubation. The specific POC production rate (μ_{POC}) was calculated as:

$$\mu_{POC} = \frac{\ln\left(\frac{R_m - R_b}{R_m - R_t}\right)}{t} \tag{2}$$

Here, R_t represents the proportion of ^{13}C relative to the total carbon pool (^{12}C + ^{13}C) in POC at time t.

R_b denotes the natural background abundance of ^{13}C in organic material (approximately 1.1%). R_m corresponds to the percentage of ^{13}C in the dissolved inorganic carbon of the medium following the addition of $NaH^{13}CO_3$.
Fractionation of carbon isotopes during photosynthetic incorporation into POC was considered negligible (Welschmeyer & Lorenzen, 1984).

2.7 Maximum quantum yield of photosystem II

Maximum quantum yield (F_v/F_m) was measured with pulse-amplitude-modulated (PAM) fluorometry on every replicate. This parameter describes the efficiency of energy conversion in photosystem II under dark-adapted conditions, and provides a standardized indicator of photosynthetic performance and stress (WATER-PAM, Heinz Walz GmbH).

$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

2.8 Normalized Metrics

Growth-normalized DMSPd production:

$$\mu_{\mathrm{DMSPd,\,corr}} = \frac{\mu_{\mathrm{DMSPd}}}{\mu_{\mathrm{POC}}}$$

This metric expresses the production of dissolved DMSP relative to biomass growth. By scaling DMSPd production to the specific growth rate of particulate organic carbon (μ_{POC}), differences in cell division or biomass accumulation between treatments are accounted for. This allows comparisons of DMSPd production efficiency across species and salinity treatments independent of absolute biomass.

D_3-DMSP loss through demethylation:

$$\%\ D_3P_{\mathrm{lost,demethyl.}} = \frac{[D_3P]_{\mathrm{added}} - [D_3P]_{\mathrm{DMS(P)t}} - [D_3P]_{\mathrm{DMS}} - [D_3P]_{\mathrm{DMSPp}}}{[D_3P]_{\mathrm{added}}} \times 100\% \qquad (3)$$

This expression quantifies the percentage of the added tracer (D_3-DMSP) that was lost from the system via demethylation. The calculation subtracts the tracer still present in measured pools (total DMS(P), DMS, and DMSPp) from the total added, and relates the remainder to the initial tracer input. This gives a direct estimate of how much of the labelled substrate was diverted into alternative pathways rather than retained in the measurable DMSP-related pools.

DMSP uptake as a fraction of tracer added:

$$\%\ \mathrm{DMSP\ uptake} = \frac{[D_3P]_{\mathrm{DMS(P)t}} - [D_3P]_{\mathrm{DMS}} - [D_3P]_{\mathrm{DMSPp}}}{[D_3P]_{\mathrm{added}}} \times 100\% \qquad (4)$$

This metric represents the proportion of tracer D_3-DMSP that was incorporated into the particulate fraction after incubation. By subtracting free DMS and DMSPp from the total measured pool, the calculation isolates the tracer retained in biomass. Expressing this relative to the

amount added provides a normalized measure of uptake efficiency that is directly comparable between treatments.

DMSP–C relative to biomass:

$$\mathrm{DMSPp{-}C} : \mathrm{POC}_{t_{24}} \;=\; \frac{n_C \times [\mathrm{DMSP}]_{t_{24}}}{\mathrm{POC}_{t_{24}}}$$

This ratio relates the concentration of DMSP-derived carbon to the total particulate organic carbon (POC) pool at t_{24}. It indicates how much of the standing algal biomass was invested in the DMSP pool. High values reflect a greater proportional allocation of cellular carbon to DMSP, which can serve roles in osmoregulation, antioxidation, and stress protection.

Tracer-derived DMSP–C relative to biomass:

$$\mathrm{DMSPup{-}C} : \mathrm{POC}_{t_{24}} \;=\; \frac{n_C \times [D_3\mathrm{P}]_{\mathrm{uptake},\, t_{24}}}{\mathrm{POC}_{t_{24}}}$$

This expression quantifies the proportion of the biomass carbon pool at t_{24} that originated specifically from tracer-derived DMSP. Unlike the previous ratio, which describes the total standing DMSP pool, this metric isolates only the fraction synthesized from the labelled substrate during the incubation. It therefore reflects the active incorporation of external carbon into the DMSP pool, relative to overall biomass accumulation.

2.9 Statistical analyses

All statistical analyses were carried out in `R` (version 4.4.2.; **R**). Linear mixed-effects models were fit in R (packages `lme4`, `lmerTest`, `nlme`, `emmeans`, `car`, `MuMIn`) using sum-to-zero contrasts (`contr.sum`), to account for the random effect of combining data where multiple measurements are done on the same acclimation salinity x species x replicate ('bottle') (Kuznetsova, Brockhoff, and Christensen, 2017; Bates et al., 2015; Pinheiro, Bates, and Team, 2025; Lenth, 2025; Fox and Weisberg, 2019; Bartoń, 2025; R Core Team, 2025). Fixed factors were species, acclimation salinity (A25, A45), and exposure salinity (E25, E45). For each response, the initial model included a random intercept for bottle. For physiological traits, paired changes (Δ =t24–t0) were analyzed per bottle. Fixed effects were tested with Type III ANOVA (Wald tests; `car::Anova`). Assumptions were checked automatically: Shapiro–Wilk test, skewness, kurtosis, Levene's test across species × acclimation × exposure, correlation of absolute residuals with fitted values, and counts of standardized residuals > 3 (with Cook's distance flagged for lm models). If responses were non-negative and showed strong right skew (skewness > 1), data was refit on a `log1p` scale and back-transformed estimated marginal means. When the random intercept variance was estimated at ≈ 0 (singular fit), it was dropped and an ordinary least squares (OLS) model (`lm`) was used for inference. If Levene's test indicated heteroskedasticity, `nlme::lme` were compared models with alternative `varIdent` structures (by species, acclimation, exposure, or their interaction) using AIC; this was done for model fit and R^2 estimation, but post-hoc tests were always

run on the inference engine (lmer/lm). Marginal and conditional R^2 (Nakagawa, Johnson, & Schielzeth, 2017) are reported from the selected model. Post-hoc tests were only run when the corresponding omnibus effect was significant. Tukey correction was used for fully crossed three-way cell means; otherwise pairwise contrasts were adjusted using the Holm-Bonferroni method to correct for multiplicity.

3 Results

3.1 Baseline physiology

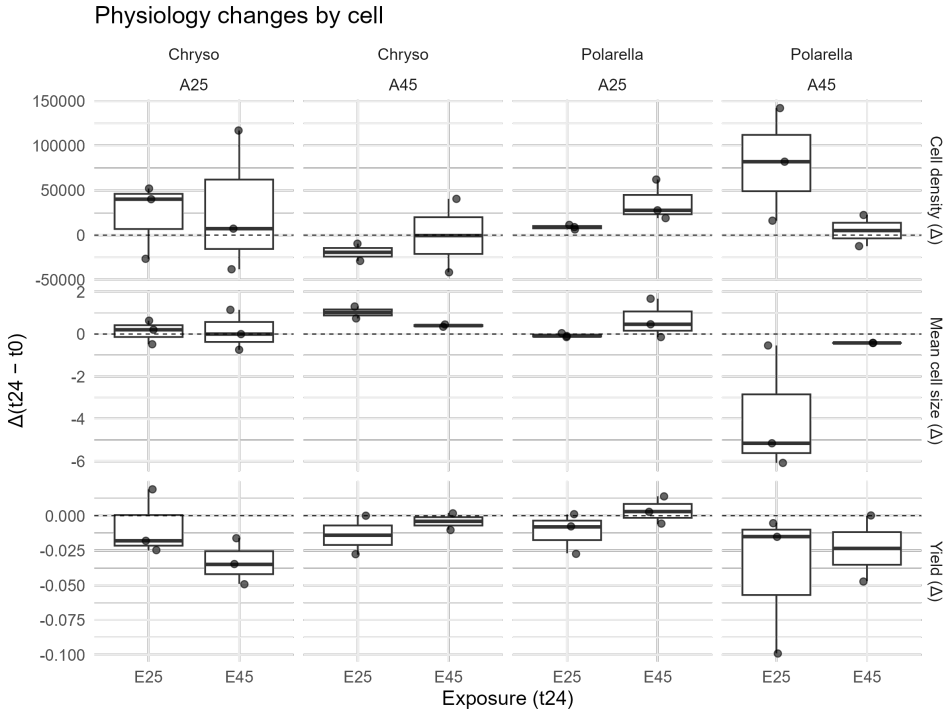

Figure 3 – Physiological responses of *Chrysochromulina sp.* and *P. glacialis* under different acclimation (A25, A45) and exposure salinities (E25, E45). Panels show changes from t0 to t24 in mean cell size (left), cell density (middle), and maximum quantum yield of photosystem II (F_v/F_m; right).

Physiological responses ($\Delta t_0 \rightarrow t_{24}$) were analysed using linear mixed-effects models with species, acclimation, and exposure as fixed factors and bottle as a random intercept. Type III ANOVA was used for omnibus tests, and post-hoc comparisons confirmed the absence of meaningful pairwise differences. See Fig. 3 for a visual representation of the results.

The change in Fv/Fm was not significantly affected by any main effects, however, the interaction effects between adaptation salinity and exposure salinity, as well as between species and exposure salinity. The three-way interaction was not significant. A post-hoc test was thus

done, however, no factors or interactions were significant.

Mean cell size was residuals deviated from normality (positive skew), but linear mixed models are robust under balanced design and inference was supported by multiple contrasts. Analysis using ANOVA showed a significant main effect of species, as well as interaction effects of adaption and exposure salinity with species. There was no interaction between adaptation and exposure salinity or a three-way interaction. Post-hoc testing revealed that *P. glacialis* acclimated to 45 ppt and with exposure salinity 45 had a greater difference in mean cell size compared to the same stock culture exposed to 25 ppt. Furthermore, *Chrysochromulina* had a greater difference in mean cell size when acclimated to 45 ppt and exposed to 25 ppt compared to *P. glacialis* cells unergoing the same treatment. Finally, between *P. glacialis* exposed to 25 ppt, the change in cell size was bigger in the 25-acclimated, compared to the 45-acclimated cultures.

All in all, no consistent pattern can be detected in the change in cell size, indicating that salinity treatments did not cause significant turgor changes that would affect cell dimensions after the duration fo 24 hours. This aligns with the general finding that physiological parameters were not consistently affected by any of the experimental effects.

3.2 DMSP and POC production

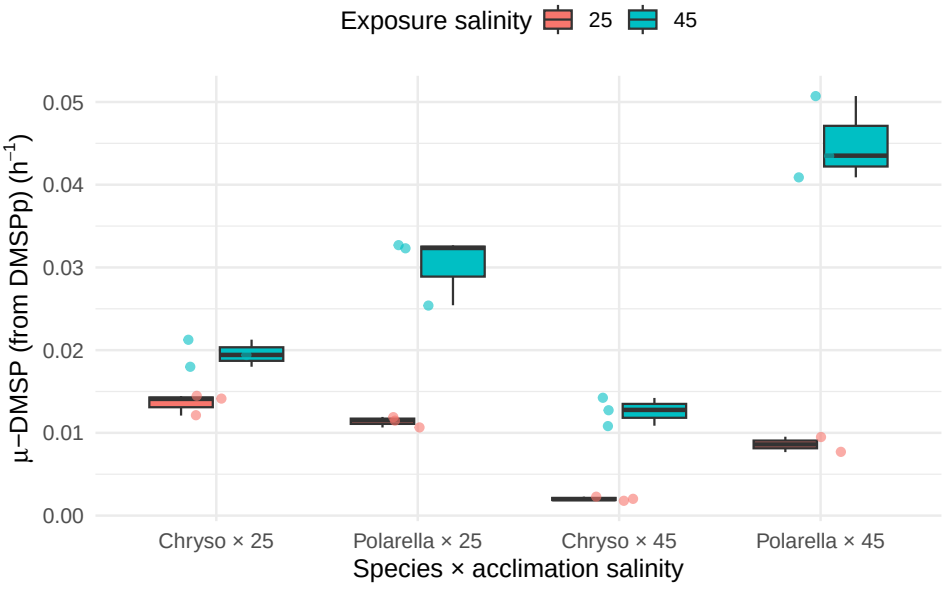

Figure 4 – Boxplots of *particulate* dimethylsulfiopropionate production (μDMSPp) across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

Particulate DMSP production rate ANOVA analysis showed that particulate μ-DMSPp data was very highly significant for all main effects except for adaptation salinity and interaction effects. Post-hoc, *P. glacialis* had a greater particulate DMSP production rate than *Chrysochromulina sp.* for almost every direct comparison. Species thus appeared to be the biggest and most consistent effect. Furthermore, an exposure salinity of 25 ppt always resulted in a lower DMSPp production rate than exposure to 45 ppt, regardless of species or acclimation salinity. Additionally, being acclimated to 25 ppt increased μDMSPp in the case of *Chrysochromulina sp.* compared to being acclimated to 45 ppt, regardless of exposure salinity. For *P. glacialis*, the effect of acclimation salinity was highly significant in the case of exposure salinity of 45, whereby the 45-acclimated cultures produced DMSPp at a higher rate than 25-acclimated cultures. Indeed, there seems to be a pattern of upregulation of DSMPp-production when receiving upshock and a downregulation of DMSPp-production when receiving a downshock in both species and in both acclimation salinities, but opposite effects of acclimation salinity in the two species, where *P. glacialis* increases its production in a higher acclimation salinity, while *Chrysochromulina sp.* decreases it.

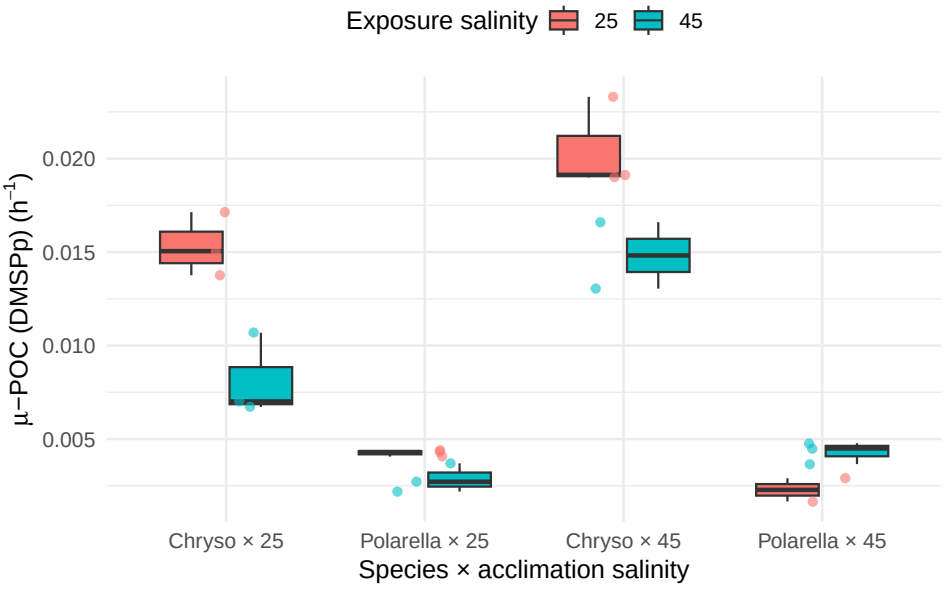

Figure 5 – Boxplots of particulate organic carbon production (μPOC) across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

Particulate organic carbon production rate For μ-POC, ANOVA showed highly significant p-values for all three main effects of species, acclimation and exposure salinity, and all two-way interactions between them, but no significant three-way interaction effects. Post hoc tests showed a significantly higher growth rate for *Chrysochromulina* compared to *P. glacialis* in all experimental conditions, which matched the observation of cell density throughout the cul-

turing. Moreover, acclimation salinity significantly affected growth rates in *Chrysochromulina sp.*, with 45 ppt-acclimated cultures growing faster than 25 ppt-acclimated cultures. *Polarella* , on the other hand, was not significantly affected by acclimation salinity. In almost all cases, with *Chrysochomulina* much more strongly than *P. glacialis*, a lower exposure salinity resulted in a higher growth rate. This shows a contrasting reaction in *Chrysochromulina sp.*; on the one hand, its growth is enhanced over long-term exposure to greater salinity, while at the same time growing better in short-term less saline environments. Thus, we can observe that *Chrysochromulina sp.* grows significantly better when exposure salinity is low, whereas *P. glacialis* grows, while not to a significant degree, better in the controls compared to the up- or downshock. See Fig. 5 for a visual representation of the data.

3.3 Normalized

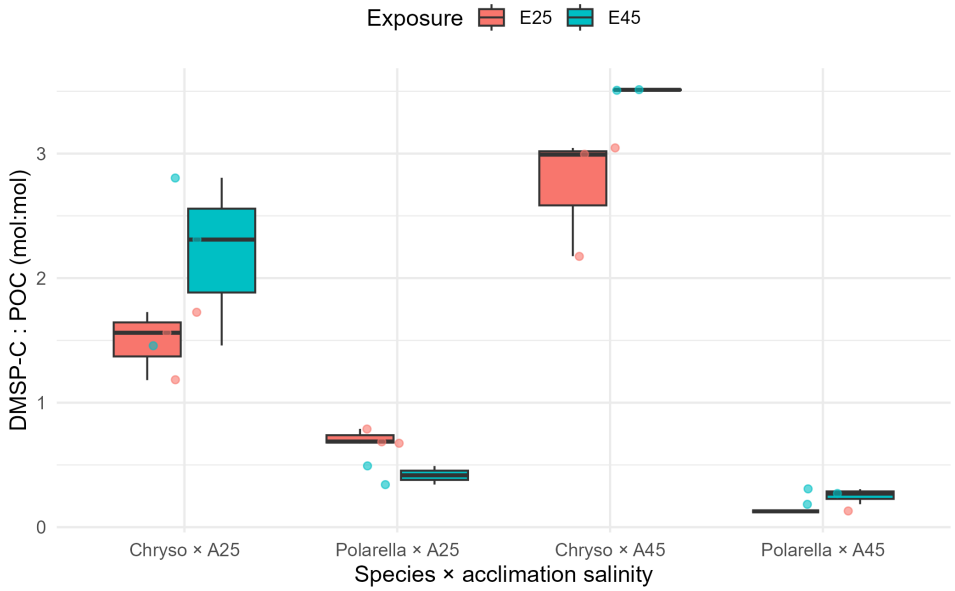

Figure 6 – Boxplots of particulate dimethylsulfiopropionate production (μDMSPp) relative to total particulate carbon production (μPOC) across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

DMSP-C : POC According to the ANOVA analysis, species had the strongest influence on DMSP-carbon relative to total assimilated carbon. Acclimation and exposure effects were less strong, but still significant. Furthermore, there were interaction effects between species and acclimation and species and exposure. Post-hoc, *Chrysochromulina sp.* had a higher DMSP-C compared to *P. glacialis* across all conditions. Moreover, *Chrysochromulina sp.* had a higher DMSP-C content when exposure salinity was high compared to when it was low, whereas in *P. glacialis*, it was insiginficantly lower in the case of osmotic shock, regardless of whether it

was up- or downshock. Finally, acclimation to a higher salinity significantly increased DMSP-C investment in *Chrysochromulina sp.*, but not in *P. glacialis*. See Fig. 6 for a visualization of the data.

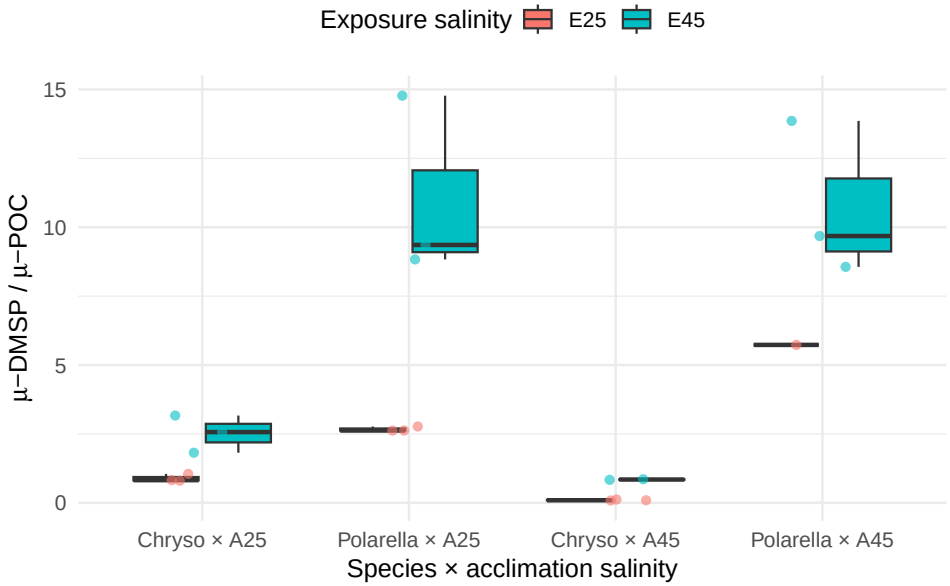

Figure 7 – Boxplots of particulate dimethylsulfiopropionate production (μDMSPp) relative to total particulate carbon production (μPOC) across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

µ-DMSP / µ-POC Normalized DMSP production rate was singular, so it was analyzed with OLS without a random intercept. Normality was violated, but OLS was retained since coefficients are unbiased and variances were homoskedastic. Model fit was good (R^2=0.87). ANOVA detected that species affected the ratio the most, although exposure salinity was also a significant main effect. Furthermore, a significant interaction between species and exposure salinity, but none of the other two-way or the three-way interactions were significant. Post-hoc, *P. glacialis* had a significantly higher ratio than *Crysochromulina* under most conditions, except for A25-E25 where it was still higher, but not significantly. Furthermore, exposure only had a significant impact on the ratio in *P. glacialis*, with an increase in the ratio under higher salinity and a decrease under lower salinity. This shows a very strong reactivity to salinity in *P. glacialis* in terms of DMSP production, with a strong relative upregulation in response to upshock, and a strong downregulation in response to downshock. The figure also shows a big difference with non-normalized µ-DMSPp, underlining the biomass differences between cultures of the two species. However, patterns remain largely the same, with a strong short- but not long-term response in both species, and a stronger reaction in *P. glacialis* compared to *Chrysochromulina sp.*. See Fig. 7 for a visualization of the data.

3.4 DMSP exudation

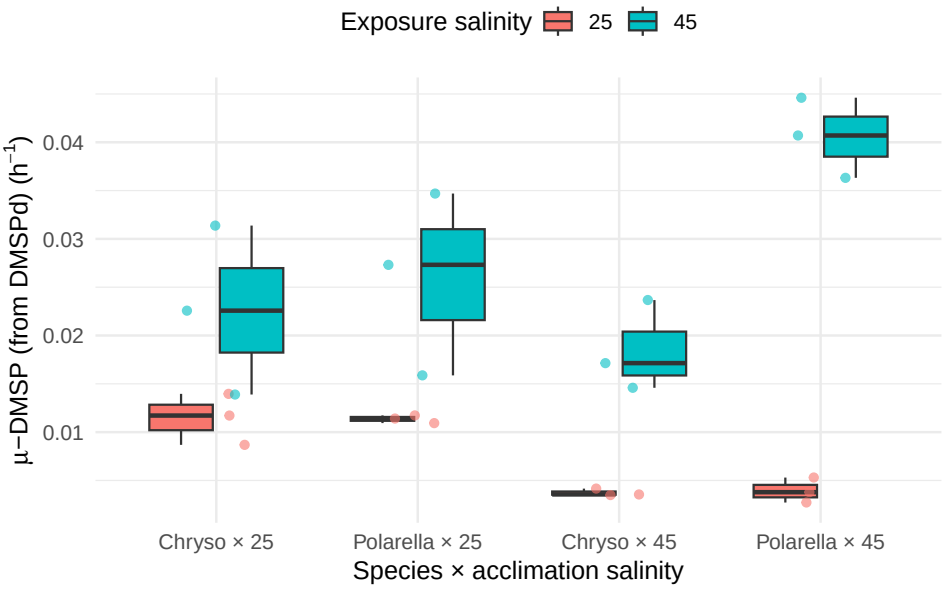

Figure 8 – Boxplots of *dissolved* dimethylsulfiopropionate production (μDMSPd) across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

Dissolved DMSP production. The random intercept was singular, therefore, an OLS model was fitted so that the random intercept could be dropped. The final model fit was high (R^2 = 0.84). ANOVA showed significant main effects for species and exposure salinity, with the latter being the strongest. Moreover, all possible two-way, and three way interaction effects were also significant. Post-hoc analysis showed that, in all cases, an exposure to 25 ppt resulted in a significantly lower μDMSPd production rate, with the strongest difference being the case of 45-acclimated *P. glacialis*. Furthermore, *P. glacialis* produced more μDMSPd than *Chrysochromulina sp.* in almost all conditions, except for for 25-acclimated control cultures, where *Chrysochromulina* produced more, although only one of these differences (A45, E45) was significant. Finally, acclimation to 25 ppt resulted in an, although not significantly, but higher DMSPd production rate across species and exposure salinity, except for the case of *P. glacialis* exposed to 45, where acclimation to 45 ppt resulted in a significantly higher DMSPd production. Thus, overall we can observe a similar pattern as for μDMSPp production rates, with both species reacting to a higher salinity by releasing more DMSP, and to a lower salinity by releasing less, both on the short-term.

Loss through demethylation. ANOVA was significant for three-way interaction. Post-hoc analysis indicated that *P. glacialis* lost significantly more tracer than *Chrysochromulina sp.* in both osmotic shock experimental conditions. This aligns with the DMSP uptake pattern; *P.*

glacialis took up less D3DMSP under these circumstances. Interpretation of this data is less reliable, however, due to large variation and confidence intervals.

DMS Production Volatile DMS release showed no significant variation between treatments (all $p > 0.25$, $R^2 = 0.24$), indicating that experimental conditions did not alter the volatile fraction.

3.5 DMSP Uptake

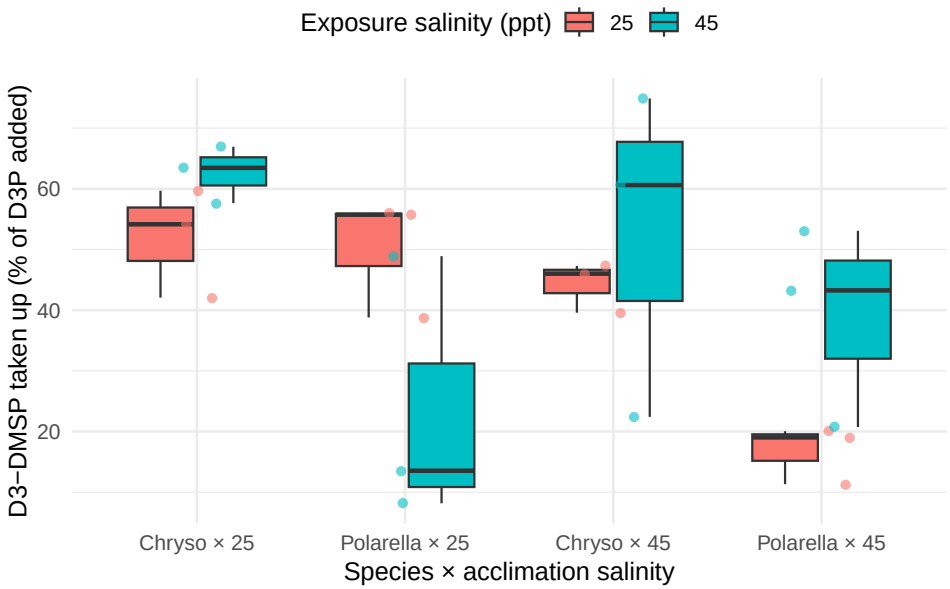

Figure 9 – Boxplots of DMSP uptake across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

Due to singularity, an OLS was used and the random intercept was dropped (R^2=0.504). ANOVA showed a significant main effect of only species, with all other factors and interactions being insignificant, except for three-way interactions. This may be due to the fact that results are hard to interpret due to the lack of initial measurements at t0. Post-hoc, an exposure to 25 ppt resulted in an insignificantly lower uptake in almost all cases, except for the case of *P. glacialis* acclimatized to 25 ppt, with these cultures instead having a significantly lower uptake when exposure salinity was high compared to when it was low (control). In all cases, *Chrysochromulina sp.* had a higher uptake than *P. glacialis*. However, these metrics are not normalized to biomass. Finally, acclimation salinities of 25 ppt also resulted in a higher uptake in every case, except for the case of *P. glacialis* exposed to 45 ppt, although none of these differences were significant. All in all, post-hoc analysis did not show a clear pattern in relation to acclimation and/or exposure salinity, however, when including insignificant differ-

ences, *Chrysochromulina sp.* appears to take up more in higher exposure salinities, whereas *P. glacialis* appears to take up less in both salinity shock experiments, whether high or low, compared to controls.

4 Discussion

This study examined the roles of DMSP synthesis, uptake and release in two miceroalgal soecies adapted to different ecological niches: the cosmopolitan haptophyte *Chrysochromulina sp.* sp. and the polar dinoflagellate *Polarella glacialis*. This is done through the addition of heavy isotopes of both carbonate (C^{13}) and DMSP (D_3DMSP) and tracing their respecitve incorporation and fate throughout the experiments using mass spectrometry according to the method specified in Stefels, Dacey, and Elzenga (2009). The experiments, allowing for both short and long term responses to salinity change to be followed using stable isotopes, showed that the two species have fundamentally different strategies in their DMSP-utilisation which may be attributed to their ecological origins.

Physiology Photosynthetic yield (Fv/Fm) is an established metric used to quantify the physiological state of the photosynthetic apparatus in plants, and commonly used to estimate plant health (Krause & Weis, 1991). Values over 0.5 indicate a photochemical apparatus functioning properly under normal sunlight conditions (Le Rouzic, 2012). With all Fv/Fm values found above this study lying in this number, and no significant differences between before and after the shock, we can conclude that the cultures did not suffer damage to photosystems. Although it is established that osmotic stress can have negative effects on photochemical apparatus (Le Rouzic, 2012), this seemed not to be the case here. This may be due to the salinity changes not being strong enough to cause damage, or possibly due to efficient preventative adaptation mechanisms being activated in the cells.

Cell size was larger in *P. glacialis*, which is indicative of this species (Montresor, Procaccini, & Stoecker, 1999), and cell density was higher in *Chrysochromulina sp.*, most likely due to faster growth and replication. Increased salinity did not cause a decrease in cell size, even after the short-term 24 hours experiment, suggesting that the cells were able to rapidly stabilize their own osmotic value, for example by producing and/or assimilating DMSP. Moreover, the stable photosynthetic yield indicates that the osmotic shock did not cause oxidative stress that would have been detectable after this period. These measurements do not, however, exclude the possibility of cell lysis or death and its impacts on DMSP measurements.

DMSP production Generally, *Chrysochromulina sp.* cells showed a consistent up- and down-regulation pattern of intracellular DMSP-content in response to salinity shifts on the short term. Moreover, the cultures were able to utilize the dissolved added DMSP to further increase their

intracellular DMSP concentration. This suggests that DMSP has an important role as a compatible solute in *Chrysochromulina sp.*, and that its levels are actively regulated through both synthesis and uptake of the compound. While there is no direct literature available on the effect of salinity on DMSP-production in *Chrysochromulina sp.*, such studies have been done on other haptophytes. For example, current results contrast with findings by Gebser and Pohnert (2013), who found that the haptophyte *Emiliania huxleyi* did not upregulate DMSP in reaction to gradually increasing salinity, but instead changed cell size. Additionally, the DSYB gene found in *Chrysochromulina tobin* (fresh-brackish water) and *Chrysochromulina sp. PCC307* (marine water) that is responsible for DMSP production was tested in *E. coli*, were tested by Curson et al. (2018) and found no effect of variation in salinity. This heterogeneity in results could, however, be attributed the different methodologies, culturing conditions and strains used by such studies.

P. glacialis showed a similar pattern in DMSPp production rates, but with stronger exaggerations between experimental salinities. This may indicate a specialized, and quick adaptation to the salinity shocks, being a brine dinoflagellate where changes in salinity occurs to extreme levels and adaptation is required for survival. However, Thomson (2000) did not find a singificant effects of salinity on DMSP production in *P. glacialis*. At the same time, findings of brine dinoflagellates have found the opposite: in the widespread polar diatom *Fragilariopsis cylindrus*, Lyon et al. (2016) found an up- and downregulation of DMSP content in response to gradual salinity changes, highlighting the osmolyte role of the compound in polar algae. This highlights once again the heterogeneity in findings related to these species. In contrast, its DMSP-C/POC ratio was lower than that of *Chrysochromulina sp.*. This may be due to the fact that *P. glacialis* cells have complex, carbon-rich structures structures (theca), which may have caused the ratio to be lower (Thomson, 2000; Montresor, Procaccini, and Stoecker, 1999). Furthermore, the difference between raw and normalized μ-DMSP rates show that *P. glacialis* does produce a high amount of DMSP, and that in the raw values, the results are harder to interpret due to the higher cell density of *Chrysochromulina sp.*, underlining the importance of normalized measurements, as well as care with interpretations of other metrics analyzed in this study.

All in all, DMSP production and incorporation appears to be influenced by environmental salinity, with both *Chrysochromulina sp.* and *P. glacialis* actively upscaling production and intracellular DMSP concentration, supporting the hypothesis that DMSP is a compatible solute needed to balance out osmolarity, preventing cell shrinkage or lysis.

DMSP exudation During the experiment, both species exhibited a significant amount of DMSP release into the medium, especially when placed in higher salinities, whether on the short- or long-term. This pattern overlaps closely with DMSP production rates. This may indicate an active exudation of the compound, which has been observed in both dinoflagellate and Prymnesiophyceae at varying, species-dependent intensities (Laroche et al., 1999). In this

case, exudation happened in response to nutrient depletion, supporting the overflow hypothesis. Additionally, one study had found

Under both acclimation regimes, *P. glacialis* reacted more strongly to experimental salinity, with a higher amount of dissolved DMSP present under higher upshock and a decreased amount under downshock. The first explanation for these observations could be that *Chrysochromulina sp.*, showing a osmolarity-dependent DMSP cycling in general, is releasing the DMSP that has accumulated inside the cells when adapting to a drop in salinity in order to balance internal osmotic value. However, this was not the case for any of the species; μ-DMSPd was always higher in higher exposure salinities compared to lower salinities across both species and acclimation salinities. One observation that does partially support this view is that for *Chrysochromulina sp.*, the average DMSPd was lower in the 45 ppt-acclimated cultures, indicating a greater preservation of DMSPd overall. However, opposing this view is the greatest production rates being in the 45-acclimated control groups of *P. glacialis*.

A further hypothesis could be that for *P. glacialis*, a sudden increase in brine levels is experienced by the cells as a signal for the danger of freezing, which can harm and kill cells (Toda et al., 2023). Thus, the cryprotectant features of DMSP may be employed through the simultanous upscaling of both intra- and extracellular DMSP-concentrations. While concrete evidence for a high DMSP level in surrounding sea water aiding in cryoprotection is not available, DMSO has, for instance, been shown to protect lysosomes from cell lysis, provided the compound is present in approximately equal amounts both in the membranes and in the surrounding medium (Klbik et al., 2022). Releasing DMSP into the environment directly, which can likely be relatively easily modified by the algae placed in the small volume inside a brine pocket, could potentially lower the freezing point of the surrounding water.

The species-independent production of DMSPd may then be due to cell lysis, which this study would be unable to detect. Although not explicitly statistically analyzed in this study, raw DMS and demethylated D3P were highly variable between bottles, indicating possible local cell lysis or physical disturbance which could have influenced DMSPd results. However, Berdalet, Llaveria, and Simó (2011) found that physical swirling of microalgal cultures can significantly increase dissolved DMSP levels in the medium by active exudation; Shaken cultures grew 55% slower and reached 46% of still-culture cell abundance, yet cellular DMSP (per cell and per biovolume) rose 20% under turbulence and remained elevated. This points to overflow under unbalanced growth rather than damage. The authors argue for the overflow hypothesis, Since throughout this study, cultures were physically regularly lightly agitated in order to prevent cell clumping and settling, this may have increased DMSP release into the medium. For a more accurate understanding of dissolved DMSP production, agitated and non-agitated cultures could be compared.

Furthermore, cell lysis should be monitored, and DMSPd metrics should be normalized

against total biomass per sample as well as growth rates as was done for particulate DMSP. Moreover, for a better understanding of how much DMSP is truly released in these conditions, DMS (see Appendix, Fig. **??** and DMSPd measurements should be combined. Finally, the same process should be investigated in axenic cultures, where the bacterial conversion of DMSPd to DMS cannot influence the final results. Nevertheless, intact, DMSP-permeable membranes were found by on study in the polar diatom *Fragilariopsis cylindrus*, through the release of DMSP into the dissolved pool in strongly hyposaline conditions. This was proven using SYTOX-positive cells, which were detected in 45% of the population undergoing hyposaline osmotic shock. This suggests that there is indeed a mechanism by which DMSP is transported through membranes that is not due to cell lysis or death (Lyon et al., 2016).

DMSP uptake It must be noted that initial measurements of the exact added D3DMSP were not done. There may, therefore, have been differences between bottles in the amount added. This was partly accounted for through the use of a linear mixed model for statistical analysis, but should be noted nevertheless.

This study has shown that, with the exact amount depending on species and osmotic pressures, both *Chrysochromulina sp.* and *P. glacialis* are capable of taking up DMSP added to the medium. Specifically, *Chrysochromulina sp.* shows uptake patterns consistent with the hypothesis that it uses DMSP as a compatible solute, taking up more when placed in a more saline environment compared to controls in the low-salinity acclimated group, and taking up less when placed in downshock compared to controls in the high salinity acclimated group. This suggests that this genus opportunistically supplements its own DMSP production using DMSP present in its immediate environment. Considering the fact that *Chrysochromulina sp.* is a bloom-forming species, this may have evolved to be an adaptive mechanism to simultaneously reduce the amount of cellular labour demanded by DMSP production by foraging on the large concentrations of DMSP released by dead cells. Interestingly, DMSP uptake appears to be a short-term response rather than a long-term adaptation, as the acclimation salinity did not have a significant effect on DMSP-uptake in *Chrysochromulina sp.*.

In the case of *P. glacialis*, the pattern is different, with control cultures taking up significantly more DMSP than experimental cultures in both acclimation groups, regardless of osmotic pressure. This could be due to a short-term protective reaction in response to shock, for example through reduced interaction with the environment. *P. glacialis* has a tendency to form cysts under stressful conditions (Royer, 2021; Montresor, Procaccini, and Stoecker, 1999; Stoecker et al., 1997), with one study having found cysts in *P. glacialis* cultures during salinity experiments (Thomson, 2000). This hypothesis would be supported by the observation of string-like aggregations (See Fig. 14) in the mother culture, as well as slow growth, pointing towards the possibility of a fraction of the cultures being in an inactive state. This would not be detected in the measures, as the active fraction could still produce DMSP and show reactions

to experimental conditions. For instance, the specific lower uptake during both shocks could point towards a specific protection mechanism against environmental stressors. Observations under a bright-field microscope (See Fig. 13) did not show any indication of the typical spikes formed by *P. glacialis* cysts, although flagella were not visible either. This does not exclude the possibility of encystment, however, as the sample was taken before the start of any osmotic shock experiments were carried out, and very few cells were observed directly.

DMSP, being a zwitterion, requires special transport mechanisms to be able to cross cell membranes. Bacteria have been shown to possess such ABC transporters (Li et al., 2023), enabling them to consume algal DMSP. However, since no such transporters have been detected in eukaryotes thus far (Li et al., 2023), the question of how they were able to incorporate it remains unanswered. While there is a possibility that D3DMSP was used up by the bacteria in the microsphere around the algae (Cirri & Pohnert, 2019), and appearing in measurements as if it was taken up, the clear up- and downscaling of DMSPp production in response to salinity hint towards an adaptive ability of these cells to take up the compound. Furthermore, similar observations have been made in the non-DMSP producing *Thalassiosira weissflogii*, which was shown to regulate the amount of intracellular DMSP in accordance with osmotic stress (Petrou & Nielsen, 2018), sulphur depletion in the case of heterotrophic algae species (Vila-Costa et al., 2006), in tropical waters (Fernandez et al., 2021), and moderate light levels in the case of polar species (Ruiz-González et al., 2012). Furthermore, DMSP ingestion was found to occur in heterotrophic dinoflagellates as a source of intracellular sulphur compounds (Lee et al., 2012). Uptake was also detected in *Cylindrotheca closterium*, a benthic diatom abundant in intertidal sediments, which took up more under higher light, and excreted DMSP under low salinity. To confirm this, however, these experiments should be repeated with axenic cultures.

One study on the benthic diatom *Cylindrotheca closterium* in increasingly saline media, found that DMSP production plateaued at 55 PSU, and was increasingly replaced by proline (Van Bergeijk, Van Der Zee, & Stal, 2003). This finding underlines the importance of tracing DSMP production along with other possible compatible solutes in understanding its role specific to the organism. This may, for example, differ with nutrient availibility. According to Thomson (2000), for example, *Polarella glacialis* likely produced betaine glycine, another common algae-produced osmolyte (Stefels, 2000), preferentially over DMSP under nitrogen-replete conditions. This would explain their findings of no clear relationship between DMSPp and salinity.

The simulatenous production and release of DMSP may, then, be a short-term physiological response to a metabolic response that is safer to overshoot in response to harsh osmotic shock to prevent plasnolysis, and consequently require regulation through the later release of the compound into the environment to prevent cell damage from water to prevent osmotic lysis. Since 24 hours is a limited time, this may explain the lack of (strong) effect of acclimation salinity on DMSP production rates and release in both species, whereas exposure salinity had a much greater effect.

4.1 Conclusions

Overall, the results confirmed the hypothesis that the effect of osmotic stress on DMSP production and uptake is highly species-dependent, with both *Chrysochromulina sp.* and *P. glacialis* showing a strong reactivity to salinity in its DMSP production, underlining the osmoprotective role of the compound. Differences in production rates are likely due to the specialized need for the brine dinoflagellate *P. glacialis* to adapt to extreme salinity changes and thus a pressure to evolve an efficient reaction to osmotic stress. The simultaneous release of the compound into the medium may be explained by various factors, such as the need to modulate internal concentrations by compensating for overshoot, cryoprotection, and bacterial symbiosis. Moreover, the hypothesis of the ability of these phytoplankton to take up DMSP from the environment was also confirmed. These results, in combination with the heterogeneity in findings in the literature, confirm that DMSP plays fundamentally different physiological roles in different species, likely due to evolutionary adaptations to respective ecological niches. These results highlight the importance of considering ecological and evolutionary aspects of species in climate models incorporating DMS, as well as underscore the importance of further research into the precise mechanisms that govern DMSP-uptake, production and exudation in phytoplankton. Furthermore, both species show signs of DMSP production to regulate overflow, further studies on normalized biomass and carbon should confirm this. In light of the ongoing and projected climate-driven changes to Arctic sea ice, results from this study suggest that the early onset of autotrophic blooms under thinner, melt-pond-covered ice may have vast implications for the timing and magnitude of DMS pulses in the Arctic (Lee et al., 2012).

References

Arrigo, K. R. (2014). Sea ice ecosystems. *Annual Review of Marine Science*, *6*(Volume 6, 2014), 439–467.

Bartoń, K. (2025). *Mumin: Multi-model inference.*

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Berdalet, E., Llaveria, G., & Simó, R. (2011). Modulation of dimethylsulfoniopropionate (DMSP) concentration in an *Alexandrium minutum* (Dinophyceae) culture by small-scale turbulence: A link to toxin production? *Harmful Algae*, *11*, 88–95.

Bullock, H. A., Luo, H., & Whitman, W. B. (2017). Evolution of dimethylsulfoniopropionate metabolism in marine phytoplankton and bacteria. *Frontiers in Microbiology*, *8*, 637.

Caruana, A., Turner, S., Steinke, M., & Malin, G. (2009). Dms and dmsp production by dinoflagellates – is plastid type the key? [Paper presented at the 8th International Conference on Modern and Fossil Dinoflagellates, Liverpool, UK].

Charlson, R. J., Lovelock, J. E., Andreae, M. O., & Warren, S. G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature*, *326*(6114), 655–661.

Cirri, E., & Pohnert, G. (2019). Algae- bacteria interactions that balance the planktonic microbiome. *New Phytologist*, *223*(1), 100–106.

Curson, A. R., Williams, B. T., Pinchbeck, B. J., Sims, L. P., Martínez, A. B., Rivera, P. P. L., Kumaresan, D., Mercadé, E., Spurgin, L. G., Carrión, O., et al. (2018). Dsyb catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton. *Nature Microbiology*, *3*(4), 430–439.

Fernandez, E., Ostrowski, M., Siboni, N., Seymour, J. R., & Petrou, K. (2021). Uptake of dimethylsulfoniopropionate (DMSP) by natural microbial communities of the great barrier reef (GBR), australia. *Microorganisms*, *9*(9), 1891.

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (Third). Sage.

Gao, C., Fernandez, V. I., Lee, K. S., Fenizia, S., Pohnert, G., Seymour, J. R., Raina, J.-B., & Stocker, R. (2020). Single-cell bacterial transcription measurements reveal the importance of dimethylsulfoniopropionate (DMSP) hotspots in ocean sulfur cycling. *Nature Communications*, *11*(1), 1942.

Gebser, B., & Pohnert, G. (2013). Synchronized regulation of different zwitterionic metabolites in the osmoadaption of phytoplankton. *Marine drugs*, *11*(6), 2168–2182.

Guillard, R. R. L. (1975). *Culture of phytoplankton for feeding marine invertebrates* (W. L. Smith & M. H. Chanley, Eds.). Springer US.

Kettle, A. J., Andreae, M. O., Amouroux, D., Andreae, T. W., Bates, T. S., Berresheim, H., Bingemer, H., Boniforti, R., Curran, M. A. J., DiTullio, G. R., Helas, G., Jones, G. B., Keller, M. D., Kiene, R. P., Leck, C., Levasseur, M., Malin, G., Maspero, M., Matrai, P., ... Uher, G. (1999). A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month. *Global Biogeochemical Cycles*, *13*(2), 399–444.

Khan, M., Gillespie, S., Razis, B., Xiao, P., Davies-Coleman, M., Percival, C., Derwent, R., Dyke, J., Ghosh, M., Lee, E., et al. (2016). A modelling study of the atmospheric chemistry of DMS using the global model, stochem-cri. *Atmospheric Environment*, *127*, 69–79.

Klbik, I., Čechová, K., Maťko, I., Lakota, J., & Šauša, O. (2022). On crystallization of water confined in liposomes and cryoprotective action of DMSO. *RSC Advances*, *12*(4), 2300–2309.

Kloster, S., Six, K., Feichter, J., Maier-Reimer, E., Roeckner, E., Wetzel, P., Stier, P., & Esch, M. (2007). Response of dimethylsulfide (DMS) in the ocean and atmosphere to global warming. *Journal of Geophysical Research: Biogeosciences*, *112*(G3).

Krause, a. G., & Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: The basics. *Annual review of plant biology*, *42*(1), 313–349.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26.

Lana, A., Bell, T. G., Simó, R., Vallina, S. M., Ballabrera-Poy, J., Kettle, A. J., Dachs, J., Bopp, L., Saltzman, E. S., Stefels, J., Johnson, J. E., & Liss, P. S. (2011). An updated climatology of surface dimethlysulfide concentrations and emission fluxes in the global ocean. *Global Biogeochemical Cycles*, *25*(1).

Laroche, D., Vézina, A., Levasseur, M., Gosselin, M., Stefels, J., Keller, M., Matrai, P., & Kwint, R. (1999). DMSP synthesis and exudation in phytoplankton:a modeling approach. *Marine Ecology Progress Series*, *180*, 37–49.

Le Rouzic, B. (2012). Changes in photosynthetic yield (Fv/Fm) responses of salt-marsh microalgal communities along an osmotic gradient (mont-saint-michel bay, france). *Estuarine, Coastal and Shelf Science*, *115*, 326–333.

Lee, H., Park, K.-T., Lee, K., Jeong, H. J., & Yoo, Y. D. (2012). Prey-dependent retention of dimethylsulfoniopropionate (DMSP) by mixotrophic dinoflagellates. *Environmental Microbiology*, *14*(3), 605–616.

Lenth, R. V. (2025). *Emmeans: Estimated marginal means, aka least-squares means* [R package version 1.11.2-8].

Li, C.-Y., Mausz, M. A., Murphy, A., Zhang, N., Chen, X.-L., Wang, S.-Y., Gao, C., Aguilo-Ferretjans, M. M., Silvano, E., Lidbury, I. D. E. A., Fu, H.-H., Todd, J. D., Chen, Y., & Zhang, Y.-Z. (2023). Ubiquitous occurrence of a dimethylsulfoniopropionate ABC transporter in abundant marine bacteria. *The ISME Journal*, *17*(4), 579–587.

Lyon, B. R., Bennett-Mintz, J. M., Lee, P. A., Janech, M. G., & DiTullio, G. R. (2016). Role of dimethylsulfoniopropionate as an osmoprotectant following gradual salinity shifts in the sea-ice diatom *Fragilariopsis cylindrus*. *Environmental Chemistry*, *13*(2), 181–194.

Montresor, M., Procaccini, G., & Stoecker, D. K. (1999). Polarella glacialis, gen. nov., sp. nov. (dinophyceae): Suessiaceae are still alive! *Journal of Phycology*, *35*(1), 186–197.

Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface*, *14*(134), 20170213.

Petrou, K., & Nielsen, D. A. (2018). Uptake of dimethylsulphoniopropionate (DMSP) by the diatom *Thalassiosira weissflogii*: A model to investigate the cellular function of DMSP. *Biogeochemistry*, *141*(2), 265–271.

Pinheiro, J. C., Bates, D. M., & Team, R. C. (2025). *Nlme: Linear and nonlinear mixed effects models*.

Pistone, K., Eisenman, I., & Ramanathan, V. (2014). Observational determination of albedo decrease caused by vanishing Arctic sea ice. *Proceedings of the National Academy of Sciences*, *111*(9), 3322–3326.

R Core Team. (2025). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria.

Royer, C. (2021). *Impact of environmental drivers and phytoplankton diversity on the dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) cell quotas: Laboratory experiments and natural variability*. Universite de Liege (Belgium).

Ruiz-González, C., Galí, M., Sintes, E., Herndl, G. J., Gasol, J. M., & Simó, R. (2012). Sunlight effects on the osmotrophic uptake of DMSP-sulfur and leucine by polar phytoplankton. *PLOS ONE*, *7*(9), e45545.

Scarratt, M., Levasseur, M., Michaud, S., Cantin, G., Gosselin, M., & De Mora, S. (2002). Influence of phytoplankton taxonomic profile on the distribution of dimethylsulfide and

dimethylsulfoniopropionate in the northwest Atlantic. *Marine Ecology Progress Series*, *244*, 49–61.

Shaw, D. K., Sekar, J., & Ramalingam, P. V. (2022). Recent insights into oceanic dimethylsulfoniopropionate biosynthesis and catabolism. *Environmental Microbiology*, *24*(6), 2669–2700.

Simó, R. (2001). Production of atmospheric sulfur by oceanic plankton: Biogeochemical, ecological and evolutionary links. *Trends in Ecology & Evolution*, *16*(6), 287–294.

Stefels, J. (2000). Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *Journal of Sea Research*, *43*(3), 183–197.

Stefels, J., Dacey, J. W., & Elzenga, J. T. M. (2009). In vivo DMSP-biosynthesis measurements using stable-isotope incorporation and proton-transfer-reaction mass spectrometry (PTR-MS). *Limnology and Oceanography: Methods*, *7*(8), 595–611.

Stefels, J., Steinke, M., Turner, S., Malin, G., & Belviso, S. (2007). Environmental constraints on the production and removal of the climatically active gas dimethylsulphide (DMS) and implications for ecosystem modelling. *Biogeochemistry*, *83*(1), 245–275.

Stoecker, D. K., Gustafson, D. E., Merrell, J. R., Black, M. M. D., & Baier, C. T. (1997). EXCYSTMENT AND GROWTH OF CHRYSOPHYTES AND DINOFLAGELLATES AT LOW TEMPERATURES AND HIGH SALINITIES IN ANTARCTIC SEA-ICE[1]. *Journal of Phycology*, *33*(4), 585–595.

Thomson, P. G. (2000). *Ecophysiology of the brine dinoflagellate, polarella glacialis, and antarctic fast ice brine communities* [Doctoral dissertation, University of Tasmania].

Toda, K., Obolkin, V., Ohira, S.-I., & Saeki, K. (2023). Abundant production of dimethylsulfoniopropionate as a cryoprotectant by freshwater phytoplanktonic dinoflagellates in ice-covered lake baikal. *Communications Biology*, *6*(1), 1194.

Torstensson, A., Dinasquet, J., Chierici, M., Fransson, A., Riemann, L., & Wulff, A. (2015). Physicochemical control of bacterial and protist community composition and diversity in Antarctic sea ice. *Environmental Microbiology*, *17*(10), 3869–3881.

Van Bergeijk, S. A., Van Der Zee, C., & Stal, L. J. (2003). Uptake and excretion of dimethylsulphoniopropionate is driven by salinity changes in the marine benthic diatom *Cylindrotheca closterium*. *European Journal of Phycology*, *38*(4), 341–349.

van Rijssel, M., & Gieskes, W. W. (2002). Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of *Emiliania huxleyi* (prymnesiophyceae). *Journal of Sea Research*, *48*(1), 17–27.

Vila-Costa, M., Simó, R., Harada, H., Gasol, J. M., Slezak, D., & Kiene, R. P. (2006). Dimethylsulfoniopropionate uptake by marine phytoplankton. *Science (New York, N.Y.)*, *314*(5799), 652–654.

Welschmeyer, N. A., & Lorenzen, C. J. (1984). Carbon-14 labeling of phytoplankton carbon and chlorophyll a carbon: Determination of specific growth rates 1. *Limnology and Oceanography*, *29*(1), 135–145.

Wittek, B., Carnat, G., Delille, B., Tison, J.-L., & Gypens, N. (2020). Dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) cell quotas variations arising from sea

ice shifts of salinity and temperature in the prymnesiophyceae phaeocystis antarctica. *Environmental Chemistry*, *17*(7), 509–523.

5 Acknowledgments

I would like to express my sincere gratitude to Dr. Jacqueline Stefels for her supervision and support throughout the research project. Many thanks also go to Dr. Maria van Leeuwe for her help in the laboratory.

6 Data Availability

All code has been made available on GitHub: https://github.com/fmurakeozykis/DMSP_Osmotic_Shock.

7 AI Statement

I acknowledge the use of ChatGPT and Perplexity AI as tools to aid in troubleshooting R code, to identify points of improvement in writing, and find relevant references. All improvements that were included in my final thesis have been reviewed and rewritten in my own words.

8 Appendix

8.1 Supporting Figures

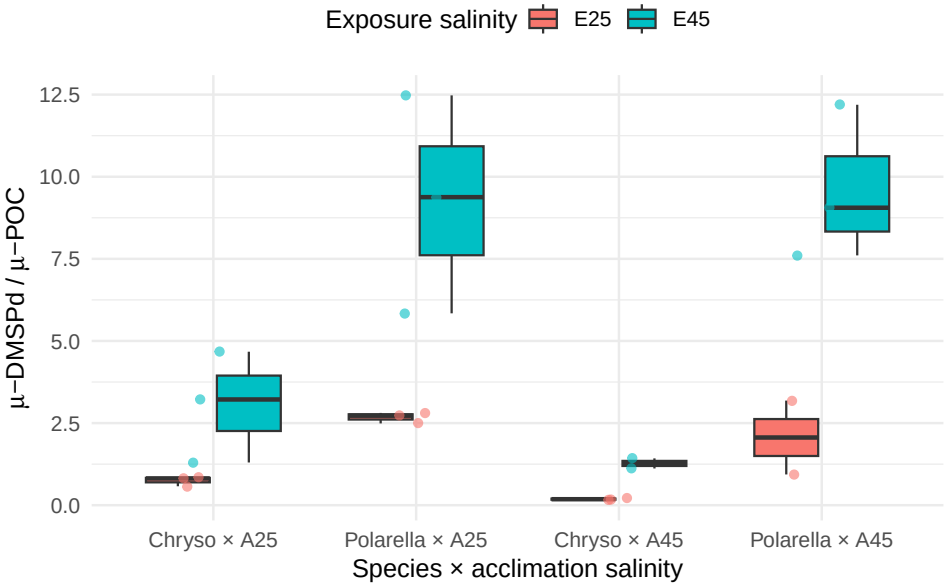

Figure 10 – Boxplots of dissolved DMSP production rate as a fraction of total particular carbon incorporation rate across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

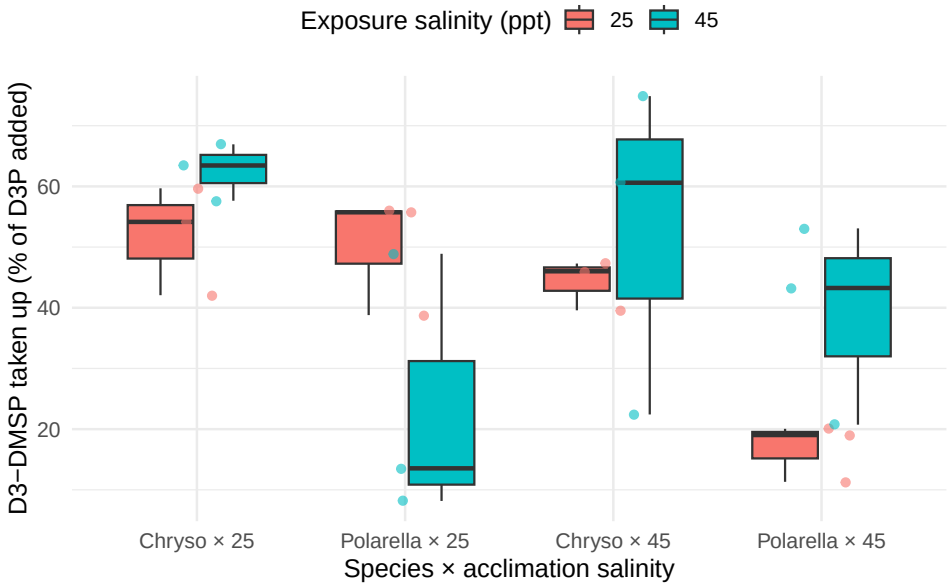

Figure 11 – Boxplots of DMS production rate across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

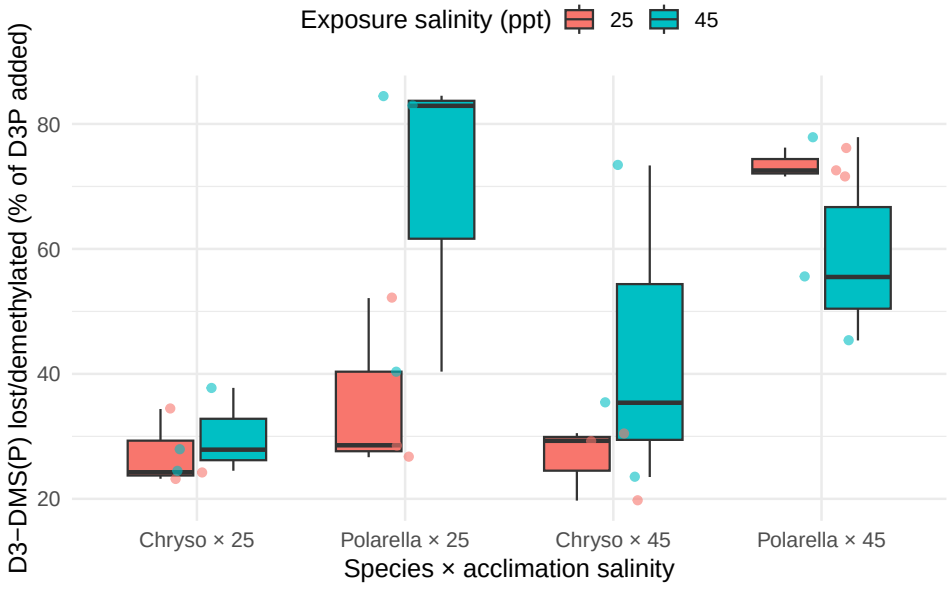

Figure 12 – Boxplots of added D3-DMSP loss across species, acclimation salinities (A25, A45), and exposure conditions (E25, E45). Groups with a final salinity of 45 ppt are depicted in blue, those with a final salinity of 25 are depicted in red. Individual data points have been added in corresponding colours.

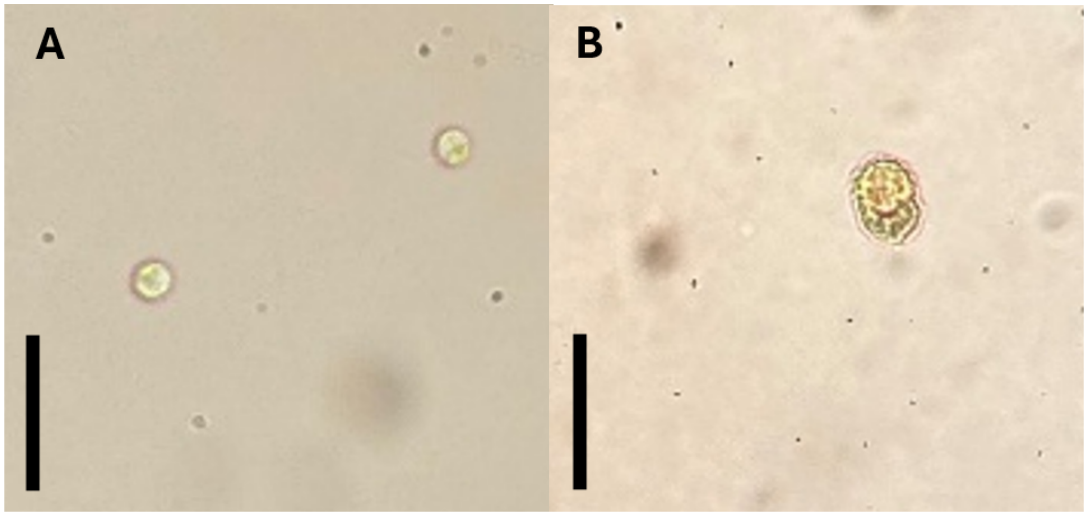

Figure 13 – Light micrographs of *Chrysochromulina sp.*chromulina sp. (A) and Polarella glacialis (B) cells (day 3 after inoculation of *Chrysochromulina sp.*). Scale bars: 25 µm.

Figure 14 – Photograph of *P. glacialis* mother culture, featuring string-like aggregation, as well as clumping together and sinking.