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ORIGINAL ARTICLE

Response of dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) cell quotas to oxidative stress in three phytoplankton species

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Several phytoplankton species produce the metabolites dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) but their intracellular roles need to be better understood. To improve the understanding of the DMSP antioxidant function suggested by Sunda *et al*. (2002), we exposed the diatom *Skeletonema costatum*, the Prymnesiophyceae *Phaeocystis globosa* and the dinoflagellate *Heterocapsa triquetra* to experimental treatments known to cause potential oxidative stress (high light intensities (HL); HL with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU); menadione sodium bisulfite (MSB)). DMSP and DMSO concentrations decreased after 6 h in all treatments indicating an interaction with Reactive Oxygen Species (ROS) produced. DMSP and DMSO-to-cell ratios in control conditions were higher for *H. triquetra*, while being unable to grow under HL. DMSP and DMSO-to-cell carbon were the highest for *P. globosa*, while the other species had similar values. During long-term treatment, these ratios were not increased in high-light grown cells of *P. globosa* and *S. costatum*. Overall, this illustrates that (1) the DMSP- and DMSO-to-cell or carbon seems to be not indicative of the capability of the species to tolerate an oxidative stress, (2) these molecules could react with ROS and lower their cellular concentration, but no clues demonstrated that these molecules are part of the antioxidant response of the cell.

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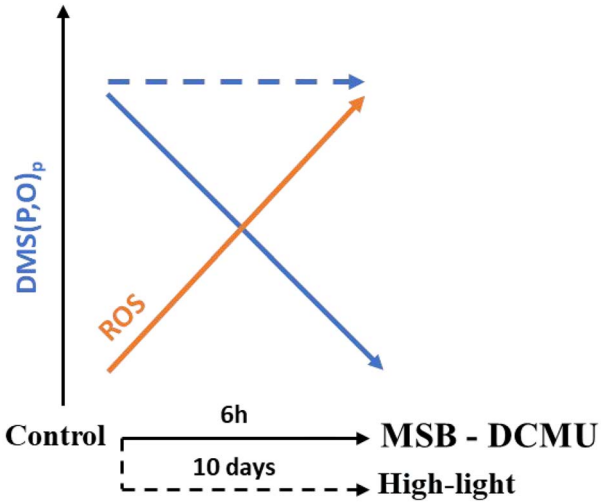
KEYWORDS: DMSP; DMSO; antioxidant; reactive oxygen species; light stress; Menadione; DCMU; Skeletonema costatum; Phaeocystis globosa; Heterocapsa triquetra

Highlights:

- DMS(P,O) might act as antioxidants during high-light and chemical treatments.
- DMS(P,O) might simply react with ROS due to their relative high abundance in cell.
- DMS(P,O) are not indicative of the cell tolerance to a subsequent oxidative stress.

Graphical Abstract



INTRODUCTION

Light, temperature and nutrient availability can be highly dynamic in aquatic ecosystems, varying at short (from seconds to hours; e.g. light) and long timescales (from day to season; e.g. temperature, nutrients or light) (Litchman and Klausmeier, 2001; Müller *et al*., 2001; Jahns and Holzwarth, 2012; Erickson *et al*., 2015). Hence in phytoplanktonic cells light-harvesting capacity has to be continuously adjusted to meet the cellular energetic demands, which in turn depend on the nutritional status of the cell (Goss and Jakob, 2010). With the evolution of processes such as photosynthesis or respiration, it has been established that all oxygen-metabolizing organisms produce reactive oxygen species (ROS) (Apel and Hirt, 2004; Lesser, 2006; Diaz and Plummer, 2018). ROS are a group of free radicals, reactive molecules and ions derived from molecular dioxygen (O_2; Sharma *et al*., 2012). In phototrophic organisms, ROS are mainly produced within the chloroplasts by: (1) energy transfer from excited chlorophyll (Chl) to O_2, leading to the formation of singlet oxygen ($^1O_2$) at the photosystem II (PSII) (Jahns and Holzwarth, 2012; Ruban *et al*., 2012; Pospíšil, 2016); and (2) direct reduction of oxygen at the acceptor side of photosystem I (PSI) (Mehler reaction), leading to the formation of superoxide radicals ($O_2^{\cdot-}$). The latter can be subsequently dismutated to hydrogen peroxide (H_2O_2) and further hydroxyl radical ($OH\cdot$) in presence of transition metal via the Haber-Weiss/Fenton reaction (Mallick and Mohn, 2000; Apel and Hirt, 2004; Jahns and Holzwarth, 2012; Pospíšil, 2016). These ROS are scavenged by enzymatic antioxidants, such as dismutases, catalases and peroxidases (Apel and Hirt, 2004; Asada, 2006), and non-enzymatic antioxidant compounds comprising ascorbate, glutathione, α-tocopherol, flavonoids, alkaloids and carotenoids (Dummermuth *et al*., 2003; Lesser, 2006). However, under adverse environmental conditions (i.e. high light intensity), the tight equilibrium between ROS production and the antioxidant network can be destabilized, and ROS in excess cause damages to proteins, lipids, carbohydrates, deoxyribonucleic acid (DNA), and ultimately trigger cell death (Apel and Hirt, 2004; Lesser, 2006; Van Alstyne, 2008; Gardner *et al*. 2016).

The dimethylsulfoniopropionate (DMSP), dimethylsulfoxide (DMSO) (here after DMS(P,O)) are biogenic sulfur

molecules that play a key role in the cycling of dimethylsulfide (DMS), a climate active gas (Liss *et al*., 1997; Stefels *et al*., 2007). DMS(P,O) are ubiquitous in seawater and produced by a large variety of micro- and macroalgae as well as some angiosperms and corals (Keller *et al*., 1989; Stefels, 2000; Simó and Vila-Costa, 2006; Hatton and Wilson, 2007; Raina *et al*., 2013; Borges and Champenois, 2017; McParland and Levine, 2019). DMS(P,O) may act as cryoprotectants, osmolytes (Kirst *et al*. 1996; Bucciarelli and Sunda, 2003), zooplankton deterrents (Wolfe *et al*. 1997; Strom *et al*. 2003), signaling compounds (Stefels, 2000; Seymour *et al*., 2010) or as ballast in algal cells (Lavoie *et al*., 2015). In addition, both DMS and its precursors DMS(P,O) are suspected to act as antioxidant molecules because: (1) the potential for DMSP accumulation in chloroplasts is in line with the ROS production in this cellular compartment (Trossat *et al*., 1998; Raina *et al*., 2017; Curson *et al*., 2018); (2) they have been associated with oxidative stress caused by high light intensity, UV-radiation, nutrient limitation or hyposalinity (Karsten *et al*., 1992; Stefels and van Leeuwe, 1998; Sunda *et al*., 2002; Bucciarelli and Sunda, 2003; Husband *et al*., 2012; Deschaseaux *et al*., 2014; Gardner *et al*., 2016); (3) the exogenous addition of DMSP and acrylate on plant leaves have been shown to reduce oxidative damages (Husband *et al*., 2012); and (4) they can readily scavenge ROS, in particular OH• (Scaduto, 1995; Lee and De Mora, 1999; Sunda *et al*., 2002). The antioxidant capacity of the DMSP pathway would be partly regulated by the activity of DMSP-lyase (DL) as the enzyme cleavage products, DMS and acrylate, are ∼60 and ∼20 times more efficient in OH• scavenging than DMSP (Sunda *et al*., 2002). In addition, DMS could also react with $^1O_2$ (Wilkinson *et al*., 1995). Finally, DMS released by the activation of the DMS(P,O) pathway would act as a negative feedback mechanism on daily dose of solar and UV radiation by enhancing cloud albedo and thereby decreasing the incoming solar radiation, supporting a potential climate-cooling feedback loop (CLAW hypothesis, Charlson *et al*., 1987; Sunda *et al*., 2002). Within marine phytoplankton, the Prymnesiophyceae and the dinoflagellates are considered as high-DMSP producers, while the diatoms are low-DMSP producers even if a high variability within each group is observed (Keller *et al*., 1989; Stefels *et al*., 2007; McParland and Levine, 2019). Also, the DL activity has been found only in some Prymnesiophyceae and dinoflagellates (Stefels *et al*., 2007; Caruana and Malin, 2014; Mohapatra *et al*., 2014 ; Alcolombri *et al*., 2015). Overall, this suggests that the contribution of DMS(P,O) to the antioxidant network might differ among phytoplankton species.

Studies aiming at improving the knowledge on DMS(P,O) cell quotas and their regulation according to abiotic parameters are necessary to better estimate the DMS(P,O) concentrations based on Chl-*a* and the phytoplankton composition. The clarification of the biological role of DMS(P,O) within the phytoplankton cell can also improve our understanding of how any climate feedback loop might operate (Ayers and Cainey, 2007) and ultimately, will help to better assess the DMS fluxes in ocean–atmosphere modeling systems. In order to improve our understanding of the antioxidant role played by DMS(P,O) in marine phytoplankton, we investigated the impact of oxidative stress on DMS(P,O) cellular concentrations in three phytoplankton species, characterized by different DMS(P,O):Chl-*a* contents. We expect an upregulation, and a decrease of the DMS(P,O) concentrations, during a long-term and a short-term oxidative stress, respectively. To this end, monospecific and axenic cultures of *Skeletonema costatum* (diatom), *Phaeocystis globosa* (Prymnesiophyceae) and *Heterocapsa triquetra* (dinoflagellate) were exposed to three different experimental treatments known to cause a potential oxidative stress and consisting in: (1) a short- and long-term light stress; (2) a short-term exposition to menadione sodium bisulfite (MSB), a prooxidant molecule; and (3) a short-term light stress in presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a chemical agent blocking the photosynthetic electron transport.

MATERIAL AND METHODS

Algal species and culture conditions

The phytoplankton species studied were the diatom *S. costatum* isolated from the Southern North Sea; the Prymnesiophyceae *P. globosa* RCC1719 originating from the Roscoff Culture Collection (English Channel, France); and the dinoflagellate *H. triquetra* RCC4800 originating from the Roscoff Culture Collection (English Channel, France). Prymnesiophyceae and dinoflagellates exhibit a DL activity (Stefels and Dijkhuizen, 1996; Caruana and Malin, 2014). For all experiments, cells were cultured axenically in F/2 medium (Guillard and Ryther, 1962) made with 0.2 µm filtered and autoclaved natural seawater (collected at the Belgian Coastal Zone). Silica (Na_2SiO_3; final concentration 107 µmol L^{-1}) was added in the culture medium for *S. costatum*. Batch cultures of all the species were grown to the exponential growth phase in 2 L Nalgene bottles containing 1 L of F/2 medium. Cultures were maintained at 15°C under cool white fluorescent bulbs providing a total light intensity of 100 µmol photons m^{-2} s^{-1} (12 h:12 h light:dark cycle) in an Aralab Fitoclima S600 incubator. Light intensities were determined between 400 and 700 nm using a LI-250

light meter (Li-Cor, USA) with a US-SQS/A light sensor (Walz, Germany).

Experimental treatments

Three experimental treatments were designed to assess the impact of ROS production on DMS(P,O) cellular concentrations in the phytoplankton species investigated: (1) a high light (HL) stress; (2) a chemical stress with MSB; and (3) a stress combining the use of DCMU and high light intensity. For each treatment, the temperature was kept at 15°C.

During the long-term HL stress, cells cultured at 100 µmol photons m^{-2} s^{-1} (control; I0) were exposed to light intensities of 600 (I1) and 1200 (I2) µmol photons m^{-2} s^{-1} (12 h:12 h light:dark cycle) for up to 15 days. Cellular density, Chl-*a* and DMS(P,O) contents were analyzed at mid-exponential growth stage (days 8–10) of this long-term stress.

A short-term HL treatment of 6 h at 1200 µmol photons m^{-2} s^{-1} was applied to cells cultured at 100 µmol photons m^{-2} s^{-1} to determine the chlorophyll concentrations and fluorescence, ROS production and lipid peroxidation (see 3.3 Analyses).

During the second experimental treatment, cells were exposed in the dark for 6 h to 25 µmol L^{-1} of MSB diluted in F/2 medium. This water-soluble compound is commonly used as a chemical agent causing oxidative stress in plants and microalgae (e.g. Sun *et al*., 1999; Borges *et al*. 2009; Roberty *et al*. 2016). Once incorporated in the cell, MSB reacts with a variety of reductive enzymes and in presence of O_2, the unstable semiquinones formed enter into a redox cycle, causing the reformation of quinones with the concomitant generation of $O_2^{\bullet-}$ and H_2O_2 (Hassan & Fridovich, 1979). The MSB concentration applied was determined experimentally on the basis of photosynthetic activity measurements (ΦPSII) in dark adapted samples (see 3.3.3 Chlorophyll fluorescence measurements). A treatment of 6 h at 25 µmol L^{-1} was chosen because it moderately impacted the photophysiology of the species investigated (i.e. by 25–50%).

And finally, for the third experimental treatment, cells were exposed for 6 h to 1200 µmol photon m^{-2} s^{-1} in presence of 10 nmol L^{-1} DCMU. This inhibitor competes for the binding site of plastoquinone Q_B and blocks the electron flux from PSII, promoting the formation of ROS within the chloroplasts (Haynes *et al*., 2000; Baker 2008). Based on the photosynthetic activity measurement (ΦPSII) after 30 min in dark adapted samples (see 3.3.3 Chlorophyll fluorescence measurements), the concentration chosen in this study inhibited PSII activity by 60, 70 and 40% in *S. costatum*, *P. globosa* and *H. triquetra*, respectively.

Analyses

The assessment of the potential oxidative stress applied was studied by the analyses of the cellular Chl-*a* quota, the Chl-*a* fluorescence, the ROS production and the cellular damages with the LPO. Those observations were conducted in parallel with the DMS(P,O) cellular quota.

Cell volume and cellular carbon concentration

The cellular concentration (cell L^{-1}) for the long-term HL treatment was determined at mid-exponential growth stage with an inverted microscope (Leitz fluovert) by using the Utermöhl sedimentation procedure on samples fixed with lugol-gluteraldehyde (10 µL mL^{-1}) (Hasle, 1978). A minimum of 400 cells around the slide were counted to have a 10% maximum error within a confidence interval of 95% (Lund *et al*., 1958).

For the short-term DCMU treatment, the cellular density and cell biovolume (μm^3) were obtained using a Z2 Coulter Particle Count and Size Analyser Version 1.01 with known volume of culture mixed with 10 mL of isoton (demineralized water with 9 g L^{-1} of NaCl and 0.5% v:v of formaldehyde). The biovolume (μm^3) for the long-term HL treatment was calculated by measuring with an inverted microscope (Leitz fluovert) the dimensions of cells according to Hillebrand *et al*. (1999) and converted into biomass per cell (pgC $cell^{-1}$) with the equations proposed by Menden-Deuer and Lessard (2000).

Chlorophyll concentrations

For the long-term HL treatment, a determined volume of the phytoplankton cultures was filtered on Whatman glass microfiber filters GF/F 25 mm and immediately frozen and stored at −20°C until analysis (within 1 month after sampling). Chl-*a* was then extracted at 4°C in acetone 90% (v:v) and measured fluorometrically using a Kontron Instruments SFM 25 (Strickland and Parsons, 1972). Chl-*a* concentrations (µg mL^{-1}) were determined using a Chl-*a* standard solution (1000 µg L^{-1}; Chl-*a* analytical standard, Merck).

For the MSB and DCMU short-term treatments, Chl-*tot* (Chl-*a* + Chl-c_2) from concentrated aliquots of cultures (3600 x g for 3 min) were extracted in ice-cold 100% MeOH in presence of 0.5 mL of glass beads (710–1180 µm; Sigma-Aldrich, USA). Samples were then vortexed during 5 min at 30 Hz and at 4°C using a Tissue Lyser II (Qiagen, Germany). After debris removal (centrifugation 10 000 x g 10 min with a MicroStar 17 (VWR, Belgium)), Chl-*tot* (µg mL^{-1}) were determined by using a SP2000 spectrophotometer (Safas, Monaco) and the equations of Ritchie (2006). The Chl-*tot* concentrations

were determined at the beginning and the end of the treatment.

Chlorophyll fluorescence measurements

In vivo Chl-*a* fluorescence measurements were performed at room temperature using a fluorescence imaging system (SpeedZen, BeamBio, France) described in Vega de Luna *et al*. (2019). Briefly, aliquots of the cultures were harvested and concentrated by gentle centrifugation to reach 10 µg Chl-*tot* mL^{-1} in fresh F/2 medium. The maximum quantum yield of PSII was calculated as F_V/F_M, where $F_V = F_M - F_0$, F_0 is the initial fluorescence level in dark-adapted sample (~10 min) and F_M is the maximum fluorescence level after a saturating pulse of light (150 ms at 4000 µmol photons m^{-2} s^{-1}). The effective photochemical quantum yield (ΦPSII) was calculated as $(F_{M'}\text{-}F)/F_{M'}$, where F is the fluorescence signal and $F_{M'}$ is the maximum fluorescence level obtained with a saturating pulse under the light (after 3 min at 230 µmol photons m^{-2} s^{-1}) (Genty *et al*., 1989). The Chl-*a* fluorescence measurements were performed at 0 and 6 h.

ROS production

ROS production was monitored by using carboxy-H_2DCFDA (6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate) (Molecular Probes, Life technologies) during the MSB treatment and the Amplex Red reagent (Molecular probes, Life technologies, USA) during the short-term HL and DCMU treatments. For both measurements, aliquots of cultures were harvested and concentrated by gentle centrifugation to contain 10 µg Chl-*tot* mL^{-1} in fresh F/2 medium. For the AmplexRed treatment, 150 µmol L^{-1} of DTPA (diethylenetriamine pentaacetate) was added to the culture medium at least 24 h prior to the analysis to form complexes with trace metals in order to prevent their reaction with $O_2^{\bullet-}$ (Saragosti *et al*., 2010). ROS production was normalized with initial Chl-*tot* concentrations at t0h.

Carboxy-H_2DCFDA is a general oxidative stress indicator. When this nonpolar compound enters the cells, it is deacetylated by esterases to carboxy-DCFH. The latter is then converted by various reactive species produced within the cell into carboxy-DCF, a fluorescent compound, whose concentration is measured by spectrofluorometry. Conditions of this assay were similar to those described in Roberty *et al*. (2016). Briefly, 1 mL of each culture was incubated with 25 µmol L^{-1} carboxy-H2DCFDA for 30 min in the dark. Cells were then washed and resuspended into 1 mL of fresh F/2 medium and placed in a Binder KB115 incubator (Binder, Germany) set to the treatment conditions. The fluorescence of the samples was then measured in black 96-well microplates (Greiner Bio-One) at 528 nm with a 485 nm excitation wavelength provided by a Synergy Mx spectrofluorometer (Biotek, USA). The measurement was performed at 0 and 6 h.

The relative production of ROS during the short-term HL and DCMU treatments was evaluated by using the Amplex Red reagent (Molecular probes, Life technologies, USA). This colorless probe reacts with H_2O_2 in the presence of peroxidase and forms a fluorescent compound, resorufin. As described in Roberty *et al*. (2015), aliquots of cultures were combined with Amplex Red (100 µmol L^{-1}) and horseradish peroxidase (0.2 U mL^{-1}), and placed in a Binder KB115 incubator (Binder, Germany) set to the treatment conditions. Then, samples were centrifuged, and the fluorescence emitted by the supernatant in black 96-well microplates was measured at 590 nm with a 540 nm excitation wavelength provided by a Synergy Mx spectrofluorometer (Biotek, USA). Concentrations of H_2O_2 were calculated by comparing fluorescence emitted by the samples to a H_2O_2 standard curve (0–10 µmol L^{-1}). As the Amplex Red (AR) reagent is sensitive to photo-oxidation, a Rose Pink filter (Lee Filters, Andover, UK) was used during experimental treatments to exclude wavelengths of light strongly absorbed by the reagent, and the experimental treatment was also limited to 3 h. Various controls were performed: without AR, and with AR (and DCMU) in the dark to evaluate basal cellular ROS production.

Lipid peroxidation assay

The level of lipid peroxidation (LPO; mmol t-BuOOH:g Chl-*tot*) was assessed in phytoplankton cells exposed to experimental treatments by using the PeroxiDetect Kit (Sigma Aldrich, USA). Aliquots of the cultures were harvested and concentrated to obtain a final Chl-*tot* concentration of 20 µg mL^{-1} in fresh F/2 medium. LPO was measured using a methanolic reagent containing xylenol orange and butylated hydroxytoluene (BHT). The determination of LPO was performed following manufacturer's instructions at the beginning and the end of the short-term treatments. Then, the absorbance of the samples was measured at 560 nm using a SP2000 spectrophotometer (Safas, Monaco). LPO was normalized with initial Chl-*tot* concentrations at t0h.

DMS(P,O) analysis

The DMS(P,O) analyses were performed at mid-exponential growth stage for the long-term HL acclimation, and at the beginning and the end of the MSB and DCMU treatments. Three biological replicates of

particulate DMS(P,O) ($DMS(P,O)_p$) were obtained by the difference between 10 mL of unfiltered seawater samples (total DMS(P,O)—$DMS(P,O)_t$) and dissolved DMS(P,O) ($DMS(P,O)_d$). $DMS(P,O)_d$ was obtained by gentle filtration of 15 mL and only the first 10 mL of filtrate were collected to avoid cell destruction at the end of the filtration that could release DMSP (Kiene & Slezak, 2006). Samples were then microwaved individually till boiling to inhibit the DL activity that converts DMSP into DMS (Kinsey and Kieber, 2016) and acidified with 5 μL mL^{-1} of 50% H_2SO_4 (del Valle *et al*. 2011), to arrest any biological activity (Curran *et al*, 1998). Samples were crimped after cooling with gas tight PFTE coated silicone septa and kept 24 h at room temperature in the dark to allow the DMS to degas or oxidize (Kiene and Slezak, 2006). Then, samples were stored at 4°C until GC analysis. The samples were sparged to remove the potential DMS and left for 20 min. A total of 5 mL of 12 M NaOH was added to the 10 mL samples to obtain a pH > 12 and quantitatively cleave DMSP into DMS for 24 h (Dacey and Blough, 1987; Stefels, 2009). The DMS(P,O) concentrations were determined using an Agilent 7890B purge and trap gas chromatography (GC) (Agilent column 30 m long, 0.32 mm internal diameter, 0.25 μm film thickness) equipped with sulfur selective Flame Photometric Detector (FPD) and the carrier gas was He (2 mL min^{-1}). For the DMSO analysis, 5 mL HCl 37% (HCl 37% Normapur, VWR) and 1 mL $TiCl_3$ (30%, Merck) (Kiene & Gerard, 1994; Deschaseaux *et al*., 2014) were added into the precedent vial yet analyzed (Champenois and Borges, 2019). After 48 h at room temperature, 3 mL of 12 M NaOH was added to avoid injecting acid fumes into the GC (Kiene & Gerard, 1994). The same procedure was applied for the calibration. The DMSP used was obtained from Research Plus and the DMSO from 99,9% pure stock solution (Merck). Working solutions were prepared with the successive dilution in MilliQ water but DMSP and DMSO were diluted in the same vial. Calibration curves were made weekly to ensure the GC stability for the detector by fitting a quadratic curve for the FPD. The average precision was 5 and 8% for DMSP and DMSO calibration, respectively. DMS(P,O) concentrations as well as normalization by cells and cell carbon were used to analyze the data.

Statistics

To investigate the correlation between the variables, the Pearson's r coefficient and its P value was used. In case of deviation of normality by the Shapiro–Wilk test ($P < 0.05$), the nonparametric Spearman's ρ coefficient was applied. The parametric paired-samples Student t-test was used to compare two related groups (i.e. only between t0h and t(x)h) on the same continuous and dependent variable. The assumption of normality was checked using the Shapiro–Wilk test. In case of deviation of the normality ($P < 0.05$), the Wilcoxon t-test was applied. These statistics analyses were performed using JASP software (van Doorn *et al*. (2021), Version 0.11.1) and the assumptions were based on Goss-Sampson (2020). The Kaiser-Meyer-Olkin index (>0.6) and Bartlett sphericity test ($P < 0.05$) were used to ensure the application of Principal Component Analysis (PCA) while outliers were not detected. PCA was performed on $DMS(P,O)_p$ contents (nmol L^{-1}), Chl-*tot* (μg L^{-1}), F_V/F_M, ΦPSII, ROS production (mole:g Chl-*tot*; fluorescence:μg Chl-*tot*) and LPO (mmol:g Chl-*tot*), using JMP Pro 14.

RESULTS

High light stress

The exposure of low light acclimated cells (i.e. 100 μmol photons m^{-2} s^{-1}) to 1200 μmol photons m^{-2} s^{-1} for 6 h strongly impacted the photosynthetic activities of the three species investigated. The maximal photochemical quantum yield (F_V/F_M) was inhibited by 81, 46 and 66%, and the ΦPSII values were decreased by 45%, 48% and 65% for *S. costatum*, *P. globosa* and *H. triquetra*, respectively ($P < 0.01$; Fig. 1A, B). The extracellular ROS production increased significantly by 3.0 for *S. costatum*, 2.2 for *P. globosa* and 2.7 for *H. triquetra* ($P < 0.05$; Fig. 1C), but the pool of peroxidised lipids remained unchanged at the end of the experimental treatment (Fig. 1D). The short-term light stress did not have any significant impact on the chlorophyll content of the species investigated neither (data not shown).

In the long term, HL treatments (I1 and I2) did not impact the cell density observed of *S. costatum* and *P. globosa*, on the contrary to *H. triquetra* that was unable to grow at 1200 μmol photons m^{-2} s^{-1} (Fig. 2A). In contrast, the cellular Chl-*a* concentrations (Chl-a:C [g:g]) decreased significantly with increasing light intensities ($P < 0.05$; Fig. 2B). As a consequence, the $DMS(P,O)_p$ concentrations relative to Chl-*a* or cellular quota showed opposite trends with high variability between the species investigated. The $DMSP_p$:cell (fmolS:cell) were similar between the treatments for *S. costatum*, while ratios were significantly lower at I1 for *P. globosa* (but not at I2) and *H. triquetra* compared to I0 ($P < 0.05$; Fig. 2C). The $DMSO_p$:cell (fmolS:cell) did not change with light intensity whatever the species (Fig. 2D). When reported by chlorophyll amount, $DMSP_p$ ($DMSP_p$:Chl-*a*) contents

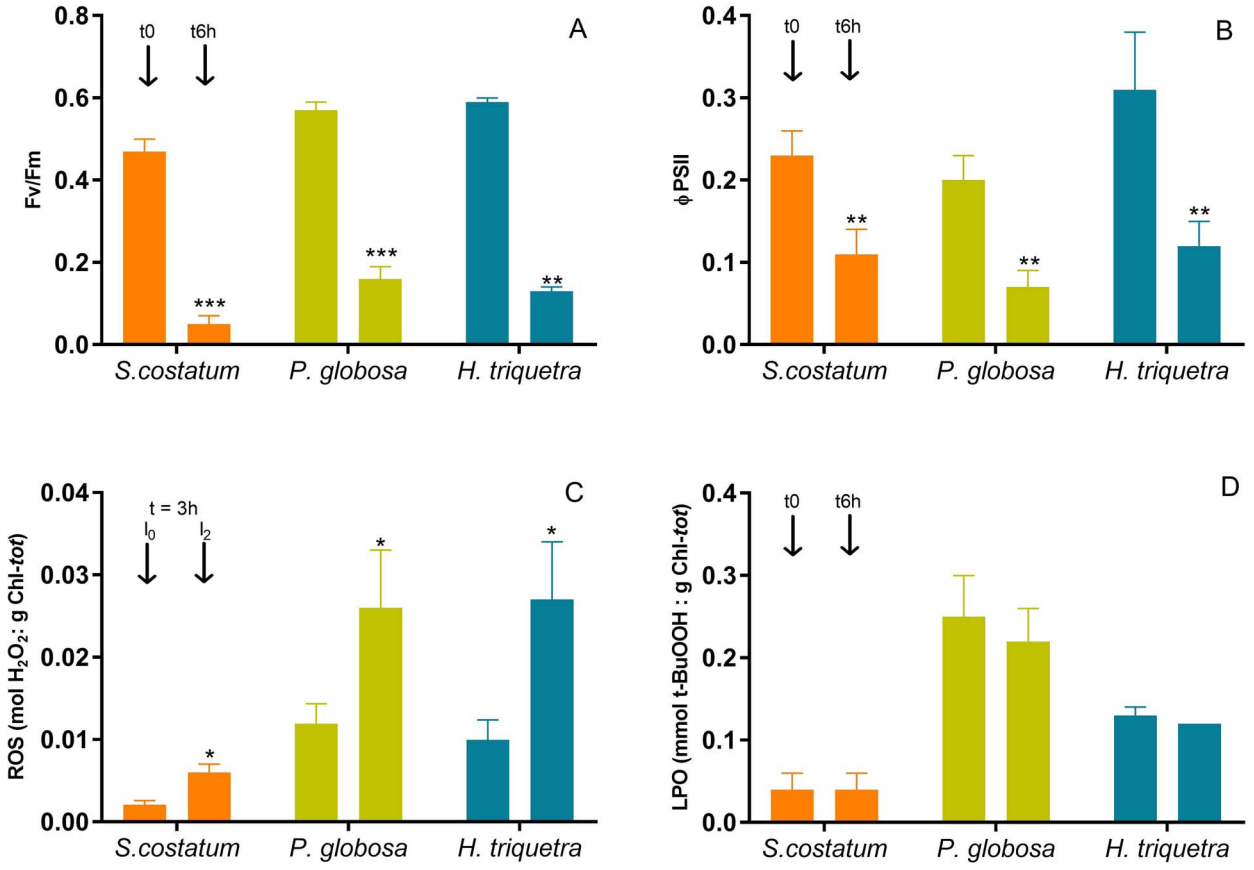


Fig. 1. Evolution of (A) Maximum quantum yield of PSII (Fv/Fm), (B) Effective photochemical quantum yield of PSII (ΦPSII), (C) Reactive oxygen species (ROS) (mol H_2O_2:g Chl-*tot*) at the beginning and after 3 h, (D) Lipid peroxidation (LPO) (mmol t-BuOOH:g Chl-*tot*) with increasing light intensity from 100 to 1200 µmol photon m^{-2} s^{-1} during 6 h for the three species *S. costatum*, *P. globosa* and *H. triquetra*. ROS analysis was performed after 3 h. Error bars represent SD calculated from triplicates biological samples. Asterisks denote significant differences between the time point 0 and 6 h, or after 3 h between the control conditions at 100 and 1 200 µmol photon m^{-2} s^{-1} (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

were positively correlated with light intensities ($R^2 = 0.74$ and $P < 0.01$ for *S. costatum*; $R^2 = 0.55$ and $P < 0.05$ for *P. globosa*; and $R^2 = 0.90$ and $P < 0.01$ for *H. triquetra*). The $DMSP_p$:Chl-*a* ratio doubled from I1 for *P. globosa* and *H. triquetra* but at I2 for *S. costatum* ($P < 0.05$; Fig. 2E). The $DMSO_p$:Chl-*a* ratio of *S. costatum* was significantly impacted by the light treatments and a 3- and a 4-fold increase of ratio was observed at I1 and I2, respectively ($P < 0.01$; Fig. 2F). A similar but not significant trend was observed in *P. globosa* and *H. triquetra* (Fig. 2F).

The calculation of $DMSP_p$-to-cell carbon ($DMSP_p$:C) at I0 led to higher values ($P < 0.01$) for *P. globosa* (4.5 ± 0.6 mmol g^{-1}), while *S. costatum* (0.6 ± 0.1 mmol g^{-1}) and *H. triquetra* (0.4 ± 0.1 mmol g^{-1}) had similar ratio ($P = 0.08$) (Fig. 2G). The same trend was observed for the $DMSO_p$-to-cell carbon ($DMSO_p$:C), with 0.7 ± 0.4 mmol g^{-1} for the Prymnesiophyceae, and 0.03 and 0.05 ± 0.02 mmol g^{-1} for *S. costatum* and *H. triquetra*, respectively, and with no significant difference between the species ($P > 0.05$; Fig. 2H). Also, HL treatments did not impact the cell biovolume of the three species (Supplementary Table 2B) with similar $DMSP_p$:C and $DMSO_p$:C between the light treatments (Fig. 2G, H). Significant differences were only observed at I1 for *P. globosa* and *H. triquetra* regarding their $DMSP_p$:C ($P < 0.05$; Fig. 2G).

DCMU treatment

Cultures of the three phytoplankton species were exposed to a photosynthetic photon flux density (PPFD) of 1200 µmol photons m^{-2} s^{-1} (I2) in presence of 10 nmol L^{-1} of DCMU. As expected, the treatment strongly impacted the photosynthetic efficiency in the three species investigated. After 6 h, F_V/F_M values decreased on average by 81, 93 and 77% in *S. costatum*, *P. globosa* and *H. triquetra* ($P < 0.001$; Fig. 3A) and the ΦPSII was totally inhibited (100%) in *P. globosa*, and at about 75% in the two remaining species ($P < 0.001$; Fig. 3B).

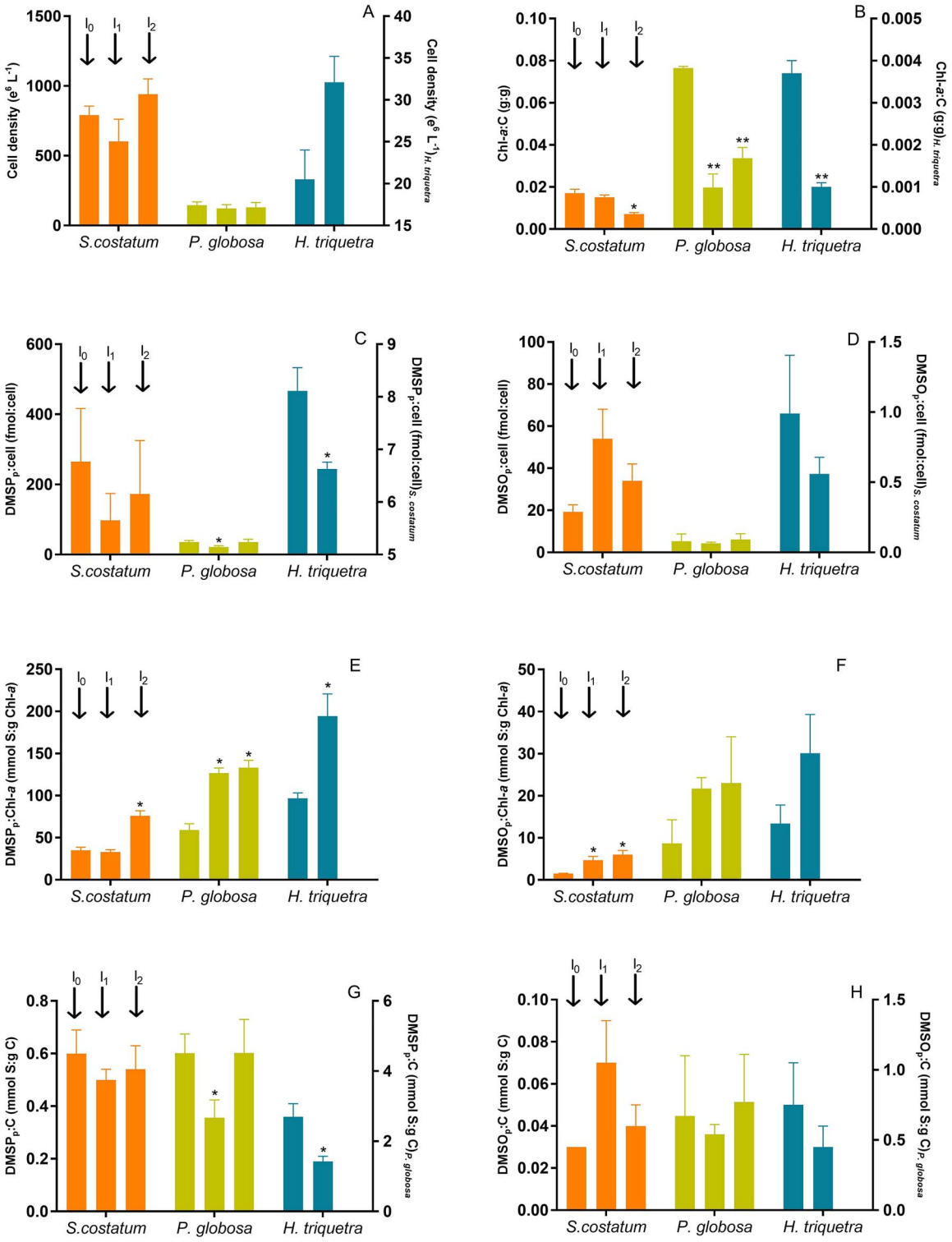


Fig. 2. Evolution of (A) Cellular density ($\times 10^6$ cells L^{-1}); (B) Cellular Chlorophyll-*a* content (Chl-*a*:C) (g:g), (C) the $DMSP_p$:Cell ratio (fmolS:cell); (D) the $DMSO_p$:Cell ratio (fmolS:cell); (E) the $DMSP_p$:Chl-*a* ratio (mmolS:g Chl-*a*), (F) the $DMSO_p$:Chl *a* ratio (mmolS:g Chl-*a*); (G) the $DMSP_p$:C ratio (mmolS:g C), (F) the $DMSO_p$:C ratio (mmolS:g C) at three light intensities of 100 (left bar), 600 (central bar) and 1200 (right bar) µmol photon m^{-2} s^{-1} during the long-term HL treatment for the three species *S. costatum*, *P. globosa* and *H. triquetra*. Error bars represent SD calculated from triplicates biological samples. Asterisks denote significant differences between the control and the light intensity considered ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

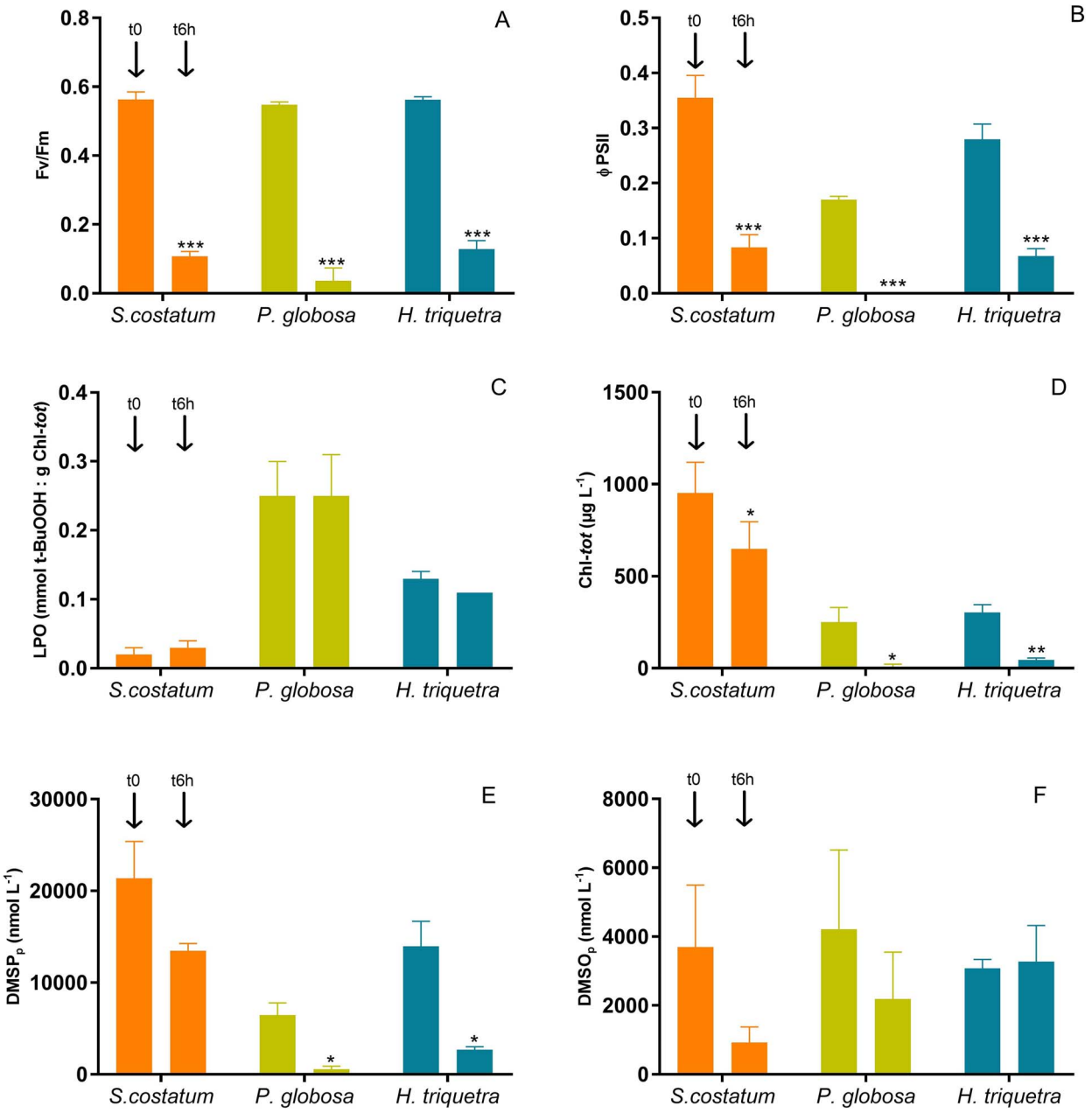


Fig. 3. Evolution of (A) Maximum quantum yield of PSII (Fv/Fm), (B) Effective photochemical quantum yield of PSII (ΦPSII), (C) Lipid Peroxidation (LPO) (mmol t-BuOOH:g Chl-*tot*), (D) Chlorophyll-*tot* (Chl-*tot*) concentration (µg L^{-1}), (E) the $DMSP_p$ concentration (nmol L^{-1}), and (F) the $DMSO_p$ concentration (nmol L^{-1}) with 10 nmol L^{-1} DCMU + HL (1 200 µmol photon m^{-2} s^{-1}) or in dark during 6 h for the three species *S. costatum*, *P. globosa* and *H. triquetra*. Error bars represent SD calculated from triplicates biological samples. Asterisks denote a significant difference between the two time-point (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

The contributions of the HL treatment alone (controls without DCMU but exposed to I2) to the decrease of the later parameter accounted for 65, 44 and 75% in *S. costatum*, *P. globosa* and *H. triquetra* (data not shown). The treatment with DCMU also resulted in a significantly higher production of H_2O_2 comparatively to the HL treatment alone (33, 51 and 48% for *S. costatum*, *P. globosa* and *H. triquetra*, respectively; Fig. 4A). However, it is important to note that the cellular production of H_2O_2 in the dark in presence of DCMU was already

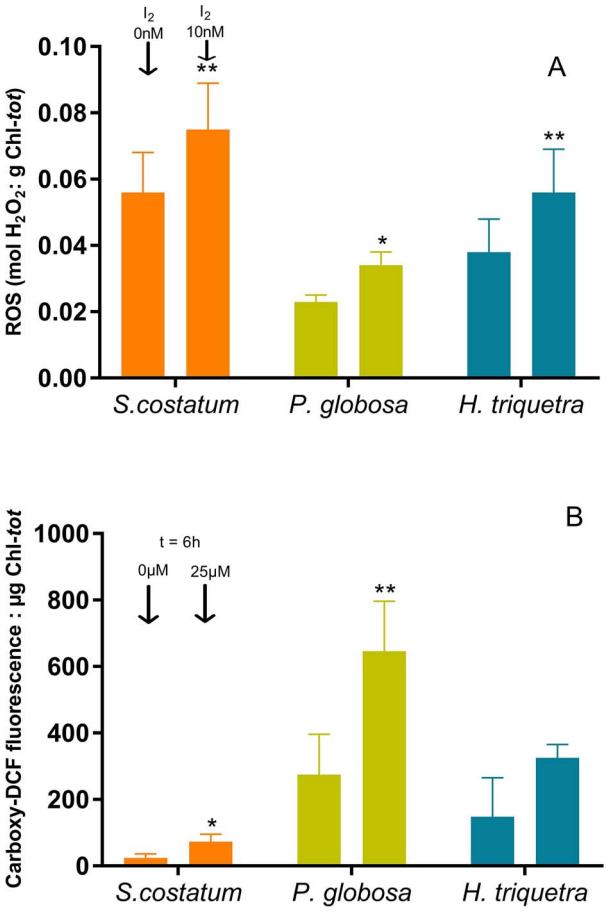


Fig. 4. Evolution of (A) Reactive oxygen species (ROS) concentration (mol H_2O_2:g Chl-*tot*), with 0 or 10 nmol L^{-1} DCMU + HL (1 200 µmol photon m^{-2} s^{-1}) during 6 h for the three species *S. costatum*, *P. globosa* and *H. triquetra*; and (B) Reactive oxygen species (ROS) production (DCF-fluorescence:µg Chl-*tot*) with 0 or 25 µmol L^{-1} MSB during 6 h in the dark for the three species *S. costatum*, *P. globosa* and *H. triquetra*. Error bars represent SD calculated from triplicates biological samples. Asterisks denote significant differences between the two time-point (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

high (Supplementary Fig. 1A), thus indicating that a nonspecific effect of DCMU stimulated the extracellular H_2O_2 production.

The peroxidised lipids content remained constant during the treatment, for the three species investigated (Fig. 3C). On the contrary, Chl-*tot* concentrations decrease significantly by 32, 97 and 85% in *S. costatum*, *P. globosa* and *H. triquetra*, respectively ($P < 0.05$; Fig. 3D). The $DMSP_p$- and $DMSO_p$:Chl-*tot* ratios were not significantly impacted by the treatment (Supplementary Fig. 1B, C). The cell fractions collected at the start and the end of the experimental treatment came from the same cultures and cell concentrations did not vary significantly between the two time-points or between dark and treated samples for *S. costatum* and *P. globosa* (Supplementary Table 1). Significant variation was found for *H. triquetra* between t0h and t6h but not between dark and treated samples (Supplementary Table 1). We thus directly compared raw $DMSP_p$ and $DMSO_p$ data (i.e. non-normalized to Chl-*tot*) that revealed that the $DMSP_p$ content decreased by 37, 91 and 81% in *S. costatum*, *P. globosa* and *H. triquetra*, respectively (Fig. 3E) and the $DMSO_p$ content declined by 75 and 48% in *S. costatum* and *P. globosa* but not in *H. triquetra* (Fig. 3F).

MSB treatment

The exposure of phytoplankton cell cultures to 25 µmol L^{-1} MSB for 6 h resulted in the increase of the intracellular ROS concentration by 3.2, 2.5 and 3.0 compared to control concentrations (i.e. without MSB), in *S. costatum*, *P. globosa* and *H. triquetra*, respectively (Fig. 4B). The increased ROS concentration very likely impacted the photosynthetic apparatus in two of the three species. F_V/F_M was inhibited by more than 50% in *S. costatum* and *P. globosa* ($P < 0.05$; Fig. 5A) and ΦPSII decreased by 77 and 100% in *S. costatum* and *P. globosa*, respectively ($P < 0.05$; Fig. 5B). The photosynthetic activity of *H. triquetra* was unaffected by the treatment. The pool of peroxidised lipids remained stable for the diatom and the Prymnesiophyceae, while a slight decrease of 28% was observed for the dinoflagellate ($P < 0.01$; Fig. 5C). The treatment with MSB did not significantly affect the Chl-*tot* content, except in *H. triquetra* where it decreased significantly ($P < 0.01$; Fig. 5D). The $DMSP_p$:Chl-*tot* ratio varied significantly in *H. triquetra* only ($P < 0.05$; Supplementary Fig. 2A) and the $DMSO_p$:Chl-*tot* ratio remained stable in the three species investigated (Supplementary Fig. 2B). Since the DCMU treatment did not impact the cellular density, we can conclude the same hypothesis for the MSB treatment and analyze raw $DMSP_p$ and $DMSO_p$ data. The $DMSP_p$ concentration decreased by 65, 88 and 28% in *S. costatum*, *P. globosa* and *H. triquetra*, respectively (Fig. 5E) and the $DMSO_p$ content decreased by 79 and 40% in *S. costatum* and *P. globosa*, respectively, but increased by 33% in *H. triquetra* (Fig. 5F).

PCA

We further explored the similarities between all the variables combining the three experimental treatments applied. For HL treatment, the parameters correspond to the short-term treatment at t0h and t6h, while the Chl-*a* and DMS(P,O) concentrations were from the long-term treatment at I0 (LL) and I2 (HL) to ensure a correct comparison. Figure 6 shows the distribution of the data within an orthogonal 2D-space along the

first two PCs explaining 59.1% of the variance. The first PC has a large positive and linear association with three variables ($DMSP_p$, Chl and ΦPSII). This first component primarily measures strain's photosynthetic phenotype. The second PC has a positive association with ROS, $DMSO_p$, LPO and Fv/Fm (although Fv/Fm and LPO have also positive and negative association with PC1, respectively), reflecting the phenotype in terms of oxidative stress. This analysis further shows that some variables are uncorrelated to each other (i.e. Chl and Fv/Fm; ROS and $DMSP_p$; LPO and $DMSO_p$; LPO and Fv/Fm; LPO and ΦPSII), while Chl and LPO are anti-correlated.

This analysis also showed different visual separation in the distribution of the data related to each species regarding the controls and each treatment. When considering the control samples of each treatment (i.e. LL, MSB and DCMU 0 h), the data related to *H. triquetra* and *P. globosa* are closer to each other than *S. costatum*, characterized with more scattered data points for MSB and DCMU controls (Fig. 6). Data related to the HL treatment (6 h) were relatively well clustered, indicating that the cellular response to the treatment was similar between species. The same conclusion can be drawn for *H. triquetra* and *P. globosa* at the end of the treatment with DCMU, while *S. costatum* showed a more distinct response. And finally, the distribution of the data related to the MSB treatment was more scattered, indicating more species-specific responses to this treatment.

DISCUSSION

This study evaluated the impact of potential oxidative stress on $DMSP_p$ and $DMSO_p$ content in three phytoplankton species. ROS relative production is discussed further with no distinction between the methodologies used. Although all the experimental treatments did not impact $DMSP_p$ and $DMSO_p$ content in a similar way, short-terms oxidative stress (treatments with DCMU and MSB) were found to decrease DMSP, suggesting that this sulfur compound possibly interacts with ROS.

$DMSP_p$ and $DMSO_p$ contents vary among phytoplankton species investigated

As previously reported in the literature (i.e. Keller *et al.*, 1989), $DMSP_p$:Chl-*a* ratios measured in control conditions (i.e. I0; 100 µmol photons m^{-2} s^{-1} and 15°C) were found to differ between species investigated, with the diatom possessing much less DMSP:Chl-*a* than the dinoflagellate and the Prymnesiophyceae. DMSP:Chl-*a* ratios measured in our cultures also agreed with previous studies conducted on *H. triquetra* (122.7 ± 27.7 mmolS:g Chl-*a* in average) and *P. globosa* (82.3 ± 11.5 mmolS:g Chl-*a*), maintained in similar environmental conditions (Keller *et al.*, 1989; Niki *et al.*, 2000; Speeckaert *et al.*, 2018, 2019; Stefels *et al.* 2007). For *S. costatum*, values were similar to those of Speeckaert *et al.* (2018), who applied the same methodology on the same strain, but differed from other studies reporting lower DMSP:Chl-*a* ratio (4.5 to 11.8 mmolS:g Chl-*a*, in average; Keller and Korjeff-Bellows 1996; Sunda *et al.*, 2007; Stefels *et al.*, 2007; Spielmeyer *et al.*, 2011; Speeckaert *et al.*, 2019). The $DMSO_p$-to-chlorophyll-*a* ($DMSO_p$:Chl-*a*) ratios measured in this study were a bit higher than values reported in the literature, with 0.2 ± 0.2 for the diatoms, 1.5 ± 0.4 for the Prymnesiophyceae and 3.9 ± 4.3 mmolS:g Chl-*a* for the dinoflagellates (Simó *et al.*, 1998; Hatton and Wilson, 2007; Bucciarelli *et al.*, 2013; Speeckaert *et al.*, 2019). The common technique for DMSP and DMSO determination we applied in this study, with base cleavage prior to DMSO analysis (Champenois et Borges, 2019) have also some drawbacks as it might induce an increase in the DMSO pool due to the cleavage of DMSOP (Thume *et al.*, 2018). However, even considering 100% cleavage efficiency, this would not lead to significant addition in the DMSO pool since the DMSOP-to-cell measured are very low (0.024 ± 0.005 fmol:cell; Thume *et al.*, 2018).

Reporting $DMSP_p$- and $DMSO_p$-to-chlorophyll-*a* ratio is not convenient for oxidative stress experiments since the physiological conditions of the algal cells (i.e. growth stage) and the environmental constraints (i.e. salinity, temperature, nutrient limitation and light intensity) were found to affect DMSP (Stefels, 2000; Sunda *et al.*, 2002; Bucciarelli and Sunda, 2003) and chlorophyll cellular contents (Brunet *et al.*, 2011). Even if lugol-gluteraldehyde fixation caused significant changes in cell size and biomass predictions (Menden-Deuer *et al.*, 2001), it is preferable to report DMSP-to-cell or DMSP-to-cell carbon ratios for studies focusing on the physiological roles of DMSP and DMSO.

Similar to $DMSP_p$:Chl-*a*, we observed much less $DMSP_p$:cell for the diatom than for the Prymnesiophyceae or the dinoflagellate in control conditions. Values of these ratios are in the same order of magnitude than those found in the literature, with an average ratio of 3.6 ± 0.1, 17.0 ± 1.0 and 605.6 ± 244.7 fmolS:cell for *S. costatum*, *P. globosa* and *H. triquetra*, respectively (Keller *et al.*, 1989; Niki *et al.*, 2000; Speeckaert *et al.*, 2018, 2019). As the $DMSO_p$ comes from the oxidation of $DMSP_p$, it is not surprising that the trends observed between species for $DMSP_p$:cell are similar for $DMSO_p$:cell. Values obtained in this study was higher than data reported previously

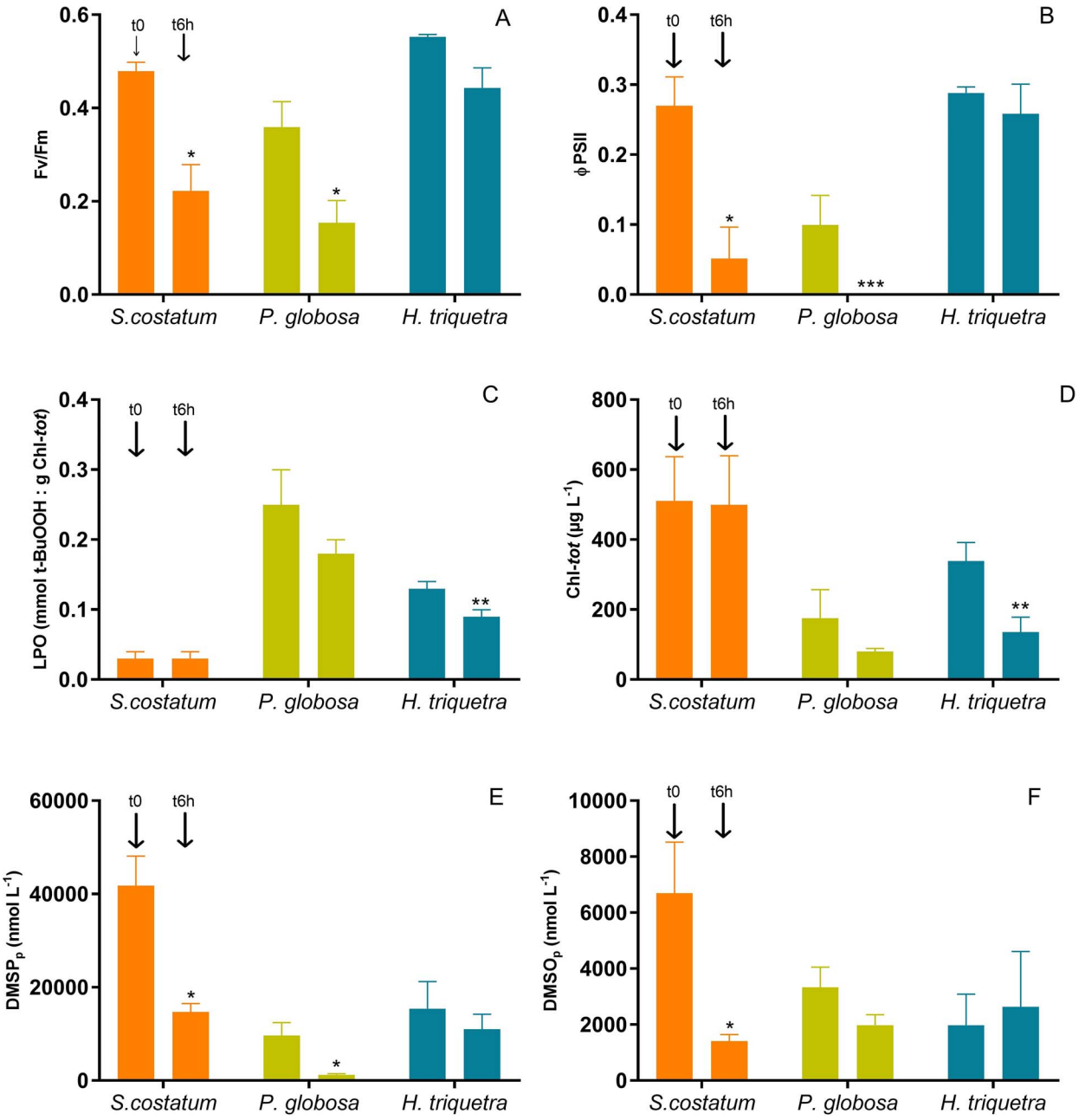


Fig. 5. Evolution of (A) Maximum quantum yield of PSII (Fv/Fm), (B) Effective photochemical quantum yield of PSII (ΦPSII), (C) Lipid Peroxidation (LPO) (mmol t-BuOOH:g Chl-*tot*), (D) Chlorophyll-*tot* (Chl-*tot*) concentration (µg L^{-1}), (E) the $DMSP_p$ concentration (nmol L^{-1}), and (F) the $DMSO_p$ concentration (nmol L^{-1}) with 25 µmol L^{-1} MSB during 6 h for the three species *S. costatum*, *P. globosa* and *H. triquetra*. Error bars represent SD calculated from triplicates biological samples. Asterisks denote significant differences between the two time-point (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

(i.e. 0.02 ± 0.01, 0.2 ± 0.1 and 23.9 ± 33.6 fmolS:cell for diatoms, Prymnesiophyceae and dinoflagellates; Simó *et al.*, 1998; Hatton and Wilson, 2007; Bucciarelli *et al.*, 2013; Speeckaert *et al.*, 2019).

Similar comparison can be drawn for $DMSP_p$ and $DMSO_p$:C with higher value obtained for the Prymnesiophyceae, while the dinoflagellate and the diatom had similar ratios (Fig. 2G, F). Nevertheless, the literature reported higher value for $DMSP_p$:C for the dinoflagellate but in the same range for the two other species: 0.2 ± 0.1, 1.8 ± 0.6 and 2.6 ± 1.4 mmol g^{-1} for *S. costatum*, *P. globosa* and *H. triquetra* (Keller *et al.*,

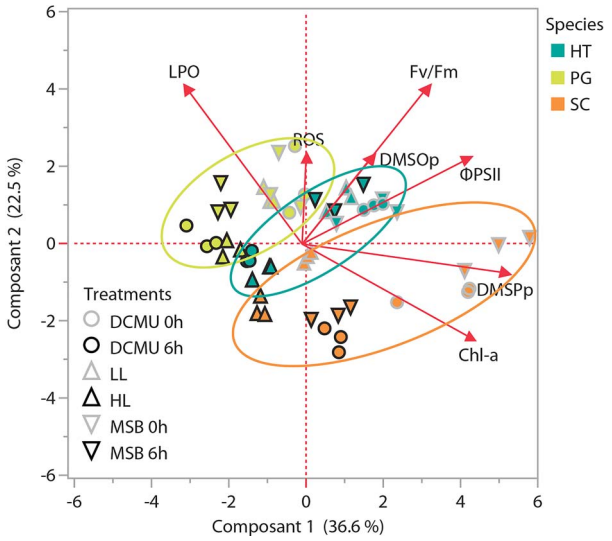


Fig. 6. Principal component analysis (PCA) combining the three treatments at t0h and t6h for the short-term treatments and at I0 (LL) and I2 (HL) for the long-term HL treatment for the three species *S. costatum* (SC), *P. globosa* (PG) and *H. triquetra* (HT). The variables used are $DMSP_p$ and $DMSO_p$ concentrations, Reactive oxygen species concentration (ROS), Lipid peroxidation (LPO), Chlorophyll concentration (Chl), the maximum quantum yield of photosystem II (Fv/Fm) and the effective photochemical quantum yield of the photosystem II (ΦPSII).

1989; Niki *et al.*, 2000; Speeckaert *et al.*, 2018, 2019). $DMSO_p$:C did not follow the same trend with higher values for the diatom and the dinoflagellate than the Prymnesiophyceae: 0.08 ± 0.15, 0.03 ± 0.02 and 0.12 ± 0.16 mmol g^{-1} (Simó *et al.*, 1998; Hatton and Wilson, 2007; Bucciarelli *et al.*, 2013; Speeckaert *et al.*, 2019).

DMSP and DMSO might not be part of the antioxidant network

The experimental treatments involving changes in light intensity showed contrasting results. Three to six hours after the beginning of the HL treatment, cells from all species investigated displayed a sharp decrease of the photosynthetic efficiency (i.e. F_V/F_M and ΦPSII; Fig. 1A, B), indicating a photoinhibition phenomenon very likely caused by photodamages to PSII reactions centers (Murata *et al.*, 2007). This physiological state is conducive to an increased production of ROS (Fig. 1C), potentially causing oxidative stress and important cellular damages. In this context, we would have expected increased levels of lipid peroxidation during the treatments, but those remained stable (Fig. 1D). Since the peroxidation of polyunsaturated fatty acids is mainly caused by $^1O_2$ and $OH^\bullet$ (Farmer et Mueller, 2013), we cannot rule out the possibility that $O_2^{\bullet-}$ and H_2O_2 were the main ROS produced during the treatment. In this context, it would have been interesting to also monitor other biomarkers of oxidative stress such as protein carbonylation or ubiquitination (Sharma *et al.*, 2012; Roberty *et al.* 2016).

In contrast to the previous observation, the results of the DCMU+HL treatment indicate that cells potentially suffered from oxidative stress. Indeed, ROS production was enhanced for the three species (Fig. 4A) due to the strong inhibition of photosynthesis (Fig. 3A, B), and Chl-*tot* concentrations were drastically reduced (Fig. 3D). Different hypotheses could explain the concomitant decrease of $DMSP_p$ concentrations during the treatment (Fig. 3E): (1) this molecule interacts with ROS because of its antioxidant properties; (2) this is the result of collateral damages since DMSP is present in relatively high concentration within the cell; (3) this is related to the DL activity in *P. globosa* and *H. triquetra*, which might be promoted due to the higher light intensity (Harada *et al.*, 2004; Bell *et al.*, 2007), or the potential oxidative stress (Sunda *et al.*, 2007). In support of the first two assumptions is the location of the DMSP production site within the chloroplasts (Raina *et al.*, 2017; Curson *et al.*, 2018), which is also the main cellular site impacted during the HL short-term treatments, and the results obtained during the experimental treatment involving MSB. The latter produces $O_2^{\bullet-}$ that will spontaneously or enzymatically be converted into H_2O_2 (Hassan & Fridovich, 1979), and $OH^\bullet$ in presence of transition metals (Apel and Hirt, 2004). The production of ROS by MSB occurs mainly in the cytosol but H_2O_2 can diffuse to the chloroplasts and cause damages to the photosynthetic apparatus. As this experimental treatment was conducted in the dark, ROS produced by MSB were very likely the cause of the decline of the photosynthetic efficiency (i.e. F_V/F_M and ΦPSII in *S. costatum* and *P. globosa*; Fig. 5A, B) and of the $DMSP_p$ concentrations, further supporting the first two hypotheses. At the opposite, *H. triquetra* seemed not to suffer from MSB at this concentration, because of the cellulose thecae characterizing the armored *H. triquetra* (Caruana, 2010). The thecae may act as a physical barrier decreasing the passive diffusion of the molecule within the cell, which is consistent with the higher concentration used on another dinoflagellate species by Roberty *et al.* (2016).

ROS produced in the cells can also act as signaling molecules. Thanks to its relative stability and its half-life (1 ms; Møller *et al.*, 2007), H_2O_2 can diffuse over a "large" distance within the cell and regulate gene expression by the activation of proteins signaling pathways associated with acclimation processes or programmed cell death (Sharma *et al.*, 2012; Pospíšil, 2016). For instance, H_2O_2 formed in the thylakoid membranes can lead to the

regulation of the PSII antennae size during the acclimation response (Borisova-Mubarakshina *et al*., 2015). ROS can also indirectly transmit a signal from the chloroplasts to the nucleus through products of protein oxidation or lipid peroxidation (Fischer *et al*., 2012). Data obtained during the long-term exposure to highest light intensity indicate that ROS produced early (see short-term HL treatment) could have led to photoacclimation in *S. costatum* and *P. globosa*, and possibly to cell death in *H. triquetra*. Indeed, while *H. triquetra* was unable to grow at the highest light intensity, the two other species showed similar cell density and cell biovolume than the controls, which is consistent with the results of Darroch *et al*. (2015) on *E. huxleyi*. The lower cellular Chl-*a* concentration observed (Fig. 2A, B) is related to a well-known strategy allowing photosynthetic cells to decrease the excitation pressure over the light-harvesting complexes and photosystems (Brunet *et al*., 2011), which could also involve the adjustment of the relative amount of accessory pigments (Chl-*c* and fucoxanthin) and/or the size and the number of photosynthetic units (Nymark *et al*., 2009).

The $DMSP_p$:cell ratios of *S. costatum* were similar among the different light levels, while it decreased at I1 (but not at I2) for *P. globosa*, demonstrating that cells of these two species have reached a new redox equilibrium thanks to the adjustment of the photosynthetic apparatus. Furthermore, while *H. triquetra* was characterized by a much higher $DMSP_p$:cell ratio than the two other species, the dinoflagellate was not able to grow at I2. Also, *S. costatum* and *H. triquetra* had similar $DMSP_p$:C ratio, with the diatom surviving all the treatments, while the dinoflagellate did not. These last observations indicate that the cellular DMSP concentration do not provide any information about the antioxidant capacity of the cell to a subsequent oxidative stress (also suggested by the PCA, Fig. 6). In the same context, a recent study using stable isotope incorporation into DMSP produced by natural phytoplanktonic communities showed that DMSP production on a diel timescale was coupled to carbon fixation rather than being stimulated at high light intensity, thus indicating that the regulation of DMSP production is not linked to photooxidative stress (Archer *et al*., 2018). Also considering the definition of an antioxidant as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell 1995), our observations rather support the hypothesis that DMSP and DMSO are simply damaged by ROS and do not exhibit any antioxidant property.

Further studies addressing the antioxidant role of DMSP should include other components of the antioxidant network (i.e. enzymatic antioxidants, carotenoids and cellular buffers), the DMS(P,O) by-products (i.e. acrylate, methane sulfonate (MSA), methane sulfinic acid (MSNA), and DMS), the potential DMSO reduction activity (DRA) and the DL activity to better understand their interactions. For instance, recent findings demonstrated diatoms' ability to produce flavonoids which display relevant antioxidant capacity and act as signaling compounds able to upregulate cellular defenses under high light intensity (i.e. at 600 µmol photons m^{-2} s^{-1} during 6 h) (Pietta, 2000; Goiris *et al*., 2015; Smerilli *et al*., 2019). DRA has been shown to be widespread in phytoplankton, participating in the DMS production (Fuse *et al*., 1995; Spiese *et al*., 2009), and increases under stress conditions in marine algae (Spiese and Tatarkov, 2014). The DL activity has also been correlated with photoprotective pigments (Steinke *et al*., 2002; Harada *et al*., 2004), higher light intensities encountered in the upper sea layer (Harada *et al*., 2004; Bell *et al*., 2007) and oxidative stress caused by a nitrogen limitation (Sunda *et al*., 2007). A better understanding of the mechanisms and the conditions controlling the activation of DL in phytoplankton should also be addressed to provide better insights on the involvement of this enzyme and DMSP in the regulation of the antioxidant network (Stefels *et al*., 2007). The common technique for DMSP and DMSO determination does not measure the fluxes between DMS(P,O) and its by-products (i.e. DMSO, DMS, acrylate, MSA and MSNA) (Stefels *et al*., 2007). Recent studies are now working with incorporation of stable isotope (D_2O or $NaH^{13}CO_3$) into DMSP to measure *de novo* DMSP synthesis rates (Stefels *et al*., 2009; Archer *et al*., 2018), which would be useful in studies aiming at deciphering the physiological roles of DMSP and its derivates.

CONCLUSION

This study highlights that cellular $DMSP_p$ and $DMSO_p$ contents decrease when phytoplankton cells are subjected to high-light and chemical stresses generating ROS. The initial $DMSP_p$ and $DMSO_p$ to cell or carbon ratios were found to vary between species investigated and were not indicative of the capability of the cell/species to tolerate a subsequent oxidative stress. Furthermore, $DMSP_p$ and $DMSO_p$ cellular content were not increased in HL grown cells (i.e. long-term treatment). Overall, these results do not allow to conclude that DMSP and DMSO have antioxidant properties (*stricto sensu*). These molecules could simply react with ROS produced because of their relative high abundance in cell, similarly as for proteins or lipids, or because of DLA and DRA activities. Further studies monitoring more constituents of the antioxidant network (i.e. enzymes, carotenoids, redox buffer) along with the metabolic pathway of DMSP (DMS(P,O)

by-products, DL and DR activity) are needed to better grasp the physiological functions of DMSP.

SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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