Contrasting effects of acidification and warming on dimethylsulfide concentrations during a temperate estuarine fall bloom mesocosm experiment

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Abstract. The effects of ocean acidification and warming on the concentrations of dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) were investigated during a mesocosm experiment in the Lower St. Lawrence Estuary (LSLE) in the fall of 2014. Twelve mesocosms covering a range of pH (pH on the total hydrogen ion concentration scale) from 8.0 to 7.2, corresponding to a range of CO₂ partial pressures (pCO₂) from 440 to 2900 µatm, at two temperatures (in situ and +5 °C; 10 °C and 15 °C) was monitored during 13 days. All mesocosms were characterized by the rapid development of a diatom bloom dominated by Skeletonema costatum, followed by its decline upon the exhaustion of nitrate and silicic acid. Neither the acidification nor the warming resulted in a significant impact on the abundance of bacteria over the experiment. However, warming the water by 5 °C resulted in a significant increase of the average bacterial production (BP) in all 15 °C mesocosms as compared to 10 °C, with no detectable effect of pCO₂ on BP. Variations in total DMSP (DMSPt = particulate + dissolved DMSP) concentrations tracked the development of the bloom although the rise in DMSPt persisted for a few days after the peaks in chlorophyll a. Average concentrations of DMSPt were not affected by acidification or warming. Initially low concentrations of DMS (< 1 nmol L⁻¹) increased to reach peak values ranging from 30 to 130 nmol L⁻¹ towards the end of the experiment. Increasing the pCO₂ reduced the averaged DMS concentrations by 66 % and 69 % at 10 °C and 15 °C, respectively, over the duration of the experiment. On the other hand, a 5 °C warming increased DMS concentrations by an average of 240 % as compared to in situ temperature, resulting in a positive offset of the adverse pCO₂ impact. Significant positive correlations found between bacterial production rates and concentrations of DMS throughout our experiment point towards temperature-associated enhancement of bacterial DMSP metabolism as a likely driver for the mitigating effect of warming on the negative impact of acidification on the net production of DMS in the LSLE and potentially the global ocean.
1. Introduction

Dimethylsulfide (DMS) is ubiquitous in productive estuarine, coastal and marine surface waters (Barnard et al., 1982; Iverson et al., 1989; Kiene and Service, 1991; Cantin et al., 1996; Kettle et al., 1999). With an estimated average 28.1 Tg of sulfur (S) being transferred to the atmosphere annually (Lana et al., 2011), DMS emissions constitute the largest natural source of tropospheric S (Lovelock et al., 1972; Andreae 1990; Bates et al., 1992). The oxidation of atmospheric DMS yields hygroscopic sulfate (SO$_{4}^{2-}$) aerosols that directly scatter incoming solar radiation and act as nuclei upon which cloud droplets can condense and grow, thereby potentially impacting cloud albedo and the radiative properties of the atmosphere (Charlson et al., 1987; Andreae and Crutzen 1997; Liss and Lovelock, 2007; Woodhouse et al., 2013). The scale of the impact of biogenic SO$_{4}^{2-}$ particles on global climate, however, remains uncertain (Carslaw et al., 2010; Quinn and Bates, 2011, Quinn et al., 2017). The strength of DMS emissions depends on wind- and temperature-driven transfer processes (Nightingale et al., 2000) but mostly on its net production in the surface mixed layer of the ocean (Malin and Kirst, 1997). Net changes in the aqueous DMS inventory are largely governed by microbial food webs (see reviews by Simó, 2001; Stefels et al., 2007) whose productivity is potentially sensitive to modifications in the habitats that sustain them. Given the complexity of the biological cycling of DMS, understanding how climate change related stressors could impact the production of this climate-active gas is a worthy but formidable challenge.

DMS stems, for the most part, from the enzymatic breakdown of dimethylsulfiniopropionate (DMSP) (Cantoni and Anderson, 1956), a metabolite produced by several groups of phytoplankton, with an extensive range in intracellular quotas between taxa (Keller et al., 1989; Stefels et al., 2007). Several species of the classes Haptophyceae and Dinophyceae are amongst the most prolific DMSP producers, but certain members of Bacillariophyceae (diatoms) and Chrysophyceae can also produce significant amounts of DMSP (Stefels et al., 2007). The biosynthesis of DMSP is highly constrained by abiotic factors and its up- or down-regulation may allow cells to cope with environmental shifts in temperature, salinity, nutrients and light intensity (Kirst et al., 1991; Karsten et al., 1996; Sunda et al., 2002), while its de novo synthesis and exudation may also serve as a sink for excess carbon (C) and sulfur (S) under unfavourable growth conditions (Stefels, 2000). Beyond active exudation in healthy cells (Laroche et al., 1999), cellular or particulate DMSP (DMSP$_p$) can be transferred to the water column as dissolved DMSP (DMSP$_d$) through viral lysis (Hill et al., 1998; Malin et al., 1998), autolysis (Nguyen et al., 1988; Stefels and Van Boeckel, 1993), and grazing by micro-, meso- and macrozooplankton (Dacey and Wakeham, 1986; Wolfe and Steinke, 1996). The turnover rate of DMSP$_d$ in the water column is generally very rapid (a few hours to days) as this compound represents sources of C and reduced S for the growth of microbial organisms (Kiene and Linn, 2000). Heterotrophic bacteria mediate most of the turnover of S-DMSP$_d$ through pathways that constrain the overall production of DMS: (1) enzymatic cleavage of DMSP$_d$ that yields DMS; (2) demethylation/ demethiolation of DMSP$_d$ that yields methanethiol (MeSH); (3) production of dissolved non-volatile S compounds, including SO$_2^{2-}$, following oxidation of DMSP$_d$; (4) intracellular accumulation of DMSP$_d$ with no further metabolismization (Kiene et al., 1999, 2000; Kiene and Linn, 2000; Yoch, 2002). A compilation of $^{35}$S-DMSP$_d$ tracer studies conducted with natural microbial populations shows that microbial DMS yields rarely exceed 40% of consumed
DMSP\textsubscript{d} in surface coastal and oceanic waters (see review table in Lizotte et al., 2017). Another potential fate of DMSP\textsubscript{d} is its uptake by non-DMSP producing eukaryotic phytoplankton such as certain diatoms (Vila-Costa et al., 2006b; Ruiz-González et al., 2012) and cyanobacteria such as *Synechococcus* and *Prochlorococcus* (Malmstrom et al., 2005; Vila-Costa et al., 2006b), but the overall turnover of DMSP\textsubscript{d} seems to be dominated by heterotrophic organisms.

Whereas the role of bacteria in the production of DMS via DMSP\textsubscript{d} is well recognized, an increasing number of studies have shown that the phytoplankton-mediated enzymatic conversion of total DMSP (DMSP\textsubscript{t}) into DMS can also be significant when communities are dominated by DMSP-lyase producing phytoplankton groups such as Dinophyceae and Haptophyceae (Niki et al., 2000; Steinke et al., 2002; Stefels et al., 2007; Lizotte et al., 2012), particularly under high doses of solar radiation (Toole and Siegel, 2004; Toole et al., 2006, 2008; Vallina et al., 2008). Removal processes of DMS from surface waters include photo-oxidation, bacterial degradation and efflux across the air-sea interface, the individual intensity of which depends on several factors such as light intensity, wind velocity, the depth of the surface mixed layer and the gross production of DMS (Brimblecombe and Shooter, 1986; Simó and Pedros-Alió, 1999; Nightingale et al., 2000; Hatton et al., 2004; Simó, 2004). Overall, production and turnover of DMS and its precursor DMSP are unequivocally linked with microbial activity, both autotrophic and heterotrophic. The associated biological processes and interactions amongst these microorganisms have been shown to be sensitive to fluctuations in abiotic factors and may thus be further modulated by multiple drivers of climate change.

Since the pre-industrial era, atmospheric CO\textsubscript{2} concentrations have risen from 280 ppm, and, according to the business-as-usual scenario RCP 8.5 and global ocean circulation models, are expected to reach 850–1370 ppm by 2100 (IPCC, 2013). The oceans have already absorbed about 28% of the anthropogenic CO\textsubscript{2} emitted to the atmosphere (Le Quéré et al., 2015), leading to a pH decrease of 0.11 units in surface waters (Gattuso et al., 2015), a phenomenon called ocean acidification (OA). An additional decrease of pH by 0.3–0.4 units is expected by the end of this century, and could reach 0.8 units by 2300 (Caldeira and Wickett, 2005; Doney et al., 2009; Feely et al., 2009). In addition to the oceanic sink, a similar fraction of anthropogenic CO\textsubscript{2} emissions has been captured by terrestrial vegetation, while the anthropogenic CO\textsubscript{2} remaining (45% of total emissions) in the atmosphere (Le Quéré et al., 2013) has led to an estimated increased greenhouse effect of 0.3–0.6 W m\textsuperscript{-2} globally over the past 135 years (Roemmich et al., 2015). Ninety percent of this excess heat has been absorbed by the ocean, increasing sea surface temperatures (SST) ~0.1 °C per decade since 1951 and could increase SST by 3–5 °C before 2100 (IPCC, 2013). Leading experts in the field of global change have called upon the scientific community to address critical knowledge gaps, among which, a top priority remains the assessment of the impact of multiple environmental stressors on marine microorganisms (Riebesell and Gattuso, 2015).

The sensitivity of natural planktonic assemblages to OA, along with their production of DMSP and DMS, has been investigated in several experimental studies (see review table in Hussherr et al., 2017). The majority of these experiments have shown a decrease in both DMSP and DMS concentrations with increasing pCO\textsubscript{2} (Hopkins et al., 2010; Avgoustidi et al., 2012; Park et al., 2014; Webb et al., 2015). The decrease in DMSP production has largely been attributed to the deleterious impact of decreasing pH on the coccolithophore *Emiliania huxleyi*, the dominant DMSP producer in several of these studies. Nevertheless, OA does not always result in a concomitant decrease in DMSP and DMS production. For example, the pCO\textsubscript{2}-
induced decrease in DMS reported by Archer et al. (2013) in Arctic waters was accompanied by an increase in DMSP concentrations, indicating that DMS production is at least partly dependent on the turnover of DMSP, rather than on the DMSP pool. A modeling study showed that the specific implementation of the negative effect of OA on DMS net production in a coupled ocean-atmosphere model reduces global DMS production by 18 ± 3 %, resulting in an additional warming of 0.23–0.48 K by 2100 under the A1B scenario (Six et al., 2013). Schwinger et al. (2017) further showed that the OA-induced decreases in oceanic DMS emissions could result in a transient global warming of 0.30 K, mostly resulting from a reduction of cloud albedo. These first attempts to model the potential effect of OA on climate through its impact on DMS oceanic production show that OA may significantly affect climate by reducing marine emissions of DMS but also highlight the importance of carefully assessing the robustness of the DMS-OA negative relationship. This is particularly relevant considering that some experiments reveal a neutral or positive effect of increasing pCO\textsubscript{2} on DMS net production (Vogt et al., 2008; Kim et al., 2010; Hopkins and Archer, 2014). Regional or seasonal differences in phytoplankton taxonomy, microzooplankton grazing, and bacterial activity have been proposed as key drivers of the discrepancies between these experimental results. Whereas studies of the impact of OA on DMS cycling have gained momentum, the importance of assessing how combined drivers of change may impact the structure and the functioning of ocean ecosystems, using multifactorial approaches, is now increasingly recognized (Boyd et al., 2015; 2018; Riebesell and Gattuso, 2015; Gunderson et al., 2016). Thus far, only two mesocosm studies assessed the combined effect of OA and warming on DMS dynamics by natural plankton assemblages. The two studies, both conducted with coastal waters, led to contrasting results. The first study showed an 80 % increase in DMS concentrations under high pCO\textsubscript{2} conditions (900 ppm vs. 400 ppm), and a reduction by 20 % of this stimulating effect when the increase in pCO\textsubscript{2} was accompanied by a 3 °C warming (Kim et al., 2010). However, the absence of a specific stand-alone warming treatment did not allow the authors to assess the sole impact of temperature on DMS net production. The second study showed decreasing DMS concentrations under both acidification and greenhouse conditions, with the lowest DMS concentrations measured under combined acidification and warming treatments (Park et al., 2014). The authors attributed these contrasting responses to differences in the phytoplankton assemblages, DMSP-related algal physiological characteristics, and microzooplankton grazing. Nevertheless, questions remain as to the combined effect of pCO\textsubscript{2} and warming on DMS net production since the temperature treatments were not conducted over the full range of pCO\textsubscript{2} tested (Kim et al., 2010; Park et al., 2014).

The combined influence of acidification and warming on the dynamics of the St. Lawrence Estuary phytoplankton fall bloom was investigated during a full factorial mesocosm experiment (Bénard et al., 2018). During this experiment, a bloom of Skeletonema costatum developed in all mesocosms, independently of the pCO\textsubscript{2} gradient (from 440 to 2900 µatm) and temperatures tested (10 and 15 °C). The increase in pCO\textsubscript{2} had no influence on the bloom but warming accelerated the growth rate of the diatoms and hastened the decline of the bloom (Bénard et al., 2018). Here, we report on the impacts of acidification and warming on DMSP and DMS concentrations with a focus on the dynamics of heterotrophic bacteria, a component of the marine food web known to affect the turnover of DMSP and DMS.
2. Material and methods

2.1 Mesocosm setup

The mesocosm experimental setup is described in detail in Bénard et al. (2018). Briefly, mesocosm experiments were conducted at the ISMER marine research station of Rimouski (Québec, Canada) in the fall of 2014. The twelve 2600 L cylindrical (2.67 m × 1.4 m), conical bottom, mesocosms were housed in two temperature-controlled, full-size shipping containers each containing six mesocosms (Aquabio tech Inc., Québec, Canada). Each mesocosm is mixed by a propeller secured near the top of each enclosure to ensure homogeneous vertical mixing of the water column. The mesocosms are sealed by a Plexiglas cover transmitting 50–85 % of solar UVB (280–315 nm), 85–90 % of UVA (315–400 nm), and 90 % of photosynthetically active radiation (PAR; 400–700 nm) of the natural incident light. Independent temperature probes (AQBT-TM) were installed in each mesocosm, recording temperature every 15 minutes and either triggering a resistance heater (Process Technology TTA1.8215) or a glycol refrigeration system activated by an automated pump. The pH of the mesocosms was measured every 15 minutes by Hach® PD1P1 probes (± 0.02 pH units) linked to Hach® SC200 controllers. To maintain pH, two reservoirs of artificial seawater were equilibrated with pure CO₂ before the start of the experiment and positive deviations from the target pH values in each mesocosm activated peristaltic pumps that injected the CO₂ supersaturated seawater into the mesocosm water. This control system was able to maintain the pH in the mesocosms within ± 0.02 pH units of the targeted values during the initial bloom development by lowering the pH, but it could not increase the pH during the declining phase of the bloom.

2.2 Experimental approach

Prior to the onset of the experiment, all the mesocosms were meticulously washed with diluted Virkon™, an anti-viral and anti-bacterial solution, according to the manufacturer’s instructions (Antec International Limited), and thoroughly rinsed. The experimental approach is also detailed in Bénard et al. (2018). To fill the mesocosms, water from ~5 m depth was collected near the Rimouski harbour (48° 28' 39.9" N, 68° 31' 03.0" W) on the 27th of September 2014 (day -5). Initial conditions were: practical salinity (S_p) = 26.52, temperature = 10 °C, nitrate (NO₃⁻) = 12.8 ± 0.6 µmol L⁻¹, silicic acid (Si(OH)₄) = 16 ± 2 µmol L⁻¹, and soluble reactive phosphate (SRP) = 1.4 ± 0.3 µmol L⁻¹. Following its collection, the water was screened through a 250 µm mesh while the mesocosms were simultaneously gravity-filled by a custom made “octopus” tubing system. The initial in situ temperature of 10 °C was maintained in all mesocosms for the first 24 h (day -4). On day -3, the six mesocosms in one of the containers were gradually heated to 15 °C while the mesocosms in the other container were maintained at 10 °C. No manipulations were carried on day -2 to avoid excessive stress, and acidification was carried out on day -1. The mesocosms were initially set to cover a gradient of pHᵢ (total proton concentration scale) of ~8.0 to 7.2 corresponding to a range of pCO₂ from 440 to 2900 µatm. Two mesocosms, one in each container (at each temperature), were not pH-controlled to assess the effect of freely fluctuating pH condition. These two mesocosms were called drifters since the in-situ pH was allowed to drift over time throughout the bloom development. To achieve the initially targeted pHᵢ, CO₂-
saturated artificial seawater was added to mesocosms M1, M3, M5, M7, M8, M10 (pH_T 7.2–7.6) while mesocosms M2, M4, M6, M9, M11, M12 (pH_T 7.8–8.0 and the drifters) were openly mixed to allow CO_2 degassing. Then, the automatic system controlling the occasional addition of CO_2-saturated artificial seawater maintained the pH equal or below the targeted pH, except for the drifters.

2.3 Seawater analysis

Daily sampling of the mesocosms was carried out between 05:00 and 08:00 every day (EDT) as described in Bénard et al. (2018). Samples for carbonate chemistry, nutrients, DMSP and DMS were collected directly from the mesocosms prior to filling of 20 L carboys from which seawater for the determination of chlorophyll a (Chl a), bacterial abundance, and bacterial production (BP) was subsampled. Samples were collected directly from the mesocosms and the artificial seawater tank on days -3, 3 and 13 for practical salinity determinations. The samples were collected in 250 mL plastic bottles and stored in the dark until analysis was carried out on a Guildline Autosal 8400B salinometer in the months following the experiment.

2.3.1 Carbonate chemistry and nutrients

Analytical methods used to determine the carbonate parameters are described in detail in Bénard et al. (2018). Briefly, pH was determined every day by transferring samples from the mesocosms to 125 mL plastic bottles without headspace. The samples were analyzed within hours of collection on a Hewlett-Packard UV-Visible diode array spectrophotometer (HP-8453A) and a 5 cm quartz cell using phenol red (PR; Robert-Baldo et al., 1985) and m-cresol purple (mCP; Clayton and Byrne, 1993) as indicators after equilibration to 25.0 ± 0.1 °C in a thermostated bath. The pH on the total proton scale (pH_T) was calculated according to Byrne (1987), with the salinity of the sample and the HSO_4^- association constants given by Dickson (1990). The reproducibility of pH measurements, based on replicate measurements of the same samples and values derived from both indicators, was on the order of 0.003. Total alkalinity (TA) samples were collected every 3–4 days in 250 mL glass bottles to which a few crystals of HgCl_2 were added before sealing with ground glass stoppers and Apiezon® Type-M high-vacuum grease. The TA determinations were carried out within one day of sampling by open-cell automated potentiometric titration (TitriLab 865, Radiometer®) with a pH combination electrode (pHC2001, Red Rod®) and a dilute (0.025 M) HCl titrant solution calibrated against Certified Reference Materials (CRM Batch#94, provided by A. G. Dickson, Scripps Institute of Oceanography, La Jolla, USA). The average relative error, calculated from the average relative standard deviation on replicate standards and sample analyses, was < 0.15 %. The computed pH_T at 25 °C, measured TA, silicic acid and SRP concentrations were used to calculate the in situ pH_T, pCO_2 and saturation state of the water in each mesocosm using CO_2SYS (Pierrot et al., 2006) and the carbonic acid dissociation constants of Cai and Wang (1998).

The samples for the determination of NO_3^-, Si(OH)_4, and SRP were filtered through Whatman GF/F filters, collected in acid washed polyethylene tubes and stored at -20 °C. Analysis was carried out using a Bran and Luebbe Autoanalyzer III using the colorimetric methods of Hansen and Koroleff (2007). The analytical detection limit was 0.03 µmol L^-1 for NO_3^-, plus nitrite (NO_2^-), 0.02 µmol L^-1 for NO_2^-, 0.1 µmol L^-1 for Si(OH)_4, and 0.05 µmol L^-1 for SRP.
2.3.2 Biological variables

Chl a determination methods are presented in Bénard et al. (2018). Succinctly, duplicate 100 mL samples were filtered onto Whatman GF/F filters. The filters were soaked in a 90% acetone solution at 4 °C in the dark for 24 h, the solution was then analyzed by a 10-AU Turner Designs fluorometer (acidification method: Parsons et al., 1984). The analytical detection limit for Chl a was 0.05 µg L⁻¹.

Samples for the determination of free-living heterotrophic bacteria were kept in sterile cryogenic polypropylene vials and fixed with glutaraldehyde Grade I (final concentration = 0.5%, Sigma Aldrich; Marie et al., 2005). Duplicate samples were placed at 4 °C in the dark for 30 min, then frozen at -80 °C until analysis by a FACS Calibur flow cytometer (Becton Dickinson) equipped with a 488 nm argon laser. Before enumeration, the samples were stained with SYBR Green I (0.1% final concentration, Invitrogen Inc.) to which 600 µl of a Tris-EDTA 10 × buffer of pH 8 were added (Laboratoire MAT; Belzile et al., 2008). Fluoresbrite beads (diameter 1 µm, Polysciences) were also added to the sample as an internal standard. The green fluorescence of SYBR Green I was measured at 525 ± 5 nm. Bacterial abundance was determined as the sum of low and high nucleic (LNA and HNA) counts (Annane et al., 2015).

Bacterial production was estimated in each mesocosm on days 0, 2, 4, 6, 8, 10, 11 and 13 by measuring incorporation rates of tritiated thymidine (³H-TdR), using an incubation and filtration protocol based on Fuhrman and Azam (1980, 1982). Twenty mL water subsamples were transferred from glass Erlenmeyers to five sterile glass vials; three as “measured” values and two as blanks. In all blank vials, 0.2 mL of formaldehyde 37% were added, immediately after the sampling to stop all biological activities. Then, 1 mL of ³H-TdR solution (4 µmol L⁻¹), prepared from commercial solution (63 Curie mmol⁻¹; 1 mCurie mL⁻¹, 10 µmol L⁻¹ ³H-TdR, MP Biomedicals), was added in all vials. Samples were incubated 2.5 h at experimental temperatures (10 or 15 °C), and then 0.2 mL of formaldehyde 37% were immediately added in the three “measure” vials. Bacteria were then collected by filtration (diameter 25 mm; 0.2 µm porosity) and filters were treated according to Fuhrman and Azam (1980, 1982). ³H-TdR incorporation was measured using a scintillation counter (Beckman LS5801) and results were expressed in dpm. Blank values were subtracted to “measured” values to remove background radioactivity. ³H-TdR incorporation rates were converted in mole of ³H-TdR incorporated per unit of volume and time, before converting to rate of carbon production using the carbon conversion factor of Bell (1993).

2.3.3 DMSP and DMS concentrations

For the quantification of DMSP, duplicate 3.5 mL samples of seawater were collected into 5 mL polyethylene tubes. Samples were preserved by adding 50 µL of a 50% sulfuric acid solution (H₂SO₄) to the tubes before storage at 4 °C in the dark until analysis in the following months. Samples for the quantification of DMSP were taken daily, but a technical problem during storage and transport of the samples led to a loss of all samples. The DMSP samples were injected into a purge and trap (PnT) system before being completely flushed using 1–5 mL Milli-Q™ water into the helium purged chamber heated to 70 °C. DMSP concentrations were determined by a mole to mole conversion to DMS following hydrolysis with a 5 M NaOH solution injected...
in the chamber prior to the sample, and trapping the gas sample in a loop immersed in liquid nitrogen. The loop was then heated in a water bath to release the trapped sample and analyzed using a Varian 3800 Gas Chromatograph equipped with a pulsed flame photometric detector (PFPD, Varian 3800) and a detection limit of 0.9 nmol L\(^{-1}\) (Scarratt et al., 2000; Lizotte et al., 2012).

Samples for the quantification of DMS were directly collected from the mesocosms into 20 mL glass vials with a butyl septum and aluminum crimp. The samples were kept in the dark at 4 °C until analysis was carried out within hours of collection using the PnT system described above.

### 2.4 Statistical analyses

The statistical analyses were performed using the nlme package in R (R Core Team, 2016). The data were analyzed using a general least squares (gls) approach to test the linear effects of the two treatments (temperature, pCO\(_2\)), and their interaction on the variables (Paul et al., 2016; Hussher et al., 2017; Bénard et al., 2018). The analyses were conducted on the averages of the measured parameters over the whole duration of the experiment, and separate regressions for pCO\(_2\) were performed for each temperature when the latter had a significant effect. The residuals were checked for normality using a Shapiro-Wilk test (p > 0.05) and data were transformed (square root or natural logarithm) if necessary. In addition, squared Pearson’s correlation coefficients (r\(^2\)) with a significance level of 0.05 were used to evaluate correlations between key variables.

### 3. Results

#### 3.1 Physical and chemical conditions during the experiments

\(S_p\) was 26.52 ± 0.03 on day -4 in all mesocosms and remained constant throughout the experiment, averaging 26.54 ± 0.02 on day 13 (Bénard et al., 2018). The temperature of the mesocosms in each container remained within ± 0.1 °C of the target temperature throughout the experiment and averaged 10.04 ± 0.02 °C for mesocosms M1 through M6, and 15.0 ± 0.1 °C for mesocosms M7 through M12 (Fig. 1a). The pH\(_T\) remained relatively stable throughout the experiment in the pH-controlled treatments, but decreased slightly as the experiment progressed, deviating by an average of -0.14 ± 0.07 units relative to the target pH\(_T\) on the last day (Fig. 1b). The pH variations corresponded to changes in pCO\(_2\) from an average of 1340 ± 150 µatm on day -3, and ranged from 564 to 2902 µatm at 10 °C and from 363 to 2884 µatm at 15 °C on day 0 following the acidification (Fig. 1c). The in situ pH\(_T\) in the drifters (M6 and M11) increased from 7.896 and 7.862 on day 0, at 10 °C and 15 °C respectively, to 8.307 and 8.554 on day 13, reflecting the balance between CO\(_2\) uptake and metabolic CO\(_2\) production over the duration of the experiment. On the last day, pCO\(_2\) in all mesocosms ranged from 186 to 3695 µatm at 10 °C and from 90 to 3480 µatm at 15 °C.

Nitrate (NO\(_3^-\)) and silicic acid (Si(OH)\(_4\)) concentrations averaged 9.1 ± 0.5 µmol L\(^{-1}\) and 13.4 ± 0.3 µmol L\(^{-1}\) on day 0, respectively (Bénard et al., 2018). The two nutrients displayed a similar temporal depletion pattern following the development of the phytoplankton bloom. NO\(_3^-\) concentrations reached undetectable levels (< 0.03 µmol L\(^{-1}\)) in all mesocosms by day 5.
Likewise, Si(OH)_4 fell below the detection limit (< 0.1 µmol L⁻¹) between day 1 and 5 in all mesocosms except for those whose pH was set at 7.2 and 7.6 at 10 °C (M5 and M3) and in which Si(OH)_4 depletion occurred on day 9.

### 3.2 Phytoplankton, bacterial abundance and production

Chl a concentrations were below 1 µg L⁻¹ following the filling of the mesocosms (day -4), and had already increased to an average of 5.9 ± 0.6 µg L⁻¹ on day 0 (Fig. 2a). At 10 °C, Chl a quickly increased to reach maximum concentrations around 27 ± 2 µg L⁻¹ on day 3 ± 2, and decreased progressively until the end of the experiment. Increasing the temperature by 5 °C resulted in a more rapid development of the bloom and a speedier decrease of Chl a concentrations during the declining phase of the bloom. The maximum Chl a concentration reached at the peak of the bloom was, however, not significantly affected by the difference in temperature. We found no significant effect of the pCO₂ gradient on the mean Chl a concentrations measured over the days 0–13, nor during the development phase and the declining phase of the bloom as described in Bénard et al. (2018) (Fig. 2a–b; Table 1).

The free-living bacterial abundance was ~1.2 × 10⁹ cells L⁻¹ on day -4, and increased rapidly to reach 3.1 ± 0.6 × 10⁹ cells L⁻¹ on day 0 (Fig. 2c). This initial increase in abundance probably resulted from the release of dissolved organic matter (DOM) during pumping of the seawater and filling of the mesocosms. The subsequent decrease in bacterial abundance during the development phase of the bloom suggests that the initial pool of DOM was fully utilized and that freshly released DOM was scarce. As expected, bacterial abundance increased during the declining phase of the bloom at 10 °C. Under warmer conditions, bacterial abundance decreased earlier during the initial bloom development than what was observed at 10 °C, but was also marked by an earlier peak during the decline of the bloom, then was followed by a second, more variable peak in abundance. These daily variations in abundances probably reflect changes in the balance between bacterial growth and loss by grazing.

When averaged over the experiment, we observed no effect of the treatments on the mean bacterial abundance (Fig. 2c–d; Table 1). At 10 °C, bacterial production was low at the beginning of the experiment and increased gradually during the development and declining phases of the bloom to reach peaks values of 9.3 ± 0.9 µg C L⁻¹ d⁻¹ (Fig. 2e). Bacterial production increased faster at 15 °C and reached maximal production rates of 19 ± 1 µg C L⁻¹ d⁻¹ on day 11. Results of the gls model show no effect of the pCO₂ gradient on bacterial production, but a positive effect of warming was observable throughout the experiment (Fig. 2f; Table 1).

### 3.3 DMSP, and DMS

At in situ temperature, DMSP, concentrations averaged 9 ± 2 nmol L⁻¹ on day 0 and increased regularly in all mesocosms up to day 10 before they plateaued or slightly decreased over the last 2–3 days (Fig. 3a). These results reveal that DMSP accumulation persisted for several days after the bloom peaks, to reach a maximum value between days 8–13 of 366 ± 22 nmol L⁻¹. At 15 °C, DMSP concentrations similarly increased after the maximum Chl a concentrations were reached, but increased faster than at in situ temperature. The maximum DMSP, concentrations were 396 ± 19 nmol L⁻¹ at 15 °C, a value
that is not statistically different from the peak values measured at 10 °C (Fig 4a; Table 2). A greater loss of DMSP took place in the last days of the experiment at 15 °C. By day 13, 79 ± 3 % of the peak DMSP, concentration was lost in the 15 °C mesocosms, while 19 ± 4 % of the peak DMSP, concentration was lost at 10 °C. When averaged over the duration of the experiment, the mean DMSP, concentrations were not significantly affected by the pCO₂ gradient, the temperatures or the interaction between these two factors (Fig. 3b; Table 1).

Over the 13 days, the DMSP;Chl a ratio averaged 11.4 ± 0.4 nmol (µg Chl a)⁻¹ at 10 °C and was not affected by increasing pCO₂ (Fig. 5a; Table 1). Due to the aforementioned mismatch between the peaks in Chl a and DMSP, the average DMSP;Chl a ratios were significantly higher at 15 °C, averaging 19 ± 1 nmol (µg Chl a)⁻¹ over the experiment (Fig. 5a; Table 1). However, we found no significant relationship between DMSP;Chl a and the pCO₂ gradient.

Initial DMS concentrations were below the detection limit on day 0 (< 0.9 nmol L⁻¹) and slowly increased during the first 7 days, while most of the build-up took place after day 8 in all treatments (Fig. 3b). The net accumulation of DMS was faster at 15 °C than at 10 °C, with higher daily DMS concentrations at 15 °C compared to 10 °C from day 3 until day 13. At the end of the experiment, DMS concentrations averaged 21 ± 4 nmol L⁻¹ at 10 °C and 74 ± 14 nmol L⁻¹ at 15 °C. Over the full duration of the experiment, we found significant negative effects of increasing pCO₂ on mean DMS concentrations at the two temperatures tested (Fig. 3c; Table 1). At 10 °C, we measured a ~67 % reduction of mean DMS concentrations from the drifter relative to the most acidified treatment (~345 ppm vs ~3200 ppm), with values decreasing from 10 ± 2 nmol L⁻¹ to 3.2 ± 0.8 nmol L⁻¹. At 15 °C, the mean DMS concentrations decreased by roughly the same percentage (~69 %) as pCO₂ increased from the drifter to the most acidified treatment (~130 ppm vs ~3130 ppm). Nevertheless, the mean DMS concentrations were higher at 15 °C, ranging from 34 ± 13 nmol L⁻¹ to 11 ± 3 nmol L⁻¹ (Fig. 3c; Table 1). Similarly, the peak DMS concentrations decreased linearly with increasing pCO₂ at both temperatures and concentrations were always higher at 15 than at 10 °C for any given pCO₂ (Fig. 4b; Table 2).

The DMS:Chl a ratios remained below 1 nmol (µg Chl a)⁻¹ during the first 8 days in all mesocosms as DMS concentrations were low, but increased exponentially at 15 °C in the following days. At 10 °C, the DMS:Chl a ratio averaged 0.43 ± 0.7 nmol (µg Chl a)⁻¹ over the 13 days and was not affected by the pCO₂ gradient. At 15 °C, the DMS:Chl a ratios were not significantly affected by the pCO₂ gradient, but were significantly higher in the warmer treatment (Fig. 5b; Table 1).

The DMS:DMSP, ratio exhibited the same general pattern as the DMS, i.e. low and stable values during the first 8 days, and increasing values between days 8–13 (Fig. 3e). The natural logarithm of the DMS:DMSP, ratio was not affected by the pCO₂ gradient at 10 °C when averaged over the 13 days experiment, but a significant decrease of the DMS:DMSP, ratios was observed with increasing pCO₂ at 15 compared to 10 °C (Fig. 3f; Table 1).
4. Discussion

4.1 General characteristics

As far as we know, this study is the first full factorial mesocosm experiment where all pCO$_2$ treatments (pH$_T$ from 8.0 to 7.2) were replicated at two different temperatures (in situ and +5 °C), to assess the impact of ocean acidification and warming on the dynamics of DMSP and DMS concentrations during a phytoplankton bloom. A diatom bloom dominated by *Skeletonema costatum* developed in all mesocosms, regardless of the treatments. This chain-forming centric diatom is a cosmopolitan species in coastal and estuarine systems and a frequent bloomer in the Lower St. Lawrence Estuary (LSLE) (Kim et al., 2004; Starr et al., 2004; Annane et al., 2015). The 13 days where treatments were applied allowed us to capture the development and declining phases of the bloom. The impacts of the treatments on the dynamics of the bloom during these two phases are described in greater details in Bénard et al. (2018). Briefly, the acidification had no detectable effect on the development rate of the diatom bloom and on the maximum Chl $a$ concentrations reached. However, increasing the water temperature by 5 °C increased the growth rate of the diatoms, shortening the development phase of the bloom, from 4–7 days at 10 °C to 1–4 days at 15 °C. However, these changes in the bloom timing did not alter the overall primary production throughout the experiment. Hereafter, we discuss how increasing pCO$_2$ (lowering the pH) affected DMSP and DMS concentrations and how a 5 °C increase in temperature altered the impacts of the pCO$_2$ gradient during the experiment.

4.2. DMSP dynamics

The buildup of the phytoplankton biomass during the bloom development was coupled with a rapid increase in DMSP$_1$ concentrations (Fig. 3a). Assuming that *S. costatum* was responsible for most of the DMSP production, our results indicate a low sensitivity of the DMSP synthesis pathway to acidification in this species. The net accumulation of DMSP$_1$ persisted several days after the peaks in Chl $a$, indicating a decoupling between DMSP synthesis, algal growth and nitrogen metabolism (Bénard et al., 2018).

4.2.1 Effects of acidification on DMSP

At in situ temperature, the averaged DMSP$_1$ concentrations were not affected by the increase in pCO$_2$ (Fig. 3b; Table 1). The lack of significant changes in the DMSP$_1$:Chl $a$ ratio as a function of the pCO$_2$ gradient also supports this conclusion (Fig. 5a; Table 1). This result is consistent with those of previous studies that showed a relatively weak effect of an increase in pCO$_2$ on DMSP concentrations (Vogt et al., 2008; Lee et al., 2009; Avgoustidi et al., 2012; Archer et al., 2013; Webb et al., 2015). Furthermore, much like the patterns observed at 10 °C, there was no relationship between the concentrations of DMSP$_1$ and the pCO$_2$ gradient observable at 15 °C (Table 1).
4.2.2 Effects of warming on DMSP

In contrast to the absence of effects of acidification on DMSP, warming has been previously shown to affect DMSP concentrations in nature. Results from shipboard incubation experiments conducted in the North Atlantic have revealed an increase in particulate DMSP (DMSPₚ) concentrations due to a 4 °C warming (Lee et al., 2009). During this last study, the higher DMSPₚ concentrations were attributed to a temperature-induced shift in community structure toward species with higher cellular DMSP content. During our study, the pCO₂ and temperature treatments did not alter the structure of the community (Bénard et al., 2018). Most of the DMSP synthesis was likely linked to the numerically dominant diatoms, as all other algal groups identified contributed to less than 10 % of the total algal abundance (see Fig. 6 in Bénard et al., 2018). Our results thus suggest that DMSP synthesis by *S. costatum* during the nitrate-replete growth phase was not significantly affected by warming. Rather, it is the accelerated growth rate of *S. costatum* that promoted the concurrent accumulation of biomass and DMSP, while the higher DMSPₚ:Chl a ratio observable at 15 °C may be explained by the faster degradation of cells under warming.

Several empty frustules were found during the last days of the experiment at 15 °C, suggesting a loss of integrity of the cells and potential increase of the release of intracellular dissolved organic matter, including DMSP. The increase in the abundance of bacteria and in bacterial production (Fig. 2c, e) during that period also suggest that more dissolved organic matter was produced during the decline of the bloom, as previously reported (Engel et al., 2004a, 2004b). During our experiment, transparent exopolymer particles (TEP) concentrations increased during this period (Gaaloul, 2017), adding to the evidence for heightened DOM production by the decaying bloom, with a potential increase in DMSP metabolization by heterotrophic bacteria under warming.

4.3 DMS dynamics

DMS concentrations remained very low during the development phase of the bloom (day 8) and increased in the latter days of the experiment. Most of the DMS accumulation in the mesocosms took place between days 8–13 and likely originated from DMSP that may have been released during cell lysis (Kwint and Kramer, 1995), or upon zooplankton grazing (Cantin et al., 1996). Unbalanced growth and photosynthesis of algal cells under nitrogen deficiency during that period may also be responsible for a greater production and active exudation of DMSP (Stefels et al., 2000; Kettles et al., 2014).

4.3.1 Effects of acidification on DMS

At in-situ temperature, we observed a significant linear decrease in DMS concentrations (both averaged over the full duration of the experiment and peak concentrations) with increasing pCO₂ (Figs. 3c, 4b; Tables 1 and 2). Several earlier mesocosm experiments have shown similar decreasing trends of DMS concentrations with increasing pCO₂ (Hopkins et al., 2010; Archer et al., 2013; Park et al., 2014; Webb et al., 2015, 2016). In these studies, the pCO₂-induced decreases in DMS were generally attributed to changes in the microbial community speciation and structure, or to microzooplankton grazing, although decreases in bacterial DMSP-to-DMS conversion or increases in DMS consumption have also been suggested (Archer et al., 2013;
Hussherr et al., (2017). During our study, the decrease in DMS concentrations with increasing pCO₂ cannot be directly attributed to a decrease in DMSP, since this pool was not affected by the pCO₂ gradient (Figs. 3b, 4a; Tables 1 and 2). In Park et al. (2014), the increase in pCO₂ led to the reduction in the abundance of Alexandrium spp., an active DMSP and DMSP-Lyase (DLA) producer, and a concomitant reduction of the associated microzooplankton grazing. As Alexandrium spp. was less numerous, the associated attenuation of microzooplankton grazing resulted in a reduction of the mixing of DMSP and DLA, leading to lesser DMSP-to-DMS conversion. Given the strong contribution of S. costatum to the bloom, a species with no reported DLA, it can be assumed that most, if not all, of the DMS produced was driven by bacterial processes following DMSP release by the diatoms. Thus, the decrease in DMS concentrations in our study could have been the result of altered bacterial mediation; either through reduced bacterial production of DMS or heightened bacterial consumption of DMS. Whereas a reduction in bacterial uptake of DMSP is unlikely, given that the bacterial abundance and production were unaffected by the pCO₂ gradient (Table 1), the observed decrease in DMS concentrations could imply that at higher pCO₂ the bacterial yields of DMS are abated. The relative proportion of DMSP consumed by bacteria and further cleaved into DMS is closely tied to bacterial demand in carbon and sulfur as well as to the availability of DMSP relative to other sources of reduced sulfur in the environment (Levasseur et al., 1996; Kiene et al., 2000; Pinhassi et al., 2005). The absence of a significant pCO₂ effect on the concentrations of DMSP during this study may be interpreted as a pCO₂-related alteration of the microbially-mediated fate of consumed DMSP. Unfortunately, in the absence of detailed ³⁵S-DMSP₄ bioassays, it is impossible to confirm the outcome of the DMSP metabolic pathways including the DMSP-to-DMS conversion efficiency in relation to the pCO₂ gradient. A few studies (Grossart et al., 2006; Engel et al., 2014; Webb et al., 2015;) have reported enhanced bacterial abundance and production at high pCO₂, especially for attached bacteria as opposed to free-living (Grossart et al., 2006). However, regardless of the temperature treatment, neither the abundance nor the activity of bacteria seemed to be significantly impacted by pCO₂ in this study. A pCO₂-induced increase in bacterial DMS turnover could also explain the decrease in DMS concentrations, but several studies suggest that bacterial DMS consumption in natural systems is often tightly coupled to DMS production itself (Simó, 2001, 2004). Furthermore, while one laboratory study reported that non-limiting supplies of DMS may be used as a substrate by several members of Bacteroidetes (Green et al., 2011), another study showed that only a subset of the natural microbial population may turnover naturally-occurring levels of DMS (Vila-Costa et al., 2006b). Nevertheless, the sensitivity of these DMS-consuming bacteria to decreasing pH remains unknown. Likewise, whereas we cannot exclude a potential impact of pCO₂ on DMS turnover via bacterioplankton, it is plausible that the pCO₂ gradient may have affected a widespread physiological pathway among bacteria, specifically, the metabolic breakdown of DMSP.

4.3.2 Effects of warming on DMS

A warming by 5 °C increased DMS concentrations at all pCO₂ tested, resulting in an offset of the negative pCO₂ impact when compared to the in situ temperature. This result differs from the observation of Kim et al. (2010) and Park et al. (2014) in two ways. First, our results show an increase in DMS concentrations in the warmer treatment while the two previous studies reported a decrease. Second, our results confirm that a temperature effect may be measured over a large range of pCO₂. It is
noteworthy that the increase in DMS concentrations at the two temperatures tested varied from 110 % at pH 8.0 up to 370 % at pH 7.4. This highlights the scaling of the temperature effect over an extensive range of pCO₂ and the importance of simultaneously studying the impact of these two factors on DMS production. As observed at 10 °C, both the average and the peak DMS concentrations decreased linearly as pCO₂ increased in the warm treatment (Figs. 3d, 4b; Tables 1 and 2). Nevertheless, the pCO₂-induced decrease in DMS concentrations at 15 °C cannot be directly attributed to a decrease in DMSP, concentrations given that an increase in pCO₂ had no discernable effect on DMSP, concentrations. In contrast to our observations at the in situ temperature, where DMSP, continued to increase until day 12, DMSP, concentrations typically decreased from day 8 and onward (Fig. 3a). This loss in DMSP, suggests that microbial consumption of DMS exceeded DMSP algal synthesis. In light of the dominance of S. costatum, a phytoplankton taxon not known to exhibit DLA, the bulk of microbial DMSP mediation was likely associated with heterotrophic bacteria. In support of this hypothesis, the bacterial production was ~2 times higher at 15 than at 10 °C between days 8–13 (19 ± 1 µg C L⁻¹ d⁻¹ vs 9.3 ± 0.9 µg C L⁻¹ d⁻¹) (Fig. 2), and we observed a significant correlation between the quantity of DMSP, lost during Phase II (day of the DMSP, peak concentration to day 13) and the quantity of DMS produced during the same period (coefficient of determination, r² = 0.60, p < 0.01, n = 11). Assuming that all the DMSP, lost was transformed into DMS by bacteria, we calculated that DMS yields could have varied by 0.5 to 32 % across the pCO₂ gradient (mean = 13 ± 11 %). These very rough estimates of DMS yields are likely at the lower end since measured DMS concentrations also reflect losses of DMS through photo-oxidation and bacterial consumption. Nevertheless, we cannot exclude the possibility of some passive uptake of DMS by the picocyanobacterial population in the mesocosms, although this pathway is not considered to be significant in natural systems (Malmstrom et al., 2005; Vila-Costa et al., 2006a) and does not lead to the production of DMS. Our ‘minimum community’ DMS yield estimates agree with an expected range of microbial DMS yields in natural environments, from 2 % to 45 % (see review table in Lizotte et al., 2017). These gross but realistic estimates of heterotrophic bacterial DMSP-to-DMS conversions could explain the bulk of the DMS present in our study, a hypothesis also supported by the strong positive correlation (r² = 0.64, p < 0.001, n = 70) between overall DMS concentrations and bacterial production rates. Combined, these findings reinforce the idea that bacterial metabolism, rather than bacterial stocks, may significantly affect the fate of DMSP (Malmstrom et al., 2004a, 2004b, 2005; Vila et al., 2004; Vila-Costa et al., 2007; Royer et al., 2010; Lizotte et al., 2017) and that drivers of environmental change, such as temperature and pH, that can alter bacterial activity and strongly impact the gross and net production of DMS. Specific measurements of bacterial DMSP uptake and DMS yields using ³⁵S-DMSP, should be conducted to assess the impacts of pCO₂ and temperature on the microbial fate of DMSP.

4.4 Limitations

During our study, the pCO₂ changes were applied abruptly, over a day, from in situ values to pCO₂ levels exceeding the most pessimistic pCO₂ scenarios for the end of the century. Compared to our manipulation, ocean acidification will proceed at a slower temporal scale, potentially allowing species to adapt and evolve to these changing conditions (Stillman and Paganini, 2015; Schlüter et al., 2016). However, in the LSLE, the upwelling of low oxygenated waters can rapidly reduce the pHT to
~7.62, or even lower with contributions of low pH (7.12) freshwaters from the Saguenay River during the spring freshet (Mucci et al., 2017). Thus, the swift and extensive pCO2 range deployed in our experiment may seem improbable for the open ocean on the short term, but may not be inconceivable for this coastal region. However, the warming of 5 °C used in this mesocosm study possibly exceeds the upper limit of temperature increase for the end of the century in this region. In the adjacent Gulf of St. Lawrence (GSL), surface waters temperature (SST) correlates strongly with air temperature, allowing the estimation of past SST. This relationship showed that SST has increased in the GSL by 0.9 °C per century since 1873 (Galbraith et al., 2012), although additional positive anomalies of 0.25–0.75 °C per decade have been shown between 1985 and 2013 (Galbraith et al., 2016). In the LSLE, the highest temperatures occur at the end of summer / early fall, and gradually dissipate by heating the subjacent cold intermediate layer through vertical mixing (Cyr et al., 2011). The extent of the projected warming in the LSLE is unknown, but will likely result from the multifaceted interactions between heat transfer from the air and physical factors controlling the water masses. The results from our study could also be influenced by the absence of macrograzers in the mesocosms. An additional grazing pressure could limit the growth of the blooming species, reducing the amount of DMSP produced or could increase the release of DMSPd through sloppy feeding after the initial bloom (Lee et al., 2003). It is unclear how an increase in grazing pressure would have impacted the concentrations of DMS in our experiment. On the one hand, increased predation could have limited the net accumulation of DMSPp, with a possible reduction in DMS production. On the other hand, increased grazing could have favoured the release of DMSPp as DMSPd, thus increasing the availability of this substrate for microbial uptake, mediation and possible conversion into DMS. Despite the absence of reported changes in community composition in our study, many OA mesocosm experiments have described changes in DMS concentrations associated with shifts in community structure in the past (Vogt et al., Hopkins et al., 2010; Kim et al., 2010, Park et al., 2014, Webb et al., 2015). Nonetheless, our results align with those of other OA studies (Archer et al., 2013; Hussherr et al., 2017), suggesting that the mediation of heterotrophic bacteria plays a major role in DMS cycling in the absence of reported phytoplanktonic DLA, such as in a diatom dominated bloom in the LSLE.

5. Conclusions

The objective of this study was to quantify the combined impact of increases in pCO2 and temperature on the dynamics of DMS during a fall diatom bloom in the St. Lawrence Estuary. Our mesocosm experiment allowed us to capture the development and declining phases of a bloom strongly dominated by the diatom Skeletonema costatum and the related changes in bacterial abundance and production. As expected, warming accelerated the development of the bloom, but also its decline. Both DMSPp and DMS concentrations increased during the development phase of the bloom, but peak concentrations were reached as the bloom was declining. Increasing pCO2 had no discernable effect on the total amount of DMSPp produced at both temperatures tested. In contrast, increasing the pCO2 to the value forecasted for the end of this century resulted in a linear decrease in DMS concentrations by 33 % and by as much as 69 % over the full pCO2 gradient tested. These results are
consistent with previous reports that acidification has a greater impact on the processes that control the conversion of DMSP to DMS than on the production of DMSP itself. The pCO₂-induced decrease in DMS concentrations observed in this study adds to the bulk of previous studies reporting a similar trend. In diatom-dominated systems, such as the one under study in this experiment, heterotrophic processes underlying DMS production seem to be most sensitive to modifications in pCO₂. Whereas predatory grazing and its associated impacts on DMS production cannot be ruled out entirely, the decreases in DMS concentrations in response to heightened pCO₂ are likely related to reductions in bacterial-mediated DMS production, a hypothesis partly supported by the significant positive correlations found between bacterial production rates and DMS concentrations. Whereas the DMS concentrations decreased significantly with increasing pCO₂ at both 10 °C and 15 °C, warming the mesocosms by 5 °C translated into a positive offset in concentrations of DMS over the whole range of pCO₂ tested. Higher DMSP release and increased bacterial productivity in the warm treatment partially explain the stimulating effect of temperature on DMS net production. Overall, results from this full factorial mesocosm experiment suggest that warming could mitigate the expected reduction in DMS production due to ocean acidification, even increasing the net DMS production with the potential to curtail radiative forcing.

Data availability. The data have been submitted to be freely accessible via Pangaea or can be obtained by contacting the author (robin.benard.1@ulaval.ca).

Author contributions. R. Bénard was responsible for the experimental design elaboration, data sampling and processing, and the writing of this article. Several co-authors supplied specific data included in this article, and all co-authors contributed to this final version of the article.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements

The authors wish to thank the Station Aquicole-ISMER, particularly Nathalie Morin and her staff, for their support during the mesocosm experiment. We also wish to acknowledge Gilles Desmeules, Bruno Cayouette, Sylvain Blondeau, Claire Lix, Rachel Hussherr, Liliane St-Amand, Marjolaine Blais, Armelle Galine Simo Matchim and Marie-Amélie Blais for their precious help over the duration of the experiment. This study was funded by a Team grant from the Fonds de recherche du Québec – Nature et technologies (FRQNT-Équipe-165335), the Canada Foundation for Innovation, the Canada Research Chair on Ocean Biogeochemistry and Climate, and by Fisheries and Oceans Canada. This is a contribution to the research program of Québec-Océan.

References


Dickson, A. G.: Standard potential of the reaction: \( \text{AgCl(s) + 1/2H_2(g) = Ag(s) + HCl(aq)} \) and the standard acidity constant of the ion \( \text{HSO}_4^- \) in synthetic sea water from 273.15 to 318.15 K, J. Chem. Thermodyn., 22(2), 113–127, doi:10.1016/0021-9614(90)90074-Z, 1990.


Figure 1. Temporal variations over the course of the experiment for: (a) temperature, (b) pH, (c) pCO2. For symbol attribution to treatments, see legend. Adapted from Bénard et al. (2018).
Figure 2. Temporal variations, and averages over the course of the experiment (day 0 to day 13) for: (a–b) chlorophyll a (adapted from Bénard et al., 2018), (c–d) free-living bacteria abundance, (e–f) bacterial production. For symbol attribution to treatments, see legend.
Figure 3. Temporal variations, and averages over the course of the experiment (day 0 to day 13) for: (a–b) DMSP, (c–d) DMS, (e–f) the natural logarithm of the DMS:DMSP ratio. For symbol attribution to treatments, see legend.
Figure 4. (a) Maximum DMSP concentrations, (b) maximum DMS concentrations reached over the full course of the experiment (day 0 to day 13). For symbol attribution to treatments, see legend.
Figure 5. Averages over the course of the experiment (day 0 to day 13) for: (a) DMSP:Chl $a$ ratio, (b) DMS:Chl $a$ ratio. For symbol attribution to treatments, see legend.
Table 1. Results of the generalized least squares models (gls) tests for the effects of temperature, pCO₂, and their interaction over the duration of the experiment (day 0 to day 13). Separate analyses with pCO₂ as a continuous factor were performed when temperature had a significant effect. Averages of bacterial abundance and production, DMSP<sub>t</sub>, DMS, Chl<sub>a</sub>-normalized DMSP<sub>t</sub> and DMS concentrations, and DMS:DMSP<sub>t</sub> ratios are presented. Natural logarithm transformation is indicated when necessary. Significant results are in bold. *p<0.05, **p<0.01, ***p<0.001.

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Table 2. Results of the generalized least squares models (gls) tests for the effects of temperature, pCO2, and their interaction on the maximum values of the parameters measured during the experiment. Separate analyses with pCO2 as a continuous factor were performed when temperature had a significant effect. Maxima of DMSPt and DMS concentrations are presented. Significant results are in bold. *p<0.05, **p<0.01, ***p<0.001.

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<th>Response Variable</th>
<th>Factor</th>
<th>df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSPt (nmol L⁻¹)</td>
<td>Temperature</td>
<td>8</td>
<td>0.384</td>
<td>0.711</td>
</tr>
<tr>
<td></td>
<td>pCO₂</td>
<td>8</td>
<td>-0.713</td>
<td>0.496</td>
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<tr>
<td></td>
<td>pCO₂ x Temperature</td>
<td>8</td>
<td>0.300</td>
<td>0.772</td>
</tr>
<tr>
<td>DMS (nmol L⁻¹)</td>
<td>Temperature</td>
<td>8</td>
<td>6.403</td>
<td>&lt;0.001***</td>
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<tr>
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<td>pCO₂ (10°C)</td>
<td>4</td>
<td>-2.868</td>
<td>0.046*</td>
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<tr>
<td></td>
<td>pCO₂ (15°C)</td>
<td>4</td>
<td>-4.061</td>
<td>0.015*</td>
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