Effect of ocean acidification and elevated $f$CO$_2$ on trace gas production by a Baltic Sea summer phytoplankton community


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Abstract

The Baltic Sea is a unique environment as the largest body of brackish water in the world. Acidification of the surface oceans due to absorption of anthropogenic CO$_2$ emissions is an additional stressor facing the pelagic community of the already challenging Baltic Sea. To investigate its impact on trace gas biogeochemistry, a large-scale mesocosm experiment was performed off Tvärminne Research Station, Finland in summer 2012. During the second half of the experiment, dimethylsulphide (DMS) concentrations in the highest $f$CO$_2$ mesocosms
(1075 - 1333 μatm) were 34% lower than at ambient CO$_2$ (350 μatm). However, the net production (as measured by concentration change) of seven halocarbons analysed was not significantly affected by even the highest CO$_2$ levels after 5 weeks exposure. Methyl iodide (CH$_3$I) and diiodomethane (CH$_2$I$_2$) showed 15% and 57% increases in mean mesocosm concentration (3.8 ± 0.6 pmol L$^{-1}$ increasing to 4.3 ± 0.4 pmol L$^{-1}$ and 87.4 ± 14.9 pmol L$^{-1}$ increasing to 134.4 ± 24.1 pmol L$^{-1}$ respectively) during Phase II of the experiment, which were unrelated to CO$_2$ and corresponded to 30% lower Chl-$a$ concentrations compared to Phase I. No other iodoarenes increased or showed a peak, with mean chlor iodomethane (CH$_2$ClI) concentrations measured at 5.3 (± 0.9) pmol L$^{-1}$ and iodoethane (C$_2$H$_5$I) at 0.5 (± 0.1) pmol L$^{-1}$. Of the concentrations of bromoform (CHBr$_3$; mean 88.1 ± 13.2 pmol L$^{-1}$), dibromomethane (CH$_2$Br$_2$; mean 5.3 ± 0.8 pmol L$^{-1}$) and dibromochloromethane (CHBr$_2$Cl, mean 3.0 ± 0.5 pmol L$^{-1}$), only CH$_2$Br$_2$ showed a decrease of 17% between Phases I and II, with CHBr$_3$ and CHBr$_2$Cl showing similar mean concentrations in both Phases. Outside the mesocosms, an upwelling event was responsible for bringing colder, high CO$_2$, low pH water to the surface starting on day $t_{16}$ of the experiment; this variable CO$_2$ system with frequent upwelling events implies the community of the Baltic Sea is acclimated to regular significant declines in pH caused by up to 800 μatm fCO$_2$. After this upwelling, DMS concentrations declined, but halocarbon concentrations remained similar or increased compared to measurements prior to the change in conditions. Based on our findings, with future acidification of Baltic Sea waters, biogenic halocarbon emissions are likely to remain at similar values to today, however emissions of biogenic sulphur could significantly decrease from this region.

1 Introduction

Anthropogenic activity has increased the fugacity of atmospheric carbon dioxide (fCO$_2$) from 280 μatm (pre-Industrial Revolution) to over 400 μatm today (Hartmann et al., 2013). The IPCC AR5 long-term projections for atmospheric pCO$_2$ and associated changes to the climate have been established for a variety of scenarios of anthropogenic activity until the year 2300. As the largest global sink for atmospheric CO$_2$, the global ocean has absorbed an estimated 30% of excess CO$_2$ produced (Canadell et al., 2007). With atmospheric pCO$_2$ projected to possibly exceed 2000 μatm by the year 2300 (Collins et al., 2013; Cubasch et al., 2013), the ocean will take up increasing amounts of CO$_2$, with a potential lowering of surface ocean pH by over 0.8 units (Raven et al., 2005). The overall effect of acidification on the biogeochemistry of surface ocean ecosystems is
unknown and currently unquantifiable, with a wide range of potential positive and negative impacts (Doney et al., 2009; Hofmann et al., 2010; Ross et al., 2011).

A number of volatile organic compounds are produced by marine phytoplankton (Liss et al., 2014), including the climatically important trace gas dimethylsulphide (DMS, $C_2H_6S$) and a number of halogen-containing organic compounds (halocarbons) including methyl iodide (CH$_3$I) and bromoform (CHBr$_3$). These trace gases are a source of sulphate particles and halide radicals when oxidised in the atmosphere, and have important roles as ozone catalysts in the troposphere and stratosphere (O’Dowd et al., 2002; Solomon et al., 1994) and as cloud condensation nuclei (CCNs; Charlson et al., 1987).

DMS is found globally in surface waters originating from the algal-produced precursor dimethylsulphoniopropionate (DMSP, $C_5H_{10}O_2S$). Both DMS and DMSP provide the basis for major routes of sulphur and carbon flux through the marine microbial food web, and can provide up to 100% of the bacterial (Simó et al., 2009) and phytoplanktonic (Vila-Costa et al., 2006a) sulphur demand (Simó et al., 2009; Vila-Costa et al., 2006a). DMS is also a volatile compound which readily passes through the marine boundary layer to the troposphere, where oxidation results in a number of sulphur-containing particles important for atmospheric climate feedbacks (Charlson et al., 1987; Quinn and Bates, 2011); for this reason, any change in the production of DMS may have significant implications for climate regulation. Several previous acidification experiments have shown differing responses of both compounds (e.g. Avgoustidi et al., 2012; Hopkins et al., 2010; Webb et al., 2015), while others have shown delayed or more rapid responses as a direct effect of CO$_2$ (e.g. Archer et al., 2013; Vogt et al., 2008). Further, some laboratory incubations of coastal microbial communities showed increased DMS production with increased f$\text{CO}_2$ (Hopkins and Archer, 2014), but lower DMSP production. The combined picture arising from existing studies is that the response of communities to f$\text{CO}_2$ perturbation is not predictable and requires further study. Previous studies measuring DMS in the Baltic Sea measured concentrations up to 100 nmol L$^{-1}$ during the summer bloom, making the Baltic Sea a significant source of DMS (Orlikowska and Schulz-Bull, 2009).

In surface waters, halocarbons such as methyl iodide (CH$_3$I), chloriodomethane (CH$_2$ClI) and bromoform (CHBr$_3$) are produced by biological and photochemical processes: many marine microbes (for example cyanobacteria; Hughes et al., 2011, diatoms; Manley and De La Cuesta, 1997 and haptophytes; Scarratt and Moore, 1998) and macroalgae (e.g. brown-algal Fucus species; Chance et al., 2009 and red algae; Leedham et al., 2013) utilise halides from seawater and emit a range of organic and inorganic halogenated compounds. This production can lead to significant flux...
to the marine boundary layer in the order of 10 Tg iodine-containing compounds (‘iodocarbons’; O’Dowd et al., 2002) and 1 Tg bromine-containing compounds (‘bromocarbons’; Goodwin et al., 1997) into the atmosphere. The effect of acidification on halocarbon concentrations has received limited attention, but two acidification experiments measured lower concentrations of several iodocarbons while bromocarbons were unaffected by $fCO_2$ up to 3000 μatm (Hopkins et al., 2010; Webb, 2015), whereas an additional mesocosm study did not elicit significant differences from any compound up to 1400 μatm $fCO_2$ (Hopkins et al., 2013).

Measurements of the trace gases within the Baltic Sea are limited, with no prior study of DMSP concentrations in the region. The Baltic Sea is the largest body of brackish water in the world, and salinity ranges from 1 to 15. Furthermore, seasonal temperature variations of over 20 °C are common. A permanent halocline at 50-80 m separates $CO_2$-rich, bottom waters from fresher, lower $CO_2$ surface waters, and a summer thermocline at 20 m separates warmer surface waters from those below 4 °C (Janssen et al., 1999). Upwelling of bottom waters from below the summer thermocline is a common summer occurrence, replenishing the surface nutrients while simultaneously lowering surface temperature and pH (Brutemark et al., 2011). Baltic organisms are required to adapt to significant variations in environmental conditions. The species assemblage in the Baltic Sea is different to those studied during previous mesocosm experiments in the Arctic, North Sea and Korea (Brussaard et al., 2013; Engel et al., 2008; Kim et al., 2010), and are largely unstudied in terms of their community trace gas production during the summer bloom. Following the spring bloom (July-August), a low dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorous (DIP) ratio combines with high temperatures and light intensities to encourage the growth of heterocystous cyanobacteria, (Niemisto et al., 1989; Raateoja et al., 2011), in preference to nitrate-dependent groups.

Here we report the concentrations of DMS, DMSP and halocarbons from the 2012 summer post-bloom season mesocosm experiment aimed to assess the impact of elevated $fCO_2$ on the microbial community and trace gas production in the Baltic Sea. Our objective was to assess how changes in the microbial community driven by changes in $fCO_2$ impacted DMS and halocarbon concentrations. It is anticipated that any effect of $CO_2$ on the growth of different groups within the phytoplankton assemblage will result in an associated change in trace gas concentrations measured in the mesocosms as $fCO_2$ increases, which can potentially be used to predict future halocarbon and sulphur emissions from the Baltic Sea region.
2 Methods

2.1 Mesocosm design and deployment

Nine mesocosms were deployed on the 10th June 2012 (day t-10; days are numbered negative prior to CO₂ addition and positive afterward) and moored near Tvärminne Zoological Station (59° 51.5’ N, 23° 15.5’ E) in Tvärminne Storfjärden in the Baltic Sea. Each mesocosm comprised a thermoplastic polyurethane (TPU) enclosure of 17 m depth, containing approximately 54,000 L of seawater, supported by an 8m tall floating frame capped with a polyvinyl hood. For full technical details of the mesocosms see Czerny et al. (2013) and Riebesell et al. (2013). The mesocosm bags were filled by lowering through the stratified water column until fully submerged, with the opening at both ends covered by 3 mm mesh to exclude organisms larger than 3 mm such as fish and large zooplankton. The mesocosms were then left for 3 days (t-10 to t-7) with the mesh in position to allow exchange with the external water masses and ensure the mesocosm contents were representative of the phytoplankton community in the Storfjärden. On t-7 the bottom of the mesocosm was sealed with a sediment trap and the upper opening was raised to approximately 1.5 m above the water surface. Stratification within the mesocosm bags was broken up on t-5 by the use of compressed air for three and a half minutes to homogenise the water column and ensure an even distribution of inorganic nutrients at all depths. Unlike in previous experiments, there was no addition of inorganic nutrients to the mesocosms at any time during the experiment; mean inorganic nitrate, inorganic phosphate and ammonium concentrations measured across all mesocosms at the start of the experiment were 37.2 (± 18.8 s.d.) nmol L⁻¹, 323.9 (± 19.4 s.d.) nmol L⁻¹ and 413.8 (± 319.5 s.d.) nmol L⁻¹ respectively.

To obtain mesocosms with different fCO₂, the carbonate chemistry of the mesocosms was altered by the addition of different volumes of 50 μm filtered, CO₂-enriched Baltic Sea water (sourced from outside the mesocosms), to each mesocosm over a four-day period, with the first day of addition being defined as day t0. Addition of the enriched CO₂ water was by the use of a bespoke dispersal apparatus (‘Spider’) lowered through the bags to ensure even distribution throughout the water column (further details are in Riebesell et al. 2013). Measurements of salinity in the mesocosms throughout the experiment determined that three of the mesocosms were not fully sealed, and had undergone unquantifiable water exchange with the surrounding waters. These three mesocosms (M2, M4 and M9) were excluded from the analysis. Two mesocosms were designated as controls (M1 and M5) and received only filtered seawater via the Spider; four mesocosms received addition of CO₂-enriched waters, with the range of target fCO₂ levels between 600 and 1650 μatm (M7, 600...
μatm; M6, 950 μatm; M3, 1300 μatm; M8 1650 μatm). Mesocosms were randomly allocated a target \( f_{\text{CO}_2} \); a noticeable decrease in \( f_{\text{CO}_2} \) was identified in the three highest \( f_{\text{CO}_2} \) mesocosms (M6, M3 and M8) over the first half of the experiment, which required the addition of more CO\(_2\) enriched water on \( t15 \) to bring the \( f_{\text{CO}_2} \) back up to maximum concentrations (Fig. 1a; Paul et al., 2015). A summary of the \( f_{\text{CO}_2} \) in the mesocosms can be seen in Table 1. At the same time as this further CO\(_2\) addition on \( t15 \), the walls of the mesocosms were cleaned using a bespoke wiper apparatus (See Riebesell et al., 2013 for more information), followed by weekly cleaning to remove aggregations on the film which would block incoming light. Light measurements showed that over 95% of the photosynthetically active radiation (PAR) was transmitted by the clean TPU and PVC materials with 100% absorbance of UV light (Riebesell et al., 2013). Samples for most parameters were collected from the mesocosms at the same time every morning from \( t-3 \), and analysed daily or every other day.

2.2 Trace gas extraction and analysis

2.2.1 DMS and halocarbons

A depth-integrated water sampler (IWS, HYDRO-BIOS, Kiel, Germany) was used to sample the entire 17 m water column daily or alternative daily. As analysis of Chlorophyll-\( \alpha \) (Chl-\( \alpha \)) showed it to be predominantly produced in the first 10 m of the water column, trace gas analysis was conducted on only integrated samples collected from the surface 10 m, with all corresponding community parameter analyses with the exception of pigment analysis performed also to this depth.

Water samples for trace gas analysis were taken from the first IWS from each mesocosm to minimise the disturbance and bubble entrainment from taking multiple samples in the surface waters. As in Hughes et al. (2009), samples were collected in 250 mL amber glass bottles in a laminar flow with minimal disturbance to the water sample, using Tygon tubing from the outlet of the IWS. Bottles were rinsed twice before being carefully filled from the bottom with minimal stirring, and allowed to overflow the volume of the bottle approximately three times before sealing with a glass stopper to prevent bubble formation and atmospheric contact. Samples were stored below 10°C in the dark for 2 hours prior to analysis. Each day, a single sample was taken from each mesocosm, with two additional samples taken from one randomly selected mesocosm to evaluate the precision of the analysis (<4%, no further data shown).

On return to the laboratory, 40 mL of water was injected into a purge and cryotrap system (Chuck et al., 2005), filtered through a 25 mm Whatman glass fibre filter (GF/F; GE Healthcare Life Sciences, Little Chalfont, England) and purged with oxygen-free nitrogen (OFN) at 80 mL min\(^{-1}\) for 10
minutes. Each gas sample passed through a glass wool trap to remove particles and aerosols, before a dual nafion counterflow drier (180 mL min\(^{-1}\) OFN) removed water vapour from the gas stream. The gas sample was trapped in a stainless steel loop held at -150 °C in the headspace of a liquid nitrogen-filled dewar. The sample was injected by immersion of the sample loop in boiling water into an Agilent 6890 gas chromatograph equipped with a 60 m DB-VRX capillary column (0.32 mm ID, 1.8 μm film thickness, Agilent J&W Ltd) according to the programme outlined by Hopkins et al. (2010). Analysis was performed by an Agilent 5973 quadrupole mass spectrometer operated in electron ionisation, single ion mode. Liquid standards of CH\(_3\)I, diiodomethane (CH\(_2\)I\(_2\)), CH\(_2\)ClI, iodoethane (C\(_2\)H\(_5\)I), iodopropane (C\(_3\)H\(_7\)I), dibromoethane (CHBr\(_2\)Br), dibromochloromethane (CHBr\(_2\)Cl), bromoiodomethane (CH\(_2\)BrI) and DMS (Standards supplied by Sigma Aldrich Ltd, UK) were gravimetrically prepared by dilution in HPLC-grade methanol (Table 2) and used for calibration. The relative standard error was expressed as a percentage of the mean for the sample analysis, calculated for each compound using triplicate analysis each day from a single mesocosm, and was <7% for all compounds. GC-MS instrument drift was corrected by the use of a surrogate analyte standard in every sample, comprising deuterated DMS (D\(_6\)-DMS), deuterated methyl iodide (CD\(_3\)I) and \(^{13}\)C dibromoethane (\(^{13}\)C\(_2\)H\(_4\)Br\(_2\)) via the method described in Hughes et al. (2006) and Martino et al. (2005). Five-point calibrations were performed weekly for each compound with the addition of the surrogate analyte, with a single standard analysed daily to check for instrument drift; linear regression from calibrations typically produced \(r^2>0.98\). All samples measured within the mesocosms were within the concentration ranges of the calibrations (Table 2).

2.2.2 DMSP

Samples for total DMSP (DMSP\(_T\)) were collected and stored for later analysis by the acidification method of Curran et al. (1998). A 7 mL sub-sample was collected from the amber glass bottle into an 8 mL glass sample vial (Labhut, Churcham, UK), into which 0.35 μL of 50% H\(_2\)SO\(_4\) was added, before storage at ambient temperature. Particulate DMSP (DMSP\(_P\)) samples were prepared by the gravity filtration of 20 mL of sample through a 47 mm GF/F in a glass filter unit, before careful removal and folding of the GF/F into a 7 mL sample vial filled with 7 mL of Milli-Q water and 0.35 μL of H\(_2\)SO\(_4\) before storage at ambient temperature. Samples were stored for approximately 8 weeks prior to analysis. DMSP samples (total and particulate) were analysed on a PTFE purge and cryotrap system using 2 mL of the sample purged with 1 mL of 10M NaOH for 5 minutes at 80 mL min\(^{-1}\). The sample gas stream passed through a glass wool trap and Nafion counterflow (Permapure) drier before being trapped in a PTFE sample loop kept at -150 °C by suspension in the headspace of a liquid nitrogen-filled dewar and controlled by feedback from a thermocouple. Immersion in
boiling water rapidly re-volatilised the sample for injection into a Shimadzu GC2010 gas chromatograph with a Varian Chrompack CP-Sil-5CB column (30 m, 0.53 mm ID) and flame photometric detector (FPD). The GC oven was operated isothermally at 60 °C which resulted in DMS eluting at 2.1 minutes. Liquid DMSP standards were prepared and purged in the same manner as the sample to provide weekly calibrations of the entire analytical system. Involvement in the 2013 AQA 12-23 international DMS analysis proficiency test (National Measurement Institute of Australia, 2013) in February 2013 demonstrated excellent agreement between our method of DMSP analysis and the mean from thirteen laboratories measuring DMS using different methods, with a measurement error of 5%.

DMSP was not detected in any of the samples (total or particulate) collected and stored during the experiment, and it was considered likely that this was due to an unresolved issue regarding acidifying the samples for later DMSP analysis. It was considered unlikely that rates of bacterial DMSP turnover through demethylation rather than through cleavage to produce DMS (Curson et al., 2011) were sufficiently high in the Baltic Sea to remove all detectable DMSP, yet still produce measureable DMS concentrations. Also, rapid turnover of dissolved DMSP in surface waters being the cause of low DMSP<sub>T</sub> concentrations does not explain the lack of intracellular particulate-phase DMSP. Although production of DMS is possible from alternate sources, it is highly unlikely that there was a total absence of DMSP-producing phytoplankton within the mesocosms or Baltic Sea surface waters around Tvärminne; DMSP has been measured in surface waters of the Southern Baltic Sea at 22.2 nmol L<sup>-1</sup> in 2012, indicating that DMSP-producing species are present within the Baltic Sea (Cathleen Zindler, GEOMAR, Pers. Comm.).

A previous study by del Valle et al. (2011) highlighted up to 94% loss of DMSP<sub>T</sub> from acidified samples of colonial *Phaeocystis globosa* culture, and field samples dominated by colonial *Phaeocystis antarctica*. Despite filamentous, colonial cyanobacteria in the samples from Tvärminne mesocosms potentially undergoing the same process, these species did not dominate the community at only 6.6% of the total Chl-<i>a</i>, implying that the acidification method for DMSP fixation also failed for unicellular phytoplankton species. This suggests that the acidification method is unreliable in the Baltic Sea, and should be considered inadequate as the sole method of DMSP fixation in future experiments in the region. The question of its applicability in other marine waters also needs further investigation.
2.3 Measurement of carbonate chemistry and community dynamics

Water samples were collected from the 10 b m and 17 m IWS on a daily basis and analysed for carbonate chemistry, fluorometric Chl-a, phytoplankton pigments (17 m IWS only) and cell abundance to analyse the community structure and dynamics during the experiment. The carbonate system was analysed through a suite of measurements (Paul et al., 2015), including potentiometric titration for total alkalinity (TA), infrared absorption for dissolved inorganic carbon (DIC) and spectrophotometric determination for pH. For Chl-a analysis and pigment determination, 500 mL sub-samples were filtered through a GF/F and stored frozen (-20 °C for two hours for Chl-a and -80 °C for up to 6 months for pigments), before homogenisation in 90 % acetone with glass beads. After centrifuging (10 minutes at 800 x g at 4 °C) the Chl-a concentrations were determined using a Turner AU-10 fluorometer by the methods of Welschmeyer (1994), and the phytoplankton pigment concentrations by reverse phase high performance liquid chromatography (WATERS HPLC with a Varian Microsorb-MV 100-3 C8 column) as described by Barlow et al. (1997). Phytoplankton community composition was determined by the use of the CHEMTAX algorithm to convert the concentrations of marker pigments to Chl-a equivalents (Mackey et al., 1996; Schulz et al., 2013). Microbes were enumerated using a Becton Dickinson FACSCalibur flow cytometer (FCM) equipped with a 488 nm argon laser (Crawfurd et al., 2016) and counts of phytoplankton cells >20 µm were made on concentrated (50 mL) sample water, fixed with acidic Lugol’s iodine solution with an inverted microscope. Filamentous cyanobacteria were counted in 50 µm length units.

2.4 Statistical Analysis

All statistical analysis was performed using Minitab V16. In analysis of the measurements between mesocosms, one-way ANOVA was used with Tukey’s post-hoc analysis test to determine the effect of different fCO2 on concentrations measured in the mesocosms and the Baltic Sea (H0 assumes no significant difference in the mean concentrations of trace gases measured through the duration of the experiment). Spearman’s Rank Correlation Coefficients were calculated to compare the relationships between trace gas concentrations, fCO2, and a number of biological parameters, and the resulting ρ-values for each correlation are given in Supplementary table S1 for the mesocosms and S2 for the Baltic Sea data.
3 Results and Discussion

3.1 Biogeochemical changes within the mesocosms

The mesocosm experiment was split into three phases based on the temporal variation in Chl-α (Fig. 2; Paul et al., 2015) evaluated after the experiment was completed:

- Phase 0 (days t-5 to t0) – pre-CO₂ addition
- Phase I (days t1 to t16) – ‘productive phase’
- Phase II (days t17 to t30) – temperature induced autotrophic decline.

3.1.1 Physical Parameters

f/CO₂ decreased over Phase I in the three highest f/CO₂ mesocosms, mainly through air-sea gas exchange and carbon fixation by phytoplankton (Fig. 1a). All mesocosms still showed distinct differences in f/CO₂ levels throughout the experiment (Table 1), and there was no overlap of mesocosm f/CO₂ values on any given day, save for the two controls (M1 and M5). The control mesocosm f/CO₂ increased through Phase I of the experiment, likely as a result of undersaturation of the water column encouraging dissolution of atmospheric CO₂ (Paul et al., 2015). Salinity in the mesocosms remained constant throughout the experiment at 5.70 ± 0.004, and showed no variation with depth (data not shown but available in Paul et al. 2015). It remained similar to salinity in the Baltic Sea surrounding the mesocosms, which was 5.74 ± 0.14. Water temperature varied from a low of 8.6 ± 0.4 °C during Phase 0 to a high of 15.9 ± 2.2 °C measured on day t16, before decreasing once again (Fig. 1b).

Summertime upwelling events are common and well described (Gidhagen, 1987; Lehmann and Myrberg, 2008), and induce a significant temperature decrease in surface waters; such an event appears to have commenced around t16, as indicated by significantly decreasing temperatures inside and out of the mesocosms (Fig. 1b) and increased salinity in the Baltic Sea from 5.5 to 6.1 over the following 15 days to the end of the experiment. Due to the enclosed nature of the mesocosms, the upwelling affected only the temperature and not pH, f/CO₂ or the microbial community. However, the temperature decrease after t16 was likely to have had a significant effect on phytoplankton growth (and biogenic gas production), explaining the lower Chl-α in Phase II.

3.1.2 Community Dynamics

Mixing of the mesocosms and redistribution of the nutrients throughout the water column after closure (prior to t-3) did not trigger a notable increase in total Chl-α in Phase 0 as was identified in
previous mesocosm experiments; in previous mesocosm experiments, mixing redistributed nutrients from the deeper stratified layers throughout the water column. During Phase I, light availability, combined with increasing water temperatures favoured the growth of phytoplankton in all mesocosms (Paul et al. 2015), and was unlikely to be a direct result of the CO\textsubscript{2} enrichment, as no difference was identified between enriched mesocosms and controls. Mean Chl-a during Phase I was 1.98 (± 0.29) μg L\textsuperscript{-1} from all mesocosms, decreasing to 1.44 (± 0.46) μg L\textsuperscript{-1} in Phase II: this decrease was attributed to a temperature induced decreased in phytoplankton growth rates and higher grazing rates as a result of higher zooplankton reproduction rates during Phase I (Lischka et al., 2015; Paul et al., 2015). Mesocosm Chl-a decreased until the end of the experiment on r31.

The largest contributors to Chl-a in the mesocosms during the summer of 2012 were the chlorophytes and cryptophytes, with up to 40% and 21% contributions to the Chl-a respectively (Table 3; Paul et al., 2015). Significant long-term differences in abundance between mesocosms developed as a result of elevated fCO\textsubscript{2} in only two groups: picoeukaryotes I showed higher abundance at high fCO\textsubscript{2} (F=8.2, p<0.01; Crawfurd et al., 2016 and Supplementary Fig. S2), as seen in previous mesocosm experiments (Brussaard et al., 2013; Newbold et al., 2012) and picoeukaryotes III the opposite trend (F=19.6, p<0.01; (Crawfurd et al., 2016)) Crawfurd et al. this issue). Temporal variation in phytoplankton abundance was similar between all mesocosms (Supplementary Fig. S1 and S2).

Diazotrophic, filamentous cyanobacterial blooms in the Baltic Sea are an annual event in summer (Finni et al., 2001), and single-celled cyanobacteria have been found to comprise as much as 80% of the cyanobacterial biomass and 50% of the total primary production during the summer in the Baltic Sea (Stal et al., 2003). However, CHEMTAX analysis identified cyanobacteria as contributing less than 10% of the total Chl-a in the mesocosms (Crawfurd et al., 2016; Paul et al., 2015). These observations were backed up by satellite observations showing reduced cyanobacterial abundance throughout the Baltic Sea in 2012 compared to previous and later years (Oberg, 2013). It was proposed that environmental conditions of limited light availability and lower surface water temperatures during the summer of 2012 were sub-optimal for triggering a filamentous cyanobacteria bloom (Wasmund, 1997).
3.2 DMS and DMSP

3.2.1 Mesocosm DMS

A significant 34% reduction in DMS concentrations was detected in the high $f$CO$_2$ treatments during Phase II compared to the ambient $f$CO$_2$ mesocosms (F=31.7, p<0.01). Mean DMS concentrations of 5.0 (± 0.8; range 3.5 – 6.8) nmol L$^{-1}$ in the ambient treatments compared to 3.3 (± 0.3; range 2.9 – 3.9) nmol L$^{-1}$ in the 1333 and 1075 μatm mesocosms (Fig. 23a). The primary differences identified were apparent from the start of Phase II on t17, after which maximum concentrations were observed in the ambient mesocosms on t21. The relationship between DMS and increasing $f$CO$_2$ during Phase II was found to be linear (Fig. 32b), a finding also identified in previous mesocosm experiments (Archer et al., 2013; Webb et al., 2015). Furthermore, increases in DMS concentrations under high $f$CO$_2$ were delayed by three days relative to the ambient and medium $f$CO$_2$ treatments, a situation which has been observed in a previous mesocosm experiment. This was attributed to small-scale shifts in community composition and succession which could not be identified with only a once-daily measurement regime (Vogt et al., 2008). DMS measured in all mesocosms fell within the range 2.7 to 6.8 nmol L$^{-1}$ across the course of the experiment. During Phase I, no difference was identified in DMS concentrations between $f$CO$_2$ treatments with the mean of all mesocosms 3.1 (± 0.2) nmol L$^{-1}$. Concentrations in all mesocosms gradually declined from t21 until the end of DMS measurements on t31. DMS concentrations measured in the mesocosms and Baltic Sea were comparable to those measured in temperate coastal conditions in the North Sea (Turner et al., 1988), the Mauritanian upwelling (Franklin et al., 2009; Zindler et al., 2012) and South Pacific (Lee et al., 2010).

Although the majority of DMS production is presumed to be from DMSP. However, an alternative production route for DMS is available through the methylation of methanethiol (Drotar et al., 1987; Kiene and Hines, 1995; Stets et al., 2004) predominantly identified in anaerobic environments such as freshwater lake sediments (Lomans et al., 1997), saltmarsh sediments (Kiene and Visscher, 1987) and microbial mats (Visscher et al., 2003; Zinder et al., 1977). However, recent studies have also identified this pathway of DMS production from Pseudomonas deceptionensis in an aerobic environment (Carrión et al., 2015), where P. deceptionensis was unable to synthesise or catabolise DMSP, but was able to enzymatically mediate DMS production from methanethiol (MeSH). The same enzyme has also been identified in a wide range of other bacterial taxa, including the cyanobacterial Pseudanabaena, which was identified in the Baltic Sea during this and previous investigations (Stuhr, pers. comm.; Kangro et al., 2007; Nausch et al., 2009). Correlations...
between DMS and the cyanobacterial equivalent Chl-α \( (\rho = 0.42, p<0.01; \text{Supplementary Figure S1g}) \) and DMS and single-celled cyanobacteria \( (\rho = 0.58, p<0.01; \text{Supplementary Figure S2a}) \) indicate suggest that the methylation pathway may be a potential source of DMS within the Baltic Sea community. In addition to the methylation pathway, DMS production has been identified from S-methylmethionine (Bentley and Chasteen, 2004), as well as from the reduction of dimethylsulphoxide (DMSO) in both surface and deep waters by bacterial metabolism (Hatton et al., 2004). As these compounds were not measured in the mesocosms, it is impossible to determine if they were significant sources of DMS.

3.2.2 DMS and Community Interactions

Throughout Phase I, DMS showed no correlation with any measured variables of biological activity or cell abundance, and was unaffected by elevated \( f\text{CO}_2 \), indicating measured DMS net production concentrations were not directly related to the perturbation of the system and associated cellular stress (Sunda et al., 2002). Of the studied phytoplankton groupings, neither the cryptophyes or chlorophyes as the largest contributors of Chl-α were identified as significant producers of DMSP. During Phase II, DMS was negatively correlated with Chl-α in the ambient and medium \( f\text{CO}_2 \) mesocosms \( (\rho = -0.60, p<0.01) \). During Phase II, a significant correlation was seen between DMS and single-celled cyanobacteria identified predominantly as Synechococcus \( (\rho = 0.53, p<0.01; \text{Crawfurd et al. 2016 and supplementary table S1}) \) and picoeukaryotes III \( (\rho = 0.75, p<0.01) \). The peak in DMS concentrations is unlikely to be a delayed response to the increased Chl-α on \( t16 \) due to the time lag of 7 days. These higher concentrations were also likely connected to a peak in dissolved organic carbon (DOC) on \( t15 \), as well as increasing bacterial abundance during Phase II (Hornick et al., 2016), as well as a response to the mesocosm wall cleaning which took place on \( t16. \) The variation in inorganic nutrient concentrations between mesocosms at the start of the experiment did not have an effect on DMS concentrations during Phase I, and by the start of Phase II the variation between mesocosms had decreased.

In previous mesocosm experiments (Archer et al., 2013; Hopkins et al., 2010; Webb et al., 2015), DMS has shown poor correlations with many of the indicators of primary production and phytoplankton abundance, as well as showing the same trend of decreased concentrations in high \( f\text{CO}_2 \) mesocosms compared to ambient. DMS production is often uncoupled from measurements of primary production in open waters (Lana et al., 2012), and also often from production of its
precursor DMSP (Archer et al., 2009). DMS and DMSP are important sources of sulphur and carbon in the microbial food web for both bacteria and algae (Simó et al., 2002, 2009), and since microbial turnover of DMSP and DMS play a significant role in net DMS production, it is unsurprising that DMS concentrations have shown poor correlation with DMSP-producing phytoplankton groups in past experiments and open waters.

DMS concentrations have been reported lower under conditions of elevated $f$CO$_2$ compared to ambient controls, in both mesocosm experiments (Table 4) and phytoplankton monocultures (Arnold et al., 2013; Avgoustidi et al., 2012). However, the varying response of the community within each experiment limit our ability to generalise the response of algal production of DMS and DMSP in all situations due to the characteristic community dynamics of each experiment in specific geographical areas and temporal periods. Previous experiments in the temperate Raunefjord of Bergen, Norway, showed lower abundance of DMSP-producing algal species, and subsequently DMSP-dependent DMS concentrations (Avgoustidi et al., 2012; Hopkins et al., 2010; Vogt et al., 2008; Webb et al., 2015). In contrast mesocosm experiments in the Arctic and Korea have shown increased abundance of DMSP producers (Archer et al., 2013; Kim et al., 2010) but lower DMS concentrations, while incubation experiments by Hopkins and Archer (2014) showed lower DMSP production but higher DMS concentrations at high $f$CO$_2$. However, in all previous experiments with DMSP as the primary precursor of DMS, elevated $f$CO$_2$ had a less marked effect on measured DMSP concentrations than on measured DMS concentrations. Hopkins et al. (2010) suggested that ‘the perturbation of the system has a greater effect on the processes that control the conversion of DMSP to DMS rather than the initial production of DMSP itself’. This is relevant even for the current experiment, where DMSP was not identified, since processes controlling DMS concentrations were likely more affected by the change in $f$CO$_2$ than the production of precursors.

Previous mesocosm experiments have suggested significant links between increased bacterial production through greater availability of organic substrates at high $f$CO$_2$ (Engel et al., 2013; Piontek et al., 2013). Further, Endres et al. (2014) identified significant enhanced enzymatic hydrolysis of organic matter with increasing $f$CO$_2$, with higher bacterial abundance. Higher bacterial abundance will likely result in greater bacterial demand for sulphur, and therefore greater consumption of DMS and conversion to DMSO. This was suggested as a significant sink for DMS in a previous experiment (Webb et al., 2015), but during the present experiment, both bacterial abundance and bacterial production were lower at high $f$CO$_2$ (Hornick et al., 2016). However, as it has been proposed that only specialist bacterial groups are DMS consumers (Vila-Costa et al.,
2006b), and there is no determination of the DMS consumption characteristics of the bacterial community in the Baltic Sea, it is not known if this loss pathway is stimulated at this is still a potential stimulated DMS loss pathway at high $f$CO$_2$. As microbial DMS yields can vary between 5-40% depending on the sulphur and carbon demand (Kiene and Linn, 2000), a change in the bacterial sulphur requirements could change DMS turnover despite lower abundance. Synechococcus has been identified as a DMS consumer in the open ocean, but abundance of this group was negatively correlated with $f$CO$_2$, implying that DMS consumption by this group would have been lower as $f$CO$_2$ increased.

### 3.3 Iodocarbons in the mesocosms and relationships with community composition

Elevated $f$CO$_2$ did not affect the concentration of iodocarbons in the mesocosms significantly at any time during the experiment, which is in agreement with the findings of Hopkins et al. (2013) in the Arctic, but in contrast to Hopkins et al. (2010) and Webb (2015), where iodocarbons were measured significantly lower under elevated $f$CO$_2$ (Table 4). Concentrations of all iodocarbons measured in the mesocosms and the Baltic Sea fall within the range of those measured previously in the region (Table 5). Mesocosm concentrations of CH$_3$I (Fig. 34a) and C$_2$H$_5$I (Fig. 34b) showed concentration ranges of 2.91 to 6.25 and 0.23 to 0.76 pmol L$^{-1}$ respectively. CH$_3$I showed a slight increase in all mesocosms during Phase I, peaking on t16 which corresponded with higher Chl-α concentrations, and correlated throughout the entire experiment with picoeukaryote groups II ($\rho$=0.59, p<0.01) and III ($\rho$=0.23, p<0.01; Crawfurd et al., this issue 2016) and nanoeukaryotes I ($\rho$=0.37, p<0.01).

Significant differences identified between mesocosms for CH$_3$I were unrelated to elevated $f$CO$_2$ (F=3.1, p<0.05), but concentrations were on average 15% higher in Phase II than Phase I. C$_2$H$_5$I decreased slightly during Phases I and II, although concentrations of this halocarbon were close to its detection limit (0.2 pmol L$^{-1}$), remaining below 1 pmol L$^{-1}$ at all times. As this compound showed no significant effect of elevated $f$CO$_2$, and was identified by Orlikowska and Schulz-Bull (2009) as having extremely low concentrations in the Baltic Sea (Table 5), it will not be discussed further.

No correlation was found between CH$_3$I and Chl-α at any phase, and the only correlation of any phytoplankton grouping was with nanoeukaryotes II ($\rho$=0.88, p<0.01; Crawfurd et al., 2015). These CH$_3$I concentrations compare well to the 7.5 pmol L$^{-1}$ measured by Karlsson et al. (2008) during a cyanobacterial bloom in the Baltic Sea (Table 5), and the summer maximum of 16 pmol L$^{-1}$ identified by Orlikowska and Schulz-Bull (2009).
Karlsson et al. (2008) showed Baltic Sea halocarbon production occurring predominately during daylight hours, with concentrations at night decreasing by 70% compared to late afternoon. Light dependent production of CH$_3$I has been shown to take place through abiotic processes, including radical recombination of CH$_3$ and I (Moore and Zafiriou, 1994). However, since samples were integrated over the surface 10m of the water column, it was impossible to determine if photochemistry was affecting iodocarbon concentrations near the surface where some UV light was able to pass between the top of the mesocosm film material and the cover. For the same reason, photodegradation of halocarbons (Zika et al., 1984) within the mesocosms was also likely to have been significantly restricted. Thus, as photochemical production was expected to be minimal, biogenic production was likely to have been the dominant source of these compounds. Karlsson et al. (2008) identified Pseudanabaena as a key producer of CH$_3$I in the Baltic Sea. However, the abundance of Pseudanabaena was highest during Phase I of the experiment (A. Stuhr, Pers. Comm.) when CH$_3$I concentrations were lower, and as discussed previously, the abundance of these species constituted only a very small proportion of the community. Previous investigations in the laboratory have identified diatoms as significant producers of CH$_3$I (Hughes et al., 2013; Manley and De La Cuesta, 1997), and the low, steady-state abundance of the diatom populations in the mesocosms could have produced the same relatively steady-state trends in the iodocarbon concentrations.

Measured in the range 57.2 – 202.2 pmol L$^{-1}$ in the mesocosms, CH$_2$I$_2$ (Fig. 34c) showed the clearest increase in concentration during Phase II, when it peaked on t21 in all mesocosms, with a maximum of 202.2 pmol L$^{-1}$ in M5 (348 μatm). During Phase II, concentrations of CH$_2$I$_2$ were 57% higher than Phase I, and were therefore negatively correlated with Chl-$\alpha$. The peak on t21 corresponds with the peak identified in DMS on t21, and concentrations through all three phases correlate with picoeukaryotes II ($\rho$=0.62, p<0.01) and III ($\rho$=0.47, p<0.01) and nanoeukaryotes I ($\rho$=0.88, p<0.01; Crawfurd et al., 2015). CH$_2$ClI (Fig. 34d) showed no peaks during either Phase I or Phase II, remaining within the range 3.81 to 8.03 pmol L$^{-1}$, and again correlated with picoeukaryotes groups II ($\rho$=0.34, p<0.01) and III ($\rho$=0.38, p<0.01). These results may suggest that these groups possessed halo-peroxidase enzymes able to oxidise I, most likely as an anti-oxidant mechanism within the cell to remove H$_2$O$_2$ (Butler and Carter-Franklin, 2004; Pedersen et al., 1996; Theiler et al., 1978). However, given the lack of response of these compounds to elevated $f$CO$_2$ (F=1.7, p<0.01), it is unlikely that production was increased in relation to elevated $f$CO$_2$. Production of all iodocarbons increased during Phase II when total Chl-$\alpha$ decreased, particularly after the walls of the mesocosms were cleaned for the first time, releasing significant volumes of organic
aggregates into the water column. Aggregates have been suggested as a source of CH$_3$I and C$_2$H$_5$I (Hughes et al., 2008), likely through the alkylation of inorganic iodide (Urhahn and Ballschmiter, 1998) or through the breakdown of organic matter by microbial activity to supply the precursors required for iodocarbon production (Smith et al., 1992). Hughes et al. (2008) did not identify this route as a pathway for CH$_2$I$_2$ or CH$_2$ClI production, but Carpenter et al. (2005) suggested a production pathway for these compounds through the reaction of HOI with aggregated organic materials.

3.4 Bromocarbons in the mesocosms and the relationships with community composition

No effect of elevated fCO$_2$ was identified for any of the three bromocarbons, which compared with the findings from previous mesocosms where bromocarbons were studied (Hopkins et al., 2010, 2013; Webb, 2015; Table 4). Measured concentrations were comparable to those of Orlikowska and Schulz-Bull (2009) and Karlsson et al. (2008) measured in the Southern part of the Baltic Sea (Table 3). The concentrations of CHBr$_3$, CH$_2$Br$_2$ and CHBr$_2$Cl showed no major peaks of production in the mesocosms. CHBr$_3$ (Fig. 4a) decreased rapidly in all mesocosms over Phase 0 from a maximum measured concentration of 147.5 pmol L$^{-1}$ in M1 (mean of 138.3 pmol L$^{-1}$ in all mesocosms) to a mean of 85.7 ($\pm$8.2 s.d.) pmol L$^{-1}$ in all mesocosms for the period t0 to t31 (Phases I and II). The steady-state CHBr$_3$ concentrations indicated a production source, however there was no clear correlation with any measured algal groups. CH$_2$Br$_2$ concentrations (Fig. 5b, 5b) decreased steadily in all mesocosms from t-3 through to t31, over the range 4.0 to 7.7 pmol L$^{-1}$, and CHBr$_2$Cl followed a similar trend in the range 1.7 to 4.7 pmol L$^{-1}$ (Fig. 5c). Of the three bromocarbons, only CH$_2$Br$_2$ showed correlation with total Chl-$\alpha$ ($\rho$=0.52, p<0.01), and with cryptophyte ($\rho$=0.86, p<0.01) and dinoflagellate ($\rho$=0.65, p<0.01) derived Chl-$\alpha$. Concentrations of CH$_2$BrI were below detection limit for the entire experiment.

CH$_2$Br$_2$ showed positive correlation with Chl-$\alpha$ ($\rho$=0.52, p<0.01), nanoeukaryotes II ($\rho$=0.34, p<0.01) and cryptophytes ($\rho$=0.86, p<0.01; see supplementary material), whereas CHBr$_3$ and CHBr$_2$Cl showed very weak or no correlation with any indicators of primary production. Schall et al. (1997) have proposed that CHBr$_2$Cl is produced in seawater by the nucleophilic substitution of bromide by chloride in CHBr$_3$, which given the steady-state concentrations of CHBr$_3$ would explain the similar distribution of CHBr$_2$Cl concentrations. Production of all three bromocarbons was identified from large-size cyanobacteria such as *Aphanizomenon flos-aquae* by Karlsson et al. (2008), and in addition, significant correlations were
found in the Arabian Sea between the abundance of the cyanobacterium *Trichodesmium* and several bromocarbons (Roy *et al.*, 2011), and the low abundance of such bacteria in the mesocosms would explain the low variation in bromocarbon concentrations through the experiment.

Halocarbon loss processes such as nucleophilic substitution (Moore, 2006), hydrolysis (Elliott and Rowland, 1995), sea-air exchange and microbial degradation are suggested as of greater importance than production of these compounds by specific algal groups, particularly given the relatively low growth rates and low net increase in total Chl-α. Hughes *et al.* (2013) identified bacterial inhibition of CHBr₃ production in laboratory cultures of *Thalassiosira* diatoms, but that it was not subject to bacterial breakdown; which could explain the relative steady state of CHBr₃ concentrations in the mesocosms. In contrast, significant bacterial degradation of CH₂Br₂ in the same experiments could explain the steady decrease in CH₂Br₂ concentrations seen in the mesocosms. Bacterial oxidation was also identified by Goodwin *et al.* (1998) as a significant sink for CH₂Br₂. As discussed for the iodocarbons, photolysis was unlikely due to the UV absorption of the mesocosm film, and limited UV exposure of the surface waters within the mesocosm due to the mesocosm cover. The ratio of CH₂Br₂ to CHBr₃ was also unaffected by increased fCO₂, staying within the range 0.04 to 0.08. This range in ratios is consistent with that calculated by Hughes *et al.* (2009) in the surface waters of an Antarctic depth profile, and attributed to higher sea-air flux of CHBr₃ than CH₂Br₂ due to a greater concentrations gradient, despite the similar transfer velocities of the two compounds (Quack *et al.*, 2007). Using cluster analysis in a time-series in the Baltic Sea, Orlikowska and Schulz-Bull (2009) identified both these compounds as originating from different sources and different pathways of production.

Macroalgal production would not have influenced the mesocosm concentrations after the bags were sealed due to the isolation from the coastal environment, however macroalgal production into the water column prior to mesocosm installation the higher bromocarbon concentrations identified in the mesocosms during Phase 0 may have originated from macroalgal sources (Klick, 1992; Leedham *et al.*, 2013; Moore and Tokarczyk, 1993) prior to mesocosm closure could account for the high initial concentrations with concentrations decreasing through the duration of the experiment via turnover and transfer to the atmosphere.
3.5 Natural variations in Baltic Sea $f$CO$_2$ and the effect on biogenic trace gases

3.5.1 Physical variation and community dynamics

Baltic Sea deep waters have high $f$CO$_2$ and subsequently lower pH (Schneider et al., 2002), and the influx to the surface waters surrounding the mesocosms resulted in $f$CO$_2$ increasing to 725 μatm on $t$31, close to the average $f$CO$_2$ of the third highest mesocosm (M6: 868 μatm). These conditions imply that pelagic communities in the Baltic Sea are regularly exposed to rapid changes in $f$CO$_2$ and the associated pH, as well as having communities associated with the elevated $f$CO$_2$ conditions.

Chl-α followed the pattern of the mesocosms until $t$4, after which concentrations were significantly higher than any mesocosm, peaking at 6.48 μg L$^{-1}$ on $t$16, corresponding to the maximum Chl-α peak in the mesocosms and the maximum peak of temperature. As upwelled water intruded into the surface waters, the surface Chl-α was diluted with low Chl-α deep water: Chl-α in the surface 10m decreased from around $t$16 at the start of the upwelling until $t$31 when concentrations were once again equivalent to those found in the mesocosms at 1.30 μg L$^{-1}$. In addition, there was potential introduction of different algal groups to the surface, but chlorophytes and cryptophytes were the major contributors to the Chl-α in the Baltic Sea, as in the mesocosms. Cyanobacteria contributed less than 2% of the total Chl-α in the Baltic Sea (Crawfurd et al., 2016; Paul et al., 2015).

Temporal community dynamics in the Baltic Sea were very different to that in the mesocosms across the experiment, with euglenophytes, chlorophytes, diatoms and prasinophytes all showing distinct peaks at the start of Phase II, with these same peaks identified in the nanoeukaryotes I and II, and picoeukaryotes II (Crawfurd et al., 2016; Paul et al., 2015; Supplementary Figs. S1 and S2).

The decrease in abundance of many groups during Phase II was attributed to the decrease in temperature and dilution with low-abundance deep waters.

3.5.2 DMS in the Baltic Sea

The input of upwelled water into the region mid-way through the experiment significantly altered the biogeochemical properties of the waters surrounding the mesocosms, and as a result it is inappropriate to directly compare the community structure and trace gas production of the Baltic Sea and the mesocosms. The Baltic Sea samples gave a mean DMS concentration of 4.6 ± 2.6 nmol L$^{-1}$, but peaked at 11.2 nmol L$^{-1}$ on $t$16, and were within the range of previous measurements for the region (Table 5). Strong correlations were seen between DMS and Chl-α ($\rho=0.84$, $p<0.01$), with the ratio of DMS: Chl-α at 1.6 (± 0.3) nmol μg$^{-1}$. Other strong correlations were seen with euglenophytes ($\rho=0.89$, $p<0.01$), dinoflagellates ($\rho=0.61$, $p<0.05$) and nanoeukaryotes II ($\rho=0.88$, 0.83).
p<0.01), but no correlation was found between DMS and cyanobacterial abundance, or with picoeukaryotes III which was identified in the mesocosms, suggesting that DMS had a different origin in the Baltic Sea community than in the mesocosms. Once again, there was no DMSP detected in the samples.

As CO$_2$ levels increased after t17 during Phase II, the DMS concentration measured in the Baltic Sea decreased, from the peak on t16 to the lowest recorded sample of the entire experiment at 1.85 nmol L$^{-1}$. As with Chl-$\alpha$, DMS concentrations in the surface of the Baltic Sea may have been diluted with low-DMS deep water, however, the inverse relationship of DMS with CO$_2$ shown in the mesocosms may suggest that this decrease in DMS is attributed to the increase in CO$_2$ levels. Bacterial abundance was similar in the Baltic Sea as in the mesocosms (Hornick et al., 2015), however the injection of high CO$_2$ water may have stimulated bacterial consumption of DMS during the upwelling, which combined with the dilution of DMS-rich surface water could have resulted in the rapid decrease in DMS concentrations. As no discernible decrease in total bacterial abundance was identified during the upwelling, it is also possible that the upwelled water contained a different microbial community, and may potentially have introduced a higher abundance of DMS-consuming microbes. No breakdown of bacterial distributions was available with which to test this hypothesis.

### 3.5.3 Halocarbon concentrations in the Baltic Sea

Outside the mesocosms in the Baltic Sea, CH$_3$I was measured at a maximum concentration of 8.65 pmol L$^{-1}$, during Phase II, and showed limited effect of the upwelling event. Both CH$_2$I$_2$ and CH$_3$ClI showed higher concentrations in the Baltic Sea samples than the mesocosms (CH$_2$I$_2$: 373.9 pmol L$^{-1}$ and CH$_3$ClI: 18.1 pmol L$^{-1}$), and were correlated with the euglenophytes (CH$_2$I$_2$: $\rho$=0.63, p<0.05 and CH$_3$ClI: $\rho$=0.68, p<0.01) and nanoeukaryotes II (CH$_2$I$_2$: $\rho$=0.53, p<0.01 and CH$_3$ClI: $\rho$=0.58, p<0.01), but no correlation with Chl-$\alpha$. Both polyhalogenated iodinated compounds showed correlation with picoeukaryote groups II and III, indicating that production was probably not limited to a single source. These concentrations of CH$_2$I$_2$ and CH$_3$ClI compared well to those measured over a macroalgal bed in the higher saline waters of the Kattegat by Klick and Abrahamsson (1992), suggesting that macroalgae were a significant iodocarbon source in the Baltic Sea.

As with the iodocarbons, the Baltic Sea showed significantly higher concentrations of CHBr$_3$ (F=28.1, p<0.01), CH$_2$Br$_2$ (F=208.8, p<0.01) and CHBr$_3$Cl (F=23.5, p<0.01) than the mesocosms, with maximum concentrations 191.6 pmol L$^{-1}$, 10.0 pmol L$^{-1}$ and 5.0 pmol L$^{-1}$ respectively. In the Baltic Sea, only CHBr$_3$ was correlated with Chl-$\alpha$ ($\rho$=0.65, p<0.05), cyanobacteria ($\rho$=0.61, p<0.01; Paul et al., 2015) and nanoeukaryotes II ($\rho$=0.56, p<0.01; Crawfurd et al., 2016), with the other
two bromocarbons showing little to no correlations with any parameter of community activity. Production of bromocarbons from macroalgal sources (Laturnus et al., 2000; Leedham et al., 2013; Manley et al., 1992) was likely a significant contributor to the concentrations detected in the Baltic Sea; over the macroalgal beds in the Kattegat, Klick (1992) measured concentrations an order of magnitude higher than seen in this experiment for CH$_2$Br$_2$ and CHBr$_2$Cl. There was only a slight increase in bromocarbon concentrations as a result of the upwelling, indicating that the upwelled water had similar concentrations to the surface waters.

4 The Baltic Sea as a natural analogue to future ocean acidification?

Mesocosm experiments are a highly valuable tool in assessing the potential impacts of elevated CO$_2$ on complex marine communities, however they are limited in that the rapid change in $f$CO$_2$ experienced by the community may not be representative of changes in the future ocean (Passow and Riebesell, 2005). This inherent problem with mesocosm experiments can be overcome through using naturally low pH/ high CO$_2$ areas such as upwelling regions or vent sites (Hall-Spencer et al., 2008), which can give an insight into populations already living and adapted/acclimated to high CO$_2$ regimes by exposure over timescales measured in years. This mesocosm experiment was performed at such a location with a relatively low-high $f$CO$_2$ excursion, however still low compared to some sites (800 μatm compared to >2000 μatm; Hall-Spencer et al., 2008), and it was clear through the minimal variation in Chl-a between all mesocosms that the community was relatively unaffected by elevated $f$CO$_2$, although variation could be identified in some phytoplankton groups and some shifts in community composition. The upwelling event occurring mid-way through our experiment allowed comparison of the mesocosm findings with a natural analogue of the system, as well as showing the extent to which the system perturbation can occur (up to 800 μatm), but the scale and timing of these upwelling events is difficult to determine. However, it is very difficult to determine where and when an upwelling will occur, and therefore it will be hard to utilise these events as natural high CO$_2$ analogues.

In this paper, we described the temporal changes in concentrations of DMS and halocarbons in natural Baltic phytoplankton communities exposed to elevated $f$CO$_2$ treatments. In contrast to the halocarbons, concentrations of DMS were significantly lower in the highest $f$CO$_2$ treatments compared to the control. Despite very different physicochemical and biological characteristics of the Baltic Sea (e.g. salinity, community composition and nutrient concentrations), this is a very similar outcome to that seen in several other high $f$CO$_2$ experiments. The Baltic Sea trace gas
samples give a good record of trace gas production-cycling during the injection of high fCO₂ deep water into the surface community during upwelling events. For the concentrations of halocarbons, no response the measured concentrations did not change during was shown to the upwelling event in the Baltic Sea, which may indicate that emissions of organic iodine and bromine are unlikely to change with future acidification of the Baltic Sea without significant alteration to the meteorological conditions. Further studies of these compounds are important to determine rates of production and consumption to include in prognostic and predictive models. However, net production of organic sulphur within the Baltic Sea region is likely to decrease with an acidified future ocean scenario, despite the possible acclimation of the microbial community to elevated fCO₂. This will potentially impact the flux of DMS to the atmosphere over Northern Europe, and could have significant impacts on the local climate through the reduction of atmospheric sulphur aerosols. Data from a previous mesocosm experiment has been used to estimate future global changes in DMS production, and predicted that global warming would be amplified (Six et al., 2013); utilising the data from this experiment combined with those of other mesocosm, field and laboratory experiments and associated modelling provide the basis for a better understanding of the future changes in global DMS production and their climatic impacts.
The Tvärminne 2012 mesocosm experiment was part of the SOPRAN II (Surface Ocean Processes in the Anthropocene) Programme (FKZ 03F0611) and BIOACID II (Biological Impacts of Ocean Acidification) project (FKZ 03F06550), funded by the German Ministry for Education and Research (BMBF) and led by the GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany. The authors thank all participants in the SOPRAN Tvärminne experiment for their assistance, including A. Ludwig for logistical support, the diving team, and the staff of Tvärminne Zoological Research Station for hosting the experiment. We also acknowledge the captain and crew of RV ALKOR (AL394 and AL397) for their work transporting, deploying and recovering the mesocosms. This work was funded by a UK Natural Environment Research Council Directed Research Studentship (NE/H025588/1) through the UK Ocean Acidification Research Programme, with CASE funding from Plymouth Marine Laboratory. Additional funding was supplied by the EU Seventh Framework Program (FP7/2007-2013) MESOAQUA (EC Contract No. 228224).


sed metabolism of dimethylsulphoniopropionate: 


Scarratt, M. G. and Moore, R. M.: Production of methyl bromide and methyl chloride in laboratory cultures of marine


Table 1. Summary of $f$CO$_2$ and pH$_T$ (total scale) during phases 0, 1 and 2 of the mesocosm experiment.

<table>
<thead>
<tr>
<th>Mesocosm$^a$</th>
<th>Target $f$CO$_2$ (µatm)</th>
<th>Mean $f$CO$_2$ (µatm)</th>
<th>Mean pH$_T$</th>
<th>Mean $f$CO$_2$ (µatm)</th>
<th>Mean pH$_T$</th>
<th>Mean $f$CO$_2$ (µatm)</th>
<th>Mean pH$_T$</th>
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<th>Mean pH$_T$</th>
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<tr>
<td>M1</td>
<td>Control</td>
<td>331</td>
<td>7.91</td>
<td>231</td>
<td>8.00</td>
<td>328</td>
<td>7.95</td>
<td>399</td>
<td>7.86</td>
</tr>
<tr>
<td>M5</td>
<td>Control</td>
<td>334</td>
<td>7.91</td>
<td>244</td>
<td>7.98</td>
<td>329</td>
<td>7.94</td>
<td>399</td>
<td>7.52</td>
</tr>
<tr>
<td>M7</td>
<td>390</td>
<td>458</td>
<td>7.80</td>
<td>239</td>
<td>7.99</td>
<td>494</td>
<td>7.81</td>
<td>532</td>
<td>7.76</td>
</tr>
<tr>
<td>M6</td>
<td>840</td>
<td>773</td>
<td>7.63</td>
<td>236</td>
<td>7.99</td>
<td>932</td>
<td>7.59</td>
<td>855</td>
<td>7.59</td>
</tr>
<tr>
<td>M3</td>
<td>1120</td>
<td>950</td>
<td>7.56</td>
<td>243</td>
<td>7.98</td>
<td>1176</td>
<td>7.51</td>
<td>1027</td>
<td>7.52</td>
</tr>
<tr>
<td>M8</td>
<td>1400</td>
<td>1166</td>
<td>7.49</td>
<td>232</td>
<td>8.00</td>
<td>1481</td>
<td>7.43</td>
<td>1243</td>
<td>7.45</td>
</tr>
<tr>
<td>Baltic Sea</td>
<td>380</td>
<td>350</td>
<td>7.91</td>
<td>298</td>
<td>7.91</td>
<td>277</td>
<td>7.98</td>
<td>436</td>
<td>7.86</td>
</tr>
</tbody>
</table>

$^a$ listed in order of increasing $f$CO$_2$
Table 2. Calibration ranges and calculated percentage mean relative standard error for the trace gases measured in the mesocosms.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calibration range (pmol L⁻¹)</th>
<th>% Mean relative standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS</td>
<td>600 – 29300*</td>
<td>6.33</td>
</tr>
<tr>
<td>DMSP</td>
<td>2030 – 405900*</td>
<td></td>
</tr>
<tr>
<td>CH₃I</td>
<td>0.11 – 11.2</td>
<td>4.62</td>
</tr>
<tr>
<td>CH₂I₂</td>
<td>5.61 – 561.0</td>
<td>4.98</td>
</tr>
<tr>
<td>C₂H₅I</td>
<td>0.10 – 4.91</td>
<td>5.61</td>
</tr>
<tr>
<td>CH₂ClI</td>
<td>1.98 – 99.0</td>
<td>3.64</td>
</tr>
<tr>
<td>CHBr₃</td>
<td>8.61 – 816.0</td>
<td>4.03</td>
</tr>
<tr>
<td>CH₂Br₂</td>
<td>0.21 – 20.9</td>
<td>5.30</td>
</tr>
<tr>
<td>CHBr₂Cl</td>
<td>0.07 – 7.00</td>
<td>7.20</td>
</tr>
</tbody>
</table>

* throughout the rest of this paper, these measurements are given in nmol L⁻¹.
Table 3. Abundance and contributions of different phytoplankton groups to the total phytoplankton community assemblage, showing the range of measurements from total Chl-a (Paul et al., 2015), CHEMTAX analysis of derived Chl-a (Paul et al., 2015) and phytoplankton abundance (Crawford et al., 2016). Data are split into the range of all the mesocosm measurements and those from the Baltic Sea.

<table>
<thead>
<tr>
<th>Phytoplankton Taxonomy (Equivalent Chlorophyll µg L⁻¹)</th>
<th>Mesocosm</th>
<th>Baltic Sea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>% Contribution to Chl-a</td>
</tr>
<tr>
<td></td>
<td>Integrated 10 m</td>
<td>Integrated 17 m</td>
</tr>
<tr>
<td>Chl-a</td>
<td>0.9 – 2.9</td>
<td>0.9 – 2.6</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td>0.01 – 0.4</td>
<td>0.01 – 0.1</td>
</tr>
<tr>
<td><strong>Prasinophytes</strong></td>
<td>0.04 – 0.3</td>
<td>0.01 – 0.3</td>
</tr>
<tr>
<td><strong>Euglenophytes</strong></td>
<td>0.0 – 1.6</td>
<td>0.0 – 2.6</td>
</tr>
<tr>
<td><strong>Dinoflagellates</strong></td>
<td>0.0 – 0.3</td>
<td>0.04 – 0.6</td>
</tr>
<tr>
<td><strong>Diatoms</strong></td>
<td>0.1 – 0.3</td>
<td>0.04 – 0.9</td>
</tr>
<tr>
<td><strong>Chlorophytes</strong></td>
<td>0.3 – 2.0</td>
<td>0.28 – 3.1</td>
</tr>
<tr>
<td><strong>Cryptophytes</strong></td>
<td>0.1 – 1.4</td>
<td>0.1 – 1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Small Phytoplankton (&lt;10 µm) abundance I (cells mL⁻¹)</th>
<th>Mesocosm</th>
<th>Baltic Sea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>55000 – 38000</td>
<td>65000 – 470000</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td>15000 – 100000</td>
<td>10000 – 110000</td>
</tr>
<tr>
<td><strong>Picoeukaryotes I</strong></td>
<td>700 – 4000</td>
<td>600 – 4000</td>
</tr>
<tr>
<td><strong>Picoeukaryotes II</strong></td>
<td>1000 – 3000</td>
<td>1100 – 8500</td>
</tr>
<tr>
<td><strong>Picoeukaryotes III</strong></td>
<td>400 – 1400</td>
<td>270 – 1500</td>
</tr>
<tr>
<td><strong>Nanoeukaryotes I</strong></td>
<td>0 – 400</td>
<td>4 – 400</td>
</tr>
<tr>
<td><strong>Nanoeukaryotes II</strong></td>
<td>400 – 3800</td>
<td>65000 – 470000</td>
</tr>
</tbody>
</table>
Table 4. Concentration ranges of trace gases measured in the mesocosms compared to other open water ocean acidification experiments, showing the range of concentrations for each gas and the percentage change between the control and the highest f/CO₂ treatment.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Range f/CO₂ (μatm)</th>
<th>Range DMS (nmol L⁻¹)</th>
<th>CH₃I</th>
<th>CH₂I₂</th>
<th>CH₂ClI</th>
<th>CHBr₃</th>
<th>CH₂Br₂</th>
<th>CH₂Br₂Cl (pmol L⁻¹)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOPRAN Tvärminne Mesocosm (this study)</td>
<td>346 – 1333</td>
<td>Range</td>
<td>2.7-6.8</td>
<td>2.9-6.4</td>
<td>57-202</td>
<td>3.8-8.0</td>
<td>69-148</td>
<td>4.0-7.7</td>
<td>1.7-3.1</td>
</tr>
<tr>
<td>% change</td>
<td>-34</td>
<td>-0.3</td>
<td>1.3</td>
<td>-11</td>
<td>-9</td>
<td>-3</td>
<td>-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOPRAN Bergen 2011 (Webb et al., 2015)</td>
<td>280 – 3000</td>
<td>Range</td>
<td>0.1-4.9</td>
<td>4.9-32</td>
<td>5.8-321</td>
<td>9.0-123</td>
<td>64-306</td>
<td>6.3-30.8</td>
<td>3.9-14</td>
</tr>
<tr>
<td>% change</td>
<td>-60</td>
<td>-37</td>
<td>-48</td>
<td>-27</td>
<td>-2</td>
<td>-4</td>
<td>-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NERC Microbial Metagenomics Experiment, Bergen 2006 (Hopkins et al., 2010)</td>
<td>300 - 750</td>
<td>Range</td>
<td>ND-50</td>
<td>2.0-25</td>
<td>ND-750</td>
<td>ND-700</td>
<td>5.0-80</td>
<td>ND-5.5</td>
<td>0.2-1.2</td>
</tr>
<tr>
<td>% change</td>
<td>-57</td>
<td>-41</td>
<td>-33</td>
<td>-28</td>
<td>13</td>
<td>8</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPOCA Svalbard 2010 (Archer et al., 2013; Hopkins et al., 2013)</td>
<td>180 - 1420</td>
<td>Range</td>
<td>ND-14</td>
<td>0.04-10</td>
<td>0.01-2.5</td>
<td>0.3-1.6</td>
<td>35-151</td>
<td>6.3-33.3</td>
<td>1.6-4.7</td>
</tr>
<tr>
<td>% change</td>
<td>-60</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UKOA European Shelf 2011 (Hopkins and Archer, 2014)</td>
<td>340 - 1000</td>
<td>Range</td>
<td>0.5-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td>225</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korean Mesocosm Experiment 2012 (Park et al., 2014)</td>
<td>160 - 830</td>
<td>Range</td>
<td>1.0-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td>-82</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Concentration ranges of trace gases measured in the Baltic Sea compared to concentrations measured in the literature. ND – Not Detected.

<table>
<thead>
<tr>
<th>Study</th>
<th>DMS concentration range (nmol L$^{-1}$)</th>
<th>Halocarbon concentration range (pmol L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOPRAN Tvärminne Baltic Sea (This Study)</td>
<td>1.9-11</td>
<td>CH$_3$I 4.3-8.6  CH$_2$I$_2$ 66.9-374  C$_2$H$_5$I 0.6 – 1.0  CH$_3$Cl 7.0-18  CHBr$_3$ 93-192  CH$_2$Br$_2$ 7.1-10  CH$_2$Br$_2$Cl 3.3-5.0</td>
</tr>
<tr>
<td>Orlikowska and Schulz-Bull (2009)</td>
<td>0.3-120</td>
<td>CH$_3$I 1-16  CH$_2$I$_2$ 0-85  C$_2$H$_5$I 0.4 – 1.2  CH$_3$Cl 5-50  CHBr$_3$ 5.0-40  CH$_2$Br$_2$ 2.0-10  CH$_2$Br$_2$Cl 0.8-2.5</td>
</tr>
<tr>
<td>Karlsson et al. (2008)</td>
<td></td>
<td>CH$_3$I 3.0-7.5  CH$_2$I$_2$ 35-60  C$_2$H$_5$I 4.0-7.0  CH$_3$Cl 2.0-6.5</td>
</tr>
<tr>
<td>Klick and Abrahamsson (1992)</td>
<td></td>
<td>CH$_3$I 15-709  CH$_2$I$_2$ 11-74  C$_2$H$_5$I 14-585</td>
</tr>
<tr>
<td>Klick (1992)</td>
<td></td>
<td>CH$_3$I ND-243  CH$_2$I$_2$ ND-57  C$_2$H$_5$I 40-790  CH$_3$Cl ND-86  CH$_2$I$_2$Cl ND-29</td>
</tr>
<tr>
<td>Leck and Rodhe (1991)</td>
<td>0.4-2.8</td>
<td>CH$_3$I ND-3.2  CH$_2$I$_2$ ND-57  C$_2$H$_5$I 40-790  CH$_3$Cl ND-86  CH$_2$I$_2$Cl ND-29</td>
</tr>
<tr>
<td>Leck et al. (1990)</td>
<td></td>
<td>CH$_3$I ND-3.2  CH$_2$I$_2$ ND-57  C$_2$H$_5$I 40-790  CH$_3$Cl ND-86  CH$_2$I$_2$Cl ND-29</td>
</tr>
</tbody>
</table>
Figure 1. Daily measurements of (a) $f$CO$_2$, (b) mean temperature and (c) total Chlorophyll-$\alpha$ in the mesocosms and surrounding Baltic Sea waters. Dashed lines represent the three Phases of the experiment, based on the Chl-$\alpha$ data.
Figure 32. (a) Mean DMS concentrations measured daily in the mesocosms and Baltic Sea from an integrated water sample of the surface 10 m integrated from the surface 10 m. Dashed lines show the Phases of the experiment as given in Fig. 12, fCO₂ shown in the legend are mean fCO₂ across the duration of the experiment. (b) Mean DMS concentrations from each mesocosm during Phase I (crosses) and Phase II (diamonds), for ambient (blue), medium (grey) and high fCO₂ (red), with error bars showing the range of both the DMS and fCO₂.
Figure 3. **Mean concentrations (pmol L⁻¹)** of (a) CH₃I, (b) C₂H₅I, (c) CH₂I₂ and (d) CH₂ClI taken from a water sample integrated from the surface 10m. Dashed lines indicate the Phases of the experiment, as given in Fig. 2. fCO₂ shown in the legend are mean fCO₂ across the duration of the experiment.
Figure S4. Mean concentrations (pmol L\(^{-1}\)) of (a) CHBr\(_3\), (b) CH\(_2\)Br\(_2\) and (c) CHBr\(_2\)Cl taken from a water sample integrated from the surface 10m. Dashed lines indicate the phases of the experiment as defined in Fig. 2, \(f\)CO\(_2\) shown in the legend are mean \(f\)CO\(_2\) across the duration of the experiment.
Reply to Editor Comments

1. The lack of DMSP data

Thanks to you and the reviewers for taking the time to read and edit this manuscript thoroughly. I have made the changes to the manuscript highlighted in the reviewer comments and now include the revised manuscript which outlines the detailed responses we have given to these comments.

The DMSP acidification method is currently used worldwide as a simple and effective method of DMSP storage, and indeed the authors used it in a previous mesocosm experiment with good correlation with samples analysed immediately on a different GC system. However, After the Tvärminne experiment, however, additional tests in this method from the Norfolk broads in varying salinity showed it to be unreliable in some circumstances (but not all), compared to samples which were analysed immediately (Data unpublished). These additional tests, alongside the paper from del Valle et al 2011, showed that this method is unreliable, yet it is still in use, and the authors wanted to highlight these discrepancies, and suggest that significant testing is required prior to heavy reliance on the data generated by this storage method.

With regards to the DMSP issue, in the initial submitted version of the manuscript, there was a significant discussion of why DMSP was not identified during this investigation. This included turnover rates of dissolved DMSP measured in other studies, including consumption by bacteria and phytoplankton, and conversion rates calculated from other studies for DMS. One of the reviewer comments was that this section was very long (which the authors agreed), since we had no measure of turnover or conversion rates, and that we would still see some DMSP even if rates were extremely high, so this section was significantly shortened and put into the methods section. A short discussion of DMS production from methanethiol was included in the results/discussion (still a potential production route, and one in which we plan further study, hence the need to mention it in this manuscript).

With regards to the reviewer comment you highlighted, this was not addressed directly with a response, as the authors read this as a general statement. Having addressed this reviewer comment throughout the revised manuscript, our response may not have been clear in just the ‘response to reviewers’ document. Hopefully the revised tracked changed manuscript will show that we have clearly addressed these comments. The discussion of DMS responses from past experiments is quite reliant on the discussion of DMSP, as this is the predominant source of DMS, and the authors believe the discussion of DMSP in the discussion is necessary, assuming that DMSP was present but lost from the samples. I hope that our edited manuscript and this response now clearly explained to you our reasoning behind the decision that this is almost certain a methodological issue. However, if you would like us to clarify anything further please let us know.

2. Combining mesocosm and Baltic Sea data

With regards to your comment regarding combining the mesocosm and Baltic Sea data, the authors disagree, and indeed one of the reviewer’s comments was that keeping discussions of the mesocosm and the outside waters separate was appropriate, since the outside underwent its own ‘experiment’. One of the drawbacks of a mesocosm experiment is that the water is separated from the surrounding environment, in the case of this experiment for over 6 weeks. Given the nature of the water movements through the Storfjärden, after even a few days the phytoplankton population within the mesocosms could...
potentially be significantly different from the surrounding water masses (dependent on the mesocosm seed population), and therefore the results from the samples within and external to the mesocosms significantly different. This drawback is a point which detractors of mesocosm experiments draw on heavily. As a result, production of trace gases can be significantly different in the mesocosms compared to the outside waters, even if the populations are the same, given that production is based on demands of key elements available in the environment (e.g. sulphur, bromine, iodine) which can change with injections of new water masses. In the case of the halocarbons in particular, macroalgal production is of huge importance to the concentration in the water column, yet macroalgae are not present in the mesocosms, and with a delay of over several days between mesocosm closure and first sampling, sufficient time has elapsed for these gases in the mesocosm water to vent into the atmosphere or break down. The data from the Baltic Sea samples is presented in its own right as an important time series of trace gas analysis, which will add to existing (albeit limited) Baltic trace gas datasets (e.g. Orlikowska & Schulz-Bull 2009; Karlsson et al. 2008; Klick 1992). The change in CO$_2$ and pH in the external waters (to which the mesocosms were not exposed), clearly demonstrates that the community in the Baltic is already adapted to changes in CO$_2$, and therefore helps to explain the lack of change in gas concentrations in the mesocosms. Essentially, although related, measurements from the mesocosms and the Baltic Sea are part of two separate experiments undertaken at the same time, and cannot just be combined easily. It was hoped that this was discussed sufficiently in the final section, but this will be revisited to check if this is so.
The authors would like to thank the reviewer for their comments and discussions at all stages of the review process, which have improved the overall quality of the manuscript. I have addressed the reviewer’s comments individually.

1- An initial paragraph or section evaluating the overall quality of the discussion paper ("general comments"),

The manuscript is well structured and for the most parts easily readable. The results show a lack of response of gas concentrations to the experimental design and no linkage to the external conditions due to the outside undergoing its own “experiment”, i.e., upwelling. No rates are reported; not clear any were measured. Hence, the entire manuscript must be clarified that the values represent “net” values and not production, nor consumption or degradation, nor emission. Hence, I strongly suggest removing most comments from the discussion that pertain to “climate change” and simply state that concentrations remained the same regardless and emphasize that we (and especially modelers) need to have rates of production, consumption, (photo/chem)-degradation or even “net” rates to include in our prognostic and predictive models.

AR. The mentions of climate change in the discussion section are quite limited, and although the change in halocarbons was limited, the change in DMS concentrations was high between the different treatments. The mention of the Six et al. paper was important, as this study was based on one mesocosm experiment and the results of the model output would have been more significant if the results of a number of mesocosm experiments had been included. A comment has been included to the effect that the reviewer says, that rates of consumption and production of halocarbons are needed to improve model output.

I recommend publishing pending changes. I also suggest shortening some of the longish speculative paragraphs; it’s hard to explain why there is no apparent change! In general, I think the manuscript would profit if it was a bit more structured based on hypotheses, rather than being purely descriptive. You must have had some expectations when the experiment was started (and the proposal written!), especially since you had results from previous mesocosm experiments. Especially since no (?) rates were measured.

AR. No rates were measured as it was difficult given the sampling regime of the experiment, without performing additional incubation experiments. There were hypotheses prior to the experiment, mainly that halocarbon concentrations would show some really interesting and varied results under high CO2, as a diazotrophic cyanobacteria bloom occurred. As this bloom did not really occur in the mesocosms, these hypotheses did not apply, particularly since the majority of the ‘interesting results’ occurred in DMS (but no DMSP). It was therefore important to discuss the lack of DMSP for the community as a whole, to draw to light the issues with the DMSP acidification/fixing method, and not to concentrate so hard on the lack of changes within the halocarbons.

2- section addressing individual scientific questions/issues ("specific comments"),

The manuscript addresses the influence of ocean acidification on the production of dimethylsulfide (DMS) and 7 halocarbons in a Baltic Sea mesocosm experiment. The authors effectively found no differences in DMS and halocarbon concentrations over time among the various fCO2 treatments; and no obvious relationship to any other environmental (biological or chemical) variable measured. Difficult to explain without knowing whether turnover is fast. The authors found a decrease of DMS concentrations for highest fCO2 treatments vs. controls only in the last phase (when Chla declined) and none of the other detected differences in halocarbons were CO2 related. The outcome of this study is a relevant piece of information, indicating that most likely there will be no major changes to halocarbon concentrations in the Baltic Sea anytime soon, and the authors conclude that this might be due to the already well adapted community in the unstable Baltic Sea environment with regards to S, T, CO2 and many other factors. The results are interesting by themselves, and valuable for modelers, though modelers need rates. The DMS results again confirm results from a range of mesocosm studies.

3- compact listing of purely technical corrections at the very end ("technical corrections": typing errors, etc.).
Inconsistent placing of units, 10m (no space) but 486 nm (space), e.g. line 247, 248 vs 261. You might wanna check if there are more.

AR. Units checked throughout manuscript

Both DMS and DMSP are major routes of sulphur and carbon flux through the marine microbial food web. I wouldn’t call them a route, they could be called transporters, or they provide the basis for major routes. Or DMS and DMSP based metabolic pathways are the route...

AR. Changed ‘are’ to ‘provide the basis for’

Where do they state that in this reference? I think Simo et al., 2009 should be the reference for phytoplanktonic demand (pages 50-51 e.g.), and Vila Costa et al., 2006a for bacteria (page 653)? Or you put them in as combined references for both (after sulphur demand)

AR. References have been combined at the end of the sentence

That was the standard deviation at the beginning? ~50%, ~7%, and ~75%? That’s a lot to start with... Any thoughts on how that could potentially affect the outcome of the experiments on the bacterial metabolism side of halocarbon and DMS production?

AR. Certainly for the halocarbons, the difference in nutrients between mesocosms had no effect on the eventual concentrations measured throughout the experiment. Variation in nutrients did not seem to affect DMS concentrations, since the high variation was only identified during the early part of Phase 1, when DMS concentrations did not show differences between mesocosms. Since the differences in DMS were only identifiable during Phase 2, nutrient concentrations had by then showed much lower standard deviation between mesocosms.

Pigment analysis was only carried out in the full 17m depth of the mesocosms. Many other parameters (not discussed in this manuscript) were analysed through the full water depth. In the previous mesocosm experiment, trace gas concentrations were significant in the surface 10m of the water column and diluted by the extra water, so during this experiment, concentrations were taken only from the surface 10m. Flow cytometry was performed both on the surface 10 m and the full 17m depth, but due to a large number of samples, HPLC pigment analysis was only performed on the full water depth.

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AR. Precision calculated as the percent deviation and inserted in manuscript.

The del Valle et al samples were DMSPd and DMSPt; not DMSPp. The Kiene group estimates DMSPp by difference between the total and dissolved pool.

AR. It is uncertain what the reviewer is highlighting here. The samples during the mesocosm experiment were DMSPt and DMSPp, but none of them showed any DMSP within. In the manuscript, there is no distinct emphasis on DMSPp over DMSPt, as the reviewer seems to be suggesting there was. This has been clarified in the manuscript by using DMSPt instead of just DMS.

AR. changed

AR. changed

Is that shown anywhere? Otherwise please state what the precision was, and that it is not further shown.

AR. Precision calculated as the percent deviation and inserted in manuscript.

Careful. The del Valle et al samples were DMSPd and DMSPt; not DMSPp. The Kiene group estimates DMSPp by difference between the total and dissolved pool.

AR. It is uncertain what the reviewer is highlighting here. The samples during the mesocosm experiment were DMSPt and DMSPp, but none of them showed any DMSP within. In the manuscript, there is no distinct emphasis on DMSPp over DMSPt, as the reviewer seems to be suggesting there was. This has been clarified in the manuscript by using DMSPt instead of just DMS.
Line 248: Chl-a the a is superscripted
AR. Could not find this error. Most likely corrected already.

Line 300: Mixing of the mesocosms after closure prior to t-3 did not trigger a notable increase in Chl-a in Phase 1; in previous mesocosm experiments, mixing redistributed nutrients from the deeper stratified layers throughout the water column.
I get what you are saying, but I think you should add what redistributing nutrients did— I am assuming here that it lead to an increase in Chl-a?
AR. In previous mesocosm experiments, redistribution of the nutrients from below the stratified surface layers results in a significant bloom of Chl-a. However, this was not identified during this experiment, suggesting a limiting factor. The manuscript has been clarified on this point.

Line 282: “mainly through air-sea gas exchange” – isn’t that usually considered to be limited by the small surface area / volume ratio? Please comment on why this should not be the same for your analyzed gases.
AR. The sea-air exchange will still exist during the mesocosm experiment, but it will be significantly decreased due to restrictions on wind interactions due to the mesocosm walls, reduced wave action and a very low SA: vol ratio for the water in the mesocosm. It is acknowledged that the trace gases will be lost to the atmosphere in the same way as CO2, but at different rates for different compounds. This is commented on later on particularly for the bromocarbons which showed a steady decrease in concentration throughout the experiment. We know that there was a steep CO2 gradient to the atmosphere, we do not know the concentration gradients for the halocarbons or DMS: if atmospheric halocarbon concentrations are equivalent to concentrations in the mesocosms (potentially possibly in the forests of finland), there would be a significantly lower rate of halocarbon loss than CO2 loss from the mesocosms. Existing flux models cannot be used due to the constraints of the mesocosm enclosure.

Line 302: no direct result of the CO2 additions because there was no significant difference between controls and treatments?
AR. Sentence clarified with ‘as no difference was identified between enriched mesocosms and controls’

Line 309: chlorophytes (largest contributor to chl a) are not exactly known to be high DMSP or DMS producers; you may want to mention that given stated link to pico and nanoeukaryotes as possible sources. This is why bringing in the Fig, S3 as Fig 3c somewhere actually shows that there are differences among treatments.
AR. L 382, a statement was added to the DMS and community parameters section stating ‘Of the studied phytoplankton groupings, neither the cryptophyes or chlorophyes as the largest contributors of Chl-a have ever been identified as significant producers of DMSP.’
It was decided to keep figure S3 in the supplementary, as DMS is clearly disconnected from total Chl-a concentrations, during Phase 2, which is not due to changes in Chl-a. The statistics on the DMS: Chl-a ratio are also insignificant due to the high standard deviation. This plot was therefore given for interest in the supplemental, but was not considered sufficiently robust to include in the finished manuscript.

Line 311-312: so between the opposing trends for pico I and pico II, the next effect on DMS in the system is zero?
AR. This section is discussing the differences seen in the mesocosms, and is not discussing the DMS concentrations. It is not implicitly stated that these groups are directly responsible for the DMS concentrations. There are many parameters acting on the DMS concentrations on top of the changes in these groups.
Line 331: Please explain F-test or at least the H0 you used in one sentence in the methods section.

AR. Null hypothesis added to the methods section

Line 348-369: Simply there was no relationship between patterns (or lack thereof) in DMS concentrations and any other measured variable. And no rate measurements available. Please say so. Too many possibilities, too many unknowns. This section reads a bit like “filler”; sorry.

AR. This section was significantly reduced prior to online discussion. It was decided that this section was necessary to discuss the alternate production pathways of DMS that could potentially be available in the Baltic Sea. A discussion occurred as to how this could be further investigated, and the authors felt it was important to keep this section relatively whole to allow for further research into these pathways, with this manuscript as a starting point.

Line 354: synthesis should be synthesise

AR. changed

Line 358: Correlations between DMS and the cyanobacterial equivalent Chl-α 359 (p=0.42, p<0.01) indicate that the methylation pathway may be a potential source of DMS within the 360 Baltic Sea community. Reference? Data shown anywhere?

AR. The cyanobacterial equivalent Chl-a and the single celled cyanobacterial abundance are shown in Supplementary. They were previously included in the paper, but it was considered too much data for too little solid evidence. This sentence therefore keeps the idea that there MAY be a relationship between DMS and cyanobacterial activity, but does not outright state that there is. The authors feel this could be a significant area of research that needs further investigation.

Line 367: Stop! What rates of net DMS production? Did you measured or estimate them? If you did, please indicate and discuss!

AR. No rates of production were calculated, hence the ‘net’ DMS production (concentrations remain the same despite removal and addition processes). However, in this instance this has been changed to ‘measured DMS concentrations’.

Line 371: but I thought that Syn does not make DMS?! There never is high DMS concentration reported along with it in subtrop regions (DiTullio et al., others). Didn’t Vla-Costa et al. 2006 report uptake of DMSPd (not DMS) by Syn and other picoeukaryotes?

AR. Other literature has not identified Syn as a significant producer of DMS or DMSP. This statistics reported a significant correlation with cyanobacteria, which is reported here, both the single-celled and multi-celled variety (Data not shown). This section has been amended by the addition of ‘predominantly’ Synechococcus’ as it is likely there are other single celled cyanobacteria within the population aside from Syn.

Line 372: Why is it unlikely?

AR. It has never been observed previously that a DMS peak occurs 5 days after a peak in Chl-a which has been directly linked to the Chla peak. DMS and Chla concentrations are rarely coupled, indeed even DMSP is rarely coupled to Chla, so this result is not unexpected.

Line 379: just one period.

AR. removed

Line 386: “However, these experiments limit our ability to generalize”… I don’t think it’s the experiments limiting, but rather the varying responses, is that what you are saying?
AR. Essentially, yes. The mesocosm experiments have been measuring DMS, DMSP and community parameters for a number of years now, and yet still no consensus appears as to the response to community changes. Mesocosm experiments also have their distinct disadvantages in their own right. This sentence has been amended to ‘the varying response within the mesocosm experiments’

Line 410-411: no data on consumption, no bacterial rates described, then what is the basis for this statement? Confusing.
AR. This statement has been amended to ‘it is not known if this loss pathway is stimulated at high CO2’

Line 412: “Synechococcus has been identified as a DMS consumer in the open ocean” Reference, please. Syn consumed labelled DMSPd, not DMS (Vila-Costa et al 2006)
This reference to Syn has been removed, as it is a DMSP consumer, not DMS, as the reviewer states.

Line 431: Sections 3.3 and 3.4: No rates of anything for the halogenated compounds either? Just checking.
AR. No, no rates were measured, due to the sampling limitations of the mesocosm experiment.

Lines 518-522: well, was the region isolated from the coastal environment or not? You can’t have it both ways. I understand that the mesocosm bags were closed so they wouldn’t have a macroalgal component. This will come back in the discussion
AR. The water within the bags was isolated from the outside environment, but this statement was to highlight that halocarbons were likely present in high concentrations in the water column prior to the mesocosm installation and closure. This would therefore have influenced halocarbon, particularly bromocarbon concentrations at the beginning of the experiment. This section has been reworded to make this clearer.

Line 548: I agree that the comparison between the mesocosms and the outside is inappropriate. The outside underwent its own and different “experiment”

Line 557: please delete sentence about DMSp as it implies that there was none because none detected when it is an analytical issue
AR. removed

Line 558-569: given the statement in Line 548, please remove this paragraph as it mixes mesocosm conditions with outside conditions. It is pure speculation as a lot more changed with the injection of upwelled water than fCO2- i.e., particles, nutrients, DOM, etc, etc
AR. Paragraph deleted.

Line 576: Is CH2ClI really polyiodinated?
AR. polyhalogenated

Line 584: Check your manuscript for Chl-α, the α is alternating between superscript and normal
AR. Checked – all changed to italic

Line 586-590: It is above indicated that macroalgal beds were not a source. Now, it is implied that those macroalgals beds were close? or far? in location w/r to the mesocosms. And the prevailing circulation was from the beds towards the mesocosms? And waht about vertical input? The entire DMS section is predicated on upwelling, ie, water injection from below NOT lateral advection. Can't have tvertical input for one gas and horizontal input for the other one.
AR. The mesocosms were approximately 500m from the shore, however the maximum depth of the seabed was 20-25m, so macroalgae growing there would have been within a few metres of the water taken from the Baltic. There was also free-floating macroalgae in the water column which could have contributed.

The mesocosms were set up in a Fjard, which although had minimal tidal impact, had obvious signs of water movement in and out, with significant currents identified when mooring the boats to the mesocosms for sampling.

A comment was included which stated that there was limited change in bromocarbons during the upwelling, likely that the upwelled water had similar concentrations to the surface waters.

Line 593-607: good

Line 599: I think you want to stress here, that the values are high enough to be considered an already adapted site, rather than stressing that they are lower than elsewhere, correct? “ [...] at such a location with a relatively low fCO2 excursion compared to some sites [...]”, maybe rephrase to “ [...] at such a location with a relatively high fCO2 excursion, however still relatively low when compared to some sites [...]”

AR. Agreed and changed.

Line 609-611: Not all the time, only after the decline of Chl-a, right? I wouldn’t stretch it out, then.

AR. This statement was included as it was the most important finding of the mesocosm experiment, and compared to all the other mesocosm experiments.

Line 614: production was not measured, only concentrations. Please change production for cycling because the levels measured are a net result

AR. Changed

Line 615: since rates were not measured, you don't know whether was a response (ie, prodn and/or cons), only that the measured concentrations did not change

AR. Changed to ‘the measured concentrations did not change’

Line 617: no change IF under similar meteorological conditions as during this sampling

AR. Added the proviso ‘without significant alteration top the meteorological conditions’

Line 617-621: NET production or availability. Again, same issue. Also, rather simplistic as meteorology must be considered.

AR. Added ‘net’

L621-625: This is a weak concluding paragraph. It says nothing at all. Keep it honest and simple by saying that no changes in concentrations were seen and that next time it would be best to measure rates so these rates can be included in models to have better predictions!! So sorry that you didn't see any changes nor anything "exciting".

AR. This paragraph has been amended to include the reviewers comments.

Figures in general:

I find it very irritating how the units are given, e.g. fCO2/μatm. I read the “/” as ‘per’, which makes it confusing. I would very much prefer if you put fCO2 (μatm) or fCO2 [μatm]

AR. The figures have been amended to have the units in brackets
Fig 3: The Legend is misleading. It sounds as if you were showing an integration, but you are actually showing the mean from a water sample integrated from the top 10m. “Dashed lines show the Phases of the experiment as given in Fig. 2,” should be moved to the a0 part of the legend, as it is not shown in 3b.

AR. Legend amended

Supplement Figures:

Fig. S2: Top left y axis is formatted differently. Also t vs T as abbreviation for time between S1 and S2

AR. Figures have been amended.

There are two Tables 1 in the supplement.

AR. Table S2 renamed

There is a Fig. S3 that is never mentioned in the text which I suggest actually be moved into the main section as Fig 3c as it shows a difference of DMS/chl among mesocosms!

AR. The standard deviation during this figure is so high that it is a non-significant finding, and it has been established that there is no link between DMS and Chla. This figure was originally in the manuscript but was removed during the first round of reviewer comments prior to online discussion.
The authors would like to thank the reviewer for their comments and discussions at all stages of the review process, which have improved the overall quality of the manuscript. I have addressed the reviewer’s comments individually.

General comment This paper presents data from an acidification experiments conducted in large mesocosms in the Baltic Sea during the 2012 summer. The mesocosms system used here has been described in the past and used in previous successful ocean acidification experiments. This is considered as the state-of-the-art system for that type of experiments. As usual in multidisciplinary experiments, many different papers were produced, some of which are already published. This particular paper focuses on the impact of acidification on the production of biogenic trace gases (dimethylsulfide and a suite of halocarbons), but makes several references to other papers related to the same study.

Few general remarks:
1. The upwelling event that took place in the middle of the experiment (t16) certainly confused the issue by cooling the water of the mesocosms. For that reason, the changes in biogenic gases concentrations observed after this event result from both the cooling and the acidification of the water. This is recognized by the authors and properly discussed in this version of the paper.

2. Measurements made outside the mesocosms are interesting by themselves, and as they are in this version of the paper, should not be compared with the results from the mesocosms where the upwelling event only translated into a decrease in temperature, but no change in salinity and more importantly no change in plankton composition. These are two independent stories which need to be treated as such. In that regard, in situ data could be presented in a separate figure to emphasize this point. A reason to do so is that the Phases indicated in figures 1 and 2 are not relevant to the in situ measurements. This would also allow to rescale the Y-axis of figure 1c and 2a and make the changes in chl-a and DMS concentrations in the mesocosms more visible.

AR. This has previously been discussed, however it doubled the number of figures in the manuscript, while not increasing the clarity of the information displayed to a huge degree. The differences in DMS concentration in the different mesocosms is clearly visible in the current figure 3 due to the scale of the difference.

3. The lack of detectable DMSP concentrations is obviously surprising. Although the authors offer possible solutions to this conundrum, the fact remains that they are able to detect a by-product of DMSP degradation but not DMSP itself, known to be, in many circumstances, orders of magnitude higher than DMS. It is difficult to believe that 30 days worth of samples within a diverse community of phytoplankton did not generate a single detectable nmol of DMSP. Some loss can be explained through the presence of acid-sensitive species (colonial Phaeocystis etc.), but the authors rule this out themselves as an important process by specifying that this type of phytoplankton accounted for less than 10% of the community. In fact cryptophytes and chlorophytes dominated the community. Various species of these two groups are known to produce DMSP (Keller et al 1989) but not known to be sensitive to the acid treatment. As stated by the authors, a methodological problem can probably explain these results.

Specific comments
P1, 25: . . .challenged Baltic Sea.

AR. Challenging is more appropriate as the sentence is talking about the challenges present and future in the Baltic Sea encountered by phytoplankton.

P2, 55: . . .the global ocean has absorbed . .

AR. Changed
P2,41: Would it be possible to come up with a ‘dilution’ factor? Using salinity as a conservative parameter perhaps? This would allow to roughly estimate how much of the variability of the parameters measured at the surface needs to be explained by other factors (production/consumption).

AR. We do not know the salinity of the upwelling water, nor the percentage volume of the upwelled water injected into the surface system. This makes this very hard to quantify.

P4, 110: Suggestion: replace ‘Post-spring bloom’ by ‘Following the spring bloom’.

AR. Changed

P4, 114: . . .2012 summer post-bloom season. . .

AR. Changed

P5, 132: . . .such as fish. . .The removal of large zooplankton is probably more relevant here than fish.

AR. Although it took a week to get 1 small fish out...

P6, 163: . . .with 100% absorbance of UV light. . .Later in the manuscript, it is mentioned that some UV light could affect the processes taking place close to the surface in the mesocosms. This seems to be in contradiction that 100% UV is removed.

AR. UV was still able to impact the very surface waters where it did not pass through the films. There is a 1m high gap in the mesocosm design between the top of the TPU bag and the PVC rain cover, where the samples are taken from. Light is able to pass through this gap in morning and evening and hit the surface waters.

P8, 230: . . .turnover of DMSPD. . .Replace by ‘dissolved DMSP’.

AR. Changed

P8, 246: Measurements of carbonate chemistry and community dynamics.

AR. Changed

P10, 281: . . .decreased over Phase 1 in the . . .The phase numbers are not properly aligned in figure 1c (on my printed copy at least), and absent in figure 2, 3 and 4 (which are by the way wrongly numbered).

AR. Figures have been amended

P10, 287: . . .no variation with depth (data not shown). . .

AR. Added
P10, 297: . . .a significant effect on phytoplankton growth (and biogases production), explaining. . .

AR. Added

P11, 324: . . .that light availability and surface water temperatures. . .Delete ‘environmental conditions of limited’ and ‘lower’.

AR. Agreed

P11, 330: A significant 34% reduction. . .These results could be better explained taking into account the temporal variability which is significant. Actually, DMS concentrations increased as Chl concentrations decreased, and the increase in DMS was less important at high PCO2. After day 21, DMS decreased gradually in all treatments until the end of the experiment.

AR. The DMS disconnect from Chl-a is a fairly common occurrence, and it would have been a lot more interesting to discuss if DMS had been connected to Chl-a concentrations! To a degree, it is interesting that DMS peaked after the Chl-a, but without any DMSP measurements, it is difficult to know to what degree this was connected. From previous mesocosm experiments and turnover rates of DMS, the temporal delay in DMS peak after Chl-a (if it exists) is usually only 2-3 days, not over a week.

P11, 333: (Fig. 3a) to be replaced by (Fig. 2a). P11, 336: (Fig. 3b) to be replaced by (Fig. 2b).

AR. Changed

P11, 337: Furthermore, increases in DMS. . .were delayed by three days. . .This 3-day delay is not obvious in Fig. 2a. Am I missing something?

AR. The increase in DMS in the highest CO2 mesocosms started three days after that in the ambient and mid-level CO2. As the DMS increased to such a small degree in the high CO2, it is not an obvious result, however it can be seen in Fig. 2.

P12, 348: Although the majority. . .This paragraph needs an introduction sentence. As in my previous review of this paper, I still think that there is too much emphasis on a rare pathway of DMS production considering that the problem is most probably a methodological one. This paragraph is important but could be shortened.

AR. The first sentence has been amended to be more of an introduction. This paragraph has been shortened significantly from the original version, and to shorten it further would be to miss out the summary of where knowledge of the alternate pathway originates from and how it affects the results of this experiment.

P12, 358: Correlations between. . .Only one P value is presented. Should it be ‘correlation’ instead of ‘correlations’? I am also wondering if all the data were pooled (all treatments) to compute this statistic.

AR. There was also correlation between the single celled cyanobacterial abundance, which has been included, and the colonial cyanobacterial abundance (data not shown as not finalised when preparing the manuscript). The statistics are also given in the supplemental file.
The peak in DMS concentrations is unlikely to be a delayed response. But the increase in DMS coincided with the decline in Chl-a concentrations (t15-t21), something frequently observed in nature in response to higher DOC production and bacterial activity during bloom decline. My point here is that the results should be presented and discussed in term of temporal changes, not only correlations.

AR. Comments have been included as to the temporal variation in DMS concentrations between the mesocosms, and as mentioned above, it is not uncommon for there to be a complete disconnect between Chl-a and DMS, and we have no DMSP concentrations to form a connection between the two. There was an increased in DOC on t15 shortly before the DMS peak, which has been referenced to Hornick et al 2016 (this issue).

DMS and DMSP...

This is relevant. . . I don’t understand the logic here. In the absence of DMSP values, whatever the reason, I don’t think that one can conclude that ‘DMS concentrations were likely more affected by the change in ÆŠCO2 than the production of the precursors’.

AR. Final sentence deleted

DMS yields may vary from 5 to 40% depending on the S and C demand of the bacteria and the quality of DOM. There are many references on variations in DMS yields. A good starting point is the paper by Kiene and Linn 2000 (Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico. Limnol Oceanogr 45: 849-861).

AR. A comment has been included to the effect that bacterial consumption varies to a wide degree.

where some UV light was able to pass. . . This seems to be in contradiction with the statement that 100% of UV radiation was absorbed by the cover (P6, 163). This requires clarification.

AR. See comment above

The peak of CH2I2 coincided with the decline of the bloom, as observed for DMS. I am not convinced that the positive correlations observed between these compounds and the abundance of the different taxa are relevant if the production of the compounds is related to processes linked to the decline of the bloom (ex. increase in DOC).

AR. There is no direct evidence of a link between the production of these compounds, but there is also no evidence that this link does not exist. This is why this is presented as a correlation, but does not equal causation, and was not described as such here.

The cleaning of the walls of the mesocosms and the associated apparent released of DOM as mentioned here seem to be an important potential artifact. As noted, this could be very important for photochemically and microbially driven processes. This potential problem, which could also be important for DMS production, should be
discussed in more details in this paper. Would it be useful to indicate on the different figures when these cleanings took place? Overall, providing more details on the impact of these cleaning events would be of great value for colleagues planning to conduct similar long term mesocosms experiments.

AR. Cleaning during the experiments was not as regular as was hoped for, and only took place during the second part of the experiment. Because of this it is likely that the cleaning had a significant effect on DMS concentrations due to the input of DOC into the mesocosm. A comment to this effect has been included.

P16, 490: . . . indicators of algal biomass. PP was not measured here.

AR. Changed

P17/177, 503/504: . . .low net increase in total Chl-a. . .

AR. Added

P18, 550: Typo: Two dots before ‘but peaked’.

AR. Removed

P18, 558: As the CO2 levels increased during Phase II. . .As mentioned by the authors at the beginning of this section, comparing the mesocosms results with the in situ ones is inappropriate. The different Phases (0, I, II) make only sense for the mesocosms experiment where they indicate either treatments or events. They are irrelevant to the in situ measurements. Keeping this comparison is confusing.

AR. A bit of the comparison is removed. The phase has been changed to the day no.

P18, 562: . . .this decrease in DMS may also be attributed to CO2 levels. . ..

AR. Section removed

P19, 577: . . .that production was probably not limited. . .

AR. Changed

P19, 598: . . .living and acclimated to. . .

AR. Changed

P20, 603-607: These two sentences would benefit from a rewording.

AR. Last sentence has been restructured.
For the concentrations of halocarbons, . . . of the Baltic Sea. I am not sure about this conclusion. This is very speculative since deep water upwelling and ocean acidification through air-sea CO2 exchange are two different processes. Upwelling brings nutrients, microbes, etc. . . in surface water in addition to high CO2.

AR. This section has been reworded.

P 35. This should be Figure 2 (instead of 3).

AR. Changed

P 36. This should be Figure 3 (instead of 4).

AR. Changed

P 37: This should be Figure 4 (instead of 5).

AR. changed