Effect of ocean acidification and elevated fCO_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 fCO_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_3I) and diiodomethane (CH_2I_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 iodocarbons increased or showed a peak, with mean chloroiodomethane (CH_2ClI) concentrations measured at 5.3 (± 0.9) pmol L^{-1} and iodoethane (C_2H_5I) 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_2Br_2; mean 5.3 ± 0.8 pmol L^{-1}) and dibromochloromethane ($CHBr_2Cl$, mean 3.0 ± 0.5 pmol L^{-1}), only CH_2Br_2 showed a decrease of 17% between Phases I and II, with $CHBr_3$ and $CHBr_2Cl$ 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_3I) 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 ~~are~~ 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 fCO_2 (Hopkins and Archer, 2014), but lower DMSP production. The combined picture arising from existing studies is that the response of communities to fCO_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_3I), chloroiodomethane (CH_2ClI) 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 CO_2 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 fCO_2, the carbonate chemistry of the mesocosms was altered by the addition of different volumes of 50 µm filtered, CO_2-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 *t*0. Addition of the enriched CO_2 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 CO_2-enriched waters, with the range of target fCO_2 levels between 600 and 1650 µatm (M7, 600

μatm; M6, 950 μatm; M3, 1300 μatm; M8 1650 μatm). Mesocosms were randomly allocated a target fCO_2; a noticeable decrease in fCO_2 was identified in the three highest fCO_2 mesocosms (M6, M3 and M8) over the first half of the experiment, which required the addition of more CO_2 enriched water on *t*15 to bring the fCO_2 back up to maximum concentrations (Fig. 1a; Paul *et al*., 2015). A summary of the fCO_2 in the mesocosms can be seen in Table 1. At the same time as this further CO_2 addition on *t*15, 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-*a* (Chl-*a*) 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_3I, diiodomethane (CH_2I_2), CH_2ClI, iodoethane (C_2H_5I), iodopropane (C_3H_7I), $CHBr_3$, dibromoethane (CH_2Br_2), dibromochloromethane ($CHBr_2Cl$), bromoiodomethane (CH_2BrI) 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_3I) and ^{13}C dibromoethane ($^{13}C_2H_4Br_2$) 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_2SO_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_2SO_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 $DMSP_{D}$ in surface waters being the cause of low $DMSP_T$ 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^{-1} 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 $DMSP_T$ 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-*a*, 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 fCO_2 on concentrations measured in the mesocosms and the Baltic Sea (H_0 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, fCO_2, 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-*a* (Fig. 2; Paul *et al.*, 2015) evaluated after the experiment was completed:

- Phase 0 (days *t*-5 to *t*0) – pre-CO_2 addition
- Phase I (days *t*1 to *t*16) – 'productive phase'
- Phase II (days *t*17 to *t*30) – temperature induced autotrophic decline.

3.1.1 Physical Parameters

fCO_2 decreased over Phase I in the three highest fCO_2 mesocosms, mainly through air-sea gas exchange and carbon fixation by phytoplankton (Fig. 1a). All mesocosms still showed distinct differences in fCO_2 levels throughout the experiment (Table 1), and there was no overlap of mesocosm fCO_2 values on any given day, save for the two controls (M1 and M5). The control mesocosm fCO_2 increased through Phase I of the experiment, likely as a result of undersaturation of the water column encouraging dissolution of atmospheric CO_2 (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 *t*16, 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 *t*16, 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, fCO_2 or the microbial community. However, the temperature decrease after *t*16 was likely to have had a significant effect on phytoplankton growth (and biogenic gas production), explaining the lower Chl-*a* 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-*a* 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_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^{-1} from all mesocosms, decreasing to 1.44 (± 0.46) µg L^{-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 *t*31.

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_2 in only two groups: picoeukaryotes I showed higher abundance at high fCO_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 fCO_2 treatments during Phase II compared to the ambient fCO_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. 2~~3~~a). The primary differences identified were apparent from the start of Phase II on *t*17, after which maximum concentrations were observed in the ambient mesocosms on *t*21. The relationship between DMS and increasing fCO_2 during Phase II was found to be linear (Fig. ~~3~~2b), a finding also identified in previous mesocosm experiments (Archer *et al.*, 2013; Webb *et al.*, 2015). Furthermore, increases in DMS concentrations under high fCO_2 were delayed by three days relative to the ambient and medium fCO_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 fCO_2 treatments with the mean of all mesocosms 3.1 (± 0.2) nmol L^{-1}. Concentrations in all mesocosms gradually declined from *t*21 until the end of DMS measurements on *t*31. 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 t~~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, r~~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~~s~~

between DMS and the cyanobacterial equivalent Chl-*a* (ρ=0.42, p<0.01; Supplementary Figure S1g) and DMS and single-celled cyanobacteria (ρ=0.58, p<0.01; 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 fCO_2, indicating measured DMS ~~net production~~ concentrations~~was~~ 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-*a* were identified as significant producers of DMSP. During Phase II, DMS was negatively correlated with Chl-*a* in the ambient and medium fCO_2 mesocosms (ρ=-0.60, p<0.01). During Phase II, a significant correlation was seen between DMS and single-celled cyanobacteria identified predominantly as *Synechococcus* (ρ=0.53, p<0.01; Crawfurd *et al.* 2016 and supplementary table S1) and picoeukaryotes III (ρ=0.75, p<0.01). The peak in DMS concentrations is unlikely to be a delayed response to the increased Chl-*a* on *t*16 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 *t*16. ~~.~~ 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 fCO_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 fCO_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 ~~se experiments~~ 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 fCO_2. However, in all previous experiments with DMSP as the primary precursor of DMS, elevated fCO_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 fCO_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 fCO_2 (Engel *et al.*, 2013; Piontek *et al.*, 2013). Further, Endres *et al.* (2014) identified significant enhanced enzymatic hydrolysis of organic matter with increasing fCO_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 fCO_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 fCO_2. As microbial DMS yields can very 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 fCO_2, implying that DMS consumption by this group would have been lower as fCO_2 increased.~~

3.3 Iodocarbons in the mesocosms and relationships with community composition

Elevated fCO_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 fCO_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_3I (Fig. 3~~4~~a) and C_2H_5I (Fig. 3~~4~~b) showed concentration ranges of 2.91 to 6.25 and 0.23 to 0.76 pmol L^{-1} respectively. CH_3I showed a slight increase in all mesocosms during Phase I, peaking on *t*16 which corresponded with higher Chl-*a* concentrations, and correlated throughout the entire experiment with picoeukaryote groups II (ρ=0.59, p<0.01) and III (ρ=0.23, p<0.01; Crawfurd *et al.* ~~this issue~~2016) and nanoeukaryotes I (ρ=0.37, p<0.01). Significant differences identified between mesocosms for CH_3I were unrelated to elevated fCO_2 (F=3.1, p<0.05), but concentrations were on average 15% higher in Phase II than Phase I. C_2H_5I 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 fCO_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_3I and Chl-*a* at any phase, and the only correlation of any phytoplankton grouping was with nanoeukaryotes II (ρ=0.88, p<0.01; Crawfurd *et al.*, 201~~5~~6). These CH_3I 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_3I 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_3I in the Baltic Sea. However, the abundance of *Pseudanabaena* was highest during Phase I of the experiment (A. Stuhr, Pers. Comm.) when CH_3I 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_3I (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_2I_2 (Fig. 34c) showed the clearest increase in concentration during Phase II, when it peaked on *t*21 in all mesocosms, with a maximum of 202.2 pmol L^{-1} in M5 (348 µatm). During Phase II, concentrations of CH_2I_2 were 57% higher than Phase I, and were therefore negatively correlated with Chl-*a*. The peak on *t*21 corresponds with the peak identified in DMS on *t*21, and concentrations through all three phases correlate with picoeukaryotes II (ρ=0.62, p<0.01) and III (ρ=0.47, p<0.01) and nanoeukaryotes I (ρ=0.88, p<0.01; Crawfurd *et al.*, 2015). CH_2ClI (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 (ρ=0.34, p<0.01) and III (ρ=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_2O_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 fCO_2 (F=1.7, p<0.01), it is unlikely that production was increased in relation to elevated fCO_2. Production of all iodocarbons increased during Phase II when total Chl-*a* 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_3I and C_2H_5I (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_2I_2 or CH_2ClI 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_2Br_2 and $CHBr_2Cl$ showed no major peaks of production in the mesocosms. $CHBr_3$ (Fig. 4~~5~~a) 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 (±8.2 s.d.) pmol L^{-1} in all mesocosms for the period *t*0 to *t*31 (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_2Br_2 concentrations (Fig. ~~5b~~4b) decreased steadily in all mesocosms from *t*-3 through to *t*31, over the range 4.0 to 7.7 pmol L^{-1}, and $CHBr_2Cl$ followed a similar trend in the range 1.7 to 4.7 pmol L^{-1} (Fig. ~~5c~~4c). Of the three bromocarbons, only CH_2Br_2 showed correlation with total Chl-*a* (ρ=0.52, p<0.01), and with cryptophyte (ρ=0.86, p<0.01) and dinoflagellate (ρ=0.65, p<0.01) derived Chl-*a*. Concentrations of CH_2BrI were below detection limit for the entire experiment.

CH_2Br_2 showed positive correlation with Chl-*a* (ρ=0.52, p<0.01), nanoeukaryotes II (ρ=0.34, p<0.01) and cryptophytes (ρ=0.86, p<0.01; see supplementary material), whereas $CHBr_3$ and $CHBr_2Cl$ showed very weak or no correlation with any indicators of algal biomass~~primary production~~. Schall *et al.* (1997) have proposed that $CHBr_2Cl$ 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 CHBr2Cl 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-*a*. Hughes *et al.* (2013) identified bacterial inhibition of $CHBr_3$ production in laboratory cultures of *Thalassiosira* diatoms, but that it was not subject to bacterial breakdown; which could explain the relative steady state of $CHBr_3$ concentrations in the mesocosms. In contrast, significant bacterial degradation of CH_2Br_2 in the same experiments could explain the steady decrease in CH_2Br_2 concentrations seen in the mesocosms. Bacterial oxidation was also identified by Goodwin *et al.* (1998) as a significant sink for CH_2Br_2. 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 CH_2Br_2 to $CHBr_3$ was also unaffected by increased fCO_2, 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 $CHBr_3$ than CH_2Br_2 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 fCO_2 and the effect on biogenic trace gases

3.5.1 Physical variation and community dynamics

Baltic Sea deep waters have high fCO_2 and subsequently lower pH (Schneider *et al.*, 2002), and the influx to the surface waters surrounding the mesocosms resulted in fCO_2 increasing to 725 µatm on *t*31, close to the average fCO_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 fCO_2 and the associated pH, as well as having communities associated with the elevated fCO_2 conditions.

Chl-*a* 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-*a* peak in the mesocosms and the maximum peak of temperature. As upwelled water intruded into the surface waters, the surface Chl-*a* was diluted with low Chl-*a* deep water: Chl-*a* 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-*a* in the Baltic Sea, as in the mesocosms. Cyanobacteria contributed less than 2% of the total Chl-*a* 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-*a* (ρ=0.84, p<0.01), with the ratio of DMS: Chl-*a* at 1.6 (± 0.3) nmol μg^{-1}. Other strong correlations were seen with euglenophytes (ρ=0.89, p<0.01), dinoflagellates (ρ=0.61, p<0.05) and nanoeukaryotes II (ρ=0.88,

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 *t*16 to the lowest recorded sample of the entire experiment at 1.85 nmol L^{-1}. As with Chl-*a*, 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_3I 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_2I_2 and CH_2ClI showed higher concentrations in the Baltic Sea samples than the mesocosms (CH_2I_2: 373.9 pmol L^{-1} and CH_2ClI: 18.1 pmol L^{-1}), and were correlated with the euglenophytes (CH_2I_2; ρ=0.63, p<0.05 and CH_2ClI; ρ=0.68, p<0.01) and nanoeukaryotes II (CH_2I_2; ρ=0.53, p<0.01 and CH_2ClI; ρ=0.58, p<0.01), but no correlation with Chl-*a*. 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_2I_2 and CH_2ClI 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_2Br_2 (F=208.8, p<0.01) and $CHBr_2Cl$ (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-*a* (ρ=0.65, p<0.05), cyanobacteria (ρ=0.61, p<0.01; Paul *et al.*, 2015) and nanoeukaryotes II (ρ=0.56, p<0.01; Crawfurd *et al.*, 2016~~5~~), 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_2Br_2 and $CHBr_2Cl$. 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 fCO_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 fCO_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 fCO_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 fCO_2 treatments. In contrast to the halocarbons, concentrations of DMS were significantly lower in the highest fCO_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 fCO_2 experiments. The Baltic Sea trace gas

samples give a good record of trace gas ~~production~~ cycling during the injection of high fCO_2 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 fCO_2. 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.

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691 Archer, S. D., Cummings, D., Llewellyn, C. and Fishwick, J.: Phytoplankton taxa, irradiance and nutrient availability
692 determine the seasonal cycle of DMSP in temperate shelf seas, Mar. Ecol. Prog. Ser., 394, 111–124,
693 doi:10.3354/meps08284, 2009.

694 Archer, S. D., Kimmance, S. A., Stephens, J. A., Hopkins, F. E., Bellerby, R. G. J., Schulz, K. G., Piontek, J. and Engel,
695 A.: Contrasting responses of DMS and DMSP to ocean acidification in Arctic waters, Biogeosciences, 10(3), 1893–
696 1908, doi:10.5194/bg-10-1893-2013, 2013.

697 Arnold, H. E., Kerrison, P. and Steinke, M.: Interacting effects of ocean acidification and warming on growth and
698 DMS-production in the haptophyte coccolithophore *Emiliania huxleyi*., Glob. Chang. Biol., 19(4), 1007–16,
699 doi:10.1111/gcb.12105, 2013.

700 Avgoustidi, V., Nightingale, P. D., Joint, I., Steinke, M., Turner, S. M., Hopkins, F. E. and Liss, P. S.: Decreased
701 marine dimethyl sulfide production under elevated CO_2 levels in mesocosm and in vitro studies, Environ. Chem., 9(4),
702 399–404, doi:10.1071/EN11125, 2012.

703 Barlow, R. G., Cummings, D. G. and Gibb, S. W.: Improved resolution of mono- and divinyl chlorophylls a and b and
704 zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC, Mar. Ecol. Prog. Ser., 161, 303–307,
705 1997.

706 Bentley, R. and Chasteen, T. G.: Environmental VOSCs—formation and degradation of dimethyl sulfide, methanethiol
707 and related materials, Chemosphere, 55(3), 291–317, doi:10.1016/j.chemosphere.2003.12.017, 2004.

708 Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A. and Riebesell, U.:
709 Arctic microbial community dynamics influenced by elevated CO_2 levels, Biogeosciences, 10(2), 719–731,
710 doi:10.5194/bg-10-719-2013, 2013.

711 Brutemark, A., Engström-Öst, J. and Vehmaa, A.: Long-term monitoring data reveal pH dynamics, trends and
712 variability in the western Gulf of Finland, Oceanol. Hydrobiol. Stud., 40(3), 91–94, doi:10.2478/s13545-011-0034-3,
713 2011.

714 Butler, A. and Carter-Franklin, J. N.: The role of vanadium bromoperoxidase in the biosynthesis of halogenated marine
715 natural products, Nat. Prod. Rep., 21(1), 180–188, doi:10.1039/b302337k, 2004.

716 Canadell, J. G., Le Quéré, C., Raupach, M. R., Field, C. B., Buitenhuis, E. T., Ciais, P., Conway, T. J., Gillett, N. P.,
717 Houghton, R. A. and Marland, G.: Contributions to accelerating atmospheric CO_2 growth from economic activity,
718 carbon intensity, and efficiency of natural sinks., Proc. Natl. Acad. Sci. U. S. A., 104(47), 18866–18870,
719 doi:10.1073/pnas.0702737104, 2007.

720 Carpenter, L. J., Hopkins, J. R., Jones, C. E., Lewis, A. C., Parthipan, R., Wevill, D. J., Poissant, L., Pilote, M. and
721 Constant, P.: Abiotic source of reactive organic halogens in the sub-arctic atmosphere?, Environ. Sci. Technol., 39(22),
722 8812–8816 [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/16323781, 2005.

723 Carrión, O., Curson, A. R. J., Kumaresan, D., Fu, Y., Lang, A. S., Mercadé, E. and Todd, J. D.: A novel pathway
724 producing dimethylsulphide in bacteria is widespread in soil environments, Nat. Commun., 6, 6579,
725 doi:10.1038/ncomms7579, 2015.

726 Chance, R., Baker, A. R., Küpper, F. C., Hughes, C., Kloareg, B. and Malin, G.: Release and transformations of
727 inorganic iodine by marine macroalgae, Estuar. Coast. Shelf Sci., 82, 406–414, doi:10.1016/j.ecss.2009.02.004, 2009.

728 Charlson, R. J., Lovelock, J. E., Andreae, M. O. and Warren, S. G.: Oceanic phytoplankton, atmospheric sulphur, cloud
729 albedo and climate, Nature, 326(6114), 655–661 [online] Available from:
730 http://www.atmos.washington.edu/~sgw/PAPERS/1987_CLAW.pdf (Accessed 15 July 2011), 1987.

731 Chuck, A. L., Turner, S. M. and Liss, P. S.: Oceanic distributions and air-sea fluxes of biogenic halocarbons in the open
732 ocean, J. Geophys. Res., 110(C10022), doi:10.1029/2004JC002741, 2005.

733 Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Frielingstein, P., Gao, X., Gutowski, W. J., Johns, T.,
734 Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A. J. and Wehner, M.: Long-term climate change: projections,
735 commitments and irreversibility, in Climate Change 2013: The Physical Science Basis. Contribution of Working Group
736 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by T. Stocker, D. Qin, G.-
737 K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, Cambridge University
738 Press, Cambridge, UK. [online] Available from: http://dial.academielouvain.be/handle/boreal:140396 (Accessed 12
739 June 2014), 2013.

740 Crawfurd, K., Brussaard, C. P. D. and Riebesell, U.: Shifts in the microbial community in the Baltic Sea with increasing
741 CO_2, Biogeosciences, In Press, 2016.

Cubasch, U., Wuebbles, D., Chen, D., Facchini, M. C., Frame, D., Mahowald, N. and Winther, J.-G.: Introduction, in Climate Change 2013: The Physical Science Basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by T. . Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, Cambridge University Press, Cambridge, UK., 2013.

Curran, M. A. J., Jones, G. B. and Burton, H.: Spatial distribution of dimethylsulfide and dimethylsulfoniopropionate in the Australasian sector of the Southern Ocean, J. Geophys. Res., 103(D13), 16677 – 16689, 1998.

Curson, A. R. J., Todd, J. D., Sullivan, M. J. and Johnston, A. W. B.: Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes, Nat. Rev. Microbiol., 9(12), 849–859, doi:10.1038/nrmicro2653, 2011.

Czerny, J., Schulz, K. G., Krug, S. A., Ludwig, A. and Riebesell, U.: Technical Note: The determination of enclosed water volume in large flexible-wall mesocosms "KOSMOS," Biogeosciences, 10, 1937–1941, doi:10.5194/bg-10-1937-2013, 2013.

Doney, S. C., Fabry, V. J., Feely, R. A. and Kleypas, J. A.: Ocean acidification: the other CO_2 problem., Ann. Rev. Mar. Sci., 1, 169–192, doi:10.1146/annurev.marine.010908.163834, 2009.

Drotar, A., Burton, G. A., Tavernier, J. E. and Fall, R.: Widespread occurrence of bacterial thiol methyltransferases and the biogenic emission of methylated sulfur gases, Appl. Environ. Microbiol., 53(7), 1626–1631 [online] Available from: http://aem.asm.org/content/53/7/1626.short (Accessed 25 March 2014), 1987.

Elliott, S. and Rowland, F. S.: Methyl halide hydrolysis rates in natural waters, J. Atmos. Chem., 20, 229–236, 1995.

Endres, S., Galgani, L., Riebesell, U., Schulz, K.-G. and Engel, A.: Stimulated bacterial growth under elevated pCO_2: results from an off-shore mesocosm study., PLoS One, 9(6), e99228, doi:10.1371/journal.pone.0099228, 2014.

Engel, A., Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Delille, B. and Schartau, M.: Effects of CO_2 on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II), Biogeosciences, 5(2), 509–521, doi:10.5194/bg-5-509-2008, 2008.

Engel, A., Borchard, C., Piontek, J., Schulz, K. G., Riebesell, U. and Bellerby, R. G. J.: CO_2 increases 14C primary production in an Arctic plankton community, Biogeosciences, 10(3), 1291–1308, doi:10.5194/bg-10-1291-2013, 2013.

Finni, T., Kononen, K., Olsonen, R. and Wallström, K.: The History of Cyanobacterial Blooms in the Baltic Sea, AMBIO A J. Hum. Environ., 30(4), 172–178, doi:10.1579/0044-7447-30.4.172, 2001.

Franklin, D. J., Poulton, A. J., Steinke, M., Young, J., Peeken, I. and Malin, G.: Dimethylsulphide, DMSP-lyase activity and microplankton community structure inside and outside of the Mauritanian upwelling, Prog. Oceanogr., 83(1-4), 134–142, doi:10.1016/j.pocean.2009.07.011, 2009.

Gidhagen, L.: Coastal upwelling in the Baltic Sea—Satellite and in situ measurements of sea-surface temperatures indicating coastal upwelling, Estuar. Coast. Shelf Sci., 24, 449–462 [online] Available from: http://www.sciencedirect.com/science/article/pii/0272771487901272 (Accessed 16 August 2014), 1987.

Goodwin, K., Schaefer, J. K. and Oremland, R. S.: Bacterial oxidation of dibromomethane and methyl bromide in natural waters and enrichment cultures, Appl. Environ. Microbiol., 64(12), 4629 –4636 [online] Available from: http://aem.asm.org/content/64/12/4629.short (Accessed 30 July 2014), 1998.

Goodwin, K. D., North, W. J. and Lidstrom, M. E.: Production of bromoform and dibromomethane by giant kelp: factors affecting release and comparison to anthropogenic bromine sources, Limnol. Oceanogr., 42(8), 1725–1734, doi:10.4319/lo.1997.42.8.1725, 1997.

Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., Rowley, S. J., Tedesco, D. and Buia, M.-C.: Volcanic carbon dioxide vents show ecosystem effects of ocean acidification, Nature, 454(7200), 96–99, doi:10.1038/nature07051, 2008.

Hartmann, D. L., Klein Tank, A. M. G., Rusticucci, M., Alexander, L. V., Bronnimann, S., Charabi, Y., Dentener, F. J., Dlugokencky, E. J., Easterling, D. R., Kaplan, A., Soden, B. J., Thorne, P. W., Wild, M. and Zhai, P. M.: Observations: Atmosphere and Surface, in Climate Change 2013: The Physical Science Basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, Cambridge University Press, Cambridge, Cambridge, UK., 2013.

Hatton, A. D., Darroch, L. and Malin, G.: The role of dimethylsulphoxide in the marine biogeochemical cycle of dimethylsulphide, Oceanogr. Mar. Biol. An Annu. Rev., 42, 29–56, 2004.

Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T. and Sewell, M. A.: The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective, Annu. Rev. Ecol. Evol. Syst., 41(1), 127–147, doi:10.1146/annurev.ecolsys.110308.120227, 2010.

Hopkins, F. E. and Archer, S. D.: Consistent increase in dimethyl sulphide (DMS) in response to high CO_2 in five shipboard bioassays from contrasting NW European waters, Biogeosciences, 11(2), 4925 – 4940, doi:10.5194/bgd-11-2267-2014, 2014.

Hopkins, F. E., Turner, S. M., Nightingale, P. D., Steinke, M., Bakker, D. and Liss, P. S.: Ocean acidification and marine trace gas emissions., Proc. Natl. Acad. Sci. U. S. A., 107(2), 760–765, doi:10.1073/pnas.0907163107, 2010.

Hopkins, F. E., Kimmance, S. A., Stephens, J. A., Bellerby, R. G. J., Brussaard, C. P. D., Czerny, J., Schulz, K. G. and Archer, S. D.: Response of halocarbons to ocean acidification in the Arctic, Biogeosciences, 10(4), 2331–2345, doi:10.5194/bg-10-2331-2013, 2013.

Hornick, T., Bach, L. T., Crawfurd, K. J., Spilling, K., Achterberg, E. P., Brussaard, C. P. D., Riebesell, U. and Grossart, H.-P.: Ocean acidification indirectly alters trophic interaction of heterotrophic bacteria at low nutrient conditions, Biogeosciences Discuss., (March), 1–37, doi:10.5194/bg-2016-61, 2016.

Hughes, C., Malin, G., Nightingale, P. D. and Liss, P. S.: The effect of light stress on the release of volatile iodocarbons by three species of marine microalgae, Limnol. Oceanogr., 51(6), 2849–2854 [online] Available from: http://cat.inist.fr/?aModele=afficheN&cpsidt=18312251 (Accessed 26 July 2013), 2006.

Hughes, C., Malin, G., Turley, C. M., Keely, B. J., Nightingale, P. D. and Liss, P. S.: The production of volatile iodocarbons by biogenic marine aggregates, Limnol. Oceanogr., 53(2), 867–872, 2008.

Hughes, C., Chuck, A. L., Rossetti, H., Mann, P. J., Turner, S. M., Clarke, A., Chance, R. and Liss, P. S.: Seasonal cycle of seawater bromoform and dibromomethane concentrations in a coastal bay on the western Antarctic Peninsula, Global Biogeochem. Cycles, 23, doi:10.1029/2008GB003268, 2009.

Hughes, C., Franklin, D. J. and Malin, G.: Iodomethane production by two important marine cyanobacteria: Prochlorococcus marinus (CCMP 2389) and Synechococcus sp. (CCMP 2370), Mar. Chem., 125(1-4), 19–25, doi:10.1016/j.marchem.2011.01.007, 2011.

Hughes, C., Johnson, M., Utting, R., Turner, S., Malin, G., Clarke, a. and Liss, P. S.: Microbial control of bromocarbon concentrations in coastal waters of the western Antarctic Peninsula, Mar. Chem., 151, 35–46, doi:10.1016/j.marchem.2013.01.007, 2013.

Janssen, F., Schrum, C. and Backhaus, J.: A climatological data set of temperature and salinity for the Baltic Sea and the North Sea, Dtsch. Hydrogr. Zeitschrift, Supplement [online] Available from: http://link.springer.com/article/10.1007/BF02933676 (Accessed 16 August 2014), 1999.

Kangro, K., Olli, K., Tamminen, T. and Lignell, R.: Species-specific responses of a cyanobacteria-dominated phytoplankton community to artificial nutrient limitation in the Baltic Sea, Mar. Ecol. Prog. Ser., 336, 15–27

Karlsson, A., Auer, N., Schulz-Bull, D. and Abrahamsson, K.: Cyanobacterial blooms in the Baltic — A source of halocarbons, Mar. Chem., 110, 129–139, doi:10.1016/j.marchem.2008.04.010, 2008.

Kiene, R. P. and Hines, M. E.: Microbial formation of dimethyl sulfide in anoxic sphagnum peat, Appl. Environ. Microbiol., 61(7), 2720–2726 [online] Available from: http://aem.asm.org/content/61/7/2720.short (Accessed 6 February 2014), 1995.

Kiene, R. P. and Linn, L. J.: Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico, Limnol. Oceanogr., 45(4), 849–861, 2000.

Kiene, R. P. and Visscher, P. T.: Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments., Appl. Environ. Microbiol., 53(10), 2426–2434, 1987.

Kim, J.-M., Lee, K., Yang, E. J., Shin, K., Noh, J. H., Park, K.-T., Hyun, B., Jeong, H.-J., Kim, J.-H., Kim, K. Y., Kim, M., Kim, H.-C., Jang, P.-G. and Jang, M.-C.: Enhanced production of oceanic dimethylsulfide resulting from CO_2-induced grazing activity in a high CO2 world., Environ. Sci. Technol., 44(21), 8140–8143, doi:10.1021/es102028k, 2010.

Klick, S.: Seasonal variations of biogenic and anthropogenic halocarbons in seawater from a coastal site, Limnol. Oceanogr., 37(7), 1579–1585 [online] Available from: http://cat.inist.fr/?aModele=afficheN&cpsidt=4788349 (Accessed 4 August 2014), 1992.

Klick, S. and Abrahamsson, K.: Biogenic volatile iodated hydrocarbons in the ocean, J. Geophys. Res., 97(C8), 12683–12687 [online] Available from: http://www.agu.org/journals/ABS/1992/92JC00948.shtml (Accessed 26 July 2013), 1992.

Lana, A., Simó, R., Vallina, S. M. and Dachs, J.: Re-examination of global emerging patterns of ocean DMS concentration, Biogeochemistry, 110, 173–182, doi:10.1007/s10533-011-9677-9, 2012.

Laturnus, F., Giese, B., Wiencke, C. and Adams, F. C.: Low-molecular-weight organoiodine and organobromine compounds released by polar macroalgae-the influence of abiotic factors, Fresenius. J. Anal. Chem., 368, 297–302 [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/11220596, 2000.

Leck, C. and Rodhe, H.: Emissions of marine biogenic sulfur to the atmosphere of northern Europe, J. Atmos. Chem., 12, 63–86 [online] Available from: http://www.springerlink.com/index/H5472PG48150N025.pdf (Accessed 26 July 2013), 1991.

Leck, C., Larsson, U., Bågander, L. E., Johansson, S. and Hajdu, S.: Dimethyl sulfide in the Baltic Sea: annual variability in relation to biological activity, J. Geophys. Res., 95(C3), 3353–3363, doi:10.1029/JC095iC03p03353, 1990.

Lee, G., Park, J., Jang, Y., Lee, M., Kim, K. R., Oh, J. R., Kim, D., Yi, H. Il and Kim, T. Y.: Vertical variability of seawater DMS in the South Pacific Ocean and its implication for atmospheric and surface seawater DMS, Chemosphere, 78(8), 1063–1070, doi:10.1016/j.chemosphere.2009.10.054, 2010.

Leedham, E. C., Hughes, C., Keng, F. S. L., Phang, S.-M., Malin, G. and Sturges, W. T.: Emission of atmospherically significant halocarbons by naturally occurring and farmed tropical macroalgae, Biogeosciences, 10(6), 3615–3633, doi:10.5194/bg-10-3615-2013, 2013.

Lehmann, A. and Myrberg, K.: Upwelling in the Baltic Sea — A review, J. Mar. Syst., 74, S3–S12, doi:10.1016/j.jmarsys.2008.02.010, 2008.

Lischka, S., Riebesell, U., Stuhr, A. and Bermudez, J. R.: Micro- and mesozooplankton community response to increasing levels of CO_2 in the Baltic Sea: insights from a large-scale mesocosm experiment, Biogeosciences, Submitted, 2015.

Liss, P., Marandino, C. A., Dahl, E., Helmig, D., Hintsa, E. J., Hughes, C., Johnson, M., Moore, R. M., Plane, J. M. C., Quack, B., Singh, H. B., Stefels, J., von Glasow, R. and Williams, J.: Short-lived trace gases in the surface ocean and the atmosphere, in Ocean-Atmosphere Interactions of Gases and Particles, edited by P. Liss and M. Johnson, pp. 55–112., 2014.

Lomans, B. P., Smolders, A., Intven, L. M., Pol, A., Op, D. and van der Drift, C.: Formation of dimethyl sulfide and methanethiol in anoxic freshwater sediments, Appl. Environ. Microbiol., 63(12), 4741–4747, 1997.

Mackey, M. D., Mackey, D. J., Higgins, H. W. and Wright, S. W.: CHEMTAX a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton, Mar. Ecol. Prog. Ser., 144, 265–283 [online] Available from: http://www.int-res.com/abstracts/meps/v144/p265-283 (Accessed 25 March 2014), 1996.

Manley, S. L. and De La Cuesta, J. L.: Methyl iodide production from marine phytoplankton cultures, Limnol. Oceanogr., 42(1), 142–147, doi:10.4319/lo.1997.42.1.0142, 1997.

Manley, S. L., Goodwin, K. and North, W. J.: Laboratory production of bromoform, methylene bromide, and methyl iodide by macroalgae in and distribution nearshore Southern California waters, Limnol. Oceanogr., 37(8), 1652–1659, 1992.

Martino, M., Liss, P. S. and Plane, J. M. C.: The photolysis of dihalomethanes in surface seawater., Environ. Sci. Technol., 39(18), 7097–7101 [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/16201634, 2005.

Moore, R. M.: Methyl halide production and loss rates in sea water from field incubation experiments, Mar. Chem., 101(3-4), 213–219, doi:10.1016/j.marchem.2006.03.003, 2006.

Moore, R. M. and Tokarczyk, R.: Volatile biogenic halocarbons in the northwest Atlantic, Global Biogeochem. Cycles, 7(1), 195–210, 1993.

Moore, R. M. and Zafiriou, O. C.: Photochemical production of methyl iodide in seawater, J. Geophys. Res., 99(D8), 16415–16420, doi:10.1029/94JD00786, 1994.

National Measurement Institute of Australia: Proficiency Study 12-23: DMS in seawater., 2013.

889 Nausch, M., Nausch, G., Lass, H. U., Mohrholz, V., Nagel, K., Siegel, H. and Wasmund, N.: Phosphorus input by
890 upwelling in the eastern Gotland Basin (Baltic Sea) in summer and its effects on filamentous cyanobacteria, Estuar.
891 Coast. Shelf Sci., 83(4), 434–442, doi:10.1016/j.ecss.2009.04.031, 2009.

892 Newbold, L. K., Oliver, A. E., Booth, T., Tiwari, B., DeSantis, T., Maguire, M., Andersen, G., van der Gast, C. J. and
893 Whiteley, A. S.: The response of marine picoplankton to ocean acidification, Environ. Microbiol., 14(9), 2293–2307,
894 doi:10.1111/j.1462-2920.2012.02762.x, 2012.

895 Niemisto, L., Rinne, I. and Melvasalo, T.: Blue-green algae and their nitrogen fixation in the Baltic Sea in 1980, 1982
896 and 1984, Meri, 17, 1–59, 1989.

897 O'Dowd, C. D., Jimenez, J. L., Bahreini, R., Flagan, R. C., Seinfeld, J. H., Hameri, K., Pirjola, L., Kulmala, M.,
898 Jennings, S. G. and Hoffmann, T.: Marine aerosol formation from biogenic iodine emissions, Nature, 417(6889), 632–
899 636, doi:10.1038/nature00773.1.2.3.4.5.6.7.8.9.10., 2002.

900 Oberg, J.: Cyanobacterial blooms in the Baltic Sea in 2013, HELCOM Balt. Sea Environ. Fact Sheet, 2013.

901 Orlikowska, A. and Schulz-Bull, D. E.: Seasonal variations of volatile organic compounds in the coastal Baltic Sea,
902 Environ. Chem., 6, 495–507, doi:10.1071/EN09107, 2009.

903 Park, K.-T., Lee, K., Shin, K., Yang, E. J., Hyun, B., Kim, J.-M., Noh, J. H., Kim, M., Kong, B., Choi, D. H., Choi, S.-
904 J., Jang, P.-G. and Jeong, H. J.: Direct linkage between dimethyl sulfide production and microzooplankton grazing,
905 resulting from prey composition change under high partial pressure of carbon dioxide conditions., Environ. Sci.
906 Technol., 48(9), 4750–4756, doi:10.1021/es403351h, 2014.

907 Passow, U. and Riebesell, U.: Mesocosm perturbation experiments and the sensitivity of marine biological systems to
908 global change, Solas News, (1), 12–13, doi:10.1029/2003JC002120, 2005.

909 Paul, A. J., Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E., Hellemann, D., Trense, Y., Nausch,
910 M., Sswat, M. and Riebesell, U.: Effect of elevated CO2 on organic matter pools and fluxes in a summer Baltic Sea
911 plankton community., Biogeosciences, 12, 6181 – 6203, 2015.

912 Pedersen, M., Collen, J., Abrahamsson, K. and Ekdahl, A.: Production of halocarbons from seaweeds: an oxidative
913 stress reaction?, Sci. Mar., 60(Supplement 1), 257–263, 1996.

914 Piontek, J., Borchard, C., Sperling, M., Schulz, K. G., Riebesell, U. and Engel, A.: Response of bacterioplankton
915 activity in an Arctic fjord system to elevated pCO_2: results from a mesocosm perturbation study, Biogeosciences, 10,
916 297–314, doi:10.5194/bg-10-297-2013, 2013.

917 Quack, B., Peeken, I., Petrick, G. and Nachtigall, K.: Oceanic distribution and sources of bromoform and
918 dibromomethane in the Mauritanian upwelling, J. Geophys. Res., 112, C10006, doi:10.1029/2006JC003803, 2007.

919 Quinn, P. K. and Bates, T. S.: The case against climate regulation via oceanic phytoplankton sulphur emissions.,
920 Nature, 480(7375), 51–56, doi:10.1038/nature10580, 2011.

921 Raateoja, M., Kuosa, H. and Hällfors, S.: Fate of excess phosphorus in the Baltic Sea: A real driving force for
922 cyanobacterial blooms?, J. Sea Res., 65(2), 315–321, doi:10.1016/j.seares.2011.01.004, 2011.

923 Raven, J. R., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P. S., Riebesell, U., Shepherd, J., Turley, C. and
924 Watson, A.: Ocean acidification due to increasing atmospheric carbon dioxide, R. Soc. Policy Doc. 12/05, (June)
925 [online] Available from: http://eprints.uni-
926 kiel.de/7878/1/965_Raven_2005_OceanAcidificationDueToIncreasing_Monogr_pubid13120.pdf (Accessed 26 July
927 2013), 2005.

928 Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann,
929 D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R. and Schulz, K. G.: Technical Note: A mobile sea-going mesocosm
930 system – new opportunities for ocean change research, Biogeosciences, 10(3), 1835–1847, doi:10.5194/bg-10-1835-
931 2013, 2013.

932 Ross, P. M., Parker, L., O'Connor, W. A. and Bailey, E. A.: The impact of ocean acidification on reproduction, early
933 development and settlement of marine organisms, Water, 3(4), 1005–1030, doi:10.3390/w3041005, 2011.

934 Roy, R., Pratihary, A., Narvenkar, G., Mochemadkar, S., Gauns, M. and Naqvi, S. W. A.: The relationship between
935 volatile halocarbons and phytoplankton pigments during a *Trichodesmium* bloom in the coastal eastern Arabian Sea,
936 Estuar. Coast. Shelf Sci., 95(1), 110–118, doi:10.1016/j.ecss.2011.08.025, 2011.

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

phytoplankton II, Mar. Chem., 59(3-4), 311–320, doi:10.1016/S0304-4203(97)00092-3, 1998.

Schall, C., Heumann, K. G. and Kirst, G. O.: Biogenic volatile organoiodine and organobromine hydrocarbons in the Atlantic Ocean from 42°N to 72°S, Fresenius. J. Anal. Chem., 359(3), 298–305, doi:10.1007/s002160050577, 1997.

Schneider, B., Nausch, G., Kubsch, H. and Petersohn, I.: Accumulation of total CO_2 during stagnation in the Baltic Sea deep water and its relationship to nutrient and oxygen concentrations, Mar. Chem., 77, 277–291, 2002.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A. and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, Biogeosciences, 10(1), 161–180, doi:10.5194/bg-10-161-2013, 2013.

Simó, R., Archer, S. D., Pedros-Alio, C., Gilpin, L. and Stelfox-Widdicombe, C. E.: Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic, Limnol. Oceanogr., 47(1), 53–61 [online] Available from: http://cedadocs.badc.rl.ac.uk/67/ (Accessed 25 March 2014), 2002.

Simó, R., Vila-Costa, M., Alonso-Sáez, L., Cardelús, C., Guadayol, Ó., Vázquez-Dominguez, E. and Gasol, J. M.: Annual DMSP contribution to S and C fluxes through phytoplankton and bacterioplankton in a NW Mediterranean coastal site, Aquat. Microb. Ecol., 57(October), 43–55, doi:10.3354/ame01325, 2009.

Six, K. D., Kloster, S., Ilyina, T., Archer, S. D., Zhang, K. and Maier-Reimer, E.: Global warming amplified by reduced sulphur fluxes as a result of ocean acidification, Nat. Clim. Chang., 3(8), 1–4, doi:10.1038/nclimate1981, 2013.

Smith, D. C., Simon, M., Alldredge, A. L. and Azam, F.: Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution, Nature, 359, 139 – 142 [online] Available from: http://www.gso.uri.edu/dcsmith/page3/page19/assets/Smithetal92.pdf (Accessed 11 September 2014), 1992.

Solomon, S., Garcia, R. R. and Ravishankara, A. R.: On the role of iodine in ozone depletion, J. Geophys. Res., 99(D10), 20491–20499, doi:10.1029/94JD02028, 1994.

Stal, L. J., Albertano, P., Bergman, B., von Bröckel, K., Gallon, J. R., Hayes, P. K., Sivonen, K. and Walsby, A. E.: BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea—responses to a changing environment, Cont. Shelf Res., 23(17-19), 1695–1714, doi:10.1016/j.csr.2003.06.001, 2003.

Stets, E. G., Hines, M. E. and Kiene, R. P.: Thiol methylation potential in anoxic, low-pH wetland sediments and its relationship with dimethylsulfide production and organic carbon cycling., FEMS Microbiol. Ecol., 47(1), 1–11, doi:10.1016/S0168-6496(03)00219-8, 2004.

Sunda, W., Kieber, D. J., Kiene, R. P. and Huntsman, S.: An antioxidant function for DMSP and DMS in marine algae, Nature, 418(6895), 317–320, doi:10.1038/nature00851, 2002.

Theiler, R., Cook, J. C., Hager, L. P. and Siuda, J. F.: Halohydrocarbon synthesis by bromoperoxidase, Science., 202(December), 1094 – 1096, 1978.

Turner, S. M., Malin, G., Liss, P. S., Harbour, D. S. and Holligan, P. M.: The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in nearshore waters, Limnol. Oceanogr., 33(3), 364–375, 1988.

Urhahn, T. and Ballschmiter, K.: Chemistry of the biosynthesis of halogenated methanes: C1-organohalogens as pre-industrial chemical stressors in the environment?, Chemosphere, 37(6), 1017–1032, doi:10.1016/S0045-6535(98)00100-3, 1998.

del Valle, D. A., Slezak, D., Smith, C. M., Rellinger, A. N., Kieber, D. J. and Kiene, R. P.: Effect of acidification on preservation of DMSP in seawater and phytoplankton cultures: Evidence for rapid loss and cleavage of DMSP in samples containing Phaeocystis sp., Mar. Chem., 124, 57–67, doi:10.1016/j.marchem.2010.12.002, 2011.

Vila-Costa, M., Simó, R., Harada, H., Gasol, J. M., Slezak, D. and Kiene, R. P.: Dimethylsulfoniopropionate uptake by marine phytoplankton, Science, 314(5799), 652–4, doi:10.1126/science.1131043, 2006a.

Vila-Costa, M., del Valle, D. A., González, J. M., Slezak, D., Kiene, R. P., Sánchez, O. and Simó, R.: Phylogenetic identification and metabolism of marine dimethylsulfide-consuming bacteria., Environ. Microbiol., 8(12), 2189–2200, doi:10.1111/j.1462-2920.2006.01102.x, 2006b.

Visscher, P. T., Baumgartner, L. . K., Buckley, D. H., Rogers, D. R., Hogan, M. E., Raleigh, C. D., Turk, K. A. and Des Marais, D. J.: Dimethyl sulphide and methanethiol formation in microbial mats: potential pathways for biogenic

signatures, Environ. Microbiol., 5(4), 296–308 [online] Available from: http://www.ncbi.nlm.nih.gov/pubmed/12662177, 2003.

Vogt, M., Steinke, M., Turner, S. M., Paulino, A., Meyerhöfer, M., Riebesell, U., LeQuéré, C. and Liss, P. S.: Dynamics of dimethylsulphoniopropionate and dimethylsulphide under different CO_2 concentrations during a mesocosm experiment, Biogeosciences, 5(2), 407–419, doi:10.5194/bg-5-407-2008, 2008.

Wasmund, N.: Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions, Iny. Rev. ges. Hydrobiol., 82(2), 169–184 [online] Available from: http://onlinelibrary.wiley.com/doi/10.1002/iroh.19970820205/full (Accessed 25 March 2014), 1997.

Webb, A.: The effects of elevated CO_2 and ocean acidification on the production of marine biogenic trace gases, PhD Thesis, Univ. East Angl., (March), 2015.

Webb, A. L., Malin, G., Hopkins, F. E., Ho, K.-L., Riebesell, U., Schulz, K., Larsen, A. and Liss, P.: Ocean acidification has different effects on the production of dimethylsulphide and dimethylsulphoniopropionate measured in cultures of Emiliania huxleyi RCC1229 and mesocosm study: a comparison of laboratory monocultures and community interactions, Environ. Chem., EN14268, doi:http://dx.doi.org/10.1071/EN14268, 2015.

Welschmeyer, N. A.: Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments, Limnol. Oceanogr., 39(8), 1985–1992, 1994.

Zika, R. G., Gidel, L. T. and Davis, D. D.: A comparison of photolysis and substitution decomposition rates of methyl iodide in the ocean, Geophys. Res. Lett., 11(4), 353–356, 1984.

Zinder, S. H., Doemel, W. N. and Brock, T. D.: Production of volatile sulfur compounds during the decomposition of algal mats, Appl. Environ. Microbiol., 34(6), 859–861 [online] Available from: http://aem.asm.org/content/34/6/859.short (Accessed 25 March 2014), 1977.

Zindler, C., Peeken, I., Marandino, C. A. and Bange, H. W.: Environmental control on the variability of DMS and DMSP in the Mauritanian upwelling region, Biogeosciences, 9, 1041–1051, doi:10.5194/bg-9-1041-2012, 2012.

1012 Table 1. Summary of fCO_2 and pH_T (total scale) during phases 0, 1 and 2 of the mesocosm
1013 experiment.

		Whole Experiment (*t*-3 to *t*31)		**Phase 0 (*t*-3 to *t*0)**		**Phase I (*t*1 –*t*16)**		**Phase II (*t*16 – *t*31)**	
Mesocosm[a]	**Target fCO_2 (µatm)**	**Mean fCO_2 (µatm)**	**Mean pH_T**	**Mean fCO_2 (µatm)**	**Mean pH_T**	**Mean fCO_2 (µatm)**	**Mean pH_T**	**Mean fCO_2 (µatm)**	**Mean pH_T**
M1	Control	331	7.91	231	8.00	328	7.95	399	7.86
M5	Control	334	7.91	244	7.98	329	7.94	399	7.52
M7	390	458	7.80	239	7.99	494	7.81	532	7.76
M6	840	773	7.63	236	7.99	932	7.59	855	7.59
M3	1120	950	7.56	243	7.98	1176	7.51	1027	7.52
M8	1400	1166	7.49	232	8.00	1481	7.43	1243	7.45
Baltic Sea	380	350	7.91	298	7.91	277	7.98	436	7.86

1014 [a] listed in order of increasing fCO_2

1015

1016 Table 2. Calibration ranges and calculated percentage mean relative standard error for the trace
1017 gases measured in the mesocosms.

Compound	Calibration range (pmol L^{-1})	% Mean relative standard error
DMS	600 – 29300*	6.33
DMSP	2030 – 405900*	
CH_3I	0.11 – 11.2	4.62
CH_2I_2	5.61 – 561.0	4.98
C_2H_5I	0.10 – 4.91	5.61
CH_2ClI	1.98 – 99.0	3.64
$CHBr_3$	8.61 – 816.0	4.03
CH_2Br_2	0.21 – 20.9	5.30
$CHBr_2Cl$	0.07 – 7.00	7.20

1018 * throughout the rest of this paper, these measurements are given in nmol L^{-1}.

1019

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 (Crawfurd et al., 2016). Data are split into the range of all the mesocosm measurements and those from the Baltic Sea.

	Mesocosm			**Baltic Sea**		
	Range Integrated 10 m	**Range Integrated 17 m**	**% Contribution to Chl-*a***	**Range Integrated 10 m**	**Range Integrated 17 m**	**% Contribution to Chl-*a***
Chl-*a*	0.9 – 2.9	0.9 – 2.6	100	1.3 – 6.5	1.12 – 5.5	100
Phytoplankton Taxonomy (Equivalent Chlorophyll µg L^{-1})						
Cyanobacteria		0.01 – 0.4	8		0.0 – 0.1	1
Prasinophytes		0.04 – 0.3	7		0.01 – 0.3	4
Euglenophytes		0.0 – 1.6	15		0.0 – 2.6	21
Dinoflagellates		0.0 – 0.3	3		0.04 – 0.6	9
Diatoms		0.1 – 0.3	7		0.04 – 0.9	9
Chlorophytes		0.3 – 2.0	40		0.28 – 3.1	41
Cryptophytes		0.1 – 1.4	21		0.1 – 1.0	15
Small Phytoplankton (<10 µm) abundance ~~/~~ (cells mL^{-1})						
Cyanobacteria	55000 – 380000	65000 – 470000		30000 – 180000	30000 – 250000	
Picoeukaryotes I	15000 – 100000	17000 – 111000		5000 – 70000	6100 – 78000	
Picoeukaryotes II	700 – 4000	600 – 4000		400 – 3000	460 – 3700	
Picoeukaryotes III	1000 - 9000	1100 – 8500		1000 – 6000	950 – 7500	
Nanoeukaryotes I	400 – 1400	270 – 1500		200 – 4000	210 – 4100	
Nanoeukaryotes II	0 – 400	4 – 400		100 – 1100	60 – 1300	

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 fCO_2 treatment.

	Range fCO_2		**DMS**	**CH_3I**	**CH_2I_2**	**CH_2ClI**	**$CHBr_3$**	**CH_2Br_2**	**CH_2Br_2Cl**
	(µatm)		**(nmol L^{-1})**			**(pmol L^{-1})**			
SOPRAN Tvärminne Mesocosm (this study)	346 – 1333	Range	2.7-6.8	2.9-6.4	57-202	3.8-8.0	69-148	4.0-7.7	1.7-3.1
		% change	-34	-0.3	1.3	-11	-9	-3	-4
SOPRAN Bergen 2011 (Webb *et al.*, 2015)	280 – 3000	Range	0.1-4.9	4.9-32	5.8-321	9.0-123	64-306	6.3-30.8	3.9-14
		% change	-60	-37	-48	-27	-2	-4	-6
NERC Microbial Metagenomics Experiment, Bergen 2006 (Hopkins *et al.*, 2010)	300 - 750	Range	ND-50	2.0-25	ND-750	ND-700	5.0-80	ND-5.5	0.2-1.2
		% change	-57	-41	-33	-28	13	8	22
EPOCA Svalbard 2010 (Archer *et al.*, 2013; Hopkins *et al.*, 2013)	180 - 1420	Range	ND-14	0.04-10	0.01-2.5	0.3-1.6	35-151	6.3-33.3	1.6-4.7
		% change	-60	NS		NS	NS	NS	NS
UKOA European Shelf 2011 (Hopkins and Archer, 2014)	340 - 1000	Range	0.5-12						
		% change	225						
Korean Mesocosm Experiment 2012 (Park *et al.*, 2014)	160 - 830	Range	1.0-100						
		% change	-82						

Table 5. Concentration ranges of trace gases measured in the Baltic Sea compared to concentrations measured in the literature. ND – Not Detected.

Study	DMS concentration range (nmol L^{-1})	Halocarbon concentration range (pmol L^{-1})						
		CH_3I	CH_2I_2	C_2H_5I	CH_3ClI	$CHBr_3$	CH_2Br_2	CH_2Br_2Cl
SOPRAN Tvärminne Baltic Sea (This Study)	1.9-11	4.3-8.6	66.9-374	0.6 – 1.0	7.0-18	93-192	7.1-10	3.3-5.0
Orlikowska and Schulz-Bull (2009)	0.3-120	1-16	0-85	0.4 – 1.2	5-50	5.0-40	2.0-10	0.8-2.5
Karlsson *et al.* (2008)		3.0-7.5				35-60	4.0-7.0	2.0-6.5
Klick and Abrahamsson (1992)			15-709		11-74	14-585		
Klick (1992)			ND-243		ND-57	40-790	ND-86	ND-29
Leck and Rodhe (1991)	0.4-2.8							
Leck *et al.* (1990)	ND-3.2							

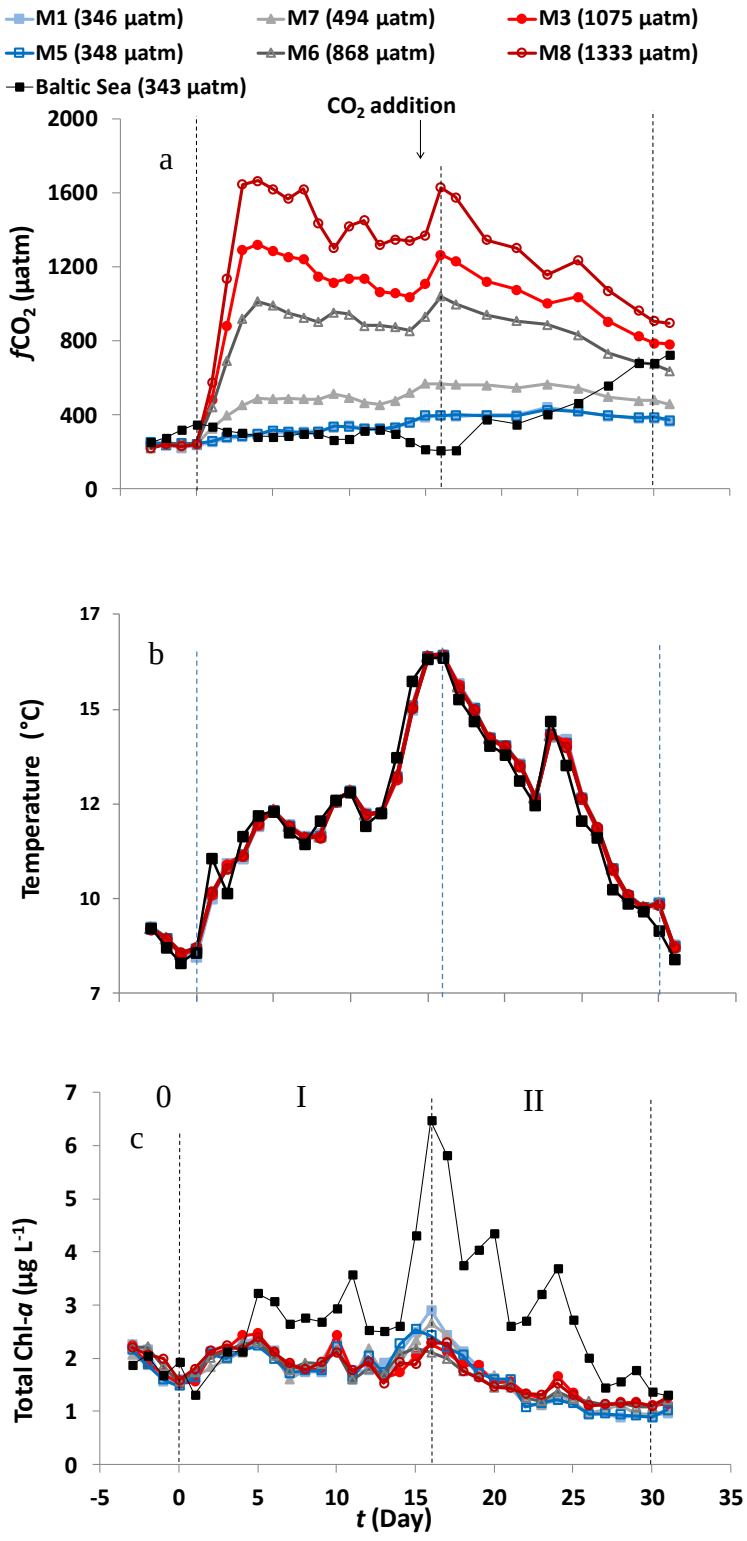

Figure 1. Daily measurements of (a) fCO_2, (b) mean temperature and (c) total Chlorophyll-*a* in the mesocosms and surrounding Baltic Sea waters. Dashed lines represent the three Phases of the experiment, based on the Chl-*a* data.

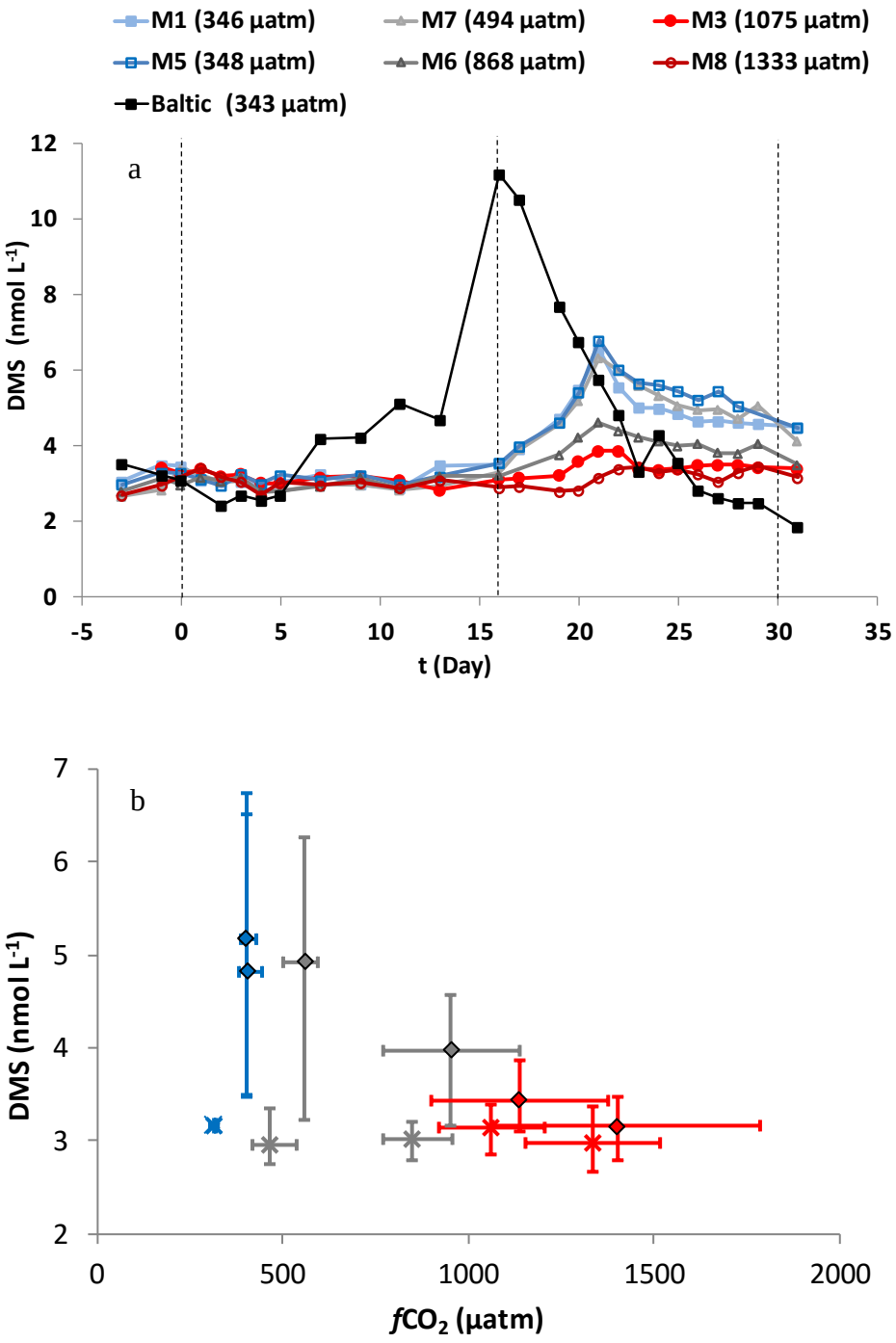

Figure ~~3~~2. (a) Mean DMS concentrations measured daily in the mesocosms and Baltic Sea from an integrated water sample of the surface 10m~~integrated from the surface 10 m~~. Dashed lines show the Phases of the experiment as given in Fig. 1~~2~~, fCO_2 shown in the legend are mean fCO_2 across the 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 fCO_2 (red), with error bars showing the range of both the DMS and fCO_2.

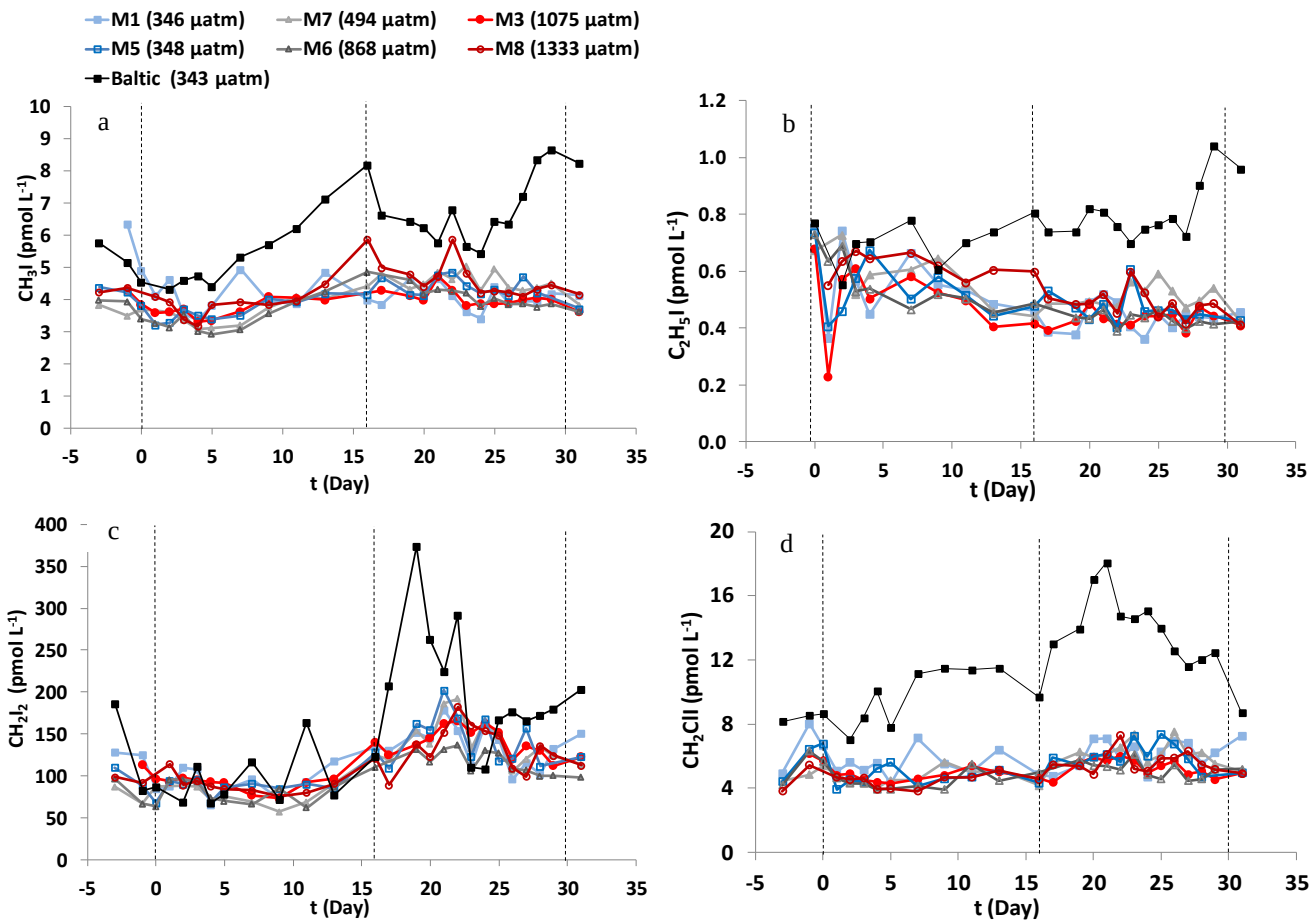

Figure 3. Mean ~~C~~concentrations (pmol L^{-1}) of (a) CH_3I, (b) C_2H_5I, (c) CH_2I_2 and (d) CH_2ClI taken from a water sample integrated from the surface 10m. Dashed lines indicate the Phases of the experiment, as given in Fig. 2. fCO_2 shown in the legend are mean fCO_2 across the duration of the experiment.

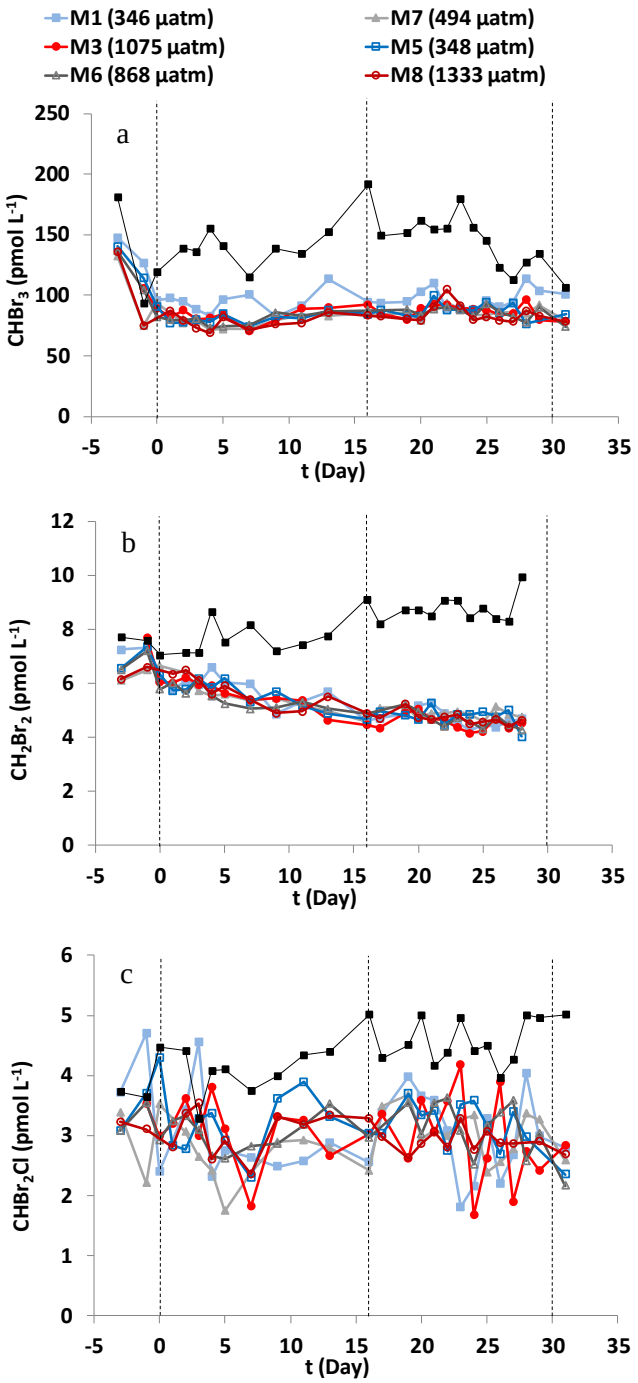

Figure ~~5~~4. Mean c~~C~~oncentrations (pmol L^{-1}) of (a) $CHBr_3$, (b) CH_2Br_2 and (c) $CHBr_2Cl$ taken from a water sample integrated from the surface 10m. Dashed lines indicate the phases of the experiment as defined in Fig. 2, fCO_2 shown in the legend are mean fCO_2 across the duration of the 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.).

Line 247, 248: 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

Line 70: "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'

Line 72: 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

Line 142: 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.

Line 169: replace ; with a ,

AR. changed

Line 171: why no comparable pigment analysis, what's the rationale behind it?

AR. 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.

Line 179: 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.

Line 237: 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 DMSP.

Line 247: 17mIWS space needed

AR. changed

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 412 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.

P12, 373: 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).

P13, 379: . . .2009). DMS and DMSP. . .

AR. Changed

P13, 398: 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

P13, 405: and therefore lower DMS microbial yield from DMSP and/or greater consumption of DMS and conversion to DMSO. 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.

P15, 441: . . .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

P15, 455: 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.

P15, 466: 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.

P20, 615: 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