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


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Abstract

The Baltic Sea is a unique environment as the largest body of brackish water in the world. Acidification of the surface oceans due to absorption of anthropogenic CO$_2$ emissions is an additional stressor facing the pelagic community of the already challenging Baltic Sea. To investigate its impact on trace gas biogeochemistry, a large-scale mesocosm experiment was performed off Tvärminne Research Station, Finland in summer 2012. During the second half of the experiment, dimethylsulphide (DMS) concentrations in the highest fCO$_2$ mesocosms (1075 -
1333 μatm) were 34% lower than at ambient CO₂ (350 μatm). However the net production (as measured by concentration change) of seven halocarbons analysed was not significantly affected by even the highest CO₂ levels after 5 weeks exposure. Methyl iodide (CH₃I) and diiodomethane (CH₂I₂) 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 CO₂ and corresponded to 30% lower Chl-a concentrations compared to Phase I. No other iodocarbons increased or showed a peak, with mean chloroiodomethane (CH₂ClI) concentrations measured at 5.3 (± 0.9) pmol L⁻¹ and iodoethane (C₂H₅I) at 0.5 (± 0.1) pmol L⁻¹. Of the concentrations of bromoform (CHBr₃; mean 88.1 ± 13.2 pmol L⁻¹), dibromomethane (CH₂Br₂; mean 5.3 ± 0.8 pmol L⁻¹) and dibromochloromethane (CHBr₂Cl, mean 3.0 ± 0.5 pmol L⁻¹), only CH₂Br₂ showed a decrease of 17% between Phases I and II, with CHBr₃ and CHBr₂Cl showing similar mean concentrations in both Phases. Outside the mesocosms, an upwelling event was responsible for bringing colder, high CO₂, low pH water to the surface starting on day t16 of the experiment; this variable CO₂ 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 fCO₂. 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 (fCO₂) from 280 μatm (pre-Industrial Revolution) to over 400 μatm today (Hartmann et al., 2013). The IPCC AR5 long-term projections for atmospheric pCO₂ 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 CO₂, the global oceans have absorbed an estimated 30% of excess CO₂ produced (Canadell et al., 2007). With atmospheric pCO₂ 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 CO₂, 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\textsubscript{2}H\textsubscript{6}S) and a number of halogen-containing organic compounds (halocarbons) including methyl iodide (CH\textsubscript{3}I) and bromoform (CHBr\textsubscript{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\textsubscript{5}H\textsubscript{10}O\textsubscript{2}S). Both DMS and DMSP are 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. 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\textsubscript{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\textsubscript{2} (Hopkins and Archer, 2014), but lower DMSP production. The combined picture arising from existing studies is that the response of communities to fCO\textsubscript{2} perturbation is not predictable and requires further study. Previous studies measuring DMS in the Baltic Sea measured concentrations up to 100 nmol L\textsuperscript{-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\textsubscript{3}I), chloroiodomethane (CH\textsubscript{2}CII) and bromoform (CHBr\textsubscript{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. Post-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 season mesocosm experiment aimed to assess the impact of elevated fCO$_2$ on the microbial community and trace gas production in the Baltic Sea. Our objective was to assess how changes in the microbial community driven by changes in fCO$_2$ impacted DMS and halocarbon concentrations. It is anticipated that any effect of CO$_2$ on the growth of different groups within the phytoplankton assemblage will result in an associated change in trace gas concentrations measured in the mesocosms as fCO$_2$ increases, which can potentially be used to predict future halocarbon and sulphur emissions from the Baltic Sea region.
2 Methods

2.1 Mesocosm design and deployment

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

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

2.2 Trace gas extraction and analysis

2.2.1 DMS and halocarbons

A depth-integrated water sampler (IWS, HYDRO-BIOS, Kiel, Germany) was used to sample the entire
17 m water column daily or alternative daily. As analysis of Chlorophyll-α (Chl-α) 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.

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 CH₃I, diiodomethane (CH₂I₂), CH₂ClI, iodoethane (C₂H₅I), iodopropane (C₃H₇I), CHBr₃, dibromoethane (CH₂Br₂), dibromochloromethane (CHBr₂Cl), bromoiodomethane (CH₂BrI) 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 (D₆-DMS), deuterated methyl iodide (CD₃I) and ¹³C dibromoethane (¹³C₂H₄Br₂) 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 r²>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 (DMSPₜ) 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, Churham, UK), into which 0.35 µL of 50% H₂SO₄ was added, before storage at ambient temperature. Particulate DMSP (DMSPₚ) 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 H₂SO₄ 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 revolatilised 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 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 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 community dynamics

Water samples were collected from the 10m and 17mIWS on a daily basis and analysed for carbonate chemistry, fluorometric Chl-$a$, phytoplankton pigments (17m 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-α analysis and pigment determination, 500 mL sub-samples were filtered through a GF/F and stored frozen (-20 °C for two hours for Chl-α 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-α 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-α 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 (Crawford et al., 2015) and counts of phytoplankton cells >20 µm were made on concentrated (50 mL) sample water, fixed with acidic Lugol’s iodine solution with an inverted microscope. Filamentous cyanobacteria were counted in 50 µm length units.

2.4 Statistical Analysis

All statistical analysis was performed using Minitab V16. In analysis of the measurements between mesocosms, one-way ANOVA was used with Tukey’s post-hoc analysis test to determine the effect of different fCO₂ on concentrations measured in the mesocosms and the Baltic Sea. Spearman’s Rank Correlation Coefficients were calculated to compare the relationships between trace gas concentrations, fCO₂, and a number of biological parameters, and the resulting ρ-values for each correlation are given in Supplementary table S1 for the mesocosms and S2 for the Baltic Sea data.

3 Results and Discussion

3.1 Biogeochemical changes within the mesocosms

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

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

fCO₂ decreased over Phase I in the three highest fCO₂ mesocosms, mainly through air-sea gas exchange and carbon fixation by phytoplankton (Fig. 1a). All mesocosms still showed distinct differences in fCO₂ levels throughout the experiment (Table 1), and there was no overlap of mesocosm fCO₂ values on any given day, save for the two controls (M1 and M5). The control mesocosm fCO₂ increased through Phase I of the experiment, likely as a result of undersaturation of the water column encouraging dissolution of atmospheric CO₂ (Paul et al., 2015). Salinity in the mesocosms remained constant throughout the experiment at 5.70 ± 0.004, and showed no variation with depth. It remained similar to salinity in the Baltic Sea surrounding the mesocosms, which was 5.74 ± 0.14. Water temperature varied from a low of 8.6 ± 0.4 °C during Phase 0 to a high of 15.9 ± 2.2 °C measured on day t16, before decreasing once again (Fig. 1b).

Summertime upwelling events are common and well described (Gidhagen, 1987; Lehmann and Myrberg, 2008), and induce a significant temperature decrease in surface waters; such an event appears to have commenced around t16, as indicated by significantly decreasing temperatures inside and out of the mesocosms (Fig. 1b) and increased salinity in the Baltic Sea from 5.5 to 6.1 over the following 15 days to the end of the experiment. Due to the enclosed nature of the mesocosms, the upwelling affected only the temperature and not pH, fCO₂ or the microbial community. However, the temperature decrease after t16 was likely to have had a significant effect on phytoplankton growth, explaining the lower Chl-a in Phase II.

3.1.2 Community Dynamics

Mixing of the mesocosms after closure prior to t-3 did not trigger a notable increase in Chl-a in Phase 0; 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 CO₂ enrichment. 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 t31.

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 fCO₂ in only two groups: picoeukaryotes I showed higher abundance at high fCO₂ (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. 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., 2015; 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 fCO₂ treatments during 
Phase II compared to the ambient fCO₂ 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. 3a). The primary differences identified were 
apparent from the start of Phase II on t17, after which maximum concentrations were observed in the 
ambient mesocosms on t21. The relationship between DMS and increasing fCO₂ during Phase II was 
found to be linear (Fig. 3b), a finding also identified in previous mesocosm experiments (Archer et al., 
2013; Webb et al., 2015). Furthermore, increases in DMS concentrations under high fCO₂ were 
delayed by three days relative to the ambient and medium fCO₂ 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 fCO₂ treatments with the mean of all mesocosms 3.1 (± 0.2) nmol L⁻¹. Concentrations in all mesocosms gradually declined from t21 until the end of DMS measurements on t31. DMS concentrations measured in the mesocosms and Baltic Sea were comparable to those measured in temperate coastal conditions in the North Sea (Turner et al., 1988), the Mauritanian upwelling (Franklin et al., 2009; Zindler et al., 2012) and South Pacific (Lee et al., 2010).

Although the majority of DMS production is presumed to be from DMSP, an alternative production route for DMS is available through the methylation of methanethiol (Drotar et al., 1987; Kiene and Hines, 1995; Stets et al., 2004) predominantly identified in anaerobic environments such as freshwater lake sediments (Lomans et al., 1997), saltmarsh sediments (Kiene and Visscher, 1987) and microbial mats (Visscher et al., 2003; Zinder et al., 1977). However, recent studies have identified this pathway of DMS production from Pseudomonas deceptionensis in an aerobic environment (Carrión et al., 2015), where P. deceptionensis was unable to synthesis or catabolise DMSP, but was able to enzymatically mediate DMS production from methanethiol (MeSH). The same enzyme has also been identified in a wide range of other bacterial taxa, including the cyanobacterial Pseudanabaena, which was identified in the Baltic Sea during this and previous investigations (Stuhr, pers. comm.; Kangro et al., 2007; Nausch et al., 2009). Correlations between DMS and the cyanobacterial equivalent Chl-α (ρ=0.42, p<0.01) indicate 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 fCO₂, indicating DMS net production was not directly related to the perturbation of the system and associated cellular stress (Sunda et al., 2002). During Phase II, DMS was negatively correlated with Chl-α in the ambient and medium fCO₂ mesocosms (ρ=-0.60, p<0.01). During Phase II, a significant correlation was seen between DMS and single-celled cyanobacteria identified 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-α on t16.
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, these 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 DMSP 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 fCO₂ (Hornick et al., 2015). 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, this is still a potential stimulated DMS loss pathway at high fCO₂. Synechococcus has been identified as a DMS consumer in the open ocean, but abundance of this group was negatively correlated with fCO₂, implying that DMS consumption by this group would have been lower as fCO₂ increased.

3.3 Iodocarbons in the mesocosms and relationships with community composition

Elevated fCO₂ 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 fCO₂ (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 CH₃I (Fig. 4a) and C₂H₃I (Fig. 4b) showed concentration ranges of 2.91 to 6.25 and 0.23 to 0.76 pmol L⁻¹ respectively. CH₃I showed a slight increase in all mesocosms during Phase I, peaking on t16 which corresponded with higher Chl-α concentrations, and correlated throughout the entire experiment with picoeukaryote groups II (ρ=0.59, p<0.01) and III (ρ=0.23, p<0.01; Crawfurd et al. this issue) and nanoeukaryotes I (ρ=0.37, p<0.01). Significant differences identified between mesocosms for CH₃I were unrelated to elevated fCO₂ (F=3.1, p<0.05), but concentrations were on average 15% higher in Phase II than Phase I. C₂H₃I 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 fCO₂, 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 CH₃I and Chl-α at any phase, and the only correlation of any phytoplankton grouping was with nanoeukaryotes II (ρ=0.88, p<0.01; Crawfurd et al., 2015). These CH₃I 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 CH₃I has been shown to take place through abiotic processes, including
radical recombination of CH$_3$ and I (Moore and Zafiriou, 1994). However since samples were integrated over the surface 10m of the water column, it was impossible to determine if photochemistry was affecting iodocarbon concentrations near the surface where some UV light was able to pass between the top of the mesocosm film material and the cover. For the same reason, photodegradation of halocarbons (Zika et al., 1984) within the mesocosms was also likely to have been significantly restricted. Thus, as photochemical production was expected to be minimal, biogenic production was likely to have been the dominant source of these compounds. Karlsson et al. (2008) identified Pseudanabaena as a key producer of CH$_3$I in the Baltic Sea. However the abundance of Pseudanabaena was highest during Phase I of the experiment (A. Stuhr, Pers. Comm.) when CH$_3$I concentrations were lower, and as discussed previously, the abundance of these species constituted only a very small proportion of the community. Previous investigations in the laboratory have identified diatoms as significant producers of CH$_3$I (Hughes et al., 2013; Manley and De La Cuesta, 1997), and the low, steady-state abundance of the diatom populations in the mesocosms could have produced the same relatively steady-state trends in the iodocarbon concentrations.

Measured in the range 57.2 – 202.2 pmol L$^{-1}$ in the mesocosms, CH$_2$I$_2$ (Fig. 4c) showed the clearest increase in concentration during Phase II, when it peaked on t21 in all mesocosms, with a maximum of 202.2 pmol L$^{-1}$ in M5 (348 μatm). During Phase II, concentrations of CH$_2$I$_2$ were 57% higher than Phase I, and were therefore negatively correlated with Chl-a. The peak on t21 corresponds with the peak identified in DMS on t21, and concentrations through all three phases correlate with picoeukaryotes II ($\rho=0.62$, $p<0.01$) and III ($\rho=0.47$, $p<0.01$) and nanoeukaryotes I ($\rho=0.88$, $p<0.01$; Crawfurd et al., 2015). CH$_2$ClI (Fig. 4d) showed no peaks during either Phase I or Phase II, remaining within the range 3.81 to 8.03 pmol L$^{-1}$, and again correlated with picoeukaryotes groups II ($\rho=0.34$, $p<0.01$) and III ($\rho=0.38$, $p<0.01$). These results may suggest that these groups possessed haloperoxidase 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 f$CO_2$ ($F=1.7$, $p<0.01$), it is unlikely that production was increased in relation to elevated f$CO_2$. Production of all iodocarbons increased during Phase II when total Chl-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$_3$I and C$_2$H$_5$I (Hughes et al., 2008), likely through the alkylation of inorganic iodide (Urhahn and Ballschmiter, 1998) or through the breakdown of organic matter by microbial activity to supply the precursors required for iodocarbon production (Smith et al., 1992). Hughes et al. (2008) did not identify this route as a pathway for CH$_3$I$_2$ or CH$_2$ClI production, but
Carpenter et al. (2005) suggested a production pathway for these compounds through the reaction of HOI with aggregated organic materials.

3.4 Bromocarbons in the mesocosms and the relationships with community composition

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

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

Halocarbon loss processes such as nucleophilic substitution (Moore, 2006), hydrolysis (Elliott and Rowland, 1995), sea-air exchange and microbial degradation are suggested as of greater importance than production of these compounds by specific algal groups, particularly given the relatively low
growth rates and total Chl-α. 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$_2$Br$_2$ in the same experiments could explain the steady decrease in CH$_2$Br$_2$ concentrations seen in the mesocosms. Bacterial oxidation was also identified by Goodwin et al. (1998) as a significant sink for CH$_2$Br$_2$. 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$_2$Br$_2$ 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$_2$Br$_2$ 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 due to the isolation from the coastal environment, however 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, with concentrations decreasing through 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 t31, 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-α followed the pattern of the mesocosms until t4, after which concentrations were significantly higher than any mesocosm, peaking at 6.48 μg L$^{-1}$ on t16, corresponding to the maximum Chl-α peak in the mesocosms and the maximum peak of temperature. As upwelled water intruded into the surface waters, the surface Chl-α was diluted with low Chl-α deep water: Chl-α in the surface 10m decreased
from around t16 at the start of the upwelling until t31 when concentrations were once again equivalent
to those found in the mesocosms at 1.30 ȝg L \(^{-1}\). In addition there was potential introduction of different
algal groups to the surface, but chlorophytes and crytophytes 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., 2015; 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 t16, and were within the range of previous measurements for the region (Table 5).
Strong correlations were seen between DMS and Chl-a (\(\rho=0.84, p<0.01\)), with the ratio of DMS: Chl-a
at 1.6 (± 0.3) nmol ȝg \(^{-1}\). Other strong correlations were seen with euglenophytes (\(\rho=0.89, p<0.01\)),
dinoflagellates (\(\rho=0.61, p<0.05\)) and nanoeukaryotes II (\(\rho=0.88, p<0.01\)), 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 during Phase II, the DMS concentration measured in the Baltic Sea decreased,
from the peak on t16 to the lowest recorded sample of the entire experiment at 1.85 nmol L \(^{-1}\). As with
Chl-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$_3$I was measured at a maximum concentration of 8.65 pmol L$^{-1}$, during Phase II, and showed limited effect of the upwelling event. Both CH$_3$I and CH$_2$ClI showed higher concentrations in the Baltic Sea samples than the mesocosms (CH$_3$I: 373.9 pmol L$^{-1}$ and CH$_2$ClI: 18.1 pmol L$^{-1}$), and were correlated with the euglenophytes (CH$_3$I: $\rho=0.63$, p<0.05 and CH$_2$ClI: $\rho=0.68$, p<0.01) and nanoeukaryotes II (CH$_3$I: $\rho=0.53$, p<0.01 and CH$_2$ClI: $\rho=0.58$, p<0.01), but no correlation with Chl-a. Both polyiodinated compounds showed correlation with picoeukaryote groups II and III, indicating that production was not limited to a single source. These concentrations of CH$_3$I and CH$_2$ClI compared well to those measured over a macroalgal bed in the higher saline waters of the Kattegat by Klick and Abrahamsson (1992), suggesting that macroalgae were a significant iodocarbon source in the Baltic Sea.

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

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 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 fCO$_2$ excursion 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-$\alpha$ 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). However, it is very difficult to determine where and when an upwelling will occur, and therefore 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 during the injection of high fCO$_2$ deep water into the surface community during upwelling events. For the concentrations of halocarbons, no response 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. However, 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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Table 1. Summary of fCO₂ and pHₜ (total scale) during phases 0, 1 and 2 of the mesocosm experiment.

<table>
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<tr>
<th>Mesocosm*</th>
<th>Target fCO₂/µatm</th>
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<tr>
<td>M1</td>
<td>Control</td>
<td>331</td>
<td>7.91</td>
<td>231</td>
<td>8.00</td>
<td>328</td>
<td>7.95</td>
<td>399</td>
<td>7.86</td>
</tr>
<tr>
<td>M5</td>
<td>Control</td>
<td>334</td>
<td>7.91</td>
<td>244</td>
<td>7.98</td>
<td>329</td>
<td>7.94</td>
<td>399</td>
<td>7.52</td>
</tr>
<tr>
<td>M7</td>
<td>390</td>
<td>458</td>
<td>7.80</td>
<td>239</td>
<td>7.99</td>
<td>494</td>
<td>7.81</td>
<td>532</td>
<td>7.76</td>
</tr>
<tr>
<td>M6</td>
<td>840</td>
<td>773</td>
<td>7.63</td>
<td>236</td>
<td>7.99</td>
<td>932</td>
<td>7.59</td>
<td>855</td>
<td>7.59</td>
</tr>
<tr>
<td>M3</td>
<td>1120</td>
<td>950</td>
<td>7.56</td>
<td>243</td>
<td>7.98</td>
<td>1176</td>
<td>7.51</td>
<td>1027</td>
<td>7.52</td>
</tr>
<tr>
<td>M8</td>
<td>1400</td>
<td>1166</td>
<td>7.49</td>
<td>232</td>
<td>8.00</td>
<td>1481</td>
<td>7.43</td>
<td>1243</td>
<td>7.45</td>
</tr>
<tr>
<td>Baltic Sea</td>
<td>380</td>
<td>350</td>
<td>7.91</td>
<td>298</td>
<td>7.91</td>
<td>277</td>
<td>7.98</td>
<td>436</td>
<td>7.86</td>
</tr>
</tbody>
</table>

*a listed in order of increasing fCO₂
Table 2. Calibration ranges and calculated percentage mean relative standard error for the trace gases measured in the mesocosms.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calibration range / pmol L$^{-1}$</th>
<th>% Mean relative standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMS</td>
<td>600 – 29300*</td>
<td>6.33</td>
</tr>
<tr>
<td>DMSP</td>
<td>2030 – 405900*</td>
<td></td>
</tr>
<tr>
<td>CH$_3$I</td>
<td>0.11 – 11.2</td>
<td>4.62</td>
</tr>
<tr>
<td>CH$_2$I$_2$</td>
<td>5.61 – 561.0</td>
<td>4.98</td>
</tr>
<tr>
<td>C$_2$H$_5$I</td>
<td>0.10 – 4.91</td>
<td>5.61</td>
</tr>
<tr>
<td>CH$_3$Cl</td>
<td>1.98 – 99.0</td>
<td>3.64</td>
</tr>
<tr>
<td>CHBr$_3$</td>
<td>8.61 – 816.0</td>
<td>4.03</td>
</tr>
<tr>
<td>CH$_2$Br$_2$</td>
<td>0.21 – 20.9</td>
<td>5.30</td>
</tr>
<tr>
<td>CHBr$_2$Cl</td>
<td>0.07 – 7.00</td>
<td>7.20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Phytoplankton Taxonomy / Equivalent Chlorophyll µg L⁻¹</th>
<th>Mesocosm</th>
<th>Baltic Sea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range Integrated 10 m</td>
<td>Range Integrated 17 m</td>
</tr>
<tr>
<td>Chl-a</td>
<td>0.9 - 2.9</td>
<td>0.9 - 2.6</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0.01 - 0.4</td>
<td>8</td>
</tr>
<tr>
<td>Prasinophytes</td>
<td>0.04 - 0.3</td>
<td>7</td>
</tr>
<tr>
<td>Euglenophytes</td>
<td>0.0 - 1.6</td>
<td>15</td>
</tr>
<tr>
<td>Dinoflagellates</td>
<td>0.0 - 0.3</td>
<td>3</td>
</tr>
<tr>
<td>Diatoms</td>
<td>0.1 - 0.3</td>
<td>7</td>
</tr>
<tr>
<td>Chlorophytes</td>
<td>0.3 - 2.0</td>
<td>40</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td>0.1 - 1.4</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Small Phytoplankton (&lt;10 µm) abundance / cells mL⁻¹</th>
<th>Mesocosm</th>
<th>Baltic Sea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range Integrated 10 m</td>
<td>Range Integrated 17 m</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>55000 - 580000</td>
<td>63000 - 470000</td>
</tr>
<tr>
<td>Picoeukaryotes I</td>
<td>15000 - 100000</td>
<td>17000 - 111000</td>
</tr>
<tr>
<td>Picoeukaryotes II</td>
<td>700 - 40000</td>
<td>600 - 40000</td>
</tr>
<tr>
<td>Nanoeukaryotes I</td>
<td>1000 - 9000</td>
<td>1100 - 8900</td>
</tr>
<tr>
<td>Nanoeukaryotes II</td>
<td>400 - 1400</td>
<td>270 - 1500</td>
</tr>
<tr>
<td>Nanoeukaryotes II</td>
<td>0 - 400</td>
<td>4 - 400</td>
</tr>
</tbody>
</table>
Table 4. Concentration ranges of trace gases measured in the mesocosms compared to other open water ocean acidification experiments, showing the range of concentrations for each gas and the percentage change between the control and the highest fCO$_2$ treatment.

<table>
<thead>
<tr>
<th>Range fCO$_2$ / $\mu$atm</th>
<th>DMS / nmol L$^{-1}$</th>
<th>CH$_4$ / pmol L$^{-1}$</th>
<th>CH$_3$I / pmol L$^{-1}$</th>
<th>CH$_2$Cl / pmol L$^{-1}$</th>
<th>CHBrCl / pmol L$^{-1}$</th>
<th>CH$_2$Br$_2$ / pmol L$^{-1}$</th>
<th>CH$_2$BrCl / pmol L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOPRAN Tvärminne Mesocosm (this study)</td>
<td>346 – 1333 Range</td>
<td>2.3-6.8</td>
<td>2.9-8.4</td>
<td>57-202</td>
<td>5.8-8.0</td>
<td>60-148</td>
<td>4.0-7.7</td>
</tr>
<tr>
<td>% change</td>
<td>-34</td>
<td>-0.3</td>
<td>1.3</td>
<td>-11</td>
<td>-9</td>
<td>-3</td>
<td>-4</td>
</tr>
<tr>
<td>SOPRAN Bergen 2011 (Webb et al., 2015)</td>
<td>280 – 3000 Range</td>
<td>0.1-4.9</td>
<td>4.9-32</td>
<td>5.8-521</td>
<td>9.0-125</td>
<td>64-506</td>
<td>6.3-30.8</td>
</tr>
<tr>
<td>% change</td>
<td>-60</td>
<td>-37</td>
<td>-48</td>
<td>-27</td>
<td>-2</td>
<td>-4</td>
<td>-6</td>
</tr>
<tr>
<td>NERC Microbial Metagenomics Experiment, Bergen 2006 (Hopkins et al., 2010)</td>
<td>300 – 750 Range</td>
<td>ND-50</td>
<td>2.0-25</td>
<td>ND-750</td>
<td>ND-700</td>
<td>5.0-80</td>
<td>ND-5.5</td>
</tr>
<tr>
<td>% change</td>
<td>-57</td>
<td>-41</td>
<td>-33</td>
<td>-26</td>
<td>13</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>EPOCA Svalbard 2010 (Archer et al., 2013; Hopkins et al., 2010)</td>
<td>180 – 1420 Range</td>
<td>ND-14</td>
<td>0.04-10</td>
<td>0.01-2.5</td>
<td>0.3-1.6</td>
<td>35-151</td>
<td>6.3-33.3</td>
</tr>
<tr>
<td>% change</td>
<td>-60</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>UKOA European Shelf 2011 (Hopkins and Archer, 2014)</td>
<td>340 – 1000 Range</td>
<td>0.5-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>225</td>
<td></td>
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</tr>
<tr>
<td>Korean Mesocosm Experiment 2012 (Park et al., 2014)</td>
<td>160 – 830 Range</td>
<td>1.0-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Study</th>
<th>DMS concentration range / nmol L(^{-1})</th>
<th>Halocarbon concentration range / pmol L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH(_3)I</td>
<td>CH(_2)I</td>
</tr>
<tr>
<td>SOPRAN Tvärminne Baltic Sea (This Study)</td>
<td>1.9-11</td>
<td>4.5-8.6</td>
</tr>
<tr>
<td>Orlikowska and Schulz-Bull (2009)</td>
<td>0.3-120</td>
<td>1-16</td>
</tr>
<tr>
<td>Karlsson et al. (2008)</td>
<td>3.0-7.5</td>
<td>35-60</td>
</tr>
<tr>
<td>Klick and Abrahamsson (1992)</td>
<td>15-709</td>
<td>14-585</td>
</tr>
<tr>
<td>Klick (1992)</td>
<td>ND-243</td>
<td>ND-97</td>
</tr>
<tr>
<td>Leck and Rolle (1991)</td>
<td>0.4-2.8</td>
<td>ND-3.2</td>
</tr>
<tr>
<td>Leck et al. (1990)</td>
<td>ND-3.2</td>
<td></td>
</tr>
</tbody>
</table>
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-$\sigma$ data.
Figure 3. (a) Integrated DMS concentrations measured daily in the mesocosms and Baltic Sea from the surface 10 m and (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$_2$. Dashed lines show 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 4. Concentrations (pmol L$^{-1}$) of (a) CH$_3$I, (b) C$_2$H$_5$I, (c) CH$_2$I$_2$ and (d) CH$_2$ClI. 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. Concentrations (pmol L\(^{-1}\)) of (a) CH\(_3\)Br, (b) CH\(_2\)Br\(_2\) and (c) CHBr\(_2\)Cl. 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.