Effect of CO_2 enrichment on phytoplankton photosynthesis in the North Atlantic sub-tropical gyre

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Abstract

The effects of changes in CO_2 concentration in seawater on phytoplankton community structure and photosynthesis were studied in the North Atlantic sub-tropical gyre. Three shipboard incubations were conducted for 48 h at ~760 ppm CO_2 and control (360 ppm CO_2) from 49°N to 7°N during October and November 2010. Elevated CO_2 caused a decrease in pH to ~7.94 compared to ~8.27 in the control. During one experiment, the biomass of nano- and picoeukaryotes increased under CO_2 enrichment, but primary production decreased relative to the control. In two of the experiments the biomass was dominated by dinoflagellates, and there was a significant increase in the maximum photosynthetic rate (P_m^B) and light-limited slope of photosynthesis (α^B) at CO_2 concentrations of 750 ppm relative to the controls. 77 K emission spectroscopy showed that the higher photosynthetic rates measured under CO_2 enrichment increased the connection of reversible photosystem antennae, which resulted in an increase in light harvesting efficiency and carbon fixation.

1. Introduction

There is global concern over increases in the CO_2 concentration of seawater and the effects of ocean acidification on the functionality and productivity of marine ecosystems (Riebesell, 2008). Increases in atmospheric CO_2 from 280 to 370 ppm since the industrial revolution have decreased surface ocean pH by 0.12 units (Riebesell, 2004). The "business as usual" scenario predicts that CO_2 will rise to 700 ppm over the next 100 years (Houghton, 2001), which will decrease seawater pH by a further 0.3 to 0.6 units (Riebesell, Schulz, Bellerby, Botros, Fritsche et al., 2007), bicarbonate ion (HCO_3^-) concentration by 50% (Riebesell, 2004) and raise the sea surface temperature (SST) by 2 to 6°C (Alley, Berntsen, Bindoff, Chen & others, 2007; Bopp, Monfray, Aumont, Dufresne, Le Treut et al., 2001). The projected pH shift from 8.2 to 7.7 covers the entire range of variation in pH currently observed in open ocean surface waters. The increase in SST is predicted to increase stratification and light availability of the surface ocean due to shoaling of the upper mixed layer (Rost, Zondervan & Wolf-Gladrow, 2008; Sarmiento, Slater, Barber, Bopp, Doney et al., 2004), which could directly impact the physiology of phytoplankton. These changes in pH, SST and light regime are predicted to enhance primary production (PP) (Hein & SandJensen, 1997; Riebesell, Wolfgladrow & Smetacek, 1993), especially in the North Atlantic (Bopp et al., 2001; Doney, Lindsay, Fung & John, 2006; Feng, Hare, Leblanc, Rose, Zhang et al., 2009), though this will depend on the associated dominant species and nutrient regime (Rost et al., 2008).

Information on the effects of CO_2 enrichment on phytoplankton photosynthesis in a range of organisms and different trophic systems is essential to understand potential shifts in the carbon cycle due to ocean acidification (Riebesell, 2004). Within the usual seawater pH range (8.0-8.3), 90% of the total DIC is HCO_3^-, and CO_2 is less than 1 % when the system is in equilibrium with atmospheric CO_2 (Skirrow, 1975). The total inorganic carbon concentration in seawater is 2 µmol L^{-1} and the CO_2 content is 10 µmol L^{-1}. This is not

sufficient to saturate carbon fixation by the algal photosynthetic enzyme ribulose bisphosphate carboxylase oxygenase (RuBisCO), which has half-saturation constants of 20–40 µmol L^{-1} CO_2 for eukaryotic microalgae and up to 750 µmol L^{-1} CO_2 for marine cyanobacteria (Badger, Andrews, Whitney, Ludwig, Yellowlees et al., 1998; Hopkinson, Dupont, Allen & Morel, 2011; Raven, 2011a; Raven & Johnston, 1991). Different phytoplankton groups or species have therefore evolved a preference for different forms of DIC, with some taking up CO_2 directly, whereas others draw on the pool of HCO_3^- present (Elzenga, Prins & Stefels, 2000) and or mechanisms to concentrate CO_2 or HCO_3^-. Sensitivity to CO_2 therefore varies in relation to the HCO_3^- : CO_2 preference and the affinity of phytoplankton for carbon fixation. Some species can rapidly acclimate to changes in the concentration of dissolved CO_2, or total DIC (Nimer & Merrett, 1996). From an ecosystem perspective, it has been suggested that the responses of phytoplankton to reduced pH in a high CO_2 ocean are likely to be species-specific, with potential 'winners' and 'losers' (Hinga, 2002).

There is a growing body of literature on changes in photosynthesis due to increases in CO_2 and related effects (Brading, Warner, Davey, Smith, Achterberg et al., 2011; Feng et al., 2009; Feng, Warner, Zhang, Sun, Fu et al., 2008; Flynn, Clark, Mitra, Fabian, Hansen et al., 2015). The majority of studies have been conducted on phytoplankton in laboratory culture rather than on natural samples (Feng et al., 2008; Fu, Warner, Zhang, Feng & Hutchins, 2007; King, Jenkins, Wallace, Liu, Wikfors et al., 2015; Shi, Li, Hopkinson, Hong, Li et al., 2015; Wu, Gao & Riebesell, 2010). In diatoms, some cyanobacteria and coccolithophorids, elevated CO_2 can lead to an increase in photosynthesis especially in large chain forming diatoms (Tortell & Morel, 2002; Tortell, Payne, Li, Trimborn, Rost et al., 2008), *Synechococcus* spp. (Fu et al., 2007) and *Emiliania huxleyi* (Leonardos & Geider, 2005). In the diatom *Chaetoceros muelleri* for example, it has been shown that under saturating irradiance,

maximal photosynthetic rates are stimulated by increasing CO_2 availability (Ihnken, Roberts & Beardall, 2011). For *Phaeodactylum tricornutum* grown at elevated CO_2 (1000 ppm) corresponding to 7.8 pH, there was greater photoinhibition of the electron transport rate from photosystem II (PSII) under high irradiance, whereas non-photochemical quenching was reduced compared to low CO_2 grown cells (Wu et al., 2010).

Most studies on natural samples in the Atlantic Ocean have been conducted in eutrophic and mesotrophic environments of the North Atlantic during natural or simulated blooms. Some of these report an increase in the abundance of diatoms compared to *Phaeocystis* spp. under CO_2 enrichment (Feng et al., 2008; Riebesell et al., 2007; Tortell et al., 2008), others report that nano-phytoplankton or Prymnesiophytes replace diatoms under elevated CO_2 and temperature in the Bering Sea (Hare, Leblanc, DiTullio, Kudela, Zhang et al., 2007) and the North Atlantic (Feng et al., 2008), where there was a change from diatoms to Prymnesiophytes under elevated CO_2 and a decrease in inorganic carbon production (calcification). There have been few studies of CO_2 enrichment on changes in phytoplankton community structure and photosynthesis in the oligotrophic gyres (Egleston, Sabine & Morel, 2010), even though they occupy the largest areas of the ocean. To date there has only been one study conducted in the sub-tropical North Atlantic at the Bermuda Atlantic Time Series site (BATS), which showed no significant difference in carbon fixation rates at elevated pCO_2 (reduced pH) for phytoplankton assemblages dominated by *Prochlorococcus* sp. and *Synechococcus* sp. (Lomas, Hopkinson, Losh, Ryan, Shi et al., 2012). By contrast, in the same study nitrogen fixation rates in colonies of *Trichodesmium* increased by 54 % at pH 7.8 but decreased by 21 % at pH 8.4.

The objective of this study was to assess the effect of CO_2 enrichment on phytoplankton photosynthesis and community composition in the North Atlantic sub-tropical gyre through a series of shipboard incubation experiments. Low temperature (LT) emission

spectra were used to assess changes in major pigment-protein complexes in the oxygenic photosynthetic membranes. The LT spectra also provided information about the presence of chlorophyll-containing light harvesting antenna complexes and their functional coupling to photosystem reaction centres.

2. Methods

2.1. Study area and experimental design

Shipboard incubations were conducted aboard the *RRS James Cook* between 13 October and 21 November 2010 during the Atlantic Meridional Transect 20 (Cruise JC053). CO_2 enrichment experiments were carried out at three stations in the North Atlantic Gyre at 29°N, 34°W for experiment (Exp.) I; 18°N, 37°W for Exp. II and 7°N, 30°W for Exp. III (Fig. 1). For each Exp., 80 L of near surface seawater was collected from the ship's underway supply before dawn into a large Nalgene container. The water was sub-sampled into 18 acid cleaned (IOC, 1994) 4 L clear polycarbonate bottles for incubation, leaving a head space of 0.5 L. Nine of the bottles were bubbled with pre-mixed synthetic air with 360 ppm CO_2 (control) and the other nine were bubbled with synthetic air with 760 ppm CO_2 (CO_2 treatment). The gases were supplied to the experimental bottles from gas cylinders via nylon tubing fitted with in-line 0.2 µm sterile Acrovent filters, to prevent contamination by particulates. The nylon tubing entered the bottles through Nalgene caps with Kinesis Omni-Lok fittings and vent tubes to prevent the build-up of pressure in the bottles. To minimise the effect of bubbling on phytoplankton, each experimental bottle was bubbled for an initial 8 h period followed by a further 4 h after 24 h to maintain the CO_2 over the 48 h period. The incubations were conducted in an on-deck incubation system (78 x 60 x 68 cm) supplied with flow through sub-surface seawater from the ship's underway supply to maintain the bottles at ambient temperature. The incubation system had no light screen, but the sides of the incubator were

opaque which provided shading either side of zenith. pH, alkalinity, pCO_2, HCO_3^-, and Chl *a* were measured in triplicate at 0 (T0), 6, (T6; except Chl *a*), 12 (T12), 24 (T24) and 48 (T48) h. Samples for pico- and nanophytoplankton enumeration by flow cytometry, photosynthesis-irradiance curves and low emission spectra were measured in triplicate at T0, T24 and T48. Microscopy counts were made as single measurements at T0 and T48.

2.2. Carbonate chemistry: pH, pCO_2 and alkalinity

pH was determined spectrophotometrically onboard using 2 µmol L^{-1} of m-cresol from a 2 mmol L^{-1} stock solution which was prepared from pure sodium salt (Sigma-Aldrich, USA). This method expresses pH on the total hydrogen concentration scale with 0.01 precision (Dickson, Afghan & Anderson, 2003). Absorbance was recorded before and after addition of m-cresol at 434, 578 and 730 nm on a PerkinElmer Lambda 35 spectrophotometer in a 100 mm cuvette. Seawater temperature was measured using an Amarell Ama-Digit 15 Temperature probe, and salinity was taken from a Seabird Electronics CTD calibrated against salinity standards. For total alkalinity, samples were stored in 100 mL borosilicate dark bottles with teflon cap liners spiked with 5% mercuric chloride (6.9 nmol L^{-1}). Alkalinity was measured 2 months after the cruise using an automatic Apollo SciTech, Alkalinity Titrator (Model AS-ALK2) in 0.8 M H_2SO_4. pCO_2 was calculated from the pH, temperature, salinity and alkalinity measurements using CO2SYS software (Pierrot & Wallace, 2006), using the constants set according to Mehrbach et al. (1973) and Dickson and Millero (1987) and corrected for differences between *in situ* and measured temperature.

2.3. Phytoplankton community structure

Nano and picoeukaryote phytoplankton cells from approx. 0.2 to 10 µm were enumerated using a Becton Dickinson FACSort™ flow cytometer (Becton Dickinson, USA) equipped

with an air-cooled laser providing blue light at 488 nm, using the methods given in Tarran et al. (Tarran, Heywood & Zubkov, 2006; Tarran, Zubkov, Sleigh, Burkill & Yallop, 2001). Picoeukaryotic phytoplankton and nanophytoplankton were analysed for between 2-4 minutes to measure chlorophyll fluorescence (>650 nm), phycoerythrin fluorescence (585 ± 21 nm) and side scatter (light scattered at 90° to the plane of the laser). Using a combination of density plots as described in Tarran et al. (2001), it was possible to identify and quantify the following planktonic groups in the samples: *Prochlorococcus* spp., *Synechococcus* spp., picoeukaryotic phytoplankton (approx. 0.6 to 3 µm, including prasinophyceae, chlorophyceae, pelagophyceae) and nanoeukaryotic phytoplankton (approx. 3 to 10 µm, including cryptophyceaeae and prymnesiophyceae). Instrument flow rate was calibrated daily using Beckman Coulter Flowset fluorospheres of known concentration. Carbon biomass was calculated using median cell diameters measured for each group using the methods described in Tarran et al. (2006) and the carbon conversion factors given in (Bjørnsen, 1986) and (Booth, 1988).

Microscopy was used to enumerate diatoms and dinoflagellates. For microscopic counts, 100 mL of seawater samples were collected into brown borosilicate bottles and preserved in Lugol's iodine (final concentration of 1 %) for stations II and III. Dinoflagellates and diatoms were counted 2 months after the cruise using a Leica DM IRB microscope (Leica, Germany). 100 mL of sample was settled in composite sedimentation chambers for 24 h. Samples were counted on 26 mm diameter sedimentation slides in replicate vertical and horizontal transects using a x 20 objective. Cell volumes for these groups were calculated using approximate geometric shapes and converted to biomass using the equations given in Menden-Deuer and Lessard (2000).

For analysis of Chlorophyll-*a* (Chl *a*), 100 mL samples were filtered onto 25 mm GF/F filters (pore size 0.7 µm, Whatman). The filter was then placed in a 15 mL Falcon™

tube (BD, UK) and 10 mL of 90 % acetone was added. The tubes were then stored in the dark, in a -20°C freezer for 12 h. Chl *a* was then measured onboard fluorometrically following Holm-Hansen, Lorenzen, Holmes and Strickland (1965) using a Turner Designs, Trilogy Fluorometer.

2.4. Phytoplankton photosynthesis

2.4.1. Photosynthesis-irradiance curves

Photosynthetic-irradiance (P-E) experiments were conducted in linear photosynthetrons illuminated with 50 W, 12 V tungsten halogen lamps following the methods described in Tilstone, Figueiras, Lorenzo and Arbones (2003). Each incubator housed 16 sub-samples in 60 mL acid-cleaned polycarbonate bottles, which were inoculated with between 185 kBq (5 µCi) and 370 kBq (10 µCi) of ^{14}C labelled bicarbonate. The PE curves were conducted at the same time each day to minimise the influence of diel light history. PAR in the incubator was set to ambient levels measured over a 2 h period prior to incubation using a Skye Instruments PAR Sensor (model SKP 200). For Exps I, II and IIII these were 1319, 2166 and 2215 µE m^{-2} s^{-1}, respectively. The P-E curves were maintained at *in situ* temperature using the ship's non-toxic seawater supply. After 1.5 h incubation, the suspended material was filtered through 25 mm GF/F filters which were then exposed to 37% hydrochloric acid fumes for 12 h and then immersed in scintillation cocktail. ^{14}C disintegration time per minute (DPM) was measured using the onboard 1414 liquid scintillation counter (PerkinElmer, USA) and the external standard and the channel ratio methods to correct for quenching. Dark ^{14}C uptake was subtracted from light uptake in the other 15 light incubation cells. Photosynthetic rates were calculated from total dissolved inorganic carbon (DIC) and Chl *a*. DIC was calculated from salinity and alkalinity. The broadband light-saturated Chla-specific rate of photosynthesis, P_m^B [mg C (mg Chla)$^{-1}$ h^{-1}], and the light-limited slope, α^B [mg C (mg Chla)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1})$^{-}$

212 [1], were estimated by normalising ^{14}C uptake to Chl *a* and fitting the data to the model of Platt
213 et al. (1980). Primary production (mg C m^{-3} d^{-1}) was subsequently calculated from
214 photosynthetically active radiation (E_{PAR}), Chl *a* and the photosynthetic parameters using the
215 brand model of Tilstone et al. (2003). E_{PAR} was modelled using the approach of Gregg &
216 Carder (1990) modified to include the effects of clouds (Reed, 1977) and using wind speed
217 and percentage cloud cover from the European Centre for Medium Range Weather
218 Forecasting (ECMWF) following Smyth et al. (2005).
219
220 *2.4.2. Low temperature fluorescence emission spectra*
221 Samples were filtered onto 25 mm GF/F filters from ~1 L of sea water and processed
222 following standard protocols (Suggett, Stambler, Prasil, Kolber, Quigg et al., 2009). A 4 x 10
223 mm piece of the filter was cut and placed onto a holder, flash frozen in liquid nitrogen and
224 measured immediately using a SM-9000 spectrometer (Photon Systems Instruments, Brno).
225 The instrument is based on a Zeiss MCS CCD spectrometer; the detector has 1044 pixels, the
226 grating images from 200-980 nm, the wavelength accuracy is 0.5 nm and the spectral
227 resolution is 3.5 nm (FWHM). Two gaussian shaped lines are separated at >3.5nm, with a
228 resolution of 3.2 nm from 0.8nm dispersion by 4 pixels. Whole fluorescence emission spectra
229 were determined using an integration time of 1000 ms. The fluorescence emission of blanks
230 (seawater pre-filtered through a 0.7 μm GF/F) was subtracted and the spectra were normalized
231 to 686 nm for de-convolution (Kaňa, Kotabová, Komárek, Papageorgiou, Govindjee et al.,
232 2012).
233
234 *2.5. CTD and Nutrients.*
235 A Seabird 911 plus CTD was deployed at each station at which experiments were conducted.
236 The data was processed using SeaBird software v7.21 and up and down casts were then

merged to 1 m binned resolution. Micro-molar nutrients were analysed using a 5 channel nitrate (Brewer & Riley, 1965), nitrite (Grasshoff, 1976), phosphate, silicate (Kirkwood, 1989) and ammonia (Mantoura & Woodward, 1983) Bran & Luebbe AAIII segmented flow, colourimetric, autoanalyser. Water samples were taken from a 24 x 20 litre bottle stainless steel framed CTD / Rosette system (Seabird). These were sub-sampled into clean (acid-washed) 60 mL HDPE (Nalgene) sample bottles. Subsequent nutrient analysis was complete within 1-2 hours of sampling. Clean handling techniques were employed to avoid contamination of the samples.

2.6. Statistical analysis

Paired T-tests samples were employed to test for significant differences between treatments at T48 on individual Exp.'s and for all Exp.'s. Results of T-test analyses from all Exp.'s (I-III) at 48 hrs are given in Table 2. Results of T-test analyses from individual Exp.'s at 48 hrs are given on each sub-figure. The T-test results are given as $T_{1,19} = x$ and $P = y$ where T is the deviation of the sample mean from the normally distributed parametric mean to parametric standard deviation ratio, the sub-script numbers (1, 23) denote the degrees of freedom and P is the T-test critical significance value.

3. Results

3.1. Initial hydrographic conditions

Temperature varied from 26 ºC to 29 ºC in the surface waters of the sub-tropical Atlantic (Figs. 1, 2). These stations were typically oligotrophic with a deep thermocline at between 50 and 75 m, low nitrate and nitrite (<0.02 µM), low surface Chl *a* (0.03-0.05 mg m^{-3}) and deep Chl *a* maxima between 70 and 130 m of between 0.1 and 0.3 mg m^{-3} (Fig. 2B, C, D). The

concentrations of nitrate+nitrite and silicate in the surface waters were beyond the detection limit (Harris, 2011).

3.2. Changes in carbonate chemistry

For all experiments, by 48 h the CO_2 treatment resulted in an average concentration of 748 ppm and 7.97 pH units compared to 468 ppm and 8.14 pH units in the control (Fig. 3). For the control, bubbling pre-mixed synthetic air with a CO_2 concentration of 360 ppm to natural seawater, resulted in > 460 ppm (Fig. 3). At 48 h the difference in CO_2 content and pH between the CO_2 treatment and control was ~280 ppm and 0.17 pH units, respectively (Fig. 3). Similarly, the mean HCO_3^- in the CO_2 treatment during these Exp.'s was 2013 ppm and 1860 ppm in the control (Fig. 4A, B, C) and the mean HCO_3^- : CO_2 ratio was 2.7 in the CO_2 treatment and 3.99 in the control (Fig. 4D, E, F).

The corresponding changes in total carbon (TC) and alkalinity (TA) during each experiment are given in Table 1. For all Exp.'s, the TA in the control and CO_2 enrichment was similar and consequently there was no significant difference in TA for Exp. II & III and all Exp.'s together (Table 2). There was a significant difference in TA between CO_2 and control at T48 for Exp. I ($T = 55.25$, $P = 0.012$). By contrast, TC was significantly higher in the CO_2 treatment during Exp. II ($T = 4.42$, $P = 0.048$) & III ($T = 7.08$, $P = 0.019$) and all Exp.'s together (Table 2), but this was not significant for Exp. I. Carbonate chemistry in the bottles equilibrated at between 12 & 24 h. For all Exp.'s, there was a significant difference in CO_2, pH, HCO_3^- and HCO_3^- : CO_2 TC, TA, (Table 2) between control and CO_2 treatments. At T48 in the CO_2 treatment, the CO_2 concentration was significantly higher than at T0 (Figure 3; Table 2).

3.3. Changes in phytoplankton community structure.

286 During Exp.'s I & II there was no significant difference in Chl *a* between CO_2 and
287 control treatments (Fig. 5) and as a consequence, for all Exp.'s. there was no significant
288 difference between treatments and with T0 (Table 2). In Exp. III, Chl *a* was significantly
289 higher in the control (Fig. 5C). Of the phytoplankton groups enumerated by flow cytometry,
290 nanoeukaryotes had the highest biomass in Exp.'s I and II, whereas in Exp. III the biomass of
291 nano- and picoeukaryotes and *Synechococcus* spp. were similar (Fig. 6, 7). During all Exp.'s,
292 there was no difference in nanoeukaryotes and picoeukaryotes between control and CO_2
293 treatments (Table 2). During Exp. I, there was little change in the biomass of nanoeukaryotes
294 and picoeukaryotes over the duration of the Exp.'s and there was no significant difference
295 between control and CO_2 treatment (Fig. 6A, D). During Exp. II, the initial biomass of
296 nanoeukaryotes and picoeukaryotes decreased at 24 h followed by a slight increase at 48 h,
297 but there was no significant difference in biomass between control and CO_2 treatments at 48 h
298 (Fig. 6B, E). In Exp. III, the biomass of these groups initially decreased, but at 48 h both
299 nanoeukaryotes and picoeukaryotes were significantly higher in the control compared to the
300 CO_2 treatments and T0 (Fig. 6C, F). During all experiments there was no significant
301 difference between control and CO_2 treatments for *Synechococcus* spp. and *Prochlorococcus*
302 spp. (Fig. 7, Table 2). The biomass of *Prochlorococcus* spp. and *Synechococcus* spp.
303 decreased from T0 to T48 and T0 was significantly higher compared to the control and the
304 CO_2 treatment (Fig. 7, Table 2). For individual Exp.'s, there was no significant difference
305 between treatments for both *Synechococcus* spp. and *Prochlorococcus* spp. in Exp. I, II & III
306 (Fig. 7).

307 From the microscopy samples, there was no significant difference in diatoms biomass
308 between treatments during Exp.'s II and III (Table 2). The total biomass in Exp.'s II was low
309 3.5 and 4.5 µg C L^{-1} at 48 h in CO_2 and control treatments, respectively when *Navicula* spp.
310 accounted for the highest biomass. In Exp. II, the diatom biomass was higher and 17 and 18

311 µg C L^{-1} in CO_2 and control treatments, respectively when *Rhizosolenia* spp. and *Navicula*
312 spp. dominated (Fig. 8A, B). In this Exp. *Navicula* spp. dominated the biomass in the CO_2
313 treatment, whereas in the control *Rhizosolenia* spp. biomass was higher than any other group
314 (Fig. 8A, B). The biomass of diatoms was significantly higher in T0 compared to both the
315 control and CO_2 treatments (Fig. 8A, B, Table 2). The diatom biomass may have been
316 reduced through silicate limitation.
317 Dinoflagellates exhibited the highest biomass of all phytoplankton groups during
318 Exp.'s II and III, reaching on average 60 µg C L^{-1} under CO_2 treatment and 30 µg C L^{-1} in the
319 control, though the differences between treatments were not significant (Table 2).
320 *Gymnodinium* spp. accounted for the majority of the biomass, especially in Exp. III (Fig. 8D).
321 The dinoflagellate biomass was also significantly higher in the control at T0 compared to T48,
322 but this was not the case for the CO_2 treatment (Fig. 8C, D, Table 2). T-test was not
323 performed on individual Exp.'s, since only single samples were enumerated at T48.
324
325 *3.4. Changes in photosynthesis and emission spectra*
326 For all Exp.'s there was no significant difference in P_m^B between control and CO_2 treatment
327 (Fig. 9A, B, C, Table 2). For the individual Exp.'s, there was no difference in P_m^B between
328 control and CO_2 treatment in Exp. I (Fig. 9A), but P_m^B was significantly higher in the CO_2
329 treatment compared to the control in both Exp.'s II and III (Fig. 9B, C). In all Exp.'s, α^B was
330 significantly higher in the CO_2 treatment compared to the control (Fig. 9D, E, F, Table 2).
331 There was no significant difference in α^B in the control between T0 and T48, but α^B was
332 significantly higher at T48 in the CO_2 treatment compared to T0 (Table 2).
333 Using spectral de-convolution of LT emission spectroscopy measurements, the
334 fluorescence peak at 678 nm is associated with uncoupled chlorophyll-containing PS antenna,
335 while 686 nm represents a mixture of signals from PS II chlorophylls and terminal emitters of

phycobilisomes (PBS) (Rakhimberdieva, Vavilin, Vermaas, Elanskaya & Karapetyan, 2007). Generally, the peak area at 678 nm decreased by more than three times from T0 to T48 in all Exp.'s and in both treatments (Fig. 10, Table 3). In all of the CO_2 treatments however, there was a significantly lower signal at 678 nm (Table 3), indicating reversible antenna connection after CO_2 enrichment. In addition, the spectral emission of the PSII core antenna increased from T0 to T48 (Fig. 10).

4. Discussion

4.1. Changes in the phytoplankton community in relation to carbonate chemistry

Experiments on CO_2 enrichment of natural samples in the Atlantic Ocean have mostly focused on the interactions between diatoms, *Phaeocystis* spp. and prymnesiophytes. The sub-tropical and tropical oligotrophic gyres are dominated by Cyanobacteria which make a significant contribution to the carbon fixation in the global ocean (Bell & Kalff, 2001). In the North Atlantic gyre, the phytoplankton assemblage at the deep Chl *a* maximum is typically dominated by picoeukaryotes, *Prochloroccocus* spp. At the DCM and *Synechococcus* spp. in the suface mixed layer (Tarran et al., 2006; Zubkov, Sleigh, Tarran, Burkill & Leakey, 1998), which exist at very low (beyond detection limit) nitrate and phosphate concentrations. Under climate change scenarios of a warming ocean, increased stratification is likely to reduce nutrient concentrations in the sub-tropical gyres (Sarmiento et al., 2004). We conducted experiments with elevated CO_2 in the North Atlantic sub-tropical gyre during autumn to evaluate the effects on phytoplankton community structure and photosynthetic rates. The Exp.'s were conducted with no nutrient addition to mimic the oligotrophic conditions of the gyre. The temperature at the experimental stations was 26 to 28°C and typical of stratified sub-tropical waters where irradiance is high. During the Exp.'s, the phytoplankton community was dominated by dinoflagellates, which was 55 to 80 times higher than the other groups, but

there was no significant difference between treatments and in some cases the biomass decreased from T0 to T48 (Fig. 8).

Understanding the response of dinoflagellates to both increases in CO_2 and temperature is key to detecting climate-driven perturbations in the ecosystem. In culuture experiments on autotrophic dinoflagellates, some strains of *Alexandrium fundyense* (Hattenrath-Lehmann, Smith, Wallace, Merlo, Koch et al., 2015), *Karenia brevis* (Errera, Yvon-Lewis, Kessler & Campbell, 2014), *Karlodinium veneficum* (Fu, Place, Garcia & Hutchins, 2010), *Prorocentrum minimum* and *Heterosigma akashiwo* (Fu, Zhang, Warner, Feng, Sun et al., 2008) grow significantly faster at high pCO_2. Additionally, *Alexandrium fundyense* experiences a significant increase in cell toxicity under elevated CO_2 (Hattenrath-Lehmann et al., 2015). There have been few studies on the response of *Gymnodinium* spp. and *Gyrodinium* spp., which exhibited high biomass in this study. Most *Gymnodinium* spp. are autotrophic, though some (e.g. *G. abbreviatum*, *G. heterostriatum*) are known heterotrophs (Tomas, 1996). Similarly many *Gyrodinium* spp. are autotrophic, though some (e.g. *G. lachryma*, *G. pingue*, *G. spirale*) are heterotrophic (Tomas, 1996), so the patterns shown in Figures 8C, D may also include some heterotrophs as we were not able to identify all *Gymnodinium* spp. and *Gyrodinium* spp. to species level. Calbet, Sazhin, Nejstgaard, Berger, Tait et al. (2014) studied the response of natural phytoplankton communities in a Norwegian Fjord using mesocosm experiments and found at elevated temperature and lower pH there was no difference in *Gyrodinium* spp. abundance. Wynn-Edwards, King, Davidson, Wright, Nichols et al. (2014) found that in continuous batch culture of Antarctic strains of *Gymnodinium* spp. that there was no difference in growth rates and cell size between CO_2 enrichment to 993 ppm and ambient, but there was a significantly higher cell polyunsaturated fatty acid content at the higher CO_2 concentrations.

In our Exp.'s Diatoms had the second highest biomass, but there was no difference between the elevated CO_2 treatment and ambient (Fig. 8, Table 2). In other studies, the growth rates of the diatoms *Skeletonema costatum* (Grev.) Cleve and *Chaetoceros* spp. increased at elevated CO_2 by 100% and 24%, respectively (Beardall & Raven, 2004; Kim, Lee, Shin, Kang, Lee et al., 2006). *Prochlorococcus* spp. exhibited the third highest biomass, however there was also no significant difference in the biomass of *Prochlorococcus* spp., and that of *Synechococcus* spp., picoeukaryotes, and nanoeukaryotes except in one Exp. III, in which there was a higher biomass of both picoeukaryotes and nanoeukaryote in the control (Fig. 6). The response of *Synechococcus* spp. in our experiments on natural samples contrasts the trends observed in culture experiments. For example, Fu et al. (2007) found that 750 ppm CO_2 stimulated the growth rate of *Synechococcus* spp. The different responses between *Synechococcus* spp. and *Prochlorococcus* spp. were thought to be due to differences in inorganic carbon acquisition systems associated with carbon limitation at low pCO2 in *Synechococcus* spp. (Fu et al. 2007). In contrast, we observed no difference in biomass between treatments under limiting nutrients. Similarly in natural phytoplankton communities Lomas et al. (2012) found that under replete P and Fe there was no significant response in the carbon-fixation of *Prochlorococcus* and *Synechococcus* spp. dominated communities to elevated CO_2 and a decrease in pH. In their study, pH was adjusted by acid-base manipulation (rather than direct enrichment with CO_2) and nutrient media were added to the incubations even though nitrate and phosphate concentrations were below detection on first collection of the samples. By contrast, in mesocosm exp.'s using natural phytoplankton communities with replete nutrient concentrations, Paulino, Egge and Larsen (2008) reported a lower abundance of *Synechococcus* spp. and a higher abundance of picoeukaryotes at high CO_2. By contrast, Low-Decarie, Fussmann and Bell (2011) found in experimental mesocosms that increases in CO_2 enhanced the competitive ability of chlorophytes relative to cyanobacteria. Similarly in

410 the North Atlantic, Feng et al. (2009) suggested that nanophytoplankton (coccolithophores)
411 may further increase in abundance relative to other phytoplankton groups in the later stages of
412 the spring bloom if future CO_2 concentrations and sea temperature continue to rise.

413 During our Exp.'s, dinoflagellates, diatoms and *Prochlorococcus* spp. decreased from
414 the initial T0 biomass, which may in part be due to the nitrate and silicate limitation or
415 grazing by heterotrophic and / or mixotrophic dinoflagellates, through confinement in bottles.
416 Nitrate and nitrite were low prior to confinement in all Exp.'s (Fig. 2) and silicate was beyond
417 the detection limit. The limited silicate availability meant that biomass of diatoms was low,
418 though it did increase in Exp. III. The biomass at T0 suggests that the cyanobacteria were
419 adversely affected by containment, which may have allowed the other groups to thrive due to
420 lack of competition and / or the recycled nutrients released during the decline of the
421 cyanobacteria biomass. Despite the initial decline, there was no clear response to elevated
422 CO_2 in the phytoplankton assemblage. Most studies on changes in the phytoplankton
423 community to elevated CO_2 report significant shifts or responses, however this may provide a
424 biased view since non-significant responses are rarely published (Lomas et al., 2012).

425

426 *4.2. Effects of CO_2 enrichment on phytoplankton photosynthesis and primary production.*

427 Aquatic photosynthesis is inherently under-saturated with respect to CO_2 (Tortell, Rau
428 & Morel, 2000), therefore increases in seawater CO_2 can enhance photosynthetic carbon
429 fixation (Riebesell, 2004). Some studies have observed a 40% increase in the uptake of DIC at
430 elevated CO_2 compared to present levels (Riebesell et al., 2007). It has been suggested that
431 the photosynthesis of dinoflagellates may be limited by current water column CO_2
432 concentrations and that they may benefit from rising atmospheric pCO_2 (Eberlein, de Waal &
433 Rost, 2014). There are a number of studies that contradict this hypothesis, for example, in
434 dilute batch cultures of the calcareous dinoflagellate *Scrippsiella trochoidea* and the toxic

435 dinoflagellate *Alexandrium tamarense* grown at a range of CO_2 concentrations (180–1200
436 ppm), there was no significant difference in photosynthetic and growth rates between
437 treatments, though *A. tamarense* did exhibit greater respiration rates at higher pCO_2 (Eberlein
438 et al., 2014). In our experiments, photosynthetic rates were significantly greater in the CO_2
439 enriched treatment (Fig. 9) and the dinoflagellates were probably able to maximise carbon
440 fixation due to lack of competition, since the biomass of the other groups was low (Fig. 6, 7,
441 8A, B). In Exp.'s II and III, when the dinoflagellates *Gymnodinium* and *Gyrodinium* spp.
442 dominated the biomass, significantly higher P_m^B and α^B at elevated CO_2 were associated with
443 a lower signal of unconnected PS antennae, suggesting that these previously unconnected PS
444 antennae become connected to facilitate the higher photosynthetic rates. In addition, there was
445 also a slight increase in fluorescence signal at 686 nm, due to a lower abundance of
446 *Prochlorococcus* spp. in the CO_2 treatment compared to the control, since this group do not
447 have PBS antennae (Partensky, Hess & Vaulot, 1999).

448 Photosynthetic carbon fixation is mediated by both pH and temperature dependent
449 enzymatic reactions via the Calvin-Benson Cycle (Giordano, Beardall & Raven, 2005; Portis
450 & Parry, 2007). Varying climate change scenarios of increased CO_2, temperature and a
451 decrease in pH may affect intra-cellular DIC uptake differently in different phytoplankton
452 groups or species (Badger et al., 1998). P_m^B and α^B and temperature are tightly coupled (Uitz,
453 Huot, Bruyant, Babin & Claustre, 2008), and different phytoplankton species are adapted to a
454 narrow temperature band with sharp decrease in photosynthetic rates beyond 20°C (Eppley,
455 1972; Raven & Geider, 1988) which can be modified through the synthesis of photoprotecting
456 rather than photosynethetic pigments (Kiefer & Mitchell, 1983). The enzyme that is primarily
457 responsible for photosynthetic carbon fixation is Ribulose Bisphosphate Carboxylase
458 (RuBisCO), though this does have some catalytic limitations (Giordano et al., 2005).
459 Phytoplankton have therefore adapted two strategies to maximise the performance of

460 RuBisCO; by either altering the affinity of RuBisCO or through CO_2-concentrating
461 mechanisms (CCMs). CCMs have a higher affinity for inorganic carbon (Giordano et al.,
462 2005; Raven, 2011a; Raven, 2011b; Reinfelder, 2011; Tortell et al., 2000),and through a
463 combination of RuBisCO activity or CCM, most species achieve photosynthetic saturation at
464 environmental CO_2 levels under light and nutrient replete conditions (Raven, 1997; Raven &
465 Johnston, 1991). The differences in photosynthetic efficiency and rates between different
466 phytoplankton groups can be partially explained by CCM and / or RuBisCO kinetics (Raven,
467 1997). The effect of an increase in CO_2 and decrease in pH may select phytoplankton species
468 or genotypes based on the efficiency of RuBisCO or CCM. Dinoflagellates possess Form II
469 RubisCO (Morse, Salois, Markovic & Hastings, 1995), which has low kinetic properties and
470 therefore a low photosynthetic efficiency (Tortell et al., 2000). They are abundant in low
471 nutrient oceanic waters (Margalef, 1979), have low growth rates attributed to their high basal
472 respiration rates (Tang, 1996), do not need to compete for their position in the ecosystem and
473 therefore rely on diffusive CO_2 uptake (Giordano et al., 2005). Dinoflagellates possess less
474 efficient CCMs and/or low affinities for CO_2, and may therefore benefit from living in a high
475 CO_2 world (Reinfelder 2011; Fu et al. 2012) and be highly sensitive to CO_2 (Rost et al.,
476 2008). Form II RUBISCO has a much lower selectivity for CO_2 over O_2 (Giordano et al.,
477 2005). To compensate for this, some marine dinoflagellates, such as *Prorocentrum minimum*,
478 *Heterocapsa triquetra*, *Ceratium lineatum*, *Protoceratium reticulatum* and *Peridinium*
479 *gatunense* have a CCM linked to carbonic anhydrases (CAs), the expression of which changes
480 in response to the external CO_2 concentration (Berman-Frank, Erez & Kaplan, 1998; Brading
481 et al., 2011; Ratti, Giordano & Morse, 2007; Rost, Riebesell & Sultemeyer, 2006). This
482 suggests that an increase in CO_2 stimulated efficiency in light absorption and utilization,
483 which resulted in the higher rates of carbon fixation shown in Figure 9. Using spectral de-
484 convolution of low temperature emission spectroscopy measurements, the peak at 691 nm is

emitted from active PS II chlorophylls and 711 nm from PS I chlorophylls (Kaňa et al., 2012). The fluorescence peak at 678 nm was assigned to uncoupled chlorophyll-containing PS antenna, while 686 nm represents a mixture of signals from PS II chlorophylls and terminal emitters of phycobilisomes (PBS) (Rakhimberdieva et al., 2007). The peak at 686 nm increased by a factor of two in all Exp.'s, which indicates either an increase in PBS content or the presence of the iron-stress protein, IsiA (Chauhan, Folea, Jolley, Kouril, Lubner et al., 2011). The lower signal of unconnected PS antennae in our Exp.'s maybe indicative of CCM functionality as the dominant dinoflagellates connect more antennae to PS II to achieve the higher P_m^B and α^B rates. The PS I chlorophyll peak at 711 nm remained constant and there was no obvious difference between treatments (Fig. 10, Table 2). Although semi-quantitative, the advantage of LT spectroscopy is that when it is applied to time series and bioassays, gradual changes in phytoplankton photochemical processes can be monitored. This was the case in the Exp.'s on this cruise, where more detailed biochemical or biophysical analyses cannot usually be performed. Further work is required to ascertain whether the higher photosynethetic rates we observed in *Gymnodinium*, and *Gyrodinium* spp. under CO_2 enrichment is a species or genotypic response. This response also needs to be further invetigated under different light conditions. For example, fluctuating light can affect dinoflagellate growth and photosynthesis with constant light and high CO_2 having a negative effect on the growth and quantum yield of *Prorocentrum micans* (Zheng, Giordano & Gao, 2015), which has implications for ocean warming and high stratification in a high CO_2 world.

Cyanobacteria and chlorophytes possess Form IB RuBisCO (Badger & Price, 2003). Cyanobacteria utilize active HCO_3^- and CO_2 pumps as their CCM to facilitate CO_2 fixation and maintain rapid growth at low external DIC concentrations (Badger & Price, 2003; Badger, Price, Long & Woodger, 2006). In Exp. III, *Prochlorococcus* sp. biomass was significantly higher in the control, possibly as a result of their high affinity for CO_2 uptake at low

concentrations or grazing of *Prochlorococcus* sp. biomass by heterotrophs or mixotrophs (Fig. 3D), but the photosynthetic rates in the control were significantly lower than the CO_2 treatment, principally because the dinoflagellate biomass was far higher in this Exp. By comparison, Fu et al. (2007) also observed that the photosynthetic rate and light harvesting efficiency of *Prochlorococcus* sp. *in vivo* was not affected by increasing CO_2. Similar to the patterns we saw in Exp.'s II & III, Fu et al. (2007) also observed only slight differences in growth and photosynthetic rates of *Synechococcus* spp. at 750 ppm CO_2 and control, though this changed when the temperature was elevated by 4 ºC. By comparison, Lomas et al. (2012) observed that natural phytoplankton assemblages dominated by cyanobacteria were able to adapt to changes in pH through modification of the assimilation number, which resulted in no significant differences in photosynthetic rates. We found in two of our Exp.'s that the assimilation number was significantly higher at elevated CO_2. The duration of our experiments represent short term responses to CO_2 enrichment and do not account for longer term adaptation and evolution, which can be complex (Collins & Bell, 2006). Our experiments also do not account for interactive synergies between CO_2, temperature, nutrients and light which may affect phytoplankton, as shown by other studies in the North Atlantic (Feng et al., 2009; Hare et al., 2007). Further work is required to elucidate these dual effects on phytoplankton community structure in the North Atlantic sub-tropical gyre.

What is the consequence of increases in CO_2 on water column integrated primary production? Some studies have reported no significant changes in primary production under elevated CO_2 (Goldman, 1999; Raven & Falkowski, 1999; Tortell & Morel, 2002; Tortell et al., 2000). We found in the North Atlantic sub-tropical gyre when dinoflagellates dominated, that there was a 25% increase in primary production over all Exp.'s at 760ppm CO_2 and that this was significant in Exp.'s II & III (Fig. 11). This is similar to the findings of Hein and

534 SandJensen (1997) who suggested that increasing CO_2 concentrations in seawater would
535 increase primary production by 15 to 19%.
536
537 **5. Conclusions**
538 Three CO_2 enrichment experiments were conducted on natural phytoplankton
539 assemblages in the North Atlantic sub-tropical gyre. The dinoflagellates, *Gymnodinium,* and
540 *Gyrodinium* spp., dominated the biomass but there was no significant difference in the
541 biomass of these and other phytoplankton groups at elevated CO_2 concentrations of 760 ppm
542 and 7.94 pH compared to ambient (468 ppm CO_2 and 8.33 pH). There were however,
543 significantly higher photosynthetic rates in the elevated CO_2 treatment which was due to the
544 connection of reversible photosystem antennae at higher CO_2 concentrations, which resulted
545 in a 25% increase primary production.
546
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Figure Legends.

Fig. 1. Stations in the North Atlantic at which CO_2 enrichment experiments were conducted overlaid on MODIS-aqua Sea Surface Temperature monthly composite image for October 2010.

Fig. 2. Initial profiles of temperature (blue line), nitrate (black line, open squares) and chlorophyll a (green line, filled squares) at stations sampled for the CO_2 enrichment experiments (A.) I, (B.) II, (C.) III. Dotted line is the euphotic depth.

Fig. 3. Changes in pCO_2 during experiments (A) I, (B) II, (C) III, and in pH during experiments (D) I, (E) II, (F) III in control (open circles) and 760 ppm CO_2 (closed circles). Broken line in (C) and (F) represents phases of (space) and dark (line) during the experiments.

Fig. 4. Changes in HCO_3^- during experiments (A) I, (B) II, (C) III, and in HCO_3^- : CO_2 during experiments (D) I, (E) II, (F) III in control (open circles) and 760 ppm CO_2 (closed circles). Broken line in (C) and (F) represents phases of light **(**space**)** and dark **(**line**)** during the experiments.

Fig. 5. Changes in Chlorophyll *a* during experiments (A) I, (B) II, (C) III. Broken line in (C) represents phases of light **(**space**)** and dark **(**line**)** during the experiments.

Fig. 6. Changes in the biomass of nanoeukaryotes in experiments (A.) I, (B.) II, (C.) III, and changes in biomass of picoeukaryotes in experiments (D.) I, (E.) II, (F.) III in control (open circles) and 760 ppm CO_2 (closed circles). Broken line in (C) and (F) represents phases of light (space) and dark (line) during the experiments.

Fig. 7. Changes in the biomass of *Synechococcus* spp. in experiments (A.) I, (B.) II, (C.) III, and changes in biomass of *Prochlorococcus* spp. in experiments (D.) I, (E.) II, (F.) III in control (open circles) and 760 ppm CO_2 (closed circles). Broken line in (C) and (F) represents phases of light (space) and dark (line) during the experiments.

Fig. 8. Changes in diatom biomass during experiments (A) II and (B) III, and dinoflagellate biomass during experiments (C) II and (D) III at time(T) 0 and 48 h in control (AIR) and 760 ppm CO_2 enrichment. In (A) and (B) brown is *Navicula* spp., orange is pennate datioms, light green is *Nitszchia* spp., dark green is *Thalassiosira* spp., light blue is *Rhizosolenia* spp. and dark blue is total diatom biomass. In (C) and (D) black is *Amphidinium* spp., dark blue is *Gonyaulax* spp., light blue is *Gymnodinium* spp., green is *Gyrodinium* spp., yellow is total dinoflagellate biomass (N=1; microscope transects = 4).

Fig. 9. Changes in P_m^B [mg C (mg Chla)$^{-1}$ h^{-1}] during experiments (A.) I, (B.) II and (C.) III. Changes in α^B [mg C (mg Chla)$^{-1}$ h^{-1} (µmol m^{-2} s^{-1})$^{-1}$] during experiments (D.) I, (E.) II and

(F.) III. Broken line in (C) and (F) represents phases of light **(space)** and dark **(line)** during the experiments.

Fig. 10. Changes in low temperature emission during experiment (A) I CO_2 treatment, (B) I control, (C) II CO_2 treatment, (D) II control, (E) III CO_2 treatment, and (F) III control (N=1). Spectra are: black – phycoerythrin; red – phycocyanin; light green - unconnected antenna; dark-blue – photosystem (PS) II or phycobilisomes anchoring pigments; yellow – PSII core antenna; cyanin – PSI; light-blue – vibrational peak; grey – cumulative sum of all spectra.

Fig. 11. Changes in Primary production [mg C m^{-3} d^{-1}] at 48h in control (grey bars) and 760 ppm CO_2 (black bars) for experiments I-II.