January 21, 2016

Dr. Jean-Pierre Gattuso
Editor,
*Biogeosciences*
Email: gattuso@obs-vlfr.fr

Dear Dr. Gattuso,

Thank you for your assistance with our manuscript: "**Increasing coccolithophore abundance in the subtropical North Atlantic from 1990 to 2014**" by K. M. Krumhardt, N. S. Lovenduski, N. M. Freeman, and N. R. Bates.

We greatly appreciate the thorough comments of the two anonymous referees. We believe their suggestions and comments have greatly improved our manuscript. Both reviewers wanted us to clarify assumptions regarding the use of pigment and satellite-derived PIC data as representative of coccolithophore abundance. We have added segments of text in the Methods and Discussion sections of our proposed revised manuscript, both of which discuss in detail the reasoning behind these assumptions and offer a plenitude of references to support the reasoning. Further, we added a large section to the Discussion describing the limitations of our study. Also, in response to the referee comments we have toned down the language regarding our conclusions and clarified the use of the word "abundance" throughout the revised manuscript. We offer this document, which contains the Original Reviews, Response to Reviewers and Proposed Revisions, as well as a latex-derived "difference" version of the manuscript highlighting the changes we propose.

We look forward to hearing from you regarding these changes and await further instructions.

On behalf of my co-authors and I, thank you.

Sincerely,

Kristen Krumhardt
(and co-authors)

# Original Reviews

## Anonymous Referee #1:

GENERAL COMMENTS

The paper by Krumhardt et al. presents pigment and satellite derived data from the Bermuda Atlantic Time-Series (BATS) site in the subtropical North Atlantic to argue for an increase over time in the abundance of coccolithophores. The authors then use ancillary data from BATS to link this increase in coccolithophore 'abundance' to DIC and bicarbonate ion concentrations, and suggest that increases in these are causing increases in coccolithophore calcification, photosynthesis and growth. This is a potentially thought provoking study and could add to the growing debate on whether coccolithophores are responding positively or negatively to current changes in their growth habitat.

However, there are two fundamental and key assumptions in the paper by Krumhardt et al. which are highly questionable and unsupported by research – making the current conclusions of the paper unsound. **[1]**

Assumption 1. Haptophyte pigment concentrations are an index for coccolithophore abundance (pg 18629, lns 26-29). Krumhardt et al. assume that all of the haptophyte pigment 19'-hexanoyloxyfucoxanthin (19'-hex) originates from coccolithophores. To support this key assumption they cite a paper which examined coccolithophore species composition at BATS (Haidar and Thierstein, 2001) from SEM analysis and ignored the full haptophyte community – this reference does not establish that coccolithophores dominate the haptophyte community in subtropical waters at BATS. (Note that the authors also cite the well-known book on coccolithophores by Thierstein and Young [2004], but is not clear in which chapter they find support for their assumption). Hence, the relative contribution of coccolithophores to total 19'-hex is unknown. The authors also completely ignore any potential effects of **[2]** photo-acclimation (i.e. changes in pigment or chlorophyll per cell, or chlorophyll to carbon ratios) on pigment concentrations or pigment ratios (i.e. 19'hex:Chlorophyll-a) – the high light/low nutrient concentrations in the upper 30 m at BATS will have significant impact on the cellular levels of pigments, and these will change with seasonal changes in light, nutrients, water-column stability and species composition. Hence, can the authors definitively say that the long-term trend is an increase in coccolithophore abundance and not an increase in either total haptophyte abundance or (cellular) pigment levels?

Assumption 2. Satellite derived PIC is reliable index for coccolithophore abundance. Satellite derived PIC reflects PIC from E. huxleyi rather than PIC from other species of coccolithophore – this is partly due to the high scattering from E. huxleyi coccoliths (e.g., Balch et al. 2005 J Geophys Res 110) and the propensity for this species to shed coccoliths into the surrounding water (i.e. the reflectance mostly comes from detached coccoliths rather than cells; Balch et al. 1996 Limnol Oceanogr). The detachment rate of E. huxleyi coccoliths from the coccosphere is dependent on growth rate and nutritional status. As coccoliths have

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negligible sinking rates, detached coccoliths (PIC) not associated with living cells can remain in the water-column after growing cells have dissipated (i.e. stopped growing or lost from the upper ocean via sinking, being eaten or viral lysis). Hence, it is extremely difficult to link the concentration of PIC with living coccolithophore biomass. Can the authors definitely say that the long-term trend is an increase in abundance and not an increase in the shedding of coccoliths into the water-column? Or a taxonomic change to a more E. huxleyi dominated community which has a greater propensity to shed coccoliths? | 3 |

Supporting data (e.g., flow cytometry data on haptophyte abundances, counts of coccolithophores) is needed to fully validate the proxies (pigments, satellite PIC) used in this study. Considerable toning down of the paper could significantly alleviate the difficulties with interpreting the proxies used, and allow the paper a firmer scientific footing. For example, changing the title to a question (Are coccolithophores increasing in the subtropical N Atlantic?), presenting two lines of evidence (haptophyte pigments, satellite PIC), fully discussing the difficulty of interpreting the evidence (coccolithophore/haptophyte ratio, photo-acclimation, reflectance issues over PIC in low Chl waters, detrital PIC), the potential drivers (DIC, bicarbonate ions, nutrients, temperature etc – without circular arguments) and significantly rewording the conclusions. | 4 |

SPECIFIC COMMENTS

Pg 18626, ln 11 – 'coccolithophore pigment data' – only coccolithophore pigment data? (see comments on interpretation of the haptophyte marker 19'hex) | 5 |

Pg 18626, ln 16 (and throughout) – 'coccolithophore abundance' ? the authors do not present abundance data (i.e. cell counts) or actual biomass (i.e. carbon), but rather present pigment concentration (absolute and relative) and satellite PIC (living and detrital biomass). Hence, the abstract and throughout the paper should be more specific when using the term abundance and reflect what the authors actually mean (pigments, chlorophyll-a, PIC). | 6 |

Pg 18626, ln 17-18 – It is not obvious from the abstract that the study looked at anything other than DIC and bicarbonate ion – negative results relative to other growth-influencing factors (nutrients, light) should also be reported. | 7 |

Pg 18627, ln 4 – Please explain what 'a substantial fraction of the primary producers' means – is this a substantial fraction of primary production, chlorophyll biomass, or cell abundance? What level is substantial (5% or 95%)? | 8 |

Pg 18627, ln 20-21 – Bach et al. (2015) should also be referenced here for the substrate-inhibitor ratio argument. Also, these authors were very specific about the difficulties in applying this concept to field data when other growth limiting factors were varying – this limitation of the ratio should be mentioned here to highlight that coccolithophore growth in the natural environment is not just limited by bicarbonate/H* (but nutrient and light availability). | 9 |

Pg 18628, ln 9 – Consider replacing 'challenging' with 'impossible'. | 10 |

Pg 18628, ln 24 – Tyrrell and Taylor (1996) is part modelling and part model-field data comparison and hence this sentence should reflect that this reference is not just field data. | 11 |

Pg 18629, ln 1 – Freeman and Lovenduski (2015) have no in situ field data – it is satellite derived – a better reference is needed (e.g. Feng et al. 2009 MEPS 388) | 12 |

Pg 18629, lns 26-29 – This is a crucial assumption of the paper, which needs further validation and discussion. Neither of the references cited support that coccolithophores are the main component of the haptophyte community. | 13 |

Pg 18630, ln 23 – More detail on the HPLC method for pigment detection at BATS is needed. Was it the same throughout? Did it change (and could this have changed the pigment ratios/signatures)? As the pigments are a major focus of the paper, much more methodological information is needed. | 14 |

Pg 18630, ln 26 – Letelier et al. (1993), although this reference is seminal it is also ∼15 years old and there are many more papers on pigment ratios and using pigments to interpret phytoplankton community composition. Have the authors considered a more up to date reference with more up to date pigment ratios? | 15 |

Pg 18631, ln 1 – 'relatively constant ratios with total Chl-a'. What evidence is there that these ratios do not change with photo-acclimation and taxonomic shifts? Such potential problems should be discussed. | 16 |

Pg 18635, ln 8-10 – It is surprising to find that Prochlorococcus is only found in the deep chlorophyll maximum ? have the authors considered that this is an issue with their pigment ratios? There are two ecotypes of Prochlorococcus, one high-light living in surface waters and one low-light living around the DCM ? the two have different (divinyl) chl-a to chl-b ratios whereas the authors (as in Letelier et al. 1993) only use one ratio (a deep Prochlorococcus ratio). | 17 |

Pg 18642, ln 16 – The units on the estimated coccolithophore abundance are wrong – it should be 143 x 103 cells L-1 and not 1.43 x106 cells L-1. Note that this is ∼7 times higher than the average cell concentration (20 x 103 cells L-1) determined from (SEM) cell counts by Haidar and Thierstain (2001) at BATS. This could be taken to imply that a large fraction of the haptophyte chlorophyll is not from coccolithophores, especially given that these estimates are based on E. huxleyi which only dominates in spring and other subtropical coccolithophore species may have much lower pigment content. It should also be noted that 100 coccolith coccospheres of E. huxleyi are rarely if ever seen outside of coccolithophore blooms or culture conditions. | 18 |

Pg 18643, lns 20-22 – This line is a key statement underlining the difficulty of linking coccolithophore abundance with the growth environment and hence warrants more attention and discussion. | 19 |

Section 4.4. Potential implications – this section is very speculative and could be summarised in a few lines (especially as there is little direct evidence at present that these changes are occurring). $\boxed{20}$

# Anonymous Referee #2:

The manuscript by Krumhardt et al. used phytoplankton pigment data from the Bermuda Atlantic Time Series collected over the last 20 years to derive potential changes in coccolithophore abundances. Furthermore, they correlated observed abundances with other environmental factors such as dissolved inorganic carbon concentrations, nitrate or temperature. Finally, they also looked into temporal changes in particulate inorganic carbon, known to be produced by coccolithophores, as derived by satellite observations. Overall this is $\boxed{21}$ an interesting data compilation, however, I do not agree with the main conclusions of this manuscript (see comments below).

General Comments:

1) Concerning deriving coccolithophore abundance from pigment data the authors make two key assumptions. First, that the ratio of marker pigments to chlorophyll a, such as 19-Hex for haptophytes, is constant in time and space, thus there is no acclimation to changes in light conditions, temperature or nutrient regime. Another inherent assumption here is that all $\boxed{2 \text{ \& } 22}$ haptophytes have the same 19-Hex to chlorophyll ratio, thus changes in the dominant species will not change the ratio and thus the estimate of overall haptophyte abundance. The second assumption is that it's only coccolithophores which contribute to haptophyte biomass, but what about other non-calcifying members which appear to be extremely diverse (compare Liu et al. 2009)?

2) Pondering over the chlorophyll a contribution by haptophytes integrated over the upper 30 meters shown in figure 5 I was intrigued about the apparent increase at the end of the time series of the Gaussian filtered data set as seemingly opposed to the single data points. I digitized the data and tried to reproduce the curve, however I didn't get a pronounced increase towards the end. Also, a linear fit through that dataset had a negative slope, $\boxed{23}$ indicating a decrease in haptophyte biomass (but also see comment #5) with time, contrary to the authors observations. Are there data points missing from figure 5? Please explain.

3) Invoking increases in DIC and HCO3- concentrations for increasing haptophyte concentrations in the last 20 years doesn?t seem to make too much sense. The issue I have with this is that at a reported rate of 1.4 micro mol/kg per annum, the overall increase would be on the order of 30 micro mol/kg. That?s about a 1.5% increase in DIC or HCO3- concentration, corresponding to an increase in pCO2 by about 50 micro atmospheres. Considering measured growth rate responses of coccolithophores in culture experiments (also see comment #1) I wouldn't expect a 37% increase (also see comment #2) in haptophyte biomass $\boxed{24}$ over time (also see comment #5). The DIC correlation of coccolithophore occurance (not

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concentration!) reported in the cited paper by Rivero-Calle et al. (2015) is over a much longer time span and thus DIC increase. In this respect, the most obvious explanation for increases in overall chlorophyll a and other phytoplankton taxa contributions with time seem increasing nitrate concentrations.

4) Satellite derived PIC concentrations during the last ten years shown in figure 5 do not seem to match haptophyte abundance estimated from marker pigments neither in the upper 30 nor 140 meters of the water column. Thus, I wonder which is the better or more reliable indicator for coccolithophore abundance? | 25 |

5) A recent study by Freeman and Lovenduski (2015) reported decreasing coccolithophore calcification in the Southern Ocean during the last 15 years while another by Winter et al. (2014) reported a poleward expansion of the bloom forming coccolithophore Emiliania huxleyi during the last 30 years. Both studies are based on satellite derived PIC estimates and the most obvious difference leading to such contrasting results is the time span for which the analysis was carried out. The same issue is affecting this study and can be seen in figure 7a. Depending on the chosen start and end year for trend estimates of haptophyte abundances the answer will be different. And it is not the majority of cases which show a positive trend as the authors claim but for the majority of cases there is no statistically significant trend! | 26 |

Specific comments and suggestions

1) P18627, L14: In the last six years following the Doney et al. summary on coccolithophore responses to increasing CO2 there has been a lot of progress in terms of process understanding. I suggest incorporating some more recent reviews. Furthermore, how exactly ocean acidification is going to affect coccolithophore populations in the future is still unknown. | 27 |

2) P18628, L7: To the best of my knowledge, Schlueter et al. (2014) did not report higher calcification rates at higher in comparison to lower CO2 treatments as suggested by the authors. | 28 |

3) P18629, L28: see general comment #1 above. | 29 |

4) P18633, L12: There was a similar period in the mid 90s with higher haptophyte derived chlorophyll a in the upper 30m (see also general comment #5 above). | 30 |

5) P18640, last paragraph: The argument that Synechococcus draws down DIC which could then limit the growth of coccolithophores could be made for any autotrophic group including coccolithophores. | 31 |

6) P18643, L2: A decrease in the opal to carbonate ratio observed in sediment traps reported by Antia et al. (2001) and Deuser et al. (1995) does not necessarily imply an increase in coccolithophore abundance at the oceans' surface. First, it is a ratio and changes in diatom abundance or species composition can equally explain observed changes, and second there could also be a change in calcium carbonate/biogenic silica preservation. | 32 |

Cited references:

Liu et al. (2009), Extreme diversity in noncalcifying haptophytes explains a major pig- ment paradox in open oceans, PNAS 106: 12803-12808.

Winter et al. (2014) Poleward expansion of the coccolithophore Emiliania huxleyi, JPR 36: 316-325.

# Response to Reviewers and Proposed Revisions

We thank both reviewers for their constructive comments, which have greatly improved our original manuscript. We propose the following changes for the the revised manuscript.

## Reviewer 1

$\boxed{1}$ We thank the reviewer for pointing out that these two assumptions, if unsupported, weaken the conclusions of the paper. As such, we have added more supporting information so readers are aware of the rationale underlying each of our assumptions (see below), and we have added a large subsection to the Discussion describing the limitations of our study.

$\boxed{2}$ The reviewer is correct that we have not adequately addressed the premise that haptophytes at BATS are mainly coccolithophores. However, there are several lines of evidence that the 19'-hex-containing haptophytes at BATS are mainly coccolithophores.

### Support from published BATS data:

Coccolithophore cell count data support the assumption that our calculated concentrations of Chl $a_{hapto}$ are mainly representative of coccolithophores. Haidar and Thierstein (2001; see their Figure 8) published a short timeseries of coccolithophore cell counts at BATS from 1991 to 1993. For the reviewer, we include a figure (Figure R1) showing the correlation between the Haidar and Thierstein cell counts and our calculated Chl $a_{hapto}$ over 1991 – 1993. The correlation is large and significant ($r^2 = 0.69$; p < 0.0000001) suggesting that Chl $a_{hapto}$ at BATS is largely from coccolithophores.

### Support from the literature:

Numerous published studies also support the conclusion that Chl $a_{hapto}$ is mainly from coccolithophores. First, haptophytes differ in their pigment compositions and, therefore, major groups within the haptophyte clade can be distinguished. Zapata et al. (2004) present pigment compositions of 37 species (65 strains) of haptophyte cultures, comprising eight haptophyte "types". Only types 6, 7, and 8 contain 19'-hex. Type 6 haptophytes are coccolithophores. Zapata et al. (2004) measured pigments in coccolithohphore species Emiliania huxleyi and Gephyrocapsa oceanica, both found at BATS (see Haidar and Thierstein, 2001). Type 6 haptophytes (coccolithophores) contain only trace amounts of 19'-butanoyloxyfucoxanthin (19'-but), while type 8 (Genus Chrysochromulina) contains more significant amounts of 19'-but.

Following Letelier et al. (1993), we eliminated the 'chrysophyte' portion of the 19'-hex pigment concentration using 19'-but measurements:

$$[19\text{'-hex}]_{prymn} = [\mathrm{P}/(\mathrm{P}-\mathrm{C})][(\mathrm{h}_T - \mathrm{b}_T\mathrm{C})],$$

where $\mathrm{h}_T$ is the total concentration of 19'-hex and $\mathrm{b}_T$ is the total concentration of 19'-but. P and C are the 19'-hex:19'-but ratios found in cultures of prymnesiophytes (a haptophyte

class that contains coccolithophores, see `http://www.marinespecies.org/aphia.php?p=taxdetails&id=115057`) and chrysophytes, respectively. This process effectively reduced the portion of chlorophyll a attributed to haptophytes (as determined by 19'-hex) by subtracting out phytoplankton that also contain 19'-but. Additionally, both haptophyte type 7 and 8 species examined in Zapata et al. (2004) are mainly found in colder water marine environments (e.g., *Phaeocystis antarctica*) and therefore are unlikely to reside at BATS. Therefore, according to the compilation of haptophyte species by Zapata et al (2004), the main 19'-hex-containing haptophytes (without containing 19'-but) at BATS are mostly haptophytes type 6 – coccolithophores.

Specifically at BATS, haptophytes have been documented to be coccolithophores – "Haptophyte taxa at BATS are calcifying coccolithophorids..." (Lomas and Bates, 2004). Further, Steinberg et al. (2001) derived the phytoplankton community structure at BATS using the methods of Letelier et al. (1993) and writes: "Prynmesiophytes (presumably largely coccolithophores) are consistently abundant in the time series...". Another study by Dandonneau et al. (2006) found that 19'-hex pigment measurements correlated well with coccolithophore biovolumes in low nutrient, open ocean environments (like BATS), but were uncorrelated in coastal or open ocean eutrophic domains where other haptophytes, such as Phaeocystis, reside. Additionally, several other studies have made this same assumption (e.g., Feng et al., 2009; Kavanaugh et al., 2014).

The CHEMTAX program has been used to calculate phytoplankton community structure for diverse marine environments (e.g., Mackey et al., 1996; Pinckney et al., 2015; Riebesell et al., 2007). CHEMTAX calculates relative chlorophyll a contributions of four groups of haptophytes. (For simplicity, we used methods from Letelier et al., 1993, rather than CHEMTAX, since this study focused on a subtropical gyre environment like BATS (Andersen et al., 1996).) Nevertheless, in CHEMTAX, haptophyte groups 1 and 2 do not contain 19'-hex, so they are not included in our measurements. Haptophyte group 4 contains 19'-hex and 19'-but pigments, and thus was eliminated from our measurements, as explained above. Haptophyte group 3 (containing 19'-hex with insignificant 19'-but) is commonly assumed to be coccolithophores (e.g., see Pinckney et al., 2015). Riebesell et al. (2007) also make the assumption that this portion of haptophytes (group 3 in CHEMTAX) is coccolithophores (specifically E. huxleyi in this study).

Therefore, some studies may disagree with the conclusion that haptophytes are mostly coccolithophores (as suggested by extreme haptophyte diversity reported in Liu et al., 2009), but there are numerous studies that agree (Steinberg et al., 2001; Lomas and Bates, 2004; Dandonneau et al., 2006; Zapata et al., 2004; Riebesell et al., 2007; Feng et al., 2009; Kavanaugh et al., 2014). As for the effects of photo-acclimation and nutrient limitation on pigment concentrations and ratios, we acknowledge this possibility should be mentioned as a caveat of our study.

Therefore, in response to this comment we have added a paragraph in the Methods section of the revised manuscript about our rationale regarding the assumption that Chl $a_{hapto}$ is mainly from coccolithophores. Further, we have added a section in the Discussion about the

limitations of our study, in which we discuss the assumptions inherent in the pigment data (mentioning possible influences of non-calcifying haptophytes species, photo-acclimation, dominant species shifts, and nutrient limitation on the pigment data).

In Methods:
*"Haptophyte pigments specifically were calculated using 19'-hexanoyloxyfucoxanthin (19'-hex) pigments and 19'-butanoyloxyfucoxanthin (19'-but). Coccolithophores and other prymnesio-phytes contain 19'-hex and negligible amounts of 19'-but, while some other phytoplankton (e.g., chrysophytes and Phaeocystis) contain significant amounts of both 19'-hex and 19'-but (Zapata et al., 2004; Letelier et al., 1993). Based on the relative concentrations of 19'-but to 19'-hex measured at BATS, we subtracted out the phytoplankton pigment contribution that contains both 19'-hex and 19'-but, as in Letelier et al. (1993), and multiply the remaining 19'-hex concentration by a 19'-hex to Chl a ratio found in calcifying haptophytes (Letelier et al., 1993). The result is the haptophyte chlorophyll a fraction, which in this study, we assume to be mainly from coccolithophores. This assumption is supported by several studies. Particularly at BATS, the dominant haptophyte group has been reported to be coccolithophores by Lomas and Bates (2004) and Steinberg et al. (2001). Further, there is a significant corre-lation ($p < 0.0000001$, $r^2 = 0.69$) between coccolithophore cell counts published in Haidar and Thierstein (2001) and calculated Chl a from haptophytes at BATS (data not shown). More generally, Dandonneau et al. (2006) report that coccolithophores dominate the haptophyte community in open ocean environments, such as BATS."*

In Discussion:
*"There are several caveats of this study that must be discussed before these results can be put into context. First, a primary assumption in this paper is that the haptophyte group is mainly composed of coccolithophores. Though high haptophyte diversity has been reported in open ocean regimes (Liu et al., 2009), this does not necessarily contradict the assumption that coccolithophores are the dominant type of haptophyte. Furthermore, other studies have shown coccolithophores to be the dominant haptophyte in open ocean sites, such as BATS (see Methods; Dandonneau et al., 2006; Lomas and Bates, 2004; Steinberg et al., 2001). We therefore assume that Chl $a_{hapto}$ is mainly representative of coccolithophores. However, as with any study that derives phytoplankton community composition from signature pigments, inherent uncertainties are associated with changes in pigment content within a phytoplankton group over time.*

*Overall pigment and PIC concentration per coccolithophore cell may be influenced by environmental conditions. For example, photo-acclimation and nutrient limitation can in-voke changes in pigment composition or calcification that are not necessarily associated with changes in overall abundance (Behrenfeld et al., 2015; Paasche, 1998). Dominant species shifts within a phytoplankton group could also influence pigment and/or PIC measurements. Nevertheless, given upward trends observed for both Chl $a_{hapto}$ and PIC (Figs. 5, 8) we feel the most probable explanation of these observations is increases in overall coccolithophore abundance. However, satellite-derived PIC measurements also contain inherent uncertain-ties."*

**3** Though it is difficult to say with certainty that coccolithophores are increasing in abundance based on PIC data, we take the overall positive trends in PIC as a qualitative support for the trends we saw in the pigment data. However, we are not the first study to use satellite-derived PIC concentration as a proxy for coccolithophore abundance. For example, Gregg and Casey (2007) use satellite-derived PIC data to evaluate the simulation of coccolithophores in the NASA Ocean Biogeochemical Model. Also, Sadeghi et al. (2012) used MODIS-Aqua PIC data to validate coccolithophore abundance estimates from hyperspectral satellite sensor data. In any case, though it may only be qualitative, there appears to be an increasing occurrence of PIC in the subtropical North Atlantic. Nevertheless, we acknowledge that this is a source of uncertainty in our study. We have changed a sentence in the introduction to reflect that the PIC data is mainly qualitative.

In Introduction:
*"Therefore, satellite estimates of PIC provide a **qualitative** proxy for coccolithophore abundance."*

Additionally, Reviewer #1 brings up some interesting points regarding detachment of and detrital coccoliths, as well as potential species shifts. We have added these points to the "Limitations of our study" section in the Discussion.

In Discussion:
*"Overall pigment and PIC concentration per coccolithophore cell may be influenced by environmental conditions. For example, photo-acclimation and nutrient limitation can invoke changes in pigment composition or calcification that are not necessarily associated with changes in overall abundance (Behrenfeld et al., 2015; Paasche, 1998). Dominant species shifts within a phytoplankton group could also influence pigment and/or PIC measurements. Nevertheless, given upward trends observed for both Chl $a_{hapto}$ and PIC (Figs. 5, 8) we feel the most probable explanation of these observations is increases in overall coccolithophore abundance. However, satellite-derived PIC measurements also contain inherent uncertainties.*

*Radiance-based algorithms for deriving PIC from satellite reflectance data are formulated to capture the light scattering properties of the numerically dominant coccolithophore, E. huxleyi, but also capture detached or detrital coccoliths (Gordon et al., 2001; Balch et al., 2005). PIC concentrations in the North Atlantic subtropical gyre are comparatively low, generally $\sim$2.7 mg m$^{-3}$, compared to other coccolithophore bloom regions, which have PIC concentrations between 10 and 100 mg m$^{-3}$ (Balch et al., 2005). The low concentrations of PIC observed in the North Atlantic subtropical gyre could be within background error or nearing the sensitivity threshold of the instrument. Errors in satellite-derived PIC can arise from atmospheric correction, inclusion of other suspended minerals (such as silica; "opal contamination"), and/or the influence of chlorophyll or colored dissolved organic matter (see Balch et al., 2005). However, these errors can be minimized by binning in space and time, as we have done in this study (using monthly, 9 km data rather than daily, 4 km data). It is also curious that SeaWiFS-derived PIC data better matches the Chl $a_{hapto}$ estimates from*

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*BATS than the MODIS PIC (Fig. 5). On one hand, this may be indicative that other 19'-hex-containing haptophytes were responsible for the increase in Chl $a_{hapto}$ at BATS during the last several years of the time-series. On the other hand, the predominance of upward trends in MODIS-derived PIC for areas around BATS (Fig. 8b,d) suggests increases in calcifying haptophytes (coccolithophores). It should be noted that MODIS PIC from the BATS region (see Fig. 1) still shows a significant correlation with Chl $a_{hapto}$ (see Results, section 3.5). "*

**4** We have demonstrated that chlorophyll a from haptophytes is well correlated with coccolithophore abundance through the only coccolithophore cell counts available from BATS (Figure R1). As mentioned above, we have added more explanation about the haptophyte pigments to the Methods section. We have added an extra section to the Discussion on the limitations of this study in order to better inform the reader about these two major assumptions on which our conclusions are based. Also, we have toned down the language throughout the manuscript regarding these two assumptions. However, we prefer to keep the title, as this still describes our main conclusions.

**5** Regarding this issue, see the response **2** to the 'Assumption #1' comment above. We prefer to keep the wording "coccolithophore pigment data" in the abstract for simplicity. The additional section in the Methods section clarifies the rationale behind this assumption.

**6** This is a good point. We have clarified the use of the word "abundance" throughout the manuscript, specifying whether we are referring to pigment or PIC data.

**7** We have added more information to this sentence in the abstract, reporting the influence of nitrate (as well as the bicarbonate ion) in explaining variability in chlorophyll a from haptophytes.

In Abstract:
*"We further demonstrate that variability in coccolithophore chlorophyll a here is positively correlated with variability in nitrate and DIC (and especially the bicarbonate ion) in the upper 30 m of the water column."*

**8** We changed 'primary producers' to 'primary production', as measured by chlorophyll a. Coccolithophores can comprise between 1% to > 30% of the chlorophyll a concentration in subtropical gyre environments (this study-see Figure 2, Steinberg et al., 2001; Cortés et al., 2001). Under bloom conditions they can approach 100% of the phytoplankton community.

**9** We have added a sentence explaining that other growth limiting factors could override the [HCO3]:[H] ratio, citing Bach et al. (2015).

In Introduction:
*"Therefore, a decreasing $[HCO_3^-]/[H^+]$ ratio may eventually hinder calcification, rather than low $CO_3^{2-}$ concentrations. Even so, other factors (e.g., nutrient and light limitation) could exert a stronger control on calcification than carbonate chemistry (Bach et al., 2015)."*

**10** We appreciate the suggestion, but prefer to keep 'challenging', as laboratory studies can

often help clarify observations from the field. Using the word 'impossible' could diminish the significance of laboratory studies.

☐11☐ We have added this information to the sentence.

In introduction:
*"These results have been supported by field data from the subtropical Pacific Ocean (Cortés et al., 2001) and through a combination of field data and modeling in the NE Atlantic Ocean (Tyrrell and Taylor, 1996)."*

☐12☐ We appreciate the suggestion and have added the reference to Feng et al. (2009).

☐13☐ We have updated the references and toned down the language regarding this assumption:

In introduction:
*"Coccolithophores, a haptophyte algae, are identified using signature pigments for haptophytes (mainly 19'-hexanoyloxyfucoxanthin), of which coccolithophores are **likely** the main component (Dandonneau et al., 2006), particularly at BATS (see Methods, Haidar and Thierstein, 2001; Steinberg et al., 2001; Lomas and Bates, 2004)."*

☐14☐ The methods for HPLC analysis of phytoplankton pigments is well documented on the BATS website (`http://bats.bios.edu/bats_methods.html`). The methods have not changed over the course of the dataset. We provide the BATS website in the text. As we have already summarized the method, we see no reason to add additional technical details that are well described elsewhere. We have, however, added more explanation about the calculation of haptophyte pigments, as described above (see response ☐3☐).

☐15☐ We appreciate the suggestion, but the Letelier reference is still highly cited (at least 25 citations per year for the last two years, according to Web of Science) and the pigment ratios have been confirmed for the Atlantic subtropics by Andersen et al. (1996). Since we used the exact methods of Letelier et al. (1993), we prefer to just cite this paper.

☐16☐ As with any study that uses signature pigment ratios to determine phytoplankton composition, there are inherent uncertainties regarding photoacclimation, species shifts, etc. We have added a discussion of these points to the "Limitations of this study" section of the Discussion.

In Discussion:
*"Overall pigment and PIC concentration per coccolithophore cell may be influenced by environmental conditions. For example, photo-acclimation and nutrient limitation can invoke changes in pigment composition or calcification that are not necessarily associated with changes in overall abundance (Behrenfeld et al., 2015; Paasche, 1998). Dominant species shifts within a phytoplankton group could also influence pigment and/or PIC measurements. Nevertheless, given upward trends observed for both Chl $a_{hapto}$ and PIC (Figs. 5, 8) we feel the most probable explanation of these observations is increases in overall coccolithophore*

*abundance. However, satellite-derived PIC measurements also contain inherent uncertainties. "*

**17** This is a good point. Prochlorococcus with low amounts of chl b (high-light Prochlorococcus) would likely be grouped with Synechococcus (they both have zeaxanthin) or in the "other" category. We have added a sentence to clarify that Chl $a_{pro}$ is mainly representative of low-light Prochlorococcus.

In Results:
*"Prochlorococcus pigments (mostly low-light Prochlorococcus, see Discussion) at BATS reside mostly in the deep chlorophyll maximum at ~ 100 m of depth and are a relatively minor component of the Chl a in the upper 30 m of the euphotic zone."*

In Discussion:
*"Prochlorococcus reside mainly in the deep chlorophyll maximum, comprising a rather small portion of the Chl a in the upper 30 m at BATS (Figs. 2, 4). This could be, however, due to the relatively high Chl b to Chl a ratio used in our pigment calculations (Letelier et al., 1993), which is more representative of low-light Prochlorococcus (Partensky et al., 1999). "*

**18** We thank the reviewer for finding this error in our units. We have corrected this.

This back of the envelope calculation was intended to relate the haptophyte pigments to satellite PIC. The cell counts that were made by Haidar and Theirstein (2001) were from 1991-1993, a relatively low period of haptophyte pigments in our dataset (see Figures 1 and 5). In any case, Haidar and Theirsten display maximum abundances of ~100 x $10^3$ cells L$^{-1}$, which is on the same order of magnitude as we report here (especially given the roughness of this calculation).

We present 100 coccoliths per cell as a maximum under severe nutrient limitation. Even though it may be rarely observed in nature, laboratory results report that, under severe nutrient limitation, E. huxleyi has the capability to produce this many coccoliths (Paasche, 1998) and, thus, it should be included in our range.

**19** Figure 3 displays correlations between phytoplankton groups and their growth environment. We discuss this figure in detail (Discussion section 4.1) and speculate about multiple controls on growth. For example, haptophytes dominate when there is high DIC, high $NO_3^-$, and low temperatures. The Synechococcus group dominates under low DIC, low $NO_3^-$, and higher temperatures. We report that 52% of the variability in Chl $a_{hapto}$ may be explained by $NO_3^-$ and $HCO_3^-$. In response to this comment, we have:

1) Added a sentence to section 4.1 (where we discuss multiple linear regressions) to emphasize that other controls are likely important for determining overall haptophyte abundance;

*"A multiple linear regression of Chl $a_{hapto}$ with both $HCO_3^-$ and $NO_3^-$ explained > 50 % of the variance in Chl $a_{hapto}$ ($r^2 = 0.52$). Other factors, such as competition or grazing, could perhaps account for some of the remaining variability."*

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2) Mentioned the role of nitrate in the Abstract; and

*"We further demonstrate that variability in coccolithophore chlorophyll a here is positively correlated with variability in nitrate and DIC (and especially the bicarbonate ion) in the upper 30 m of the water column."*

3) Added a sentence to the Introduction after the discussion of in the influence of carbonate chemistry on coccolithophore growth.

*"Therefore, a decreasing $[HCO_3^-]/[H^+]$ ratio may eventually hinder calcification, rather than low $CO_3^{2-}$ concentrations. Even so, other factors (e.g., nutrient and light limitation) could exert a stronger control on calcification than carbonate chemistry (Bach et al., 2015)."*

[20] We appreciate the suggestion, but the section is only two paragraphs long. Given that our results are in line with those of Rivero-Calle et al. (2015), we feel that the discussion of these potential implications is warranted and helps put the study into a larger context. We consistently use the word "could" (e.g., "overall increases in coccolithophore abundance *could* increase DMS production.") to assure the reader that this is speculation.

## Reviewer 2

[21] We respond to specific comments below, clarifying how we arrived at the main conclusions of this manuscript.

[22] We appreciate the reviewer pointing out this assumption. Since Reviewer #1 raised the same concern, we refer Reviewer #2 to this response (see above, under 'Assumption #1', response [2]).

To summarize, Liu et al. (2009) report on extreme haptophyte diversity, but do not necessarily contradict the assumption that the dominant haptophytes at BATS are coccolithophores. First, not all haptophytes contain the pigment 19'-hex (and we subtract out the ones that contain both 19'-hex and 19'-but). Secondly, the dominant haptophyte group at BATS has been reported to be coccolithophores by several studies (Lomas and Bates, 2004; Steinberg et al., 2001). Further, there is a significant correlation ($p < 0.0000001$, $r^2=0.69$) between published coccolithophore cell counts and Chl $a_{hapto}$ (see Figure R1). Lastly, Dandonneau et al. (2006) report that coccolithophores dominate the haptophytes in open ocean environments such as BATS.

[23] We thank the reviewer for bringing this to our attention. Indeed, some of the higher value data points are cut off in the plot, as we zoomed in on the filtered time-series. Please see Figure R2, if the reviewer would like to digitize all the data. We have added an explanation to the captions of Figures 5 and 6:

*"We restricted the y-axes in panels (a) and (b) to highlight the filtered data."*

[24] DIC and $HCO_3^-$ are both highly positively correlated with Chl $a_{hapto}$. ($p = 2.03 \times 10^{-10}$

for DIC and p = 2.69 x $10^{-12}$ for $HCO_3^-$). As mentioned in the paper, multiple linear regression analysis revealed that $HCO_3^-$- and $NO_3^-$ explain 52% of the variability in Chl $a_{hapto}$. Also stated, the trends in $NO_3^-$ at BATS are only significant when the last two years are included in the data (see Figure R3). We appreciate the comment and have added an extra sentence to the abstract to clarify the influence of $NO_3^-$ in explaining the variability in Chl $a_{hapto}$. Though increases in $NO_3^-$ likely contributed to the increasing Chl $a_{hapto}$ trends during the last two years of the study period, overall $HCO_3^-$ trends appear to better match those observed in Chl $a_{hapto}$ (see Figure 7a, b compared to Figure R3).

About the DIC increase: Published studies have demonstrated that photosynthesis in coccolithophores can become carbon limited at $CO_2$ concentrations measured at BATS ($\sim$10 micromol $L^{-1}$; see Figure 2 in Riebesell, 2004). Increasing DIC ($CO_2$ and $HCO_3^-$) may help alleviate some of this carbon limitation (Bach et al., 2013). Ecosystem processes are known to be highly non-linear (e.g., Belgrano et al., 2004). Therefore, a lessening of carbon limitation in coccolithophores could increase the overall competitive ability of coccolithophores and result in higher increases in their pigments than the overall increase in DIC. Of course, this is all speculative given the trends and correlations we are observing. In the revised manuscript we assure that any sentence referring to this idea (that coccolithophores are increasing in response to increasing carbon) is clear with respect to the fact that it is only speculation.

Lastly, total Chl $a$ has not been increasing for most of the study period at BATS or in the rest of the subtropical gyre (Figures 7d and 9). Therefore, we cannot say that nitrate is causing overall increases in Chl $a$, as the reviewer suggested.

**25** This is a good point. The SeaWiFS-derived PIC data seem to better match the pigment record at BATS than does MODIS-derived PIC. We had already stated this point statistically (see section 3.5, Relating BATS pigment data to satellite products), but now this is also mentioned in the Discussion section about the limitations of this study:

*"It is also curious that SeaWiFS-derived PIC data better matches the Chl $a_{hapto}$ estimates than the MODIS PIC (Fig. 8). On one hand, this may be indicative that other 19'-hex-containing haptophytes were responsible for the increase in Chl $a_{hapto}$ at BATS during the last several years of the time-series. On the other hand, the predominance of upward trends in MODIS-derived PIC for areas around BATS (Fig. 8b,d) suggests increases in calcifying haptophytes (coccolithophores). It should be noted that MODIS PIC from the BATS region (see Fig. 1) still shows a significant correlation with Chl $a_{hapto}$ (see Results, section 3.5). "*

**26** We aimed to be clear about the length of the time-series with regard to trends (Figure 7). Of course, longer time-series are always preferred, but so far this is the longest published time-series on phytoplankton community structure for the subtropical North Atlantic. We appreciate the comment and have added this as a caveat of our study in the Discussion section (Limitations of this study).

*"Finally, we are limited in our trend analysis by the length of the time-series data. Figure 7 demonstrates that different start and end years can influence the sign and magnitude of our*

*trends in, e.g., Chl a$_{hapto}$. In this study, we report trends in pigments from 1990 to 2012 and trends in PIC from 1998 to 2014, both of which, when employing the full time-series of data, imply increases in coccolithophore populations in the subtropical North Atlantic."*

**27** We thank the reviewer pointing this out. In the revised manuscript, we have added a citation to Mackey et al. (2015) who provide recent and comprehensive overview on how coccolithophore photosynthesis, calcification, and PIC/POC ratio could be affected by ocean acidification. This article emphasizes the numerous recent papers that have predicted a reduction in coccolithophore calcification with ocean acidification.

**28** Schlüter et al. (2014) report that PIC production was restored to present-day values (no response) under high-$CO_2$ treatments. We state, "However, some recent studies show that coccolithophores have no response or even increase calcification in response to increasing $CO_2$ (e.g., Iglesias-Rodriguez et al., 2008; Schlüter et al., 2014)." Therefore, the Schlüter et al. (2014) study is an example of a study that saw no change in calcification.

**29** In the revised manuscript, we have updated the cited references with those that specifically support this assumption. Also we toned down the language by saying that coccolithophores are "likely" the main component of the 19'-hex containing haptophytes (see comment **13** above).

**30** We have modified the sentence in the revised manuscript to reflect this observation:

In Results:
*"During the mid-90s and last six years of the dataset, Chl a$_{hapto}$ was more concentrated, especially in the upper 30 m of the water column (Fig. 1b)".*

**31** Yes, but coccolithophores are the only group that have been shown have an inefficient carbon uptake system and the potential to become carbon-limited at the DIC concentrations measured at BATS (Rost et al., 2003; Riebesell, 2004). To clarify this reasoning, we have added citations to articles that support this hypothesis.

**32** The reviewer makes a good point. Given the ambiguity of interpreting changes in the opal to carbonate ratio, we have removed the sentence referring to the Antia and Deuser articles from the Discussion in the revised manuscript.

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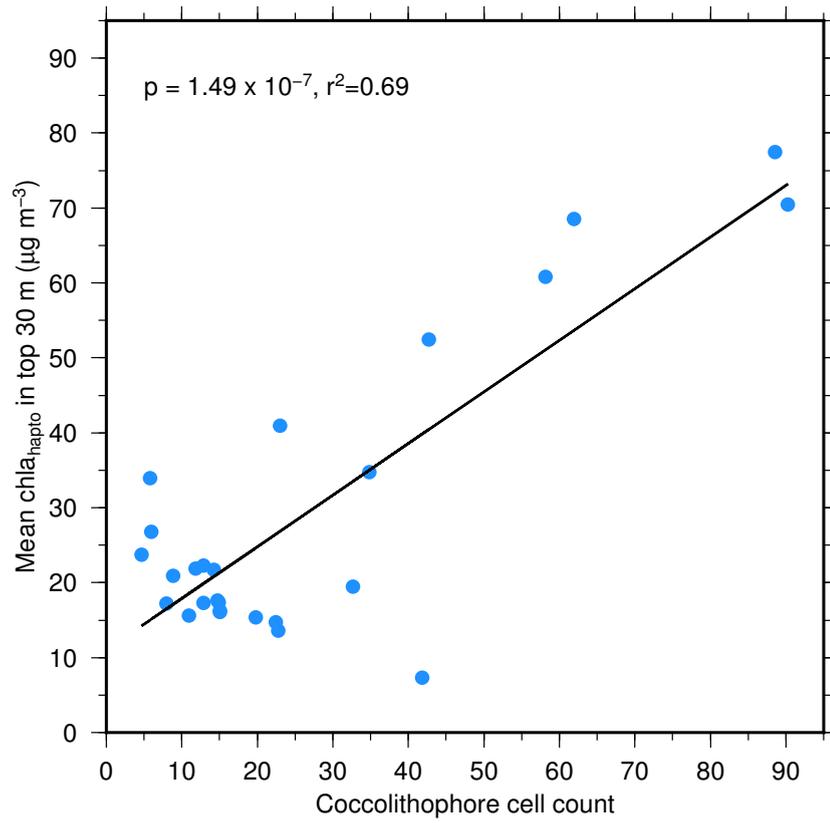
Figure R1: Mean Chl $a_{hapto}$ in the top 30 m versus coccolithophore cell counts from Haidar and Thierstein (2001). Pigment and count data (from 1991 – 1993) were resampled at monthly intervals and a least-squares line was fit to the data.
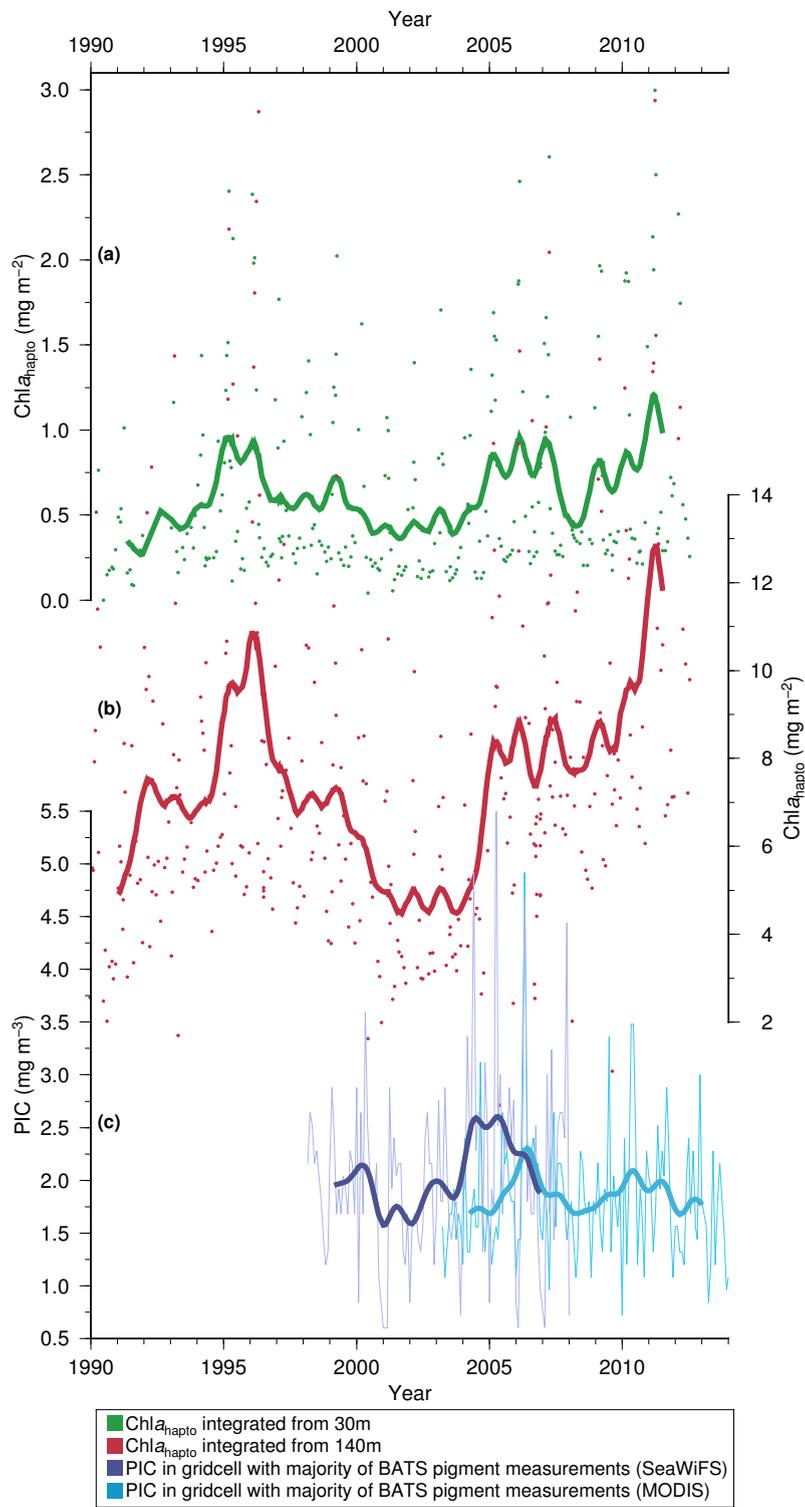
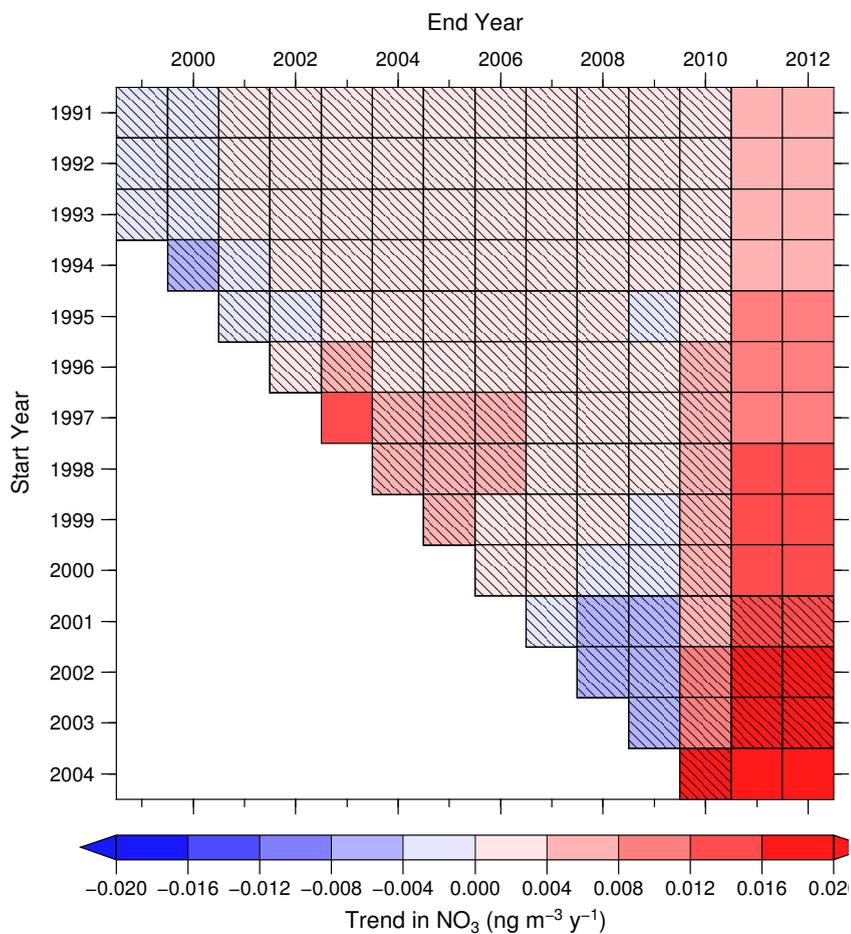Figure R2: A version of Figure 5 with all the unfiltered data points.

Figure R3: Linear trends in mean $NO_3^-$ in the upper 30 m at BATS for a range of start and end years. Boxes with hatch lines demarcate nonsignificant trends ($p > 0.05$).

# Increasing coccolithophore abundance in the subtropical North Atlantic from 1990 to 2014

**K. M. Krumhardt**[1]**, N. S. Lovenduski**[2]**, N. M. Freeman**[2]**, and N. R. Bates**[3]

[1]Environmental Studies Program and Institute of Arctic and Alpine Research, University of Colorado Boulder, Boulder, Colorado, USA
[2]Department of Atmospheric and Oceanic Sciences and Institute of Arctic and Alpine Research, University of Colorado Boulder, Boulder, Colorado, USA
[3]Bermuda Institute of Ocean Sciences, Bermuda

Correspondence to: K. M. Krumhardt (kristen.krumhardt@colorado.edu)

## Abstract

As environmental conditions evolve with rapidly increasing atmospheric $CO_2$, biological communities will change as species reorient their distributions, adapt, or alter their abundance. In the surface ocean, dissolved inorganic carbon (DIC) has been increasing over the past several decades as anthropogenic $CO_2$ dissolves into seawater, causing acidification (decreases in pH and carbonate ion concentration). Calcifying phytoplankton, such as coccolithophores, are thought to be especially vulnerable to ocean acidification. How coccolithophores will respond to increasing carbon input has been a subject of much speculation and inspired numerous laboratory and mesocosm experiments, but how they are currently responding in situ is less well documented. In this study, we use coccolithophore pigment data collected at the Bermuda Atlantic Time-series Study (BATS) site together with satellite estimates (1998–2014) of surface chlorophyll and particulate inorganic carbon (PIC) to show that coccolithophore populations in the North Atlantic Subtropical Gyre have been increasing significantly over the past two decades. Over 1991–2012, we observe a 37 % increase in euphotic zone-integrated coccolithophore pigment abundance at BATS. We further demonstrate that variability in coccolithophore ~~abundance~~ chlorophyll *a* here is positively correlated with variability in nitrate and DIC (and especially the bicarbonate ion) in the upper 30 m of the water column. Previous studies have suggested that coccolithophore photosynthesis may benefit from increasing $CO_2$, but calcification may eventually be hindered by low $pH_T$ ($< 7.7$). Given that DIC has been increasing at BATS by $\sim 1.4\ \mu mol\ kg^{-1}\ yr^{-1}$ over 1991 to 2012, we speculate that coccolithophore photosynthesis and perhaps calcification may have increased in response to anthropogenic $CO_2$ input.

## 1 Introduction

Coccolithophores are the most abundant type of calcifying phytoplankton in the ocean. Belonging to the phytoplankton group known as haptophytes, coccolithophores ~~comprise~~ generate a substantial fraction of the primary ~~producers~~ production in many diverse marine

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environments from cold, sub-polar waters to warm, tropical waters (Thierstein and Young, 2004). Coccolithophores produce calcium carbonate ($CaCO_3$) shells that sink to the deep ocean forming chalk deposits and thus are important for global biogeochemical cycling of carbon and climate feedbacks. Further, coccolithophores comprise the base of many marine food webs and are widespread throughout the world ocean (Boyd et al., 2015). Changes in coccolithophore abundance could therefore have far-reaching effects from the ecosystem level to global carbon cycling (Boyd et al., 2015).

Ocean acidification from the gradual oceanic absorption of anthropogenic $CO_2$ has been projected to impact future coccolithophore populations, possibly disrupting the formation and/or dissolution of their $CaCO_3$ shells ~~(e.g., Doney et al., 2009)~~ (e.g., Doney et al., 2009; Macke As $CO_2$ is absorbed from the atmosphere, it reacts with water releasing hydrogen ions ($H^+$) and increasing dissolved inorganic carbon (DIC). Excess $H^+$ ions and more DIC in the water column lead to a decrease in the carbonate ion ($CO_3^{2-}$) concentration in the ocean (for an overview of $CO_2$-carbonate chemistry see Bates et al., 2014). Lower $CO_3^{2-}$ concentrations decrease the saturation state of $CaCO_3$ and could result in lower calcification rates in coccolithophores. Recently, Bach (2015) proposed that a substrate-inhibitor ratio may be a better indicator of potential biocalcification rates. Bicarbonate ions ($HCO_3^-$) are the substrate for calcification in most calcifying organisms, but high concentrations of $H^+$ ions can limit calcification. Therefore, a decreasing $[HCO_3^-]/[H^+]$ ratio may eventually hinder calcification, rather than low $CO_3^{2-}$ concentrations. Even so, other factors (e.g., nutrient and light limitation) could exert a stronger control on calcification than carbonate chemistry (Bach et al., 2015). A $CO_3^{2-}$ saturation state of less than one would still, however, cause the dissolution of $CaCO_3$ shells. Speculation of how coccolithophores will respond to increasing DIC and acidification (i.e., the balance between $CaCO_3$ production vs. dissolution) has been the subject of many laboratory and mesocosm studies (e.g., Iglesias-Rodriguez et al., 2008; Schlüter et al., 2014; Riebesell et al., 2007). These, however, have yielded mixed results, highlighting the complexity of biological responses to these changing oceanic conditions.

Numerous laboratory studies indicate that acidification of oceanic waters leads to a decrease in calcification rates for coccolithophores (Riebesell et al., 2000; Sciandra et al.,

2003; Delille et al., 2005). However, some recent studies show that coccolithophores have no response or even increase calcification in response to increasing $CO_2$ (e.g., Iglesias-Rodriguez et al., 2008; Schlüter et al., 2014). Indeed, responses to elevated $CO_2$ by different species of coccolithophores vary in all directions, making the extrapolation of these laboratory results to natural populations challenging. How calcifying phytoplankton will react to continually increasing $CO_2$ may vary from region to region in the world's oceans, depending on phytoplankton assemblages (e.g., the dominant species or strain of coccolithophore), available nutrients, and temperature ~~(Sett et al. , 2014).~~ (Sett et al., 2014) . Coccolithophore responses to ocean acidification may be species specific or even vary within species (different *morphotype* responses, see Beaufort et al., 2011).

Differences in physiological mechanisms could play an important role in determining relative phytoplankton abundances under increasing DIC and acidification. For instance, nutrient uptake rates vary between phytoplankton species depending on the affinity of the transport mechanism for its substrate (e.g., phosphate ion). Laboratory experiments on the widespread coccolithophore *Emiliania huxleyi* have shown this species to have an efficient phosphate uptake system with a low half-saturation constant for phosphate, making it a superior competitor in phosphate-limited oceanic regions (Riegman et al., 2000). These results have been supported by field data from the subtropical Pacific Ocean (Cortés et al., 2001) and through a combination of field data and modeling in the NE Atlantic Ocean (Tyrrell and Taylor, 1996). For inorganic carbon uptake for photosynthesis, however, *E. huxleyi* displays a relatively high half-saturation constant compared to other phytoplankton ~~(Riebesell, 2004)~~ (Riebesell, 2004; Rost et al., 2003) , indicating that coccolithophores may benefit from increasing atmospheric $CO_2$ absorbed into the ocean. However, there are few in situ or observational studies of coccolithophore responses to increasing anthropogenic carbon ~~(e.g., Freeman and Lovenduski, 2015)~~ (e.g., Feng et al., 2009; Freeman and Lovenduski,

To test the hypothesis that coccolithophores may be responding positively to additional $CO_2$ inputs, we employed data from the Bermuda Atlantic Time-Series (BATS), a long running oceanic time series in the North Atlantic Subtropical Gyre (Sargasso Sea) located at approximately 31.7° N, 64.2° W (Fig. 1). At this site, twice-monthly and monthly hydro-

graphic and biogeochemical measurements have been made since the late 1980s (Fig. 1; Lomas et al., 2010; Bates et al., 2014). The BATS environment is characterized by Ekman down-welling and convergence, which results in an oligotrophic setting (Sarmiento and Gruber, 2006). While this area displays strong summer stratification (mixed layer depth $\sim 35\,\mathrm{m}$), seasonal overturning results in a deep mixing of the water column during winter (mixed layer depth $\sim 250\,\mathrm{m}$; Steinberg et al., 2001; Lomas et al., 2013). Though oligotrophic oceanic gyres have relatively low productivity compared to other areas of the oceans, they cover vast areas and thus are important on a global scale (Sarmiento and Gruber, 2006). Furthermore, the strong summer stratification experienced in these regions could be indicative of future trends, as increased stratification of the water column is projected with global warming (Gruber, 2011; Giovannoni and Vergin, 2012; Cabré et al., 2015). Thus, understanding phytoplankton dynamics subject to these environmental conditions is essential for accurately forecasting future ocean biogeochemistry.

Pigment analyses have been used to study the distribution, relative abundance, and assemblages of natural phytoplankton populations. Using high performance liquid chromatography (HPLC) to identify the presence and concentration of signature pigments, researchers can obtain relative components of chlorophyll *a* (Chl *a*) from phytoplankton (Letelier et al., 1993; Wright and van den Enden, 2000; Van Lenning et al., 2004). Coccolithophores, a haptophyte algae, are identified using signature pigments for haptophytes (mainly 19'-hexanoyloxyfucoxanthin), of which coccolithophores are ~~a main component (Thierstein and Young, 2004)~~ likely the main component (Dandonneau et al., 2006) , particularly at BATS ~~(Haidar and Thierstein, 2001)~~ (see Methods, Haidar and Thierstein, 2001; Steinber While HPLC pigment analyses can provide a site-specific record of phytoplankton relative abundance, satellite-based records can provide information at larger spatial scales.

Ocean color remote sensing, through the Sea-viewing Wide Field-of-view Sensor (SeaWiFS; 1997–2010) and the Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua (2002-present) platforms, has revolutionized our understanding of the ecological processes of the upper ocean on a variety of spatial and temporal scales. Satellite-estimated Chl *a* concentration has been used as a proxy for phytoplankton abundance and biomass since 1978.

While most phytoplankton are not very effective light scatterers relative to their surroundings, coccolithophores produce $CaCO_3$ shells that are highly reflective. An understanding of coccolithophore-specific water-leaving radiances and the calcite-specific backscattering cross section allows for the concentration of coccolithophore particulate inorganic carbon (PIC) to be estimated via remote sensing (Gordon et al., 2001; Balch et al., 2005; Balch and Utgoff, 2008). Therefore, satellite estimates of PIC provide a ~~suitable~~ qualitative proxy for coccolithophore abundance.

In this study, we combine pigment data from BATS along with PIC and Chl *a* measurements from the satellite record to assess recent trends in phytoplankton dynamics in the North Atlantic subtropical gyre, with a focus on coccolithophores. ~~We show~~ This data suggests that coccolithophore populations in the North Atlantic are increasing~~in abundance.~~ ~~Correlations suggest that they have been~~ . Correlations indicate that they may be responding positively to increasing inorganic carbon from anthropogenic inputs in the upper mixed layer.

## 2   Methods

### 2.1   Data sources

Pigment measurements were obtained from the BATS website (http://bats.bios.edu), resampled at regular monthly intervals using a linear interpolation between measurements, and converted to relative Chl *a* components from different phytoplankton groups as in Letelier et al. (1993). Briefly, each phytoplankton group is associated with signature pigments that have relatively constant ratios with total Chl *a*. Signature pigment concentrations from each phytoplankton group, obtained via HPLC analysis, were converted to Chl *a* concentration using these ratios. This method has been verified in the North Pacific (Letelier et al., 1993) and the North Atlantic subtropical gyres (Andersen et al., 1996). We focused on measurements from the upper water column (top 30 m), consistently within the mixed layer (Steinberg et al., 2001; Lomas et al., 2013), but also examined trends and variability inte-

grated over the depth of the euphotic zone ($\sim$140 m) to verify congruence with the top 30 m of the water column.

Haptophyte pigments specifically were calculated using 19'-hexanoyloxyfucoxanthin (19'-hex) pigments and 19'-butanoyloxyfucoxanthin (19'-but). Coccolithophores and other prymnesiophytes contain 19'-hex and negligible amounts of 19'-but, while some other phytoplankton (e.g., chrysophytes and *Phaeocystis*) contain significant amounts of both 19'-hex and 19'-but (Zapata et al., 2004; Letelier et al., 1993) . Based on the relative concentrations of 19'-but to 19'-hex measured at BATS, we subtracted out the phytoplankton pigment contribution that contains both 19'-hex and 19'-but, as in Letelier et al. (1993) , and multiply the remaining 19'-hex concentration by a 19'-hex to Chl *a* ratio found in calcifying haptophytes (Letelier et al., 1993) The result is the haptophyte chlorophyll *a* fraction, which in this study, we assume to be mainly from coccolithophores. This assumption is supported by several studies. Particularly at BATS, the dominant haptophyte group has been reported to be coccolithophores by Lomas and Bates (2004) and Steinberg et al. (2001) . Further, there is a significant correlation ($p < 0.0000001$, $r^2 = 0.69$) between coccolithophore cell counts published in Haidar and Thierstein calculated Chl *a* from haptophytes at BATS (data not shown). More generally, Dandonneau et al. (2 that coccolithophores dominate the haptophyte community in open ocean environments, such as BATS.

In order to explore whether phytoplankton population dynamics are driven by carbonate chemistry parameters, we used the Mocsy Fortran 90 package (Orr and Epitalon, 2015) to solve the full carbonate chemistry system using available measurements at BATS (Bates et al., 2012). Using dissociation constants from Lueker et al. (2000), carbonate chemistry output from Mocsy agrees with other current carbonate system packages available (Orr et al., 2014). Input includes average concentrations of total alkalinity and DIC along with mean temperature and salinity in the top 30 m. Output includes $pH_T$, $CO_3^{2-}$ concentration, bicarbonate ($HCO_3^-$) concentration, and aqueous $CO_2$ + carbonic acid ($CO_{2(aq)} + H_2CO_3 = H_2CO_3^*$).

We used satellite observations of level 3, monthly binned PIC and Chl *a* from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS; 1997–2007; limited data availability after

2007) and MODIS Aqua (2003–2014) on a 9 km (5 min) grid obtained from the NASA Ocean Colour distributed archive (http://oceancolor.gsfc.nasa.gov/). We calculated the mean satellite-derived PIC concentration in the BATS region that contains $> 95\%$ of pigment measurements (Fig. 1a).

## 2.2 Statistical analyses

### 2.2.1 Correlations

In order to identify correlations of phytoplankton pigment abundance across different species, with local environmental variables, and with satellite products, we performed correlation analysis on linearly detrended and deseasonalized (1 year boxcar smoothing) anomalies. Correlations between the main Chl *a* phytoplankton fractions present at BATS (*Prochlorococcus*, *Synechococcus*, haptophytes, and diatoms) were calculated for a variety of oceanic measurements hypothesized to influence phytoplankton abundance: DIC, alkalinity, inorganic nitrogen concentrations, temperature, salinity, and carbonate chemistry variables (see above section on carbonate chemistry). We also explored possible correlations between Chl *a* phytoplankton fractions and the mixed layer depth (MLD). We used two methods for calculating MLD. $\text{MLD}_{\text{sigma}}$ was determined to be where the potential density anomalies (sigma-theta) at depth displayed a $> 0.125\,\text{kg}\,\text{m}^{-3}$ difference from surface waters, while $\text{MLD}_{\text{temp}}$ was calculated as the location of $0.5\,^{\circ}\text{C}$ change in temperature from the surface (Monterey and Levitus, 1997). Mean density and temperature measurements within the top 10 m of the water column were used for "surface" values. We also tested correlations with the monthly mean North Atlantic Oscillation (NAO) index, obtained from NOAA National Weather Service Climate Prediction Center (http://www.cpc.ncep.noaa.gov/data/teledoc/nao.shtml).

### 2.2.2 Trends

In order to quantify temporal trends in pigment concentrations, biogeochemical measurements, and satellite data, we calculated the slope of a straight line that best fit the time-

series in a least-squares sense. When comparing trends in pigments with trends in biogeochemical measurements at BATS, we used the average values over the top 30 m of the water column. Satellite Chl *a* and PIC trend analysis was performed on a grid cell basis.

## 3 Results

### 3.1 Chl $a_{hapto}$ at BATS

~~Haptophytes (i.e., coccolithophores; Thierstein and Young, 2004; Haidar and Thierstein, 2001) are~~ Chl *a* from haptophytes (Chl $a_{hapto}$; assumed to be primarily from cocccolithophores, see Methods) is present throughout the euphotic zone at BATS and ~~display~~ displays a pronounced seasonal cycle. Concentrations of Chl ~~*a* from haptophytes (Chl~~*a*$_{hapto}$ ) surpassed $100\,\mu g\,m^{-3}$ during periods of high abundance, with $\sim 35\,\mu g$ Chl $a_{hapto}\,m^{-3}$ during ~~during~~ periods of relatively low abundance, such as between 2000 and 2004 (Fig. 1b). The bulk of haptophyte pigments occurred around $\sim 80\,m$ of depth, but pigments were also abundant in the upper 30 m, especially during spring. Haptophyte ~~abundance~~ pigment concentration is low below depths of $\sim 140\,m$. During the mid-90s and last six years of the dataset, Chl $a_{hapto}$ was more concentrated, especially in the upper 30 m of the water column (Fig. 1b).

Haptophytes comprise roughly 30 % of the Chl *a* in the upper 30 m of the water column at BATS (Fig. 2), a percentage that persists from the start of measurements ($\sim 1990$) to the end of our dataset ($\sim 2012$). However, a period of low haptophyte abundance occurred between 2000 and 2004, reducing their relative contribution to Chl *a* to 15 %. *Synechococcus* Chl *a* is variable, ranging from 20 to 70 % of the total Chl *a* in the upper 30 m. Unfortunately, signature pigment concentrations necessary to calculate *Synechococcus* Chl *a* were missing from the BATS dataset at the beginning of our time-series as well as during a five-year segment from $\sim 1997$ to $\sim 2002$ (shown as hatched area in Fig. 2). In contrast to generally high *Synechococcus* and haptophyte pigment abundance, *Prochlorococcus* and ~~diatoms~~ diatom pigments contribute relatively small fractions to the total Chl *a* in the upper 30 m.

Correlations with potentially influential oceanographic drivers can aid to explain variable abundance of different phytoplankton ~~groups~~ Chl *a* fractions in these surface waters.

## 3.2 Correlations of Chl *a* components with oceanographic measurements at BATS

Correlation coefficients between detrended, deseasonalized anomalies of Chl $a_{\text{hapto}}$, Chl *a* from *Synechococcus* (Chl $a_{\text{syn}}$), Chl *a* from *Prochlorococcus* (Chl $a_{\text{pro}}$), and Chl *a* from diatoms (Chl $a_{\text{diatoms}}$) and anomalies in other oceanographic measurements/indices are presented in Fig. 3. Chl $a_{\text{hapto}}$ shows strong positive correlations with DIC and $HCO_3^-$ (explaining nearly 20 % of the variability). Chl $a_{\text{pro}}$ showed similar, but somewhat weaker, correlations with DIC and $HCO_3^-$. Conversely, ~~*Synechococcus*~~ Chl $a_{\text{syn}}$ displayed a strong negative correlation with DIC and $HCO_3^-$ (Fig. 3). All phytoplankton pigment groups, except *Synechococcus*, were positively correlated with nitrate ($NO_3^-$) variability (measurements also include nitrite, $NO_2^-$; $NO_3^- + NO_2^-$ is referred to hereinafter as $NO_3^-$). $CO_3^{2-}$ concentration, the saturation state of aragonite ($\Omega_{\text{arag}}$), and temperature were negatively correlated with ~~haptophyte abundance~~Chl $a_{\text{hapto}}$, opposite to ~~*Synechococcus*~~Chl $a_{\text{syn}}$. Indeed, *Synechococcus* and ~~haptophytes~~ haptophyte pigments display inverse correlations for nearly every variable tested, including temperature (Fig. 3).

Temperature is negatively correlated with Chl $a_{\text{hapto}}$ (as well as Chl $a_{\text{pro}}$ and Chl $a_{\text{diatoms}}$) and positively correlated with Chl $a_{\text{syn}}$ (Fig. 3). Further, when the North Atlantic Oscillation (NAO) index is in a positive phase, temperatures over this region of the North Atlantic are generally warmer (Visbeck et al., 2001). Therefore, in line with the temperature correlations, the NAO index is negatively correlated with Chl $a_{\text{hapto}}$, opposite again to Chl $a_{\text{syn}}$. Mixed layer depth (MLD), an indicator of both temperature and nutrient availability, shows corresponding correlations with Chl *a* components. When the MLD is deeper, there is more Chl $a_{\text{hapto}}$ and less Chl $a_{\text{syn}}$. Both methods of calculating MLD ($MLD_{\text{temp}}$ and $MLD_{\text{sigma}}$) showed similar correlations (Fig. 3).

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### 3.3 Fluctuations in ~~abundance of~~ chlorophyll *a* from different phytoplankton groups

~~Haptophytes~~ Haptophyte and *Synechococcus* pigments generally show opposing correlations with the variables tested (Fig. 3). This is supported by the opposing dominance of either *Synechococus* or ~~haptophytes~~ haptophyte Chl *a* throughout the timeseries (Fig. 4). Indeed Chl $a_\text{hapto}$ and Chl $a_\text{syn}$ show a significant negative correlation ($p < 0.00001$). During periods of low haptophyte ~~abundance~~ pigment bundance (e.g., 2000–2004), *Synechococcus* pigments dominate the water column. Later in the time-series, however, *Synechococcus* ~~populations~~ pigments decline and haptophyte ~~abundance increases.~~ pigments increase. ~~*prochlorococcos*~~*Prochlorococcus* pigments (mostly low-light *Prochlorococcus*, see Discussion) at BATS reside mostly in the deep chlorophyll maximum at $\sim 100\,\text{m}$ of depth and are a relatively minor component of the Chl *a* in the upper 30 m of the euphotic zone. Diatom ~~abundance~~presence, usually associated with cold, high nutrient environments, is sporadic and generally low in this oligotrophic oceanic region of the subtropical North Atlantic, according to their signature pigments (Fig. 4).

In general, pigment analyses indicate that *Synechococcus* and haptophytes are the most abundant phytoplankton groups at BATS (Figs. 2 and 4). However, the category classified as "other" (includes chrysophytes, dinoflagellates, prasinophytes, among others) in Fig. 3 comprises a large portion ($> 30\,\%$) of the phytoplankton biomass during certain periods, indicating certain conditions may favor neither *Synechoccocus* nor haptophytes. The opposing correlations *Synechoccocus* ~~or haptophytes~~ and haptophyte pigments exhibit with DIC, $HCO_3^-$, $CO_3^{2-}$, $NO_3^-$ and temperature could lead to interesting trends in phytoplankton abundance in an ocean increasingly influenced by anthropogenic climate change.

### 3.4 Trends in Chl $a_\text{hapto}$ and total Chl *a* at BATS

A time-series of Chl $a_\text{hapto}$ at BATS shows that haptophytes (~~i.e., coccolithophores; Fig. 5a, b~~mainly coccolithophores, see Methods), have been increasing significantly since 1990 ($p < 0.01$ for 30 m integral; $p < 0.001$ for 140 m integral; Fig. 5a, b). Mean concentration

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of Chl $a_{\text{hapto}}$ in the upper 30 m of the water column has increased by 0.848 µg m$^{-3}$ yr$^{-1}$ (standard error = 0.332), corresponding to a 68 % increase over the course of the BATS time-series (1991–2012; an overall increase of 17.8 µg m$^{-3}$), while the 140 m integral of Chl $a_{\text{hapto}}$ has increased by 0.103 mg m$^{-2}$ yr$^{-1}$ (standard error = 0.0307) corresponding to a 37 % increase (an overall increase of 2.2 mg m$^{-2}$). We assess the sensitivity of these trends to interannual variability by performing trend calculations for a range of start and end years over the time-series. The resulting trend pattern shown in Fig. 7a shows mostly positive trends in ~~haptophyte abundance~~Chl $a_{\text{hapto}}$, except for end years in the 2000–2004 period. Total chlorophyll a (Chl $a_{\text{total}}$) also shows a significant positive trend over the time-series ($p < 0.05$ for 30 m integral; $p < 0.001$ for 140 m integral; Fig. 6; upper right corner of Fig. 7c). Figure 7c shows trends in Chl $a_{\text{total}}$ for a range of start and end years, displaying a different pattern than that of the trends in the Chl $a_{\text{hapto}}$ component of Chl a. For instance, for end years in the 2000–2004 period, Chl $a_{\text{total}}$ shows positive trends (but nonsignificant), whereas Chl $a_{\text{hapto}}$ shows significant negative trends (Fig. 7). Mean Chl a in the upper 30 m primarily exhibits negative trends in the later part of the time-series and most trends in Chl a are nonsignificant (Fig. 7c). Unfortunately, missing *Synechococcus* pigment data did not allow for long-term trend analysis of this group of phytoplankton (see hatched area in Fig. 2 and white area in Fig. 4).

In line with the results of our correlation analysis, the trends in HCO$_3^-$ for various start and end years show a similar pattern to the trends in Chl $a_{\text{hapto}}$ (Fig. 7b): mostly positive trends with slightly negative (but nonsignificant) trends in HCO$_3^-$ concentration for end year in the 2000–2004 period, a low point in haptophyte ~~abundance~~pigments. Conversely, the trend pattern for the substrate-inhibitor ratio, [HCO$_3^-$]/[H$^+$], is distinctly different from that of Chl $a_{\text{hapto}}$, exhibiting all negative trends (Fig. 7d). Trends in ~~calcifying haptophyte (coccolithophore)abundance~~ Chl $a_{\text{hapto}}$, assumed here to be mainly representative of coccolithophore (see Methods), can be further corroborated with particulate inorganic carbon (PIC) measurements from the satellite record.

### 3.5 Relating BATS pigment data to satellite products

Significant correlations were detected between Chl $a_{hapto}$ (30 m integral) measured at BATS and PIC derived from each satellite. The SeaWiFS-derived PIC correlated somewhat better with Chl $a_{hapto}$ than MODIS-derived PIC (SeaWiFS PIC-Chl $a_{hapto}$ $p = 0.0075$, $r^2 = 0.19$ vs. MODIS PIC-Chl $a_{hapto}$ $p = 0.050$, $r^2 = 0.12$). This difference is likely inherent in the different algorithms used to estimate PIC from each satellite (see following paragraph). Nevertheless, these correlations demonstrate correspondence between Chl $a_{hapto}$ measurements and satellite PIC, both of which ~~denote~~ relate to relative coccolithophore abundance.

Two radiance-based PIC algorithms can be used to relate water-leaving radiance to calcite absorption and scattering properties: a two-band algorithm (Balch et al., 2005) and a three-band algorithm (Gordon et al., 2001). The North Atlantic subtropical gyre exhibits relatively low PIC concentrations year-round. During the SeaWiFS/MODIS overlap period (2003–2007), PIC estimated from the two satellites revealed stark differences (Fig. 5c), possibly explained by the differences in algorithm performance in this region (i.e., sensitivity to low/background PIC concentrations). The low correspondence between the two estimates of PIC prevented the generation of a single, merged PIC time-series. We therefore report trends in PIC separately over the respective satellite eras (Fig. 8).

Chl $a$ measured at BATS (30 m integral) and Chl $a$ derived from satellite were significantly correlated ($p < 0.01$, $r^2 = 0.16$). In this case, Chl $a$ measured by satellite displayed good correspondence between the two satellite eras and could be merged into one time-series. Following the regression technique of Brown and Arrigo (2012), we generated one continuous record of Chl $a$ from 1998 to 2014 by applying linear regression over the 2003–2007 SeaWiFS/MODIS overlap period to predict these variables from 2008 to 2014 (Fig. 6c).

### 3.6 Regional trends in satellite PIC and Chl $a$

Linear trends in PIC derived from satellite observations are positive for most of the North Atlantic subtropical gyre (Fig. 8). Nearly all significant trends ($p < 0.05$) in PIC concentration are positive, especially during the MODIS era (Fig. 8c, d; 1998–2007 for SeaWiFS and

2003–2012 for MODIS). However, unlike at the BATS site, Chl *a* does not appear to be increasing in most of the gyre (Fig. 9). There are slight positive trends in Chl *a* around the BATS region (Fig. 9a), but these are not statistically significant (Fig. 9b). Indeed, most of the North Atlantic subtropical gyre shows a slight negative trend in Chl *a* or no trend at all. A trend of a subset of the satellite Chl *a* from 1998 to 2012 shows a slight, but nonsignificant, upward trend in Chl *a* in the BATS region (Fig. 1; 0.0009 mg m$^{-3}$ yr$^{-1}$, $p > 0.05$), just as the corresponding gridbox for Chl *a* at BATS in Fig. 7.

## 4 Discussion

### 4.1 Phytoplankton dynamics at BATS

In this study, we observed that coccolithophore populations, based on pigment data for haptophytes, are increasing at BATS and are positively correlated with DIC and HCO$_3^-$ (Figs. 1b, 3, 7a). We observed opposite correlations for DIC and HCO$_3^-$ with *Synechococcus*, the other major member of the phytoplankton community at BATS. Some studies have suggested that photosynthesis and growth of the coccolithophore, *E. huxleyi*, is carbon limited and could possibly benefit from increasing CO$_2$ ~~(Riebesell, 2004; Bach et al., 2013)~~ (Riebesell, 20 Since CO$_{2(aq)}$ and HCO$_3^-$ concentrations increase with increasing DIC/ocean acidification, both photosynthesis and calcification could be stimulated in coccolithophores, which primarily use CO$_2$ for photosynthesis and HCO$_3^-$ for calcification (Bach et al., 2013). The results presented in this study support the hypothesis that coccolithophores are responding positively to increasing carbon availability, perhaps increasing their competitive ability in oligotrophic settings such as BATS. However, a threshold H$^+$ ion concentration could be reached with further ocean acidification, eventually constraining coccolithophore growth.

*Synechococcus*, on the other hand, was negatively correlated with increasing carbon in the upper mixed layer and positively correlated with temperature. In laboratory experiments, *Synechococcus* showed only a slight, non-significant increase in growth rate under elevated CO$_2$ conditions, but increased growth 2.3 fold with increasing CO$_2$ and tempera-

ture (Fu et al., 2007). Sea surface temperature in the upper 30 m at BATS has not increased significantly over the time period of this study ($0.04\,^{\circ}\mathrm{C}\,\mathrm{yr}^{-1}$ trend, $p = 0.22$). However, positive temperature anomalies were recorded during the 2000–2004 period, a period of increased *Synechococcus* ~~pigment~~ abundance (and low ~~coccolithophore~~ haptophyte pigment abundance). Conditions that could favor *Synechococcus* may eventually arise with further warming, increasing the competitive ability of *Synechococcus*.

In order to examine redundancy in our correlations, we performed multiple linear regressions between Chl $a_{\mathrm{hapto}}$ and several of the driver variables with which it showed the strongest correlations (not shown). When DIC and $HCO_3^-$ were regressed together with Chl $a_{\mathrm{hapto}}$, all the statistical power of DIC was removed, indicating that $HCO_3^-$ is the primary driver of the two for Chl $a_{\mathrm{hapto}}$ variability. Further, when temperature and $NO_3^-$ were regressed together with Chl $a_{\mathrm{hapto}}$, the statistical effect of temperature was removed. This indicates that temperature is not a controlling factor for coccolithophore variability, but rather is a proxy for nutrient concentration in relation to coccolithophore growth. A multiple linear regression of Chl $a_{\mathrm{hapto}}$ with both $HCO_3^-$ and $NO_3^-$ explained $> 50\,\%$ of the variance in Chl $a_{\mathrm{hapto}}$ ($r^2 = 0.52$). Other factors, such as competition or grazing, could perhaps account for some of the remaining variability. Both $HCO_3^-$ and $NO_3^-$ have increased significantly over this time period ($p < 0.001$). However, $NO_3^-$ measurements are highly variable and near zero, making their accuracy questionable, and the trend is only significant if the last two years are included in the time-series (there were particularly high $NO_3^-$ measurements during 2011 and 2012). This is in contrast to $HCO_3^-$, which shows largely positive trends over this time period matching quite well with those of Chl $a_{\mathrm{hapto}}$ (Fig. 7).

The positive trend in $HCO_3^-$ concentration in the upper mixed layer of the water column at BATS is most likely due to increasing absorption of anthropogenic $CO_2$ from the atmosphere. The upper 30 m of the water column is particularly inundated with anthropogenic $CO_2$ in the North Atlantic (Sabine et al., 2004; Bates et al., 2012). From 1991 to 2012, DIC concentration in the upper 30 m at BATS increased by a rate of $1.4\,\mu\mathrm{mol\,kg}^{-1}\,\mathrm{yr}^{-1}$, which is roughly the expected rate of increase given the rise in atmospheric $CO_2$ (see Chapter 10 in Sarmiento and Gruber, 2006). Increasing inorganic carbon supply could also be accompa-

nied by warmer sea surface temperatures, increased stratification, and decreased nutrient supply over the next century (Cabré et al., 2015). Enriched coccolithophore growth by this additional carbon, as well as other predicted oceanic changes with global warming, could lead to shifts in phytoplankton community structure at BATS.

Coccolithophores are not the only phytoplankton that may be responding positively to additional inorganic carbon. *Trichodesmium*, the filamentous $N_2$-fixing cyanobacteria, has been shown to increase growth and $N_2$ fixation under increasing $CO_2$ (Hutchins et al., 2007), yet other drivers such as sea surface temperature, nutrients, and species diversity tend to exert more control on their growth in situ (Snow et al., 2015; Gradoville et al., 2014). *Trichodesmium* has been reported to be a common component of the phytoplankton assemblages in the subtropical North Atlantic (Carpenter et al., 2004; Agawin et al., 2013; Orcutt et al., 2001), but was not specifically resolved in this study. *Trichodesmium* contain a similar suite of pigments as *Synechococcus* (Carpenter et al., 1993; Andersen et al., 1996), and therefore could be included in the Chl $a_{syn}$ fraction of our calculations. This would aid to explain the negative correlation between Chl $a_{syn}$ and $NO_3^-$ (Fig. 3), and further explain why Chl $a_{syn}$ is more abundant than Chl $a_{hapto}$ in the upper water column during warmer, more stratified periods.

Whatever the exact components of Chl $a_{syn}$, Chl $a_{hapto}$ generally shows opposing abundance with this group. We hypothesize that when the *Synechococcus* component is abundant (perhaps due to a positive temperature anomaly), photosynthesis accompanying an increase in *Synechoccocus* draws down DIC (thus, $HCO_3^-$). Low DIC provokes carbon limitation of the coccolithophore population (Riebesell, 2004; Rost et al., 2003), hindering their competitive ability. If coccolithophores are becoming more competitive at BATS due to a lessening of carbon limitation, then they could continue to exert greater competitive stress on *Synechococcus*, which appear to be competing with coccolithophores for a similar niche. However, if the surface waters continue to warm in this region of the Atlantic, as predicted (Cabré et al., 2015), then *Synechococcus* could regain its competitive edge. Furthermore, declining $H^+$ ion concentrations could eventually constrain coccolithophore growth (Bach et al., 2013, 2015).

The *Prochlorococcus* group, designated by Chl $a_{\text{pro}}$, shows similar correlations with oceanic driver variables as for Chl $a_{\text{hapto}}$ (Fig. 3). *Prochlorococcus* have been shown to lack a $CO_{2(aq)}$ uptake mechanism and therefore rely on $HCO_3^-$ uptake for photosynthesis (Badger and Price, 2003), possibly explaining similar behavior to coccolithophores (also positively correlated with $HCO_3^-$), which use $HCO_3^-$ for calcification. *Prochlorococcus* reside mainly in the deep chlorophyll maximum, comprising a rather small portion of the ~~photosynthetic biomass~~ Chl $a$ in the upper 30 m at BATS (Figs. 2, 4). ~~However,~~ This could be, however, due to the relatively high Chl $b$ to Chl $a$ ratio used in our pigment calculations (Letelier et al., 1993), which is more representative of low-light *Prochlorococcus* (Partensky et al., 1999). Yet, since $\sim$ 2005, ~~*Prochlorococcus* have~~ Chl $a_{\text{pro}}$ has been more common in the upper 30 m, resulting in an overall positive trend in Chl $a_{\text{pro}}$ over the entire time-series ($p < 0.001$).

Consistent with colder, high nutrient environments in which diatoms are normally found, Chl $a_{\text{diatoms}}$ showed a strong positive correlation with $NO_3^-$ and a negative correlation with temperature (Fig. 3). If predicted trends in sea surface temperature and nutrient supply with further stratification are realized, then diatoms could become a reduced component of the phytoplankton assemblage at BATS (Cabré et al., 2015). Combining fine scale phytoplankton dynamics from BATS with the satellite record can help to elucidate what changes are occurring over large spatial scales.

#### 4.2 Trends Chl *a* and Chl $a_{\text{hapto}}$ in the subtropical gyre

Unlike at BATS, Chl *a* in the North Atlantic subtropical gyre is not increasing (Fig. 9). PIC, on the other hand, shows mainly positive trends over the whole gyre, in agreement with data from BATS. Together with an absence of Chl *a* trend, this implies that coccolithophores are increasing in abundance relative to other types of phytoplankton in the subtropical gyre. Accompanying this conclusion, however, are uncertainties associated with the satellite-derived PIC estimates ~~.~~

~~Radiance-based algorithms for deriving PIC from satellite reflectance data are formulated to capture the light scattering properties of the numerically dominant coccolithophore, *E. huxleyi* (Gordon et al., 2001; Balch et al., 2005).~~ PIC concentrations in the North Atlantic

subtropical gyre are comparatively low, generally ~ 2.7 mg m$^{-3}$, compared to other coccolithophore bloom regions, which have PIC concentrations between 10 and 100(Balch et al., 2005) . The low concentrations of PIC observed in the North Atlantic subtropical gyre could be within background error or nearing the sensitivity threshold of the instrument. Errors in satellite-derived PIC can arise from atmospheric correction, inclusion of other suspended minerals (such as silica; "opal contamination"), and/or the influence of chlorophyll or colored dissolved organic matter (see Balch et al., 2005) . However these problems can be minimized by binning in space and time, as we have done in this study (using monthly, 9data rather than daily, 4data). Ratios (see Section 4.3 on Limitations of this Study). Even so, ratios relating Chl $a_{\text{hapto}}$ to PIC can further elucidate confidence in satellite-derived PIC estimates.

Data on the amount of Chl $a$ per coccolithophore cell allows the calculation of cell concentration of coccolithophores in the surface waters at BATS. Using a value of 0.26 pg Chl $a$ per cell (Haxo, 1985), mean coccolithophore abundance cell concentration in the upper 30 m is $1.43 \times 10^6$ cells L$^{-1}$ $143 \times 10^3$ cells L$^{-1}$ (corresponding to the mean value of 37.3 µg Chl $a_{\text{hapto}}$ m$^{-3}$ in the upper 30 m). Employing a ratio of PIC to coccolith of 0.26 (Balch et al., 1992) and considering 15 coccoliths per cell (a minimal monolayer of coccoliths covering the cell) under nutrient replete conditions and 100 coccoliths per cell under severe nutrient limitation (Paasche, 1998), we arrive at a PIC concentration range of 0.56 to 3.73 mg PIC m$^{-3}$. This range corresponds well to the average satellite-derived PIC concentration in the BATS region (Fig. 1a) over the study period: 2.71 mg PIC m$^{-3}$ for Sea-WiFS (standard deviation = 0.50) and 2.66 mg PIC m$^{-3}$ (standard deviation = 0.39) for MODIS. The relatively high satellite-derived PIC concentration further suggests that coccolithophores may be experiencing nutrient limitation at BATS (and producing additional coccoliths in response; Paasche, 1998). Therefore, even given the multiple sources of error involved with satellite-derived PIC estimates and pigment analyses (see below), we feel the strong predominance of positive trends in PIC, along with the BATS Chl $a_{\text{hapto}}$ data, suggests that coccolithophores are proliferating in this region (Fig. 8).

### 4.3 ~~Trends in coccolithophore abundance: present and future~~<u>Limitations of this study</u>

~~This is not the only study documenting an increase in coccolithophore abundance~~ <u>There are several caveats of this study that must be discussed before these results can be put into context. First, a primary assumption in this paper is that the haptophyte group is mainly composed of coccolithophores. Though high haptophyte diversity has been reported in open ocean regimes (Liu et al., 2009) , this does not necessarily contradict the assumption that coccolithophores are the dominant type of haptophyte. Furthermore, other studies have shown coccolithophores to be the dominant haptophyte in open ocean sites, such as BATS (see Methods; Dandonneau et al., 2006; Lomas and Bates, 2004; Steinberg et al., 2001) . We therefore assume that Chl $a_{\text{hapto}}$ is mainly representative of coccolithophores. However, as with any study that derives phytoplankton community composition from signature pigments, inherent uncertainties are associated with changes in pigment content within a phytoplankton group over time.</u>

<u>Overall pigment and PIC concentration per coccolithophore cell may be influenced by environmental conditions. For example, photo-acclimation and nutrient limitation can invoke changes in pigment composition or calcification that are not necessarily associated with changes in overall abundance (Behrenfeld et al., 2015; Paasche, 1998) . Dominant species shifts within a phytoplankton group could also influence pigment and/or PIC measurements. Nevertheless, given upward trends observed for both Chl $a_{\text{hapto}}$ and PIC (Figs. 5, 8) we feel the most probable explanation of these observations is increases in overall coccolithophore abundance. However, satellite-derived PIC measurements also contain inherent uncertainties.</u>

<u>Radiance-based algorithms for deriving PIC from satellite reflectance data are formulated to capture the light scattering properties of the numerically dominant coccolithophore, *E. huxleyi*, but also capture detached or detrital coccoliths (Gordon et al., 2001; Balch et al., 2005) . PIC concentrations in the North Atlantic subtropical gyre are comparatively low, generally $\sim$2.7 mg m$^{-3}$, compared to other coccolithophore bloom regions, which have PIC concentrations</u>

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between 10 and 100 mg m$^{-3}$ (Balch et al., 2005) . The low concentrations of PIC observed in the ~~North Atlantic. ? and ? observed decreases in the opal~~North Atlantic subtropical gyre could be within background error or nearing the sensitivity threshold of the instrument. Errors in satellite-derived PIC can arise from atmospheric correction, inclusion of other suspended minerals (such as silica; "opal contamination"), and/or the influence of chlorophyll or colored dissolved organic matter (see Balch et al., 2005) . However, these errors can be minimized by binning in space and time, as we have done in this study (using monthly, 9 km data rather than daily, 4 km data). It is also curious that SeaWiFS-derived PIC data better matches the Chl ~~:~~$a_{hapto}$ estimates from BATS than the MODIS PIC (Fig. 5). On one hand, this may be indicative that other 19'-hex-containing haptophytes were responsible for the increase in Chl ~~carbonate ratio in the North Atlantic during the 1980s and 1990s~~$a_{hapto}$ at BATS during the last several years of the time-series. On the other hand, the predominance of upward trends in MODIS-derived PIC for areas around BATS (Fig. 8b,d) suggests increases in calcifying haptophytes (coccolithophores). It should be noted that MODIS PIC from the BATS region (see Fig. 1) still shows a significant correlation with Chl $a_{hapto}$ (see Results, section 3.5).

Finally, we are limited in our trend analysis by the length of the time-series data. Figure 7 demonstrates that different start and end years can influence the sign and magnitude of our trends in, e.g., Chl $a_{hapto}$. In this study, we report trends in pigments from 1990 to 2012 and trends in PIC from 1998 to 2014, both of which, when employing the full time-series of data, imply increases in coccolithophore populations in the subtropical North Atlantic.

## 4.4 Trends in coccolithophore abundance: present and future

This is not the only study to suggest that coccolithophores are increasing in abundance in the North Atlantic. Further, using Continuous Plankton Recorder ship measurements, Rivero-Calle and co-authors (2015) document an increase in coccolithophore occurrence from $\sim 2$ to $> 20\%$ in the North Atlantic from 1965 to 2010, which they attribute to increasing $CO_2$ concentrations. The data region in their study extends from $\sim 40$ to $\sim 65°$ N (subpolar gyre), just north of the subtropical gyre region focused on in this study. Thus, these studies

combined add robustness to the conclusion that coccolithophores in the North Atlantic are increasing in abundance and are likely stimulated by additional carbon from anthropogenic sources.

Ocean acidification ~~will likely~~ may eventually hinder the growth and calcification of coccolithophores, however. Recently, Bach (2015) and Bach et al. (2015) introduced the "substrate–inhibitor" ratio, describing the dependence of calcification on $HCO_3^-$ (the substrate) and $H^+$ (the inhibitor) concentrations. When this ratio falls below a critical level (i.e., intercellular to extracellular $H^+$ concentration ratio too low) then coccolithophore calcification will be hindered, unless they evolve a mechanism for coping with low pH (Bach et al., 2015). Bach et al. (2013) demonstrate that pH starts to have a negative impact below $pH_T$7.7, whereas the BATS average $pH_T$ is $\sim 8.1$. Thus, critical pH levels will not likely happen for several thousand years (Bach et al., 2015). Other factors besides carbonate chemistry, such as light availability, temperature, and nutrients, likely influence coccolithophore growth in present day oceans.

## 4.5 Potential implications

Increases in coccolithophore abundance in the North Atlantic could have far-reaching ecological, biogeochemical, and climate effects. A shift in phytoplankton community structure could change trophic dynamics, ultimately resulting in ecosystem shifts (Pörtner et al., 2014). For example, though the evolutionary purpose of coccolithophore shells is unclear, some studies speculate they could protect against grazing (see chapter on functions of coccoliths in Winter and Siesser, 1994). Shifts to relatively more coccolithophores in a phytoplankton assemblage could reduce trophic energy available for grazers. Coccolithophore shells also function as a ballast material, sinking faster due to increased weight of the $CaCO_3$ shell, and sequestering organic matter in the deep ocean (Sarmiento and Gruber, 2006; for a recent study of export at BATS, see Lomas et al., 2010). Increases in coccolithophore abundance may have a positive impact on export production, thus a negative feedback on increasing atmospheric $CO_2$. In addition to bringing carbon to the deep ocean, coccolithophores produce the marine trace gas dimethyl sulfide (DMS; Keller, 1989),

which affects cloud formation and climate. Therefore, overall increases in coccolithophore abundance could increase marine DMS production. Furthermore, DMS production by the coccolithophore, *E. huxeyi*, has been shown to increase with increasing temperature and ambient $CO_2$ (Arnold et al., 2013). Thus, changes in coccolithophore abundance could have a multitude of effects on marine ecosystems in the North Atlantic, as well as global carbon cycling and climate. These effects could be further amplified if other ocean basins show similar shifts in phytoplankton composition.

In the subtropical North Atlantic, the upper mixed layer contains particularly high levels of anthropogenic $CO_2$ (Sabine et al., 2004; Bates et al., 2012). We speculate that this rise in DIC is contributing to the increases in coccolithophore ~~abundance~~ pigments and PIC documented in this study. However, it is not clear if phytoplankton communities in other similar oceanic ecosystems, e.g., the North Pacific subtropical gyre, will show similar changes as atmospheric $CO_2$ concentrations continue to increase and inundate the upper mixed layer. The aforementioned ecosystem and carbon cycle effects of coccolithophore increases could become even more prevalent in the world's ocean, or, alternatively, coccolithophore growth could be further modulated by temperature, nutrients, and light. In any case, monitoring the response of natural coccolithophore populations to increasing DIC/ocean acidification is essential for understanding effects of anthropogenic carbon emissions on the world's oceans.

## 5 Conclusions

In this study, we ~~documented an increase in coccolithophore~~ showed that, based on pigment and satellite-derived PIC measurements, coccolithophores appear to be increasing abundance in the subtropical North Atlantic. Coccolithophores ~~appear~~ seem to be responding positively to additional inorganic carbon in the upper mixed later of water column, but are also correlated with $NO_3^-$. These results complement those of Rivero-Calle et al. (2015), who also document an increase in coccolithophore ~~populations~~ occurrence in the North Atlantic, albeit farther north, stimulated by anthropogenic $CO_2$ emissions. Increasing coc-

colithophore abundance is contrary to what numerous laboratory studies have predicted, highlighting the importance of in situ observations. Growth of coccolithophores could, however, be eventually inhibited by decreasing pH and/or other environmental effects of climate change.

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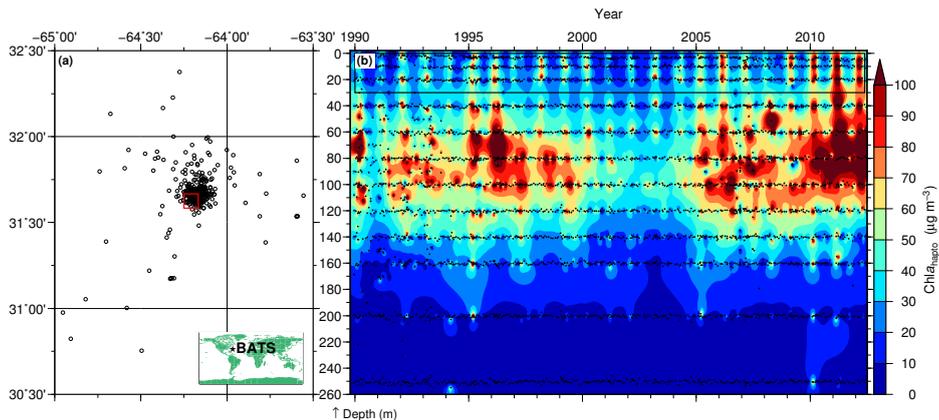
**Figure 1.** Horizontal **(a)** and vertical **(b)** distribution of pigment measurements taken at BATS (black dots) overlaid on a contour plot of HPLC-measured Chl $a_{hapto}$ at BATS. The red box in **(a)** shows the PIC grid cell containing the most BATS measurements (PIC data shown in Fig. 8), while the black box in **(b)** shows the upper 30 m of measurements used for PIC-Chl $a_{hapto}$ correlations and correlations presented in Fig. 3.
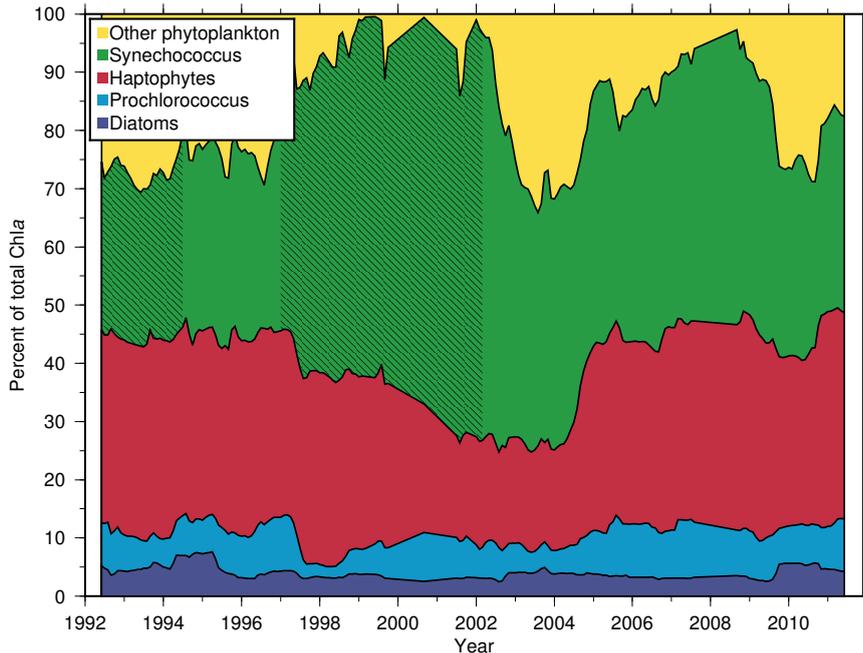
**Figure 2.** Percent of Chl *a* from main phytoplankton groups at BATS from 1992 to 2012 in the top 30 m of the water column derived from signature pigment and Chl *a*$_{total}$ concentrations, deseasonalized with a 1 year boxcar filter (purple = diatoms, blue = *Prochlorococcus*, red = haptophytes, green = *Synechococcus*, yellow = other phytoplankton). Hatched area indicates missing pigment data for *Synechococcus*.
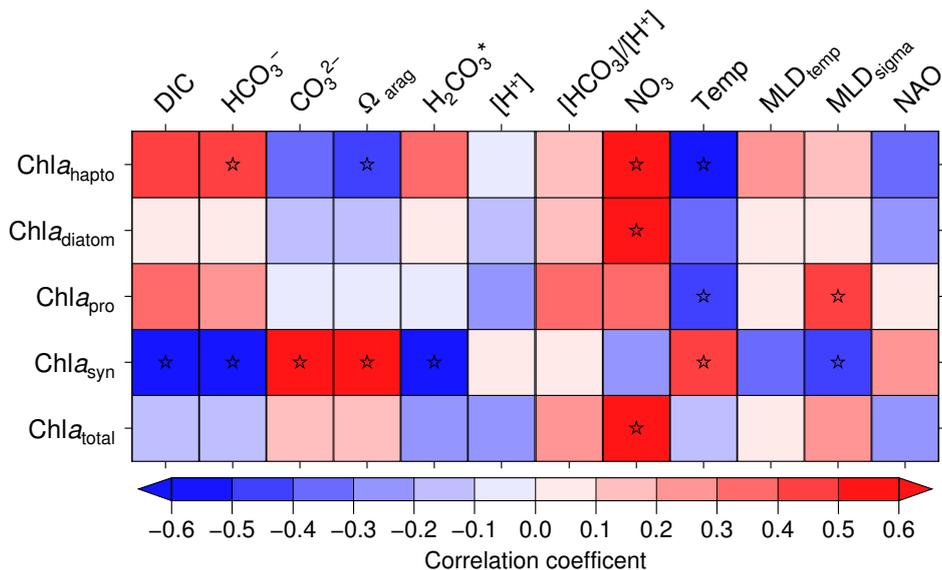
**Figure 3.** Correlation coefficients between Chl *a* components and various oceanographic measurements made in the upper 30 m of the water column at BATS, NAO index, calculated mixed layer depth (MLD; see Methods) and derived carbonate chemistry parameters. Stars indicate the absolute value of the correlation coefficient is greater than 0.4.
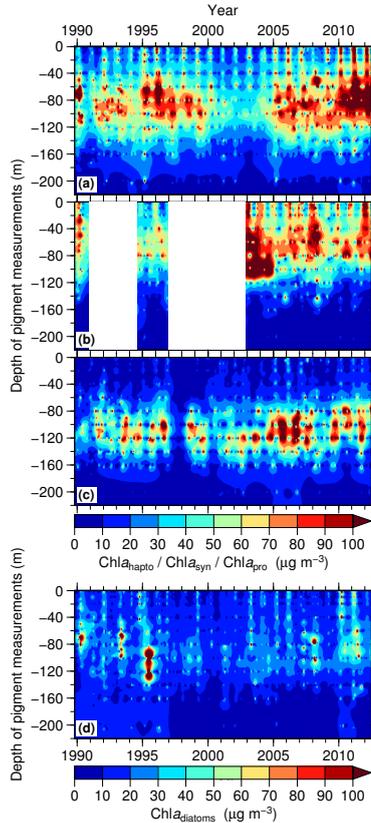
**Figure 4.** Temporal evolution of the vertically-resolved Chl $a$ concentration from the main phytoplankton present at BATS derived from signature pigments from 1990 to 2012: **(a)** Chl $a_{hapto}$, **(b)** Chl $a_{syn}$, **(c)** Chl $a_{pro}$, and **(d)** Chl $a_{diatoms}$.

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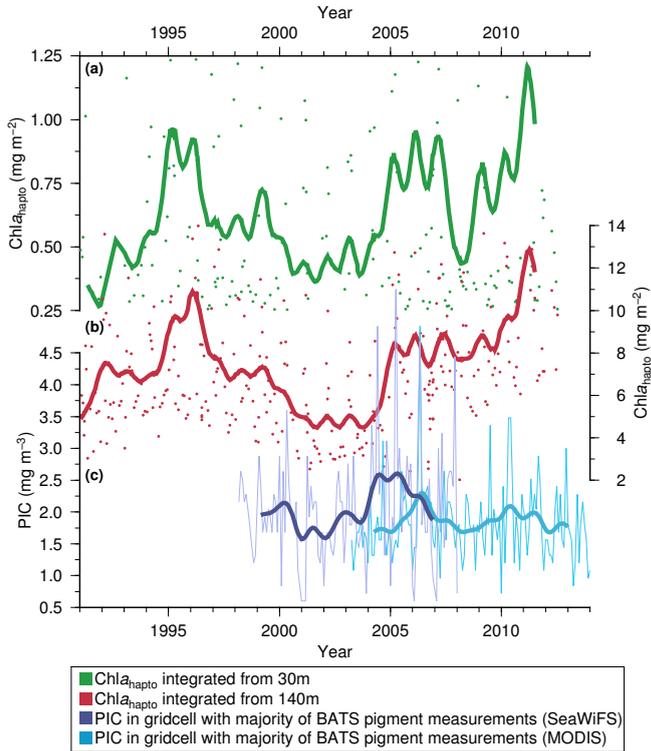
**Figure 5.** Chl $a_{hapto}$ measured at BATS and satellite-derived PIC. Chl $a_{hapto}$ integrated from 30 m is shown in **(a)**, while Chl $a_{hapto}$ integrated from 140 m depth is shown in **(b)**. PIC data shown in **(c)** was obtained from the 5 min satellite grid cell with the most BATS measurements (see Fig. 1). Bolder lines represent a 2 year Gaussian filter on the data. We restricted the y-axes in panels (a) and (b) to highlight the filtered data.
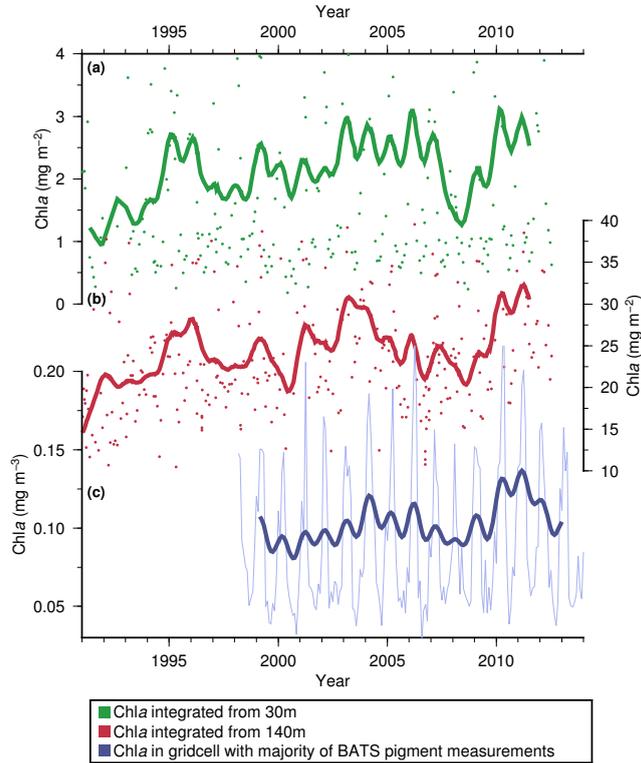
**Figure 6.** Chl $a_{total}$ measured at BATS and satellite-derived Chl $a$. Chl $a_{total}$ integrated from 30 m is shown in **(a)**, while Chl $a_{total}$ integrated from 140 m depth is shown in **(b)**. Chl $a$ data shown in **(c)** was obtained from the 5 min satellite grid cell with the most BATS measurements (see Fig. 1). Bolder lines represent a 2 year Gaussian filter on the data. We restricted the y-axes in panels (a) and (b) to highlight the filtered data.

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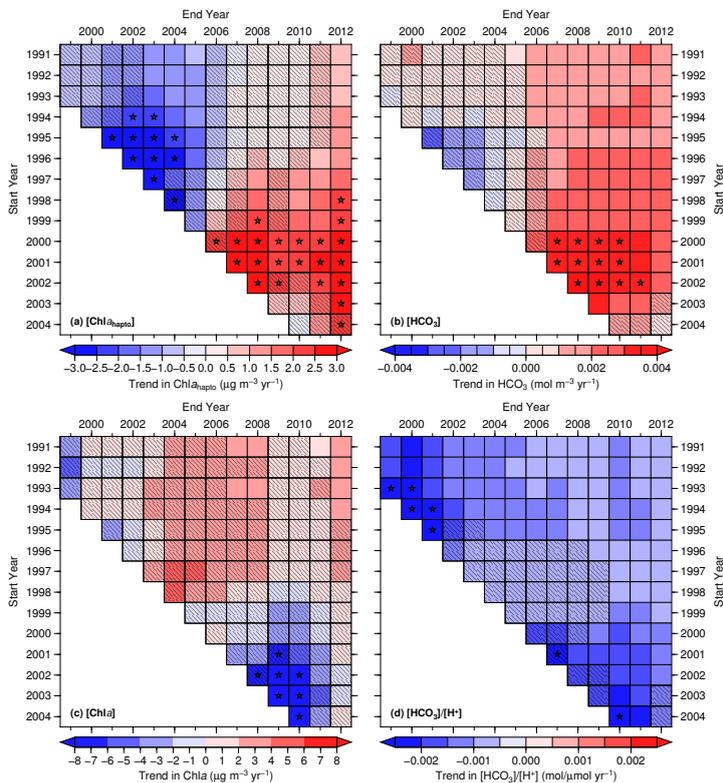
**Figure 7.** Linear trends for a range of start and end years in **(a)** Chl $a_{hapto}$, **(b)** $HCO_3^-$, **(c)** Chl $a$, and **(d)** ratio of $HCO_3 : H^+$. All trends are based on mean concentrations measured at BATS in the upper 30 m of the water column. Boxes with hatch lines demarcate nonsignificant trends. Stars indicate absolute values of trends are greater than 0.2 µg m$^{-3}$ yr$^{-1}$ for Chl $a_{hapto}$, 0.003 mol m$^{-3}$ yr$^{-1}$ for $HCO_3^-$, 6 µg m$^{-3}$ yr$^{-1}$ for Chl $a$, and 0.002 mol/µmol yr$^{-1}$ for $[HCO_3^-]/[H^+]$.
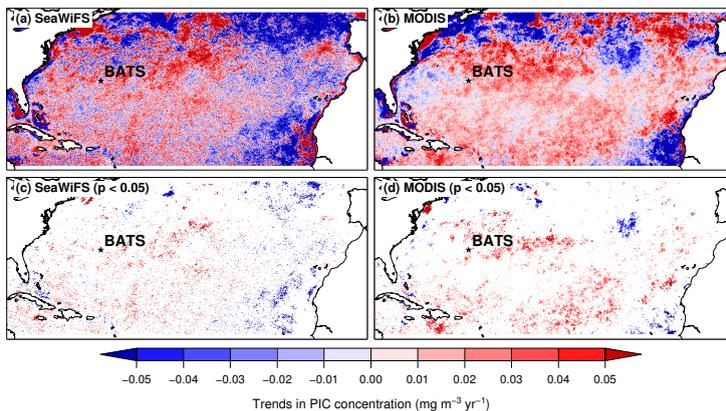
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**Figure 8.** Trends in PIC concentration derived from satellite data for **(a)** and **(c)** SeaWiFS (1998–2007), and **(b)** and **(d)** MODIS (2003–2014). Bottom panels shows significant trends ($p < 0.05$).
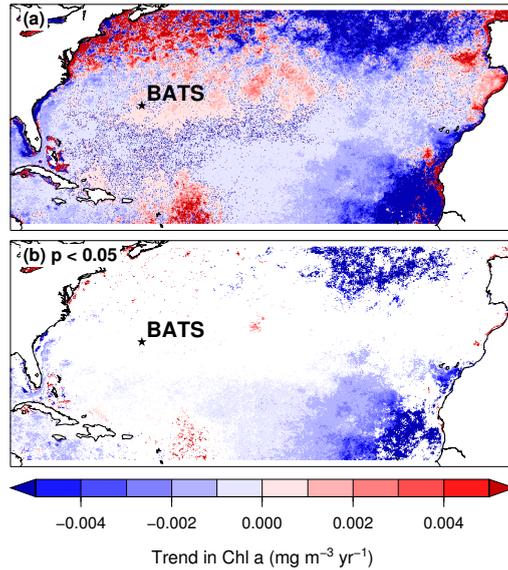
**Figure 9.** Trends in Chl *a* concentration derived from merged satellite records: SeaWiFS and MODIS (1998–2014). The bottom map **(b)** shows significant trends ($p < 0.05$).