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Phytoplankton community structure and depth distribution changes in the Cariaco Basin between 1996 and 2010

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Abstract

Phytoplankton community structure at the Cariaco Basin Time-Series site (10°30′N, 64°40′W) within the Cariaco Basin was examined using high performance liquid chromatography (HPLC) and microscopy between January 1996 to October 2006, and July 2006 to December 2010. Microscopy results from the upper 55 m indicated that the abundance of microphytoplankton (>20 μm) decreased by more than 5-fold from 1996 through 2010, accompanied by a change in diatom composition and a 4-fold reduction in large diatom cell numbers, particularly during upwelling periods. HPLC results also indicate a decline in diatoms, but not nearly to the same extent. Rather, HPLC data suggests a substantial reorganization of the phytoplankton community. Between the two time periods, there was a significant deepening of high biomass concentrations from near surface waters to depths below 55 m. At the same time, phytoplankton diversity increased by 14%, while seasonal variability in community composition declined. Vertical photopigment distributions of nanophytoplankton (<20 μm) further showed significant overall increases in coccolithophore, cryptophyte, and other phytoflagellate concentrations. The deepening in phytoplankton biomass below the mixed layer, the relative increase in smaller taxa, and the increasing homogeneity in phytoplankton distributions over time are likely a direct response to a reduction in upwelling intensity and a deepening of the euphotic zone associated with a decrease in the average trade winds of the region from 2006–2010, compared to 1996–2000. Surprisingly, the overall decline in large cells dominated by diatoms and an increase in smaller taxa has led to no significant change in the export of particulate organic carbon (POC) to depth, contrary to many studies that predict increasing stratification with future climate change scenarios will reduce POC export and carbon sequestration in the deep ocean.

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1. Introduction

The biogeochemistry and ecology of marine systems varies from diel to greater than millennial time scales in response to physical, biological, chemical, and geological forcing (e.g., Muller-Karger et al., 2010; Lomas et al., 2010; Le Quéré et al., 2010; Chavez et al., 2011; Karl et al., 2012; Racault et al., 2012). Over these scales, changes have occurred in the abundance of marine species as part of processes that reorganize food webs (Poloczanska et al., 2013; Bernhardt and Leslie, 2013; Ajani et al., 2014). These changes have direct implications for the strength and efficiency of the “biological pump”, the export and sequestration of organic carbon to the deep sea. Satellite-based and surface chlorophyll a (chl a) records suggest that over the past century, global ocean primary production and phytoplankton abundance may be declining in some parts of the ocean in response to warming sea surface temperatures and increased stratification (Behrenfeld et al., 2006; Li et al., 2009; Boyce et al., 2010; Mousing et al., 2014). Direct measurements of open ocean phytoplankton communities within the North Pacific and Atlantic Oceans show that phytoplankton have become increasingly dominated by smaller taxa (Lomas et al., 2010; Karl, 2014). The impact of these phytoplankton community transitions on food webs and particle flux processes remains complex. Mineral-ballasted phytoplankton, such as diatoms and coccolithophores, are thought to enhance the strength and efficiency of carbon export (Michaels and Silver, 1988; Armstrong et al., 2002). However, more
recently Richardson and Jackson (2007) suggested that all phytoplankton contribute to carbon export in direct proportion to primary production. In the North Pacific, while diatoms accounted for only a small portion of phytoplankton biomass, they continued to provide 9–20% of the particulate carbon flux measured at 150 m (Brzeziński et al., 2011). In contrast, in the Sargasso Sea, picophytoplankton inputs have increased particulate carbon export at 150 m depth by 60% (Lomas et al., 2010).

Although continental margins constitute only about 10% of the total ocean surface area, these regions are responsible for about 10–15% of the global marine primary production and greater than 40% of seabed carbon sequestration (Yool and Fasham, 2001; Muller-Karger et al., 2005). The Cariaco Basin, located off the coast of Venezuela, has been the site of high frequency water column sampling for marine biogeochemical and ecological observations since 1995. The observations were collected as part of the Cariaco Ocean Time-Series Program (Muller-Karger et al., 2001; Thunell et al., 2007). The southern Caribbean Sea is characterized by high seasonal biological production due to coastal, wind-driven upwelling (Rueda-Roa and Muller-Karger, 2013). In the Cariaco Basin, the water column is anoxic from about 230–250 m depth to the bottom at ~1380 m (Scranton et al., 1984; Astor et al., 2003). Anoxic waters have further resulted in the formation of an unparalleled, high resolution sediment record of past climate, hydrography, and hydrology of the Atlantic Ocean and tropical regions of the Americas (Lea et al., 2003; Hughen et al., 2004; Peterson and Haug, 2006; Black et al., 2009, 2011). The Basin thus offers an opportunity for assessments of how changes in phytoplankton diversity and size distribution in the upper water column influence the magnitude and composition of sinking particulate material that ultimately reaches the seafloor and is buried.

The Cariaco Basin has undergone marked shifts in biogeochemistry and food web structure over the past two decades (Taylor et al., 2012; Montes et al., 2013; Bates et al., 2014; Scranton et al., 2014). Since the initiation of the CARIACO Ocean Time-Series, near-surface (0–55 m) chl a concentrations (measured by fluorescence of pigments extracted in methanol) and net primary production rates (NPP, measured using 14C incubations) have declined, particularly between 2004 and 2010 (Taylor et al., 2012; Scranton et al., 2014). This is hypothesized to be the result of a measured decrease in seasonal trade wind intensity, which has reduced coastal upwelling and led to shallower mixed layer depths (Taylor et al., 2012). Microscopy observations indicate that microphytoplankton assemblages (>20 μm) within the euphotic zone have shifted from a community comprised mainly of diatoms, dinoflagellates, and coccolithophorids to one with increasing numbers of smaller taxa including cyanobacteria (Montes et al., 2013). During this period, a collapse in the sardine industry also occurred. The causes are not clear, but the phenomenon was accompanied by an increase in zooplankton biomass and an increase in particulate organic carbon export below the euphotic zone (Taylor et al., 2012). These results suggest that changes in zooplankton abundance and therefore grazing may have contributed to the decline in diatoms and to more efficient carbon export to depth.

Traditional diversity indices are usually based on species composition and the abundances of individual species. The variety and phylogenetic association of specific photosynthetic accessory pigments (chlorophylls and carotenoids) with different algal groups provides diagnostic biomarker compounds for differentiating the relative abundance of phytoplankton groups in mixed species assemblages. Microscopy and direct count methods to enumerate species may underestimate phytoplankton species richness, while larger sample volumes for HPLC photopigment analysis more likely represent diversity (Cermeño et al., 2014). Photopigments and concentrations are analogous to species and abundances and therefore further provide the ability to assess phytoplankton diversity at the community level using common diversity indices (Noble et al., 2003; Sherrard et al., 2006). Thus, photopigment-based measures offer an efficient way to quantify community or functional diversity (Moreno et al., 2012). Indeed, phytoplankton community diversity is a better indicator of enrichment and ecosystem function than species diversity (Loreau, 2000). Furthermore, functional diversity, in contrast to taxonomic diversity, is a principal factor determining ecosystem processes (Tilman et al., 1997). For measuring trends in ecosystem diversity, the absolute value of the index is less important than how the relative index changes over time, thereby providing insights into compositional changes in community structure (Sherrard et al., 2006).

In this study, we document and characterize the changes observed in the phytoplankton community structure and their vertical distributions in the Cariaco Basin from 1996 to 2010. We focus on high performance liquid chromatography (HPLC) measurements of photopigments in the upper 100 m of the water column from the CARIACO Time-Series site in the context of changes in hydrography and phytoplankton community composition measured by microscopy. This study complements the work of Taylor et al. (2012) with a more detailed analysis of changes in phytoplankton distributions and structure in the photic zone (0–100 m) and suggests taxa-specific responses to long term alterations in Cariaco Basin hydrography.

2. Materials and methods

The Cariaco Basin, located on the northeastern shelf of Venezuela in the southern Caribbean Sea, is isolated from the Caribbean Sea and Atlantic Ocean by a shallow sill of ca. 145 m depth in two channels (Lidz et al., 1969; Muller-Karger et al., 2000). The region is characterized by seasonal upwelling driven by Trade Winds, which in turn are controlled by the position of the Intertropical Convergence Zone (ITCZ). Phytoplankton production rates (>300 g C m⁻² y⁻¹) are the result of high productivity associated with the primary upwelling season that occurs from January to April each year (Muller-Karger et al., 2001). This production results in the export of large fluxes of particulate organic matter to depth (Thunell et al., 2007).

Samples were collected as part of the Cariaco Ocean Time-Series Program at a station located in the eastern Cariaco Basin (10°30'N and 64°40'W) between January 1996 and December 2010. All samples were collected using a 12 bottle rosette system equipped with a CTD (Seabird, model SBE 25) (Astor and Varela, 2013). Water samples for chl a and phaeopigment analysis were obtained on a monthly basis from 1996–2010 from the first cast of each cruise, at about 0500 local time. Samples were collected at 8 standard depths in the upper 100 m (1, 7, 15, 25, 35, 55, 75, and 100 m) following Varela [2013]. HPLC samples were drawn from the same cast and depth. During periods of low primary production (typically May through November), 2 l of water was vacuum filtered through a 47 mm glass fiber filter (Whatman GF/F). During periods of high primary production (typically December through April), water was filtered until the filter changed color or clogged (typically between 0.5 and 1.5 l). HPLC filters were stored folded in aluminum foil and frozen at ~20 °C while at sea. Once on land, samples were stored in a ~40 °C freezer until analyzed. For phytoplankton species determination by microscopy, 500 ml of seawater were collected at the same depths as for pigments (down to 55 m) into HDPE bottles and fixed with a 4% formalin solution neutralized with sodium tetraborate. Samples were analyzed at the Universidad de Oriente, Venezuela, using the Utermöhl technique (Hasle, 1978) with 100 μl sedimentation chambers and a settling period of 48 h. Identifications and enumerations by microscopy were limited to microphytoplankton (cells > 20 μm) (Muthinda et al., 2013).
HPLC analyses were conducted at three different laboratories at different times throughout the life of CARIACO program, specifically: Bermuda Biological Research Station (BBSR; from January 1996 to May 1998), Mote Marine Lab (MML; from June 1998 to October 2000), and Horn Point Laboratory (HPL; from July 2006 to December 2010). We combined the HPLC data to construct two time series; one spanning the period from Jan 1996–October 2000 (BBSR & Mote) and the other from Jul 2006–Dec 2010 (HPL) (Table 1). No samples for HPLC analysis were collected between October 2000 and July 2006. Analysis at MML and BBSR used the U.S. JGOFS BATS protocol (Knap et al., 1997) and the Wright et al. (2000) method, while HPL used the method described in Van Heuken and Thomas (2001), Hooker et al. (2005). Rondón (2009) provides additional details on the HPLC sample history and methods. All data are available online at the Cariaco Ocean Time-Series database (BCO-DMO; http://www.bco-dmo.org/data set/3235). Photopigments not common to all three databases were excluded from our analysis in order to build a consistent data set. The software ChemTax (v. 1.95; Mackey et al., 1996; Wright et al., 1996) was used to determine the relative abundance of major phytoplankton groups (Pinckney et al., 2001; Lewitus et al., 2005; Higgins et al., 2011). The initial pigment ratio matrix used for this analysis was a combination of matrices provided by Mackey et al. (1996) and Schlüter et al. (2000) (Supplement 1). The convergence procedure outlined by Latasa (2007) was used to minimize errors in algal group biomass due to inaccurate pigment ratio seed values. A two-step cluster analysis procedure based on log-likelihood distance measures of nine photopigment variables was used to define homogeneous groups for separate bins in ChemTax analyses (SPSS v. 21). Two clusters, each consisting of 838 (82%) and 185 (18%) samples were constructed in the analysis. The most important predictor for group membership was 19′-hexanoyloxyfucoxanthin. The least important was zeaxanthin.

The groups were analyzed using two analysis bins in ChemTax to provide estimates of the relative abundances of 10 algal groups (chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, haptophytes 1, 2, 3, and 4, and prasinophytes). Diagnostic pigments for the ChemTax analysis of haptophyte groups are outlined in Table 2. Here, the haptophytes 3 group was assumed to be coccolithophores. The concentrations of haptophytes 1 and 4 were <1% of total chl a and were not considered further in our analysis. The indicator pigment for prochlorophytes (di-vinyl chlorophyll a) was not quantified in two of the three pigment databases, therefore the contribution of prochlorophytes could not be accurately separated from other phytoplankton. In the database containing measures of di-vinyl chl a, divinyl chl a constituted <2% of total chl a (chl a+divinyl chl a). For the present study, we assumed that the contribution of prochlorophytes to total chl a was negligible. Photopigment concentrations and algal group abundances for each vertical profile were depth-integrated by trapezoidal rule using the midpoint between sample depths and the measured values for each sample depth. Contour plots were constructed using Surfer (v. 12) and the kriging algorithm.

Measures of diversity were calculated on a per sample basis using the individual pigments and their concentrations as variables and applying ecological diversity indices (Krebs, 1999; Clarke and Gorley 2001; Noble et al., 2003; Sherrard et al., 2006). Calculations included total number of pigments in each sample (S), pigment concentrations (N), Margalef’s species richness (d), Pielou’s evenness (J), and Shannon–Weiner Index (H’ using log2). Pigment and diversity data were heteroscedastic and could not be transformed to achieve normality. Consequently, the non-parametric Mann–Whitney U test was used for paired comparisons. MANOVA and ANOVA procedures are robust with respect to departures from normality (Underwood, 1981). The power for all tests was ≥0.80. Box-plots were used to illustrate the data’s central tendencies (mean and median), and distribution of the data between the 25th and 75th percentiles (interquartile) and 5th and 95th percentiles of all observations.

### 3. Results

Our study focused on the time intervals 1996–2000 (Time 1) and 2006–2010 (Time 2). The average depth of the photic zone (0.1% photosynthetically active radiation (PAR)) increased from 61 ± 18 m (mean ± 1 sd) to 75 ± 13 m during the two time periods (p=0.003). The average mixed layer depth, as defined by a density change with depth of 0.125 kg m⁻³ and calculated for each sampling date, also decreased slightly from 22.5 ± 11.5 to 20.0 ± 7.1. The coefficient of variation (CV=standard deviation/mean) for the mixed layer depth decreased from 0.51 in Time 1 to 0.37 in Time 2 and is indicative of a reduction in mixing intensity during Time 2, i.e., mixed layer depth maxima were consistently shallower than in Time 1.

On average, during Time 1 upwelling periods (January–April), highest HPLC-derived chl a concentrations were observed within the upper 20 m of the water column (Figs. 1 and 2). In Time 2, the chl a maxima were lower and there was a downward shift in chl a concentration to deeper in the water column (Fig. 1). As a result, there was a decrease in the fluctuations of vertically-integrated

### Table 1

Summary of parameters used in the combined HPLC data set.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection frequency</td>
<td>monthly</td>
</tr>
<tr>
<td>Collection depths</td>
<td>5 to 100 m at 10 m intervals</td>
</tr>
<tr>
<td>Total number of samples</td>
<td>930</td>
</tr>
<tr>
<td>Photopigments</td>
<td></td>
</tr>
<tr>
<td>Allixanthin</td>
<td>Fucoxanthin</td>
</tr>
<tr>
<td>19′ Butanoyloxyfucoxanthin</td>
<td>19′ Hexanoyloxyfucoxanthin</td>
</tr>
<tr>
<td>Total chlorophyll a</td>
<td>Lutein</td>
</tr>
<tr>
<td>Total chlorophyll b</td>
<td>Peridinin</td>
</tr>
<tr>
<td>Chlorophyll c₁</td>
<td>Prasinoxanthin</td>
</tr>
<tr>
<td>Chlorophyll c₁+c₂</td>
<td>Zeaxanthin</td>
</tr>
</tbody>
</table>

### Table 2

Haptophyte groups based on photosynthetic pigment composition used for ChemTax. Abbreviations: chl c₁ (chlorophyll c₁), chl c₁+c₂ (chlorophyll c₁+c₂), but-fuco (19′ butanoyloxyfucoxanthin), fuco (fucoxanthin), hex-fuco (19′ hexanoyloxyfucoxanthin), chl a (chlorophyll a).

<table>
<thead>
<tr>
<th>Group</th>
<th>Major Photosynthetic Pigments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haptophytes 1</td>
<td>chl c₁+c₂, fuco, chl a</td>
</tr>
<tr>
<td>Haptophytes 2</td>
<td>chl c₂, chl c₁+c₂, fuco, chl a</td>
</tr>
<tr>
<td>Haptophytes 3</td>
<td>chl c₃, chl c₁+c₂, fuco, hex-fuco, chl a</td>
</tr>
<tr>
<td>Haptophytes 4</td>
<td>chl c₅, chl c₁+c₂, but-fuco, fuco, hex-fuco, chl a</td>
</tr>
</tbody>
</table>
(0–100 m) chl a concentrations (CV for each time period decreased from 0.605 to 0.413). Furthermore, annual depth-integrated (0–100 m) chl a averaged for Time 1 (27.38 ± 17.23 mg chl a m$^{-2}$) was significantly lower than the average over Time 2 (33.43 ± 13.80 mg chl a m$^{-2}$) ($p < 0.01$) (Table 3), although a linear regression of approximately monthly integrated chl a concentrations over the entire dataset did not exhibit a statistically significant trend ($p = 0.307$) (Fig. 2).

When the vertically-integrated chl a concentrations were split into discrete intervals of 0–55 m (the maximum depth of microscopy measurements) and 55–100 m, chl a did not increase in the 0–55 m interval between Times 1 and 2 ($p = 0.325$). In contrast, chl a concentrations doubled in the 55–100 m interval from 5.39 ± 3.64 mg chl a m$^{-2}$ in Time 1 to 10.34 ± 5.23 mg chl a m$^{-2}$ in Time 2 ($p < 0.001$) (Table 3).

The increase in integrated HPLC derived chl a concentrations is not consistent with the chl a concentrations measured using fluorometry collected during the same casts and cruises (reported in Taylor et al., 2012). Indeed, Taylor et al. (2012) report a significant decrease in chl a concentrations for the same time-period of ~11% or 22.5 mg m$^{-2}$ integrated over the upper 100 m. In an attempt to resolve discrepancies between the two methods, we plotted corresponding paired values for HPLC-derived and fluorometry-derived measures of chl a over both time periods. While both Time 1 and Time 2 contained strong correlations between the two measurement techniques ($r^2 ≥ 0.87$), the relationships were different, with fluorometric to HPLC derived chl a ratios declining from 1.58 to 0.87, respectively. These ratio differences are due primarily to a reduction in fluorometric chl a values, as there is almost no variation in HPLC derived chl a values between the two time periods. Methodological differences could explain this discrepancy.

The fluorometric method used in this study (the "acidification method") detects a variety of related chl a compounds (mono-vinyl chl a, di-vinyl chl a, allomeric chl a, epimeric chl a, and chlorophyllide a) as well as chl b and chl c, but reports a single value for chl a that is the sum of the concentrations of all these isomers and chlorophylls (Welshmeyer, 1994; Varela, 2013). Although some HPLC methods can completely separate all these forms, two of the three HPLC methods used in this study (BBSR and MML; Time 1) were unable to quantify all of the individual forms of chl a. In contrast, the HPL laboratory (Time 2) reported HPLC derived "Total Chl a" as the sum of mono-vinyl chl a, di-vinyl chl a, allomeric chl a, epimeric chl a, and chlorophyllide a. As a result, the values reported as "Total Chl a" in the Caraco database may have been calculated differently depending on which of the three laboratories conducted the HPLC measurements. We were unable to determine a Total Chl a concentration for BBSR, MML, and the HPL databases that was consistent for all three methods. Given the more comprehensive nature of the HPL laboratory derived HPLC measurements, we argue that Time 2, likely provides the "best" comparison between HPLC and fluorometrically derived measurements of Total chl a and partially explains the differences in slope that occur between Time 1 and Time 2.

Community species composition changes and associated pigment absorption could also be responsible for some of the observed differences in Total chl a between the various methodologies employed here. For example, diatoms and dinoflagellates may contain significant amounts of chlorophyll c, which may lead to an overestimation of fluorometric chl a (Welshmeyer, 1994). Microscopic measurements of microphytoplankton abundance by Taylor et al. (2012) and in this study (see below) show that there was an abrupt reduction in diatoms, dinoflagellates, and coccolithophorids within the upper 55 m of the water column which began in 2005. Median microphytoplankton cell densities were 50- to 300-fold lower in 2005–2009 than in 1996 to 2004 (see below). Here, some of the highest fluorometric chl a measured throughout the time-series occurred during Time 1. Chl b and c also decreased between Time 1 and 2 (0.101 to 0.082 and 0.081 to 0.064 ng l$^{-1}$, respectively). Combined, these results suggest the possibility of a fluorometric overestimation in total chl a concentrations relative to HPLC in Time 1 relative to Time 2.
In the present study, our analysis primarily involves the interpretation of concentrations of accessory pigments rather than chl a. We argue that differences in "Total Chl a" concentrations between databases should have minor impacts on our ChemTax results because the error is partitioned among the individual groups resulting in smaller uncertainties (< 5%) for the concentration estimates of individual algal groups. Furthermore, at low concentrations of chl a (< 2 mg m⁻²), there is no significant difference between the two methods in either Time 1 or Time 2. Thus the findings and conclusions of this study are applicable even with the small uncertainty in the measurements of chl a concentration.

The photopigment data were analyzed using ChemTax in two separate "bins". The separation into bins was based primarily on depth intervals (< 10 m and > 10 m). This resulted in root mean square errors (RMS) of 0.132 and 0.153 after 20 separate consecutive runs for each bin. Spatiotemporal contour plots for diatoms show large blooms in January–February of each year, but the intensity of these diatom blooms is lower in Time 2 (Fig. 3a). In most all cases, major diatom blooms were limited to the mixed layer. Coccolithophore (haptophyte) blooms also decreased in intensity in Time 2, with many blooms occurring below the mixed layer (Fig. 3a). The timing of coccolithophore blooms did not follow a regular seasonal pattern. The group haptophytes 2 (haptophytes that do not have 19' butanoyloxyfucoxanthin or 19' hexanoyloxyfucoxanthin as accessory pigments) exhibited blooms throughout the upper 80 m, and highest concentrations tended to occur at depths above the mixed layer, with the exception of one major bloom during the summer of 1999 (Fig. 3a). Cryptophytes, dinoflagellates, and chlorophytes showed a much higher frequency of blooms below the mixed layer in Time 2 relative to Time 1 (Fig. 3b and c). Cyanobacteria blooms were also more common in Time 2 (Fig. 3b). For prasinophytes, the frequency and intensity of blooms was similar for both time periods with the highest concentrations below the mixed layer (Fig. 3c).

Depth integrated (0–100 m) concentrations were calculated for all the algal groups (Fig. 4). Diatoms and coccolithophores had the highest relative concentrations (~ 60–70% of total HPLC derived chl a), while all other groups had values less than 5 mg chl a m⁻² or ~ 10% each (Table 3). Overall, HPLC derived phytoplankton community composition in the upper 100 m differed significantly between the two time periods (MANOVA, p < 0.001) (Table 3, Fig. 4). The results of univariate ANOVAs for each group suggest that dinoflagellates, cryptophytes, coccolithophores, chlorophytes, and cyanobacteria significantly (p ≤ 0.05) increased in relative abundance during Time 2 by 57 to 243%, while diatom and prasinophytes concentrations remained unchanged (Table 3). Collectively, the phytoflagellates (chlorophytes, cryptophytes, dinoflagellates, haptophytes 2, and prasinophytes) showed an average increase of 7.44 mg chl a m⁻². Among these were chl b containing phytoflagellates (chlorophytes, prasinophytes), which increased by 0.91 mg chl a m⁻².

The data were further split into depth intervals of 0–55 m and 56–100 m to compare the HPLC–ChemTax results with microscopy observations (Table 3). In the upper 55 m, the abundances of diatoms declined by 33% while changes in prasinophyte and Total chl a concentrations were not significantly different between Time 1 and Time 2 (p > 0.05). All other algal groups showed significant increases in abundance ranging from 38 to 204%. The largest increases were in cryptophytes, chlorophytes, and cyanobacteria. For the 56–100 m interval, the abundances of all algal groups as well as Total chl a significantly increased (ranging from 33% to 41%) from Time 1 to Time 2. Although diatom abundance decreased in the upper 55 m, there was a 77% increase in the 56–100 m interval. These results suggest that the phytoplankton community had a significant increase in abundance in the 56–100 m depth interval.

Since there was high variability in the annual, vertically integrated concentrations for diatoms and prasinophytes, we further divided the 100 m integrated dataset into upwelling and non-upwelling periods within each of the two time intervals. Upwelling was defined as the 21 °C isotherm rising shallower than 80 m (Astor et al., 2003). For diatoms, a two-way ANOVA, with factors including upwelling vs. non-upwelling and Time 1 vs. Time 2, indicated that the concentrations in the upper 100 m were different for upwelling vs. non-upwelling events (p = 0.03) but not different between Time 1 and Time 2 (p = 0.243). A similar analysis for prasinophytes found no significant difference in concentrations for either upwelling (p = 0.221) or time period (p = 0.206). However, dinoflagellates, cryptophytes, haptophytes 2, coccolithophores, chlorophytes, and cyanobacteria all showed significant increases for Time 2 (p < 0.01), but only cryptophytes and chlorophytes were higher in average concentrations during upwelling events (p < 0.01). In contrast, cyanobacteria concentrations were higher during non-upwelling periods (p < 0.001).

Shannon diversity indices (H), calculated using pigment types and concentrations over time for the upper 100 m, suggest an overall increase in diversity from Time 1 to Time 2. In fact, photopigment diversity was significantly higher at all depths during the latter half of the time series (p < 0.05, Table 4), with the largest photopigment diversity changes occurring in the 80–100 m depth interval (Fig. 5). Diversity coefficient of variation (CV1 and CV2) at each sampling depth was significantly lower (p < 0.05; Table 4) in
Time 2 relative to Time 1, consistent with the more homogeneous chl \( a \) distribution during Time 2. Depth-integrated photopigment diversity data averaged over each of the two time periods shows that the mean (± 1 sd) diversity values significantly increased from 1.47 ± 0.27 during Time 1 to 1.68 ± 0.13 during Time 2 (\( p < 0.001 \)). The increase in diversity during Time 2 was due primarily to an increase in photopigment richness (number of different types of pigments) rather than major changes in evenness (relative photopigment concentrations) (Fig. 6).

Microphytoplankton abundance determined by microscopy was measured in the upper 55 m of the water column during the two time periods (Fig. 7). During Time 1, average microphytoplankton abundances steadily decreased from more than 250 cells ml\(^{-1}\) to less than 100 cells ml\(^{-1}\) (\( p < 0.05 \)). Diatom abundance followed a similar pattern, decreasing from more than 100 cells ml\(^{-1}\) to less than 25 cells ml\(^{-1}\) in the upper water column (Fig. 8). Time 1 was dominated by centric diatoms (\textit{Guinardia}, \textit{Leptocylindrus}, \textit{Skeletonema}) and the dinoflagellate \textit{Gymnodinium} sp. In contrast, coccolithophorids and a mix of centric and pennate diatoms (\textit{Pseudonitzchia}, \textit{Leptocylindrus}, \textit{Guinardia}) were the dominant phytoplankton components in Time 2. A multidimensional scaling analysis (MDS–ANOSIM) of microphytoplankton species count data indicated significant differences in

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Fig. 3. (a) Contour plots of ChemTax-derived algal group abundances for the two time periods. Data points are indicated by the dots on the plots. The white line shows the depth of the mixed layer. (b) Contour plots of ChemTax-derived algal group abundances for the two time periods. Data points are indicated by the dots on the plots. The white line shows the depth of the mixed layer. (c) Contour plots of ChemTax-derived algal group abundances for the two time periods. Data points are indicated by the dots on the plots. The white line shows the depth of the mixed layer.
community structure between the two time periods ($R=0.435$, $p<0.01$). However, species diversity was not significantly different in the upper 55 m between the two time periods ($p>0.05$).

4. Discussion

During the first period of our pigment data, Time 1 (1996–2000), the Cariaco Basin experienced regular seasonal upwelling driven by strong (>$6 \text{m s}^{-1}$) Trade Winds between December and April (Muller-Karger et al., 2001; Astor et al., 2003). This upwelling period accounted for ~70% of the annual depth-integrated NPP and was dominated by a microphytoplankton community comprised of diatoms, dinoflagellates, and nanophytoplankton coccolithophorids (Muller-Karger et al., 2001). By the mid to late 2000s (Time 2, 2006–2010), the physical and biogeochemical regime had changed to one with significantly reduced Trade Winds during the upwelling period (i.e., typically <6 m s$^{-1}$; Taylor et al., 2012). By 2010, average annual sea surface temperature (SST) also increased by ~1.0 °C relative to 1995 and mixed layer depths shoaled (Taylor et al., 2012). The Spanish sardine (Sardinella aurita) fishery off NE Venezuela, which supplies >50% of the Caribbean small pelagic fish catch, decreased from ~80,000 metric tons in 1988 to 40,000–60,000 mt y$^{-1}$ between 1989 and 2004, and to less than 15,000 mt y$^{-1}$ since 2005 (FAO Fisheries Global Information System 2012). The changing physical regime coupled with a reduction in large grazers appears to have played a major role in changing the Cariaco Basin biological community structure.

Water column chl $a$ concentrations derived from HPLC increased in deeper waters in the latter part of the time-series (Time 2) and were more uniform with depth, consistent with a reduction in upwelling intensity and greater light penetration associated with declining net primary production (Taylor et al., 2012; Mutshinda et al., 2013). The increase in depth-integrated (100 m) chl $a$ concentrations from Time 1 to Time 2 by 6.05 mg chl $a$ m$^{-2}$ (22.1%), which occurred mostly in the 55–100 m depth interval, likely reflects biomass accumulation closer in proximity to the deeper nutricline associated with weaker upwelling and an increased penetration of light as biomass decreased at shallower depths. Although increasing pigment concentrations at depth may reflect adaptation strategies associated with the phytoplankton community moving deeper into the water column, the relative concentrations of chl $a$ within the same depth zone should not exhibit temporal acclimation responses.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>$n$</th>
<th>$z$</th>
<th>p-Value</th>
<th>CV$_1$</th>
<th>CV$_2$</th>
<th>$n_1$</th>
<th>$n_2$</th>
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<td>85</td>
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<td>0.073</td>
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<td>55</td>
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<tr>
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<tr>
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<td>0.072</td>
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<td>53</td>
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<tr>
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<td>0.240</td>
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<tr>
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<td>&lt;0.001</td>
<td>0.273</td>
<td>0.111</td>
<td>47</td>
<td>52</td>
</tr>
</tbody>
</table>

Table 4: Comparison of photopigment diversity indices at each depth from the two time periods (1996–2000 and 2006–2010) using a Mann–Whitney U-test. The Bonferroni-corrected critical value for the 95% confidence interval for the multiple tests is 0.006. The abbreviations are total number of samples for both groups ($n$), critical $z$-value ($z$), p-value for the test, the coefficient of variation for groups 1 and 2 (CV$_1$ and CV$_2$), and the respective sample sizes ($n_1$ and $n_2$).

Consistent with the results of Taylor et al. (2012), the microcosm data show large scale reductions in overall microphytoplankton (>20 μm) cell densities and a large decrease in both diatoms and coccolithophores over the upper 55 m. However, the observed photopigment decline in diatom abundance is not nearly to the same extent and coccolithophores had a significant concentration increase of 60.1% over this same depth interval (Table 3). We argue that the high photopigment variability during Time 1 is due to the more intense upwelling that occurred early in the time series. In both time periods, diatoms were the most abundant phytoplankton (53.4 and 34.9%, respectively) followed by coccolithophores (18.7 and 24.5%, respectively). The concentrations of diatoms actually increased in the 55–100 m depth interval by 77%, suggesting a major reorganization in diatom vertical distribution between the two time periods.
nutrient availability. Finally, depth limited microscopy data misses the downward movement of diatoms and coccolithophores deeper in the water column (i.e. below 56 m). In fact, there was also a dramatic shift in diatom speciation, with centric diatoms (*Guinardia*, *Leptocylindrus*, *Skeletonema*) dominating during the first half of the time-series and a mix of centric and pennate diatoms (*Pseudonitzschia*, *Leptocylindrus*, *Guinardia*) dominating in more recent years. In microscopy, smaller sample volumes may lead to underestimations of species richness or diversity (Cermeño et al., 2014). Nonetheless, both photopigment and microscopy measurements suggest that phytoplankton communities became more homogeneously distributed over the years, with the majority of the difference due to declining diatom populations that occur during upwelling events.

Combined, the HPLC and microscopy results confirm that the Cariaco Basin has undergone a regime shift. Since the start of the Cariaco Basin Time Series in 1996, the phytoplankton community has transitioned to a higher concentration of smaller taxa located deeper in the water column and with less variability in their seasonal abundance, similar in many aspects to that observed by Taylor et al. (2012), Mutshinda et al. (2013), and Montes et al. (2013). These results are consistent with the changing hydrographic regime observed in the Cariaco Basin, which is characterized by declining wind intensities that have resulted in a mixed layer increase in temperature, particularly during upwelling events.

**Fig. 5.** Time series contour plot of photopigment diversity index ($H'$). Data points are indicated by dots on the plot and the white line shows the mixed layer depth.

**Fig. 6.** Photopigment evenness and richness for the two time periods. Values are the mean ± 1 sd.

**Fig. 7.** Box plots of microphytoplankton abundance, as determined by microscopy, integrated over the upper 55 m. The vertical solid line separates Time 1 from Time 2.

**Fig. 8.** Box plots of diatom abundance, as determined by microscopy, integrated over the upper 55 m. The vertical solid line separates Time 1 from Time 2.
periods (ca. 1 °C; Taylor et al., 2012). Increasing light penetration from 61 to 75 m over time allows for increased growth of phytoplankton at depth and the rapid decline in large diatoms results in a more homogeneous plankton community that is at the same time more species rich.

The impacts of this shift in community composition on the biogeochemistry of the Cariaco Basin are less certain. Changes in phytoplankton community structure could influence the vertical particle fluxes in the Cariaco Basin if mineral-ballasted phytoplankton, such as diatoms and coccolithophores, do disproportionately enhance organic matter fluxes to depth (Michaels and Silver, 1988; Armstrong et al., 2002). A reexamination of particle flux data showed that indeed opal and carbonate fluxes significantly increased between the two time periods by approximately 45% (0.098 versus 0.143 g opal m⁻² d⁻¹, 0.086 versus 0.128 g carbonate m⁻² d⁻¹ (Thunell et al., 2008). However, the particulate organic carbon flux remained similar (5.6 versus 5.2 mmol m⁻² d⁻¹, p = 0.46), in contrast to the results found by Taylor et al. (2012). The increase in carbonate fluxes agree with the increases of coccolithophores as assessed by HPLC. The increase in opal flux, however, may be attributed to the increase in diatom abundance in the 56–100 m interval found using HPLC in this study and with the changes in diatom composition from centric to pennate forms. Taylor et al. (2012) suggest that the increase in particle flux over time is due to an increase in zooplankton grazing pressure associated with reported increases in zooplankton biomass, which would occur with the decline in sardine grazing on zooplankton. In fact, studies in the nearby Sargasso Sea have shown that vertically-migrating zooplankton have a preference for diatoms (Schnetzer and Steinberg, 2002). Grazing rates could be such that the current sampling resolution would fail to detect diatom true abundance. The lack of change in the particulate organic carbon export is more puzzling as one might expect all particle fluxes to increase with enhanced grazing rates as well, despite declines in overall net primary production observed at the Cariaco Basin Time-series site (Taylor et al., 2012). However, the lack of particulate organic matter export to deeper waters may also be due to enhanced zooplankton activity (Steinberg et al., 2008; Lomas et al., 2010).

In the Sargasso Sea, in a decade-long (1996–2007) period, a more than 50% increase in euphotic zone integrated autotrophic biomass, prokaryotic phytoplankton, and primary production has been observed, and is hypothesized to be the result of increasing mixing rates over the course of the time-series (Lomas et al., 2010). During this same time-period, Lomas et al. (2010) found that a 60% increase in POC export at 150 m was offset by a dramatic decrease in export efficiency, such that POC fluxes measured at 300 m remained unchanged. They argued that enhanced zooplankton and bacterial mediated remineralization (e.g., Steinberg et al., 2008), coupled with the aggregation of smaller particles that are exported at the same rate but are more easily remineralized (e.g., Richardson and Jackson, 2007), may have led to the decline in deep water POC export. One hypothesis is that, while the mechanisms inducing the change in community shift differ in the Sargasso Sea and the Cariaco Basin, the end results are the same—a community structure characterized by declining diatom abundances and changes in species, increases in smaller taxa, and potentially higher zooplankton grazing results in little to no change in the magnitude of POC exported to depth.

5. Conclusions

The Cariaco Basin has undergone significant changes in phytoplankton community structure over the past 15 years, likely associated with changes in the basin’s hydrography which have resulted from shifts in the regional climate system. In this study, we demonstrate marked changes in the distribution, concentration, and diversity of the phytoplankton community that includes an increasingly diverse, yet less seasonally variable phytoplankton community that has moved deeper in the water column. The transition in the phytoplankton community appears to be in direct response to changes in the physical structure of the water column, which includes a reduction in upwelling intensity that resulted in a shallower mixed layer, and more frequent stratification. We interpret these changes as an outcome of longer term variability in climatic conditions associated with declining Trade Winds, and increasing stratification that have affected the Caribbean Sea and tropical Atlantic Ocean between 1996 and 2010. Carbonate and opal fluxes to depth have increased over the course of the time-series, while POC export rates have remained relatively stable. Despite the overall increase in POC export efficiency over the course of the time-series, the lack of a similar magnitude increase in POC export relative to the mineral components suggests that either increased zooplankton grazing has enhanced organic matter remineralization at depth or that smaller taxa, even though they contribute to particle flux, may be more efficiently remineralized. Understanding these complex linkages between phytoplankton community structure and the magnitude and composition of particle export are important next steps to understanding how large scale changes in climate impact coastal tropical ecosystems.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr.2015.03.004.

References


