Bio-optical characteristics of a red tide induced by *Mesodinium rubrum* in the Cariaco Basin, Venezuela.

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Abstract

The bio-optical changes of the water induced by red tides depend on the type of organism present, and the spectral characterization of such changes can provide useful information on the organism, abundance and distribution. Here we present results from the bio-optical characterization of a non-toxic red tide induced by the autotrophic ciliate *Mesodinium rubrum*. Particle absorption was high [$a_p(440)=1.78 \text{ m}^{-1}$], as compared to measurements done in the same region [$a_p(440)= 0.09 \pm 0.06 \text{ m}^{-1}$], with detrital components contributing roughly 11% [$a_d(440)=0.19 \text{ m}^{-1}$]. The remainder was attributed to absorption by phytoplankton pigments [$a_{ph}(440)=1.60 \text{ m}^{-1}$]. These a_{ph} values were ~ 15 times higher than typical values for these waters. High chlorophyll *a* concentrations were also measured ($52.73 \mu\text{g L}^{-1}$), together with alloxanthin ($9.52 \mu\text{g L}^{-1}$) and chlorophyll *c* ($6.25 \mu\text{g L}^{-1}$). This suite of pigment is typical of the algal class *Cryptophyceae*, from which *Mesodinium* obtains its chloroplasts. Remote sensing reflectance showed relatively low values [$R_{rs}(440) = 0.0007 \text{ sr}^{-1}$], as compared to other R_{rs} values for the region under high

bloom conditions [$R_{rs}(440) = 0.0028 \text{ sr}^{-1}$], with maxima at 388, 484, 520, 596 and 688 nm. Based on the low reflection in the green-yellow, as compared to other red tides, we propose a new band ratio [$R_{rs}(688)/R_{rs}(564)$] to identify blooms of this particular group of organisms.

Keywords: *Mesodinium*, *Cryptophyceae*, algal bloom, bio-optical properties, Southeastern Caribbean Sea.

1. Introduction

Red tides have received increased attention during the last decades due to their apparent increase throughout the world, and in particular because of their harmful effects on fisheries, aquaculture and human health. Red tides have also been used as an indicator of water quality (Hallegraeff, 1993, 1995, 2010; Anderson, 2004; Azanza *et al.*, 2005; Gilbert *et al.*, 2005; Larkin and Adams, 2007; Alvarez-Salgado *et al.*, 2008; Landsberget *et al.*, 2009; Morgan *et al.*, 2009; Pitcher and Probyn, 2011). The most visible manifestation of red tides is the change in water color, induced by the pigment contained within the organisms that comprise the bloom (Dierssen *et al.*, 2006; Roesler and Boss, 2008). There can also be increased scattering which reduces the transparency of the water column (Cannizzaro *et al.*, 2008). Indeed, in Eastern Venezuela red tide events are known as ‘turbios’ (or ‘turbid’ in English), due to the aforementioned water characteristics.

Red tides are generally noticed when the bloom is in an advanced stage and the numbers of dinoflagellates, diatoms, cyanophytes and other ciliates is well within the thousandsof cells per L (Cannizzaro *et al.*, 2008; Soto, 2013). The color of the bloom can

vary, depending on the species present, on the concentration of organisms and their distribution throughout the water column (Dierssen *et al.*, 2006; Roesler and Boss, 2008). In economically important locations, early detection networks have been put in place (e.g.; Cannizzaro *et al.*, 2008; Alvarez-Salgado *et al.*, 2008; Heil and Steidinger, 2009; Soto, 2013), where waters are consistently monitored through in situ sampling for the presence of harmful algae. Many of these monitoring techniques take advantage of the changes in the bio-optical properties of the water induced by the red tide (Millie *et al.* 1997; Kyewalyanga *et al.* 2002; Cannizzaro *et al.*, 2008; Sasaki *et al.* 2008, Anderson, 2009; Shen *et al.*, 2012). One of the advantages of using bio-optical techniques, in particular remote sensing, is that they can cover large surfaces synoptically, providing information on the extent and distribution of the bloom (Kahru and Mitchell, 1998; Stumpf *et al.*, 2003; Cannizzaro *et al.*, 2008; 2009).

Because of their important impact on resources and human health, most of the research on red tides has focused on those species which are toxic. However, there are other species of red tides which do not produce toxins, but still induce significant changes in the water quality and can thus have adverse effects in the regions where they occur, and are thus classified as Harmful Algal Blooms, or HABs. One such species is *Mesodinium rubrum*, a phototrophic ciliate that it is kleptochloroplastidic and robs the pigmented organelles from their cryptophyte algal prey (Gustafson *et al.*, 2000). Due to its ample geographic distribution (e.g. Crawford, 1989; Kyewalyanga *et al.*, 2002), *M. rubrum* has been considered a group of species, more than a single one (García-Cuetos, *et al.*, 2012). *M. rubrum* can have adverse effects on water quality if in sufficient numbers (e.g. oxygen depletion, change or disrupt food-web dynamics; Horner *et al.*, 1997; Johnson *et al.*, 2013), and has been identified as prey for *Dinophysis* spp., a dinoflagellate responsible for the

diarrheic shellfish poisoning (DSP) (Park *et al.* 2006, Nishitani *et al.* 2008; Riisgaard & Hansen 2009). Most research about this species has been done in cultures (Gustafson *et al.*, 2000; Hansen and Fenchel, 2006; Park *et al.*, 2007; Garcia-Cuetos *et al.*, 2012; Hansen *et al.*, 2012); booms of *M. rubrum* are difficult to quantify in the field because of the speed in which this ciliate aggregates and disaggregates (Crawford, 1989). It is distributed worldwide, and has also been reported in Eastern Venezuela (La Barbera-Sánchez *et al.*, 2004; Mutshinda *et al.*, 2013). To the best of our knowledge, there has not been a characterization of the bio-optical properties of red tides induced by this organism (or others) in this region. Optical characterization of *M. rubrum* has been carried out in temperate areas such as the Bedford Basin (Nova Scotia, Canada; Kyewalyanga *et al.*, 2002), Long Island Sound (USA; Dierssen *et al.*, 2015), the southern Benguela Upwelling System (Roesler *et al.*, 2004; Babin *et al.*, 2005), and Southampton Water (UK; Garcia *et al.*, 1993).

Here we present bio-optical properties of a red tide of *Mesodinium rubrum* in the Cariaco Basin which occurred in April 2008. We investigate the changes in the water color through particle absorption (a_p , a_d , a_{ph}), high performance liquid chromatography (HPLC) and in situ remote sensing reflectance (R_{rs}). These results are further compared with characteristics of this region, which has been studied under the framework of the CARIACO Ocean Time-Series project since 1995 (Muller-Karger *et al.* 2010).

2. Methods

2.1 Study Area

The Cariaco Basin is a ~1400 m deep tectonic pull-apart basin located on the continental margin of northeastern Venezuela (Figure 1). A shallow sill with two channels

(~140 m) isolates the deeper waters of this basin, and exchange with the Caribbean Sea occurs only in surface waters. The basin experiences an upwelling season as Trade Winds intensify between December and May. The rainy season extends from June to November,

Figure 1. The Cariaco Basin showing the locations of the CARIACO Ocean Time Series station and the *Mesodinium rubrum* red tide.

and surface waters are thermally stratified during this time. Due to the strong coastal upwelling and restricted circulation, the basin is anoxic below ~250 m. The basin has been the site of the CARIACO Ocean Time-Series project since 1995, which seeks to understand the biogeochemistry, sedimentology and the bio-optics of the Basin (e.g. Thunel *et al.*, 2007; Muller-Karger *et al.* 2010, 2013; Scranton *et al.* 2014). During one of the CARIACO core cruises on April 8, 2008, a red tide was observed at 14:20 h, 10.65° N, 64.44° W, roughly 30.4 km NE from the CARIACO site and 16 km W from the Araya Peninsula (Figure 1).

2.2 Hydrographic measurements

So as to be able to understand the bloom conditions, hydrographic data from the CARIACO time-series station corresponding to the same day was utilized. Specifically, salinity, temperature and mixed layer depth were looked at, together with a 7-day mean (1-8 April 2008) of satellite wind data obtained from <http://ood.cbm.usb.ve/historial/viento/> for 11° N 65° W. The methods for collection and processing of the hydrographic data are described in Astor *et al.* (2013).

2.3 Pigment analysis

All samples for this study were collected from surface waters. For chlorophyll *a* (Chl*a*), four samples of different volumes (125; 180; 50 and 50 mL) were vacuum filtered through 25mm GF/F filters, stored inside plastic centrifuge tubes and frozen immediately (-20°C) until analysis. Two samples for pigment analysis through high performance liquid chromatography (HPLC) were also collected by filtering 100 and 50 mL of water through 47 mm GF/F filters. HPLC samples were stored folded in half inside aluminum foil and frozen to -40°C until analysis.

Fluorometric estimations of Chl*a* concentrations were done using a Turner Design 10-AU-005 Fluorometer (Turner Designs, San Jose, CA, USA), using methanol instead of acetone as the extracting solvent, and sonicating the sample prior to analysis in order to break up the cells (Wright *et al.*, 1997; Astor, *et al.* 2013). HPLC Samples were analyzed at Horn Point Laboratory (HPL; Maryland, USA) following Hooker *et al.* (2005). Pigment-based size classes were calculated following Uitz *et al.*, 2006. Briefly, seven major diagnostic pigments were used to calculate the proportions of micro-, nano-, and picophytoplankton present in each sample. Pigment ratios, such as alloxanthin:Chl*a*, were calculating by dividing the HPLC-derived alloxanthin by total Chl*a*.

2.4 Sampling for organism identification

Phytoplankton species identification was done on 1L water samples also collected from the surface and kept refrigerated until analysis. Taxonomic identification was performed through an inverted microscope Axiovert Carl Zeiss (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) on live individuals; cell dimensions were measured with calibrated eyepiece micrometer. Because typical solutions (e.g. 4 % sodium tetraborate-buffered formaldehyde) used for phytoplankton preservation were not successful (organisms burst or deformed), it was not possible to determine abundance.

2.5 Particle absorption

Three water samples (two of 20 mL and one of 30mL) were collected from the surface and filtered through 25mm Whatman GF/F filters using a vacuum pump for total particle absorption [$a_p(\lambda)$]. The filters were stored frozen at -20°C in petri dishes covered by aluminum foil (to avoid light exposure) until analysis within a few days of collection. $a_p(\lambda)$ was measured using the filter pad technique (Kishino *et al.*, 1985; Mitchell and Kiefer, 1988; Astor *et al.*, 2013). Spectral optical density [$OD(\lambda)$] was measured on the filters from 380 to 780 nm at 4 nm intervals using a PR-650 spectroradiometer (Photo Research, Inc., Chatsworth, CA, USA). Subsequently, the filters were washed with 50 mL of hot (60°C $\pm$ 0.5°C) methanol in aliquots of 5 mL and re-scanned to obtain a second optical density [$OD'(\lambda)$] estimate to derive the detrital absorption coefficient (a_d), a_p and $a_d(\text{m}^{-1})$ were calculated from OD following Mitchell and Kiefer (1988) and Bricaud and Stramski (1990). Phytoplankton absorption [$a_{ph}(\lambda)$] was obtained from the difference between these absorption coefficients, as follows:

$$a_{ph}(\lambda) = a_p(\lambda) - a_d(\lambda).$$

The three different spectra from the three different samples were averaged in order to obtain a single spectrum and perform subsequent analyses. The chlorophyll-specific absorption coefficient $a_{ph}^*(\lambda)$ [$\text{m}^2 (\text{mg Chla})^{-1}$] was obtained dividing $a_{ph}(\lambda)$ by the corresponding fluorometric Chla value (Moisan and Mitchell, 1999).

For a given algal population, the “package effect index”, Q_a^* , is defined as the ratio of the actual absorption coefficient, $a_{ph}(\lambda)$, to the absorption coefficient of the same material which would be dispersed into solution, $a_{sol}(\lambda)$ (Bricaud *et al.*, 2004):

$$Q_a^* = a_{ph}(\lambda) / a_{sol}(\lambda)$$

$a_{sol}(\lambda)$ was calculated following Bidigare *et al.* (1990), which sums the contributions of all individual pigments, taking into consideration their volume-based concentrations and pigment types. This index ranges from 0 (maximal package effect) to 1 (minimal package effect; Bricaud *et al.*, 2004).

Derivative spectroscopy has been widely used in the analysis of hyperspectral data (e.g. Bidigare *et al.*, 1989; Torrecilla *et al.*, 2009; Organelli *et al.*, 2013). It can be used for separating absorption peaks caused by different algal pigments. The fourth derivative of the a_{ph} spectrum of the bloom was calculated; data was smoothed to 8 nm prior to the derivative calculation and the decrease in signal / noise ratio was avoided by averaging the derivative calculations every 8 nm.

2.6 Remote Sensing Reflectance

Remote sensing reflectance [$R_{rs}(\lambda)$] was collected using a hand-held spectroradiometer (Spectrascan PR650, PhotoResearch Inc., Chatsworth, CA, USA) following the NASA Ocean Optics Protocols for SeaWiFS Validation (Mueller and Austin, 1995). The data were processed using IDL v.6.1 with programs developed in-house (Dr. Chuanmin Hu, USF). Remote sensing reflectance was derived from water leaving radiance

(L_w) and downwelling irradiance (E_d) between 380-780 nm, at 4 nm intervals using the following relationship:

$$R_{rs} = L_w / E_d \text{ (Mobley, 1994),}$$

L_w was calculated from above-water measurements of upwelling radiance and sky radiance measured at zenith angles of 30°; E_d was measured using a cosine collector.

2.7 Comparison with CARIACO data

In order to compare $R_{rs}(\lambda)$ and $a_p(\lambda)$ from the red tide with those from natural conditions within the basin, data from the CARIACO program was used, which is freely available from SeaBASS (<http://seabass.gsfc.nasa.gov/seabasscgi/data.cgi?experiment=CARIACO>) and upon request (<http://www.imars.usf.edu/CAR/>).

Methods for the processing of CARIACO samples have been published elsewhere (e.g. Muller-Karger et al., 2001; Lorenzoni et al., 2011; 2015; Astor et al., 2011) and are consistent with the way *M. rubrum* samples were processed.

3. Results

3.1 Environmental conditions

To the naked eye, the bloom appeared dark red, forming patches of a few tens of meters. Atmospheric conditions were clear with little cloud cover (10%) and a mean wind speed of 7.5 m s⁻¹ from ENE, within what was measured for previous days (between 6 to 8 m s⁻¹). At the CARIACO station for the same day and an hour before the sighting of the red tide, surface temperature was 23.8 °C and the salinity 36.82. The mixed layer depth extended down to ~23 m, and had an average temperature of 22.9 °C; due to the intense solar heating, temporary diurnal stratification was observed in the upper 7m. Given the

proximity, we assume that conditions found at the site of the red tide were similar to those measured at the CARIACO station.

3.2 Organism and pigment concentrations

There was a single planktonic organism observed in the water samples which was identified as the ciliate *Mesodinium rubrum* (Lohmann 1908; Figure 2). Approximate measurements of individuals were 60 - 70 x 40 - 50 μm .

Results from Chl *a* and phaeopigment (Phaeo) fluorescence analyses are shown in Table 1. Chl *a* concentrations were high ($60 \mu\text{g L}^{-1}$), compared to what has been reported for the region ($1.94 \pm 1.58 \mu\text{g L}^{-1}$, average upwelling values at CARIACO for the period 1995-2013), and even higher than extremes measured at the CARIACO site ($7.30 \mu\text{g L}^{-1}$; measured during the March, 2001 cruise; <http://www.imars.usf.edu/CAR/>).

Figure 2. Live specimens of *Mesodinium rubrum* observed in phase contrast microscopy, viewed from different angles.

Table 1. Chlorophyll a and phaeopigment concentrations ($\mu\text{g L}^{-1}$) measured using fluorescence for the four different subsamples collected within the *Mesodinium rubrum* red tide observed in the Cariaco Basin.

Sub-sample	Chlorophyll ($\mu\text{g L}^{-1}$)	Phaeopigment ($\mu\text{g L}^{-1}$)	Phaeo/Chl
1	60.84	13.73	0.23
2	55.30	12.95	0.23
3	62.56	12.92	0.21
4	60.69	9.77	0.16
Mean	59.85	12.84	0.21

HPLC pigment composition is shown in Table 2. The highest concentration corresponded to total Chla ($52.7 \mu\text{g L}^{-1}$; 68% of total pigment concentration); total Chla is defined as the summed contributions of concentrations of monovinylChla, divinylChla, chlorophyllide-*a*, and the allomeric and epimeric forms of Chla. Following Chla, the next highest concentration was of chlorophyll *c* (Chlc) and carotenes. Other pigments present in relative high abundance included chlorophyll *c*₁₊₂, alloxanthin, and α - β carotenes. Pigment-based size classes, as calculated according to Uitz *et al* (2006), indicated a dominance of nanoplankton (0.99), corresponding to the Cryptophyta which reside within *M. rubrum*.

3.3 Particle absorption

$a_p(440)$ measured in the *M. rubrum* bloom was ~20 times higher than average values measured at the CARIACO ocean time-series site during upwelling [$a_p(440) = 1.78 \text{ m}^{-1}$, as compared to $a_p(440) = 0.09 \pm 0.06 \text{ m}^{-1}$]. The shape of the profiles measured during the red tide were similar to those observed during high bloom conditions ($> 3 \mu\text{g L}^{-1}$) at CARIACO (Figure 3), with maxima around 432-440 nm, decreasing towards the green - yellow band, and exhibiting the typical Chla absorption peak at 676 nm in the red. The package effect observed during the *M. rubrum* bloom was 0.65.

Table 2. Summary of phytoplankton pigment concentration ($\mu\text{g L}^{-1}$) measured using HPLC for the *M. rubrum* red tide, and in CARIACO station on the same day. Gray bars highlight the dominant pigments present in Cryptophytes. Pigment-based size classes (following Uitz et al., 2006) are also indicated at the bottom.

Pigment	CARIACO	<i>Mesodinium</i>
Total chlorophylla	0.459	52.735
Total chlorophyllb	0.052	0.001
Total chlorophyllc	0.084	6.246
Carotenenes	0.017	1.907
19'-butanoyloxyfucoxanthin	0.012	0.001
19'-hexanoyloxyfucoxanthin	0.051	0.023
Alloxanthin	0.009	9.515
Diadinoxanthin	0.017	0.043
Diatoxanthin	0.003	0.001
Fucoxanthin	0.092	0.052
Peridinin	0.012	0.001
Zeaxanthin	0.018	0.022
Monovinylchlorophylla	0.443	52.594
Divinylchlorophylla	0.001	0.001
Chlorophyllidea	0.016	0.142
Monovinylchlorophyllb	0.052	0.001
Divinylchlorophyllb	0.001	0.001
Chlorophyll c2 + chlorophyll c1 + MGDVP	0.043	6.246
Chlorophyllc3	0.041	0.001
Lutein	0.001	0.001
Neoxanthin	0.009	0.001
Violaxanthin	0.006	0.001
Total pheophytina	0.006	0.322
Total pheophorbidea	0.044	0.001
Prasincoxanthin	0.022	0.001
<i>Microplankton</i>	0.420	0.010
<i>Nanoplankton</i>	0.290	0.990
<i>Picoplankton</i>	0.280	0.000

The contribution of phytoplankton to the total particle absorption was roughly 89%, with the remaining 11% was attributed to detrital absorption (a_d). The a_d spectrum exhibited shoulders not previously noted in the CARIACO ocean time-series spectra at 540 nm and 584 nm (0.39 m^{-1} and 0.26 m^{-1} , respectively; Figure 3), likely caused by pigments remaining on the filter pad that were not fully extracted by the technique employed. Indeed, after the filter was washed with the hot methanol, it still retained a pink coloration, possibly

Figure 3. Comparison of the absorption spectra corresponding to total particles (a_p) phytoplankton, (a_{ph}) and detrital material (a_d), during (A) high phytoplankton biomass at the CARIACO Time Series station (February 2007) and (B) in *Mesodinium rubrum* red tide.

due to phycoerithryn, which is not very soluble with this solvent. In order to fully account for phytoplankton pigments from the filters, a correction to the a_d and a_{ph} spectra was applied, similar to what is done in the calculation of Fluorescence Line Height (FLH; Xing, *et al.* 2007). The calculation is to establish a baseline in the spectrum of a_d , between 480 and 652 nm, a zone where the extra shoulders appear. It is based on the ratios between the differences in wavelengths and absorption, wherein a calculated absorption establishes new baseline values for that interval. The difference between the values of a_d and the baseline, are added to the a_{ph} spectrum for the calculated interval. The corrected spectra are shown in Figure 4. Phytoplankton specific absorption $a^*_{ph}(440)$, ranged between 0.02 – 0.03 $\text{m}^{-1} (\text{mgChl } a \text{ m}^{-3})^{-1}$.

Figure 4. Absorption by phytoplankton spectrum corrected (a_{ph} , bottom) and associated fourth derivative plot (top) in the *Mesodinium rubrum* red tide. The maxima of the fourth derivative plot correspond to peaks of the a_{ph} spectrum, and are related to the absorption of the different pigments present.

Derivative spectroscopy was used to identify absorption peaks due to accessory pigments present. The peaks that were identified applying the fourth derivative to the a_{ph} spectrum are shown in Figure 4, together with the corresponding pigments which absorb at these wavelengths.

3.4 Remote Sensing Reflectance [$R_{rs}(\lambda)$]

Typical variations in remote sensing reflectance, $R_{rs}(\lambda)$, observed at the CARIACO Ocean Time-Series site, are shown in Figure 5 alongside with R_{rs} measured during the *M. rubrum* bloom. The R_{rs} spectra corresponding to low Chl *a* ($<0.2 \mu g L^{-1}$) have higher blue reflectance than those with higher Chl *a* ($>3 \mu g L^{-1}$). $R_{rs}(\lambda)$ in the *Mesodinium* bloom was

270 relatively low except in red, as compared to values observed during upwelling at the
 271 CARIACO station (e.g. March 1998 and February 2007; Figure 4). $R_{rs}(440)$ (0.0007 sr^{-1})
 272 ¹) was approximately three times lower than what has been observed at CARIACO under
 273 high biomass conditions (for example, in February 2007, $R_{rs}(440) = 0.0021 \text{ sr}^{-1}$ and $\text{Chla} =$
 274 $3.80 \mu\text{g L}^{-1}$). At the same scale of the CARIACO $R_{rs}(\lambda)$ spectra, *M. rubrum* was quite low,
 275 which gave a very dark and reddish appearance to the waters (Figure 5); two maxima were
 276 visible in the red (688 nm, 0.0013 sr^{-1}) and orange (596 nm, 0.0010 sr^{-1}), but other peaks
 277 were visible in the green (520 nm, 0.0008 sr^{-1}), blue green (484 nm, 0.0007 sr^{-1}), and
 278 purplish blue (388 nm, 0.0013 sr^{-1}). The lowest reflectance was at 660 nm 0.0002 sr^{-1} and

Figure 5. Remote sensing reflectance spectrum (R_{rs}) in CARIACO Time Series station at different levels of biomass: very high (March, 1998), high (February, 2007) and low (October, 2007), in comparison with *Mesodinium rubrum* red tide R_{rs} spectrum.

279 560 nm 0.0005 sr^{-1} (yellowish green).

280

281 4. Discussion

282 4.1 Occurrence of the bloom

283 April corresponds to the end of the upwelling season in the Cariaco Basin (Astor *et*
 284 *al.*, 1998; Muller-Karger *et al.*, 2001;2010). However, in April 2008 at the CARIACO
 285 Ocean time-series station a relatively thick mixed layer was observed (23 m) which had

relatively low temperatures (22.9 °C), indicative of upwelling for that time period. It is possible that the upwelling injected nutrients into the euphotic zone to stimulate the *M. rubrum* bloom. Indeed, some authors suggest that conditions that increase nutrient availability within the euphotic zone, such as river runoff, increased mixed layer depth and upwelling can stimulate the initiation of a *M. rubrum* bloom (Kyewalyanga *et al.*, 2002; Jiménez and Gualancañay, 2005). However, other authors suggest that *M. rubrum* thrives in environments with lower salinity and strong stratification (e.g. Cloern *et al.*, 1994; Johnson *et al.*, 2013). Salinity was not particularly low at the CARIACO site (36.82). It is also possible that the bloom of *M. rubrum* was advected from the coast, where more favorable bloom conditions dominated. The temporary stratification observed in the upper 7m, coupled with the high motility of this organism which helps form patches, likely helped maintain the bloom under the moderate sea and wind conditions that were measured on April 8, 2008 (4 in the Beaufort scale).

In general, there are few references that report *M. rubrum* in the Cariaco Basin and throughout Venezuelan waters (Mutshinda, *et al.* 2013). However, it is possible that this organism might have been undersampled, due to its fragility upon preservation (e.g. Crawford, 1989; Dierssen *et al.*, 2015). It is also possible that its abundance has been underestimated, for being considered part of the microzooplankton, and thus has gone undetected except when red tides have occurred (e.g. La Barbera–Sanchez, 2004; Flynn *et al.*, 2012). The red coloration of the water is only visible when high concentrations of this organism are present (Shen *et al.*, 2012).

4.2 Pigments, their relationship with the organism and bio-optical properties of the water

Chla concentrations measured in the *Mesodinium* bloom ($\sim 60 \mu\text{g L}^{-1}$) were significantly higher than peak concentrations measured historically at CARIACO ($7.3 \mu\text{g L}^{-1}$).

¹; <http://www.imars.usf.edu/CAR/>). Such high Chla concentrations are common for *Mesodinium rubrum* blooms (Wilkerson and Grunseich, 1990; Proenca 2004; Jiménez and Gulancañay 2005; Johnson *et al.*, 2013). *M. rubrum* obtains its symbiotic chloroplasts from prey via ingestion from nanoflagellates belonging to the class *Cryptophyceae*. It has been suggested that this species has a preference for particular clades of cryptophytes (Park *et al.*, 2007; Hansen *et al.*, 2012). The highest concentrations of HPLC pigments analyzed in this study correspond to Chla, chlorophyll c₁₊₂, alloxanthin, and α - β carotenes, all of which have been previously reported in cryptophytes (e.g. Jeffrey *et al.*, 2011). Phycoerythrin, one of the major light-harvesting pigments of cryptophytes, was not quantified via HPLC, as it requires a separate extraction process (Subramaniam *et al.* 1999, Bäsaca-Loya *et al.*, 2009). Alloxanthin can serve as either a light harvesting or photoprotective pigment, depending on the level of irradiance; the low alloxanthin:Chla ratio of 0.18 suggests that at this time it likely served photosynthetic functions (Laviale and Neveux, 2011; Rialet *et al.*, 2013). The dominance of nanoplankton in this bloom, as determined by pigment ratios (Uitz *et al.*, 2006) is misleading, and it is caused by the kleptochloroplastic nature of this ciliate, which robs the chloroplasts from its cryptophytes prey and is thus classified, using pigments, as such.

Pigment composition, cell size, and intracellular packaging can affect and modify the wavelengths and level of light absorption by phytoplankton (Platt and Sathyendranath, 1988; Morel and Andre, 1991; Roesler and Perry, 1995; Bricaud *et al.*, 2004). Major light-harvesting pigments of cryptophytes, such as Chla, Chlc and α - β carotenes, have dominant absorption peaks at wavelengths between 420-490 nm (Hoepffner and Sathyendranath, 1991). Indeed, photosynthetically usable radiation (PUR) absorbed by phytoplankton is

highest in the blue region of the spectrum (Morel and Bricaud, 1981). High absorption is also observed in the red (670-680 nm), corresponding to absorption by Chla (Hoepffner and Sathyendranath, 1991). In the *M. rubrum* samples, a_p and a_{ph} exhibited the highest absorption in the blue (410-450nm) and red (670-680nm) part of the spectrum, in agreement with the pigment composition determined via HPLC (Figure 3). The a_{ph} spectrum was similar to that of a_p , in both shape and magnitude, suggesting that phytoplankton was responsible for most of the absorption. $a_{ph}(440)$ measured in the red tide (1.59 m⁻¹) was roughly 15 times higher to what has been reported at the CARIACO time-series station in upwelling season (0.1 m⁻¹, Chla > 1.5 µg L⁻¹).

The degree of packaging effect affects the light absorbing capability of phytoplankton; a high package effect results in a decrease in absorption (Morel and Bricaud, 1981). The high package effect observed during the *M. rubrum* bloom (0.65) resulted in $a_{ph}^*(440) = 0.02 \text{ m}^2 (\text{mg Chla})^{-1}$, which was lower than what has been historically measured at CARIACO during the upwelling season [$0.048 \pm 0.026 \text{ m}^2 (\text{mg Chla})^{-1}$; Lorenzoniet al., 2011].

The spectral shape of a_{ph} was not smooth, but exhibited peaks and shoulders that corresponded to the presence of accessory pigments. The fourth-derivative, which enabled a better separation of absorption peaks for the identification of corresponding pigments, showed maxima corresponding to Chla (440, 676 nm), carotenes (464, 496 nm), phycoerythrin (540 nm), and Chlc (584, 636 nm) (Hoepffner and Sathyendranath, 1991; Jeffrey et al., 1997; Kyewalyanga et al., 2002; Dierssen et al., 2015; Figure 3), in agreement with HPLC-based pigment analysis.

The most pronounced variations in R_{rs} observed in the Cariaco Basin occur in the blue spectral region and are associated with different concentrations of Chl a ; while colored dissolved organic matter (CDOM) also contributes to the absorption of light in the blue part of the spectrum, concentrations in Cariaco are not that high (Lorenzoni et al., 2009). Variations in the green part of the spectrum are relatively small (Figure 5). In the *Mesodinium* red tide, the high Chl a concentrations resulted in low R_{rs} values in the blue, approximately three times lower than what has been observed at CARIACO under high biomass conditions. The decrease in $R_{rs}(\lambda)$ observed in the *M. rubrum* bloom waters could also be attributed to low particle backscattering (Dierssen et al., 2006; Cannizzaro et al., 2008). *Mesodinium* itself (a ciliate) is not a strong scatterer (Morel and Ahn, 1991; Whitmire et al., 2010). Other sources of scatter could include detritus. An indirect measurement of detritus (scatterer) concentration can be obtained from detrital absorption coefficients; the detrital absorption measured (Figure 3) indicate that there was relatively low detrital concentration at the time of bloom. Phaeopigments are chlorophyll degradation products that can be used as an indirect measurement of phytoplankton mortality, including grazing, as they are commonly produced by feeding zooplankton (Lorenzen, 1967). Phaeopigment to Chl a ratios were low, as compared to what has been reported for CARIACO (Taylor et al., 2012), suggesting reduced grazing pressure, which may partially be responsible for the low detrital concentrations.

The R_{rs} spectrum from the *M. rubrum* bloom was different from what has been observed for red tides induced by other organisms (e.g. Gower, et al., 1999, Dierssen et al., 2006; Ahn, et al., 2006; He et al., 2008; Shen, et al., 2012). The particular optical features of this type of algal bloom, which owes its spectrum largely to the cryptophytes it contains,

have been noted by other authors (e.g. Gower et al., 1984; Lin et al., 1984; Roesler et al., 2004; Xi et al., 2015). In those cases where diatoms or dinoflagellates are responsible for the bloom, pronounced peaks in the green (550 – 600 nm) and in the red (~685 nm) are generally visible (e.g. Cannizzaro et al., 2008). These maxima are a result of the high phytoplankton concentrations which strongly absorb in the blue (together with CDOM) and reflect in the green; water leaving radiance in this part of the spectrum is also higher if backscatterers are present (Dierssen et al., 2006; Cannizzaro et al., 2008). In the case of *Mesodinium*, the peak in the green was absent, and instead a peak was observed at around 590 nm, corresponding to the fluorescence of phycoerythrin (Exton et al., 1983). The reflectance spectra was also different from cyanobacteria red tides such as those caused by *Trichodesmium*. While this group also contains phycobiliproteins that strongly reflect in the yellow - green (575 nm), they lack a Chl *a* maximum in the red and exhibit large backscattering induced by the gas vacuoles of this organism (Subramaniam et al. 1999b).

The changes observed in the remote sensing reflectance at different wavelengths, induced by the amount of biomass and detritus present in surface waters, can be used to derive band ratios as a tool for identifying red tides (Stumpf et al., 2003; Tomlinson et al., 2004; Wynne et al., 2005; Cannizzaro et al., 2008; Shen et al., 2012). Several different techniques for remote detection have been proposed, which include the use of Chl *a* anomalies (Stumpf et al., 2003; 2008; Tomlinson et al., 2004; Wynne et al., 2005), backscatter algorithms (Cannizzaro et al., 2004; 2008), fluorescence (Cannizzaro et al., 2008; Hu et al., 2005; 2008), and band ratios (Amin et al., 2009; Carvalho et al., 2010; 2011). Identification techniques based on band ratios take advantage of the distinctive features of red tides in the red part of the spectrum (Amin et al., 2009; Carvalho et al., 2011; Soto, 2013), in the green (Sasaki et al., 2008; Dierssen et al., 2015) or the UV-blue

(Kahru and Mitchell, 1998). However, different red tide species have distinct spectral characteristics, which can make it almost impossible to use a single, standard algorithm for detection of different HABs (Shen *et al.*, 2012). Indeed, most of the aforementioned band-ratio algorithms have been developed and applied to blooms which are dominated by groups which have a strong reflectance in both the green and red parts of the spectrum (e.g. Tang, 2004, Ahn and Shanmugan, 2006; Sasaki *et al.* 2008; Shen, *et al.* 2012). In the case of *Mesodinium rubrum*, it lacks the strong R_{rs} in the green, and the traditional peak in the red, caused by the fluorescence of Chl *a*, is shifted to longer wavelengths by 10-20 nm. The enhanced phycoerythrin absorption in the green part of the spectrum (~530-570 nm) produces the corresponding dip in the reflectance (e.g. Dierssen *et al.*, 2015). The shift of the fluorescence peak to longer wavelengths (from ~ 685 to anywhere between 690 - 710 nm) has been attributed to the unique composition of the light-harvesting complexes of cryptophytes (Lin *et al.*, 1984; MacPherson and Hiller, 2003; Kaňa *et al.*, 2009; 2012). It has also been noted that this shift to longer wavelengths increases with increasing Chl *a* concentrations (Gower *et al.*, 1984). It is important to point out, however, that these special characteristics of the cryptophyte reflectance are mostly observable when concentrations of Chl *a* are high (>5-10 mg m⁻³; see Lin *et al.*, (1984) and Xi *et al.*, (2015) for comparisons of in situ and modeled cryptophyte R_{rs} with different Chl *a* concentrations).

Considering the unique spectral properties of cryptophytes, we propose the use of a simple band ratio to differentiate between waters with high concentrations of chlorophyll (> 20 mg m⁻³), induced by this type of algal bloom, from those of a normal bloom. This band ratio uses the R_{rs} peak in the red (688 nm in the case of the *M. rubrum* bloom observed in Cariaco) and the minimum in the green at 564 nm. In order to examine the performance of this band ratio, we compared our results with CARIACO Ocean Time-Series in situ R_{rs} data,

as well as with *M. rubrum* and cryptophyte R_{rs} spectra from the literature. R_{rs} spectra used for this comparison came from the work published by Xi et al. (2015 – modeled cryptophyte spectra), Babin et al. (2005 – *M. rubrum* bloom in the Benguela upwelling zone, South Africa), Lin et al. (1984 – *M. rubrum* bloom south of Vancouver Island, Canada), and from Cannizzaro and English (unpub. Data – *M. rubrum* bloom from Bayboro Harbor, St. Petersburg, FL. USA). The shape of all of these spectra compared very well with the R_{rs} spectra from the Cariaco Basin (data not shown).

For the *M. rubrum* bloom in Cariaco, the $[R_{rs}(688)/R_{rs}(564)]$ was 2.8. In non-bloom conditions measured at CARIACO ($Chla < 0.5 \text{ mg m}^{-3}$), this ratio was close to zero. During the upwelling, $[R_{rs}(688)/R_{rs}(564)]$ varied between 0.2 and 0.4 ($Chla > 1.5 \text{ mg m}^{-3}$) depending on the chlorophyll concentrations. This was nearly ten times less than what was measured during the red tide. For other *M. rubrum* or cryptophytes blooms (from Xi et al., 2015, Babin et al., 2005, Lin et al., 1984 and Cannizzaro and English, unpub. data), $[R_{rs}(688)/R_{rs}(564)]$ varied between 1.0 and 1.9. As stated earlier, the $Chla$ fluorescence peak often times shifts to longer wavelengths (see for example the spectra by Lin et al., 1984); this was specifically visible in the data from Lin et al. (1984) and Cannizzaro and English (unpub. Data; this particular bloom had concentrations of $\sim 75\text{-}150 \text{ mg m}^{-3}$; Cannizzaro, pers. Comm.). Performing the band ratio using specifically the shifted fluorescence peaks yielded the following: $[R_{rs}(700)/R_{rs}(565)] = 1.1$ (Lin et al., 1984) and $[R_{rs}(710)/R_{rs}(565)] = 4.9$ (Cannizzaro and English, unpub. Data). The minima in the green was always found centered at around 565 nm. Additional analyses were done using published reflectance spectra of other harmful algae species such as *K. brevis* (Cannizzaro et al., 2008) and *G. breve* (Dierssen et al., 2006); band ratios between the green (565 nm)

and the red (685-690 nm) in these types of blooms were well below 1, suggesting that this band ratio can discern between these types of algal blooms.

There is limited information on the remote sensing reflectance of blooms of *M. rubrum* and cryptophytes in general, and while this simple ratio would appear to be successful at differentiating cryptophytes from regular mixed phytoplankton blooms, it is necessary to further test it, in particular with remote sensing data. The unique spectral signature of cryptophytes suggests that it would be possible to differentiate these types of algal blooms using hyperspectral remote sensing reflectance $R_{rs}(\lambda)$ from upcoming satellite missions such as the Pre-Aerosol, Clouds, and ocean Ecosystem (PACE) and Hyperspectral Infrared Imager (HypIRI). It may also be possible to use current multispectral satellites, such as the Moderate Resolution Imaging Spectroradiometer (MODIS) and the Visible Infrared Imaging Radiometer Suite (VIIRS), to detect *M. rubrum* blooms though this may largely depend on the red shift of the fluorescence peak (neither satellite mission has spectral bands at around 700nm). Testing whether for these blooms a chlorophyll anomaly detection technique is appropriate (Stumpf *et al.*, 2003; Tomlinson *et al.*, 2004) would also be of interest. Unfortunately, we were not able to test the derived algorithm using satellite remote sensing data for Cariaco, as the only available ocean color satellite imagery corresponding to the time of the bloom for the region was on April 4, 2008. In this image, the concentration of Chl *a* for the coordinates of the bloom (10.65° N, 64.44° W) were 5 $\mu\text{g L}^{-1}$, significantly less than what was measured in the field. It is possible that at this time the bloom was still not formed, or that the processing algorithm (OC4v4; O'Reilly *et al.*, 2000) flagged the anomalously high chlorophyll concentrations.

5. Conclusions

Here we presented an analysis of the bio-optical properties of a *Mesodinium rubrum* bloom that was observed in the Cariaco Basin in April 2008. This red tide forming organism was present in high concentrations, which resulted in Chl_a values roughly thirty times higher than what has been typically measured under bloom conditions at the CARIACO Ocean time-series station ($\sim 60 \mu\text{g L}^{-1}$ as compared to $\sim 2 \mu\text{g L}^{-1}$). Typical *Cryptophyceae* pigment composition was observed through HPLC measurements; these pigments were identified in the phytoplankton absorption spectrum as peaks present at determined wavelengths. The particle and phytoplankton absorption spectrum were nearly identical in shape and magnitude, suggesting that most of the absorption of light was being caused by the ciliate. *M. rubrum* presents a particular R_{rs} spectrum, with low reflectivity in the green. This feature was used to develop a band ratio [$R_{rs}(688)/R_{rs}(564)$] to distinguish *Mesodinium* red tides from typical mixed blooms observed in the Cariaco Basin. Based on additional literature, this ratio seems to successfully distinguish cryptophyte-like from non-cryptophyte algal bloom conditions. It would be highly desirable to further test it using satellite-derived data for *M. rubrum* and cryptophytes algal blooms.

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