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Anatomy of a red tide bloom off the southwest coast of Florida

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ABSTRACT

A massive outbreak of *Karenia brevis* that had been ongoing for several months along the southwestern coast of Florida was sampled in early September 2005 off Sanibel Island to assess the utility of bio-optical features and ataxonomic analysis (quantification of eukaryotic and cyanobacterial picoplankton) by flow cytometry in monitoring red tide blooms. Sea-surface sampling followed aircraft visual location of discolored water. Within the most concentrated area of the bloom, chlorophyll *a* values exceeded 500 $\mu g\, l^{-1}$, and concentrations of nitrate (0.3 $\mu M \pm 0.0$) and ammonium (<0.2 μM) were depleted compared to high concentrations of total dissolved nitrogen, total dissolved phosphorus, and soluble reactive phosphorus (141 $\pm$ 34 μM, 16.5 $\pm$ 2.5 μM, and 6.44 $\pm$ 0.57 μM, respectively). Low water clarity in the bloom (Secchi depth transparency 0.3 m, K_d estimated at 4.83 m^{-1}) was strongly influenced by attenuation from dinoflagellates as well as chromophoric dissolved organic matter (CDOM). The fact that the *K. brevis* bloom occurred in lower-salinity (30 psu), high-nutrient waters implicates riverine transport of land-based nutrients as a source of nutrient supplies that fueled or sustained the bloom. Throughout ongoing efforts to advance modeling and technological capabilities that presently lack reliable predictive capability, bio-optical remote sensing via aerial flyovers along with in-water sensor data can continue to provide accurate coverage of relatively large temporal and spatial features. Flow cytometry can provide conservative (because of some cell lysis), rapid, near-real-time validation of bloom components. The concentration and position of the organisms, along with water mass scalars, can also help to diagnose factors promoting *K. brevis* bloom development and dispersion.

1. Introduction

The theme of this paper is borrowed from earlier work of Ryther et al. (1958). Both papers address problems of eutrophication in marine coastal waters; both involve observations and measurements of massive microalgal blooms; both, in the Eltonion sense (Elton, 1927), describe phenomena wherein a bloom species threatens the diversity in a natural ecosystem. A surprising outcome of the early Ryther et al. (1958) paper – that eutrophication had stimulated blooms of microalgae that were unpalatable for commercially important shellfish in coastal waters of New York – prompted the decision for engineering hydrographic changes (flushing strategies for the inlet) that set the stage for sensible conservation and improved benthic faunal production (U.S. Federal Water Pollution Control Administration, 1966; Ryther and Dunstan, 1971; Ryther and Officer, 1981). A key difference between the earlier study in New York waters and Florida "red tide" blooms of the harmful alga, *Karenia brevis*, is that the role of cultural eutrophication in stimulating these blooms remains a controversial issue (Hu et al., 2006; Brand and Compton, 2007; Lapointe and Bedford, 2007; Shrope, 2008). This paper similarly is contributed in the spirit of providing information to help facilitate initiatives to reduce the impacts of red tide blooms along the southwest coast of Florida.

The urbanized southwest coast of Florida has a long history of toxic blooms of the naked dinoflagellate *K. brevis* (Davis) G. Hansen et Moestrup, or "red tides" (Gunther et al., 1947; Rounsefell and Nelson, 1966; Brand and Compton, 2007). Brevetoxins produced by this organism promote massive fish kills and, when aerosolized, adversely affect human respiration and asphyxiate domestic animals (Lee et al., 1989). Widespread concerns are mounting along the southwest coast of Florida, where the local economy strongly depends upon tourism and recreational fisheries (Anderson et al., 2000; Larkin and Adams, 2007). Regional authorities wish to determine the causes and the sources of red tides in an attempt to stem the problem and minimize impacts.

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Two important early publications serve as guideposts for this paper. First, Ketchum and Keen (1948) reported unusually high concentrations of total phosphorus associated with red tide blooms in coastal waters off Sarasota, FL. Because these concentrations greatly exceeded the concentrations in surrounding water masses, these workers postulated that "the excessive nutrient content may be the result of terriginous contamination or fertilization of the waters". Second, Slobodkin (1953) studied long-term records of red tide events (1840–1952) and noted that after periods of heavy rainfall, major red tide events occurred, supporting the hypothesis of land drainage as a major cause for coastal outbreaks. Slobodkin (1953) specifically noted the importance of a buoyant freshwater plume in his conclusion that "a discrete mass of water, with a salinity lower than that of normal Gulf of Mexico water, is a necessary pre-requisite for the occurrence of red tide off the Florida Coast".

Our study is also contributed to assist coastal resource managers in continuing to improve early warning systems for *K. brevis* blooms. The concept of early warning systems is not new. Previous researchers proposed use of remote sensing (Lillesand and Kiefer, 1979; Yentsch, 1979; Yentsch and Phinney, 1989) and particle counting techniques such as flow cytometric analysis (Yentsch et al., 1983; Yentsch, 1989; Veldhuis and Kraay, 2000) to detect and track harmful algal blooms (HABs). Early warning systems focusing on toxin detection as well as cell numbers have been in use for decades in bivalve surveillance and aquaculture as protective measures for seafood safety (Shumway, 1990). More recently, development and dissipation of blooms of some harmful species have been tracked using real-time remote monitoring network programs (e.g. Springer et al., 2005; Cullen and Roesler, 2006); remote sensing techniques have been refined specifically to track *K. brevis* blooms (Craig et al., 2006); and the imaging-flow-cytometer, Flow-CAM®, has been used to monitor *K. brevis* abundance (Buskey and Hyatt, 2006). Although satellite tracking, real-time remote monitoring networks, and other sophisticated instrumentation continues to be developed and refined to detect, track, and forecast *K. brevis* blooms (e.g. Stæhr and Cullen, 2003; Stumpf et al., 2003; Tomlinson et al., 2004; Hu et al., 2005), these techniques often cannot be used during and shortly after major storms (Fisher et al., 2006). Moreover, sea-surface sampling efforts remain a critical component of early warning systems regardless of weather conditions (Fisher et al., 2006).

The present study provides a recent example of application of biological optics and ataxonomic analysis with flow cytometry, in concert with analysis of environmental conditions, to examine the "anatomy" of a *K. brevis* bloom—that is, the bloom components most responsible for optical attenuation of visible reflected light.

2. Materials and methods

Blooms of *K. brevis* had been ongoing along much of the southwestern coast of Florida since January 2005 when they were first detected by satellite imagery (Fisher et al., 2006). These blooms followed record rainfall and physical impacts on the watersheds of Lake Okeechobee and southwest Florida in 2004 as a result of the overlapping paths of hurricanes Charley, Frances, and Jeanne (Mallin and Corbett, 2006; Lapointe and Bedford, 2007). Massive water releases from Lake Okeechobee through the Caloosahatchee River and into coastal waters of southwest Florida via the Franklin Lock occurred in Fall of 2004 and throughout much of 2005 (Lapointe and Bedford, 2007; South Florida Water Management District, DBHYDRO database). The severe *K. brevis* bloom that developed off southwest Florida in 2005 led to a widespread hypoxic zone and mortality of benthic communities, fishes, sea turtles, birds, and manatees (Rothschild, 2005; Hu et al., 2006). The red tides persisted until late 2005 when high winds and water column mixing associated with Hurricane Wilma finally began to disperse the bloom.

In early September 2005, bloom concentrations of *K. brevis* were reported in nearshore waters off Fort Myers, FL. During the following days, we employed aircraft and sea sampling for measurements of biological optics and water chemistry both inside and outside the central region of a bloom along Lee County's coastline. Aerial photographs were taken between 10:00 h and 12:00 h on 7 September from flights at 152.4 m and 304.8 m altitude (e.g. Fig. 1), using a Sony Cyber-Shot fitted with a Carl Zeiss Vario-Tessar 3× zoom lens. Six stations were selected for sea-surface sampling based on observations from the aircraft. Surface water samples were collected on 8 September between 09:00 h and 12:00 h at six stations, each approximately 500 m off the Lee County coastline between Bonita Springs and Captiva Island (Table 1 and Fig. 2).

2.1. Environmental conditions

Surface and near-bottom water temperature, salinity, dissolved oxygen (DO), and pH were measured on 8 September using a YSI Model 650 MDS data logger coupled with a submersible Model 600 QS probe (Yellow Springs, OH). A Secchi disk was used to measure water transparency. Replicate water samples ($n = 2$) were collected, 0.25 m below the surface (hereafter designated as surface) and at near-bottom (1.0 m above bottom) using a Niskin water sampler, and held in darkness on ice during transport to the laboratory and overnight until analysis. On the following day, sub-samples of the water samples were filtered and then refrigerated or frozen according to measurement protocol. Sub-samples for flow cytometry were flown on ice to Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, ME, for analysis within appropriate time intervals.

For nutrient analyses, 100-ml sub-samples ($n = 2$) were gently filtered through 0.7 μm Whatman GF/F filters, using a 60 ml. syringe, into clean 150-ml high-density polyethylene bottles at the Harbor Branch Oceanographic Institution (HBOI) in Ft. Pierce, FL. The samples were frozen and analyzed within 28 days for ammonium, nitrate + nitrite, soluble reactive phosphorus (SRP), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) at Nutrient Analytical Services, Chesapeake Biological Laboratory, Center for Environmental Science, University of Maryland, Solomons, MD (NAS-CBL). A Technicon Auto-Analyzer II was used for determination of nitrate and SRP (detection limits, 0.01 μM and 0.02 μM, respectively), and a Technicon TRAACS 800 was used for analysis of ammonium (detection limit, 0.21 μM) following the techniques of Keefe et al. (2004). Sub-samples were also analyzed for total dissolved nitrogen and total dissolved phosphorus at NAS-CBL using a Technicon Auto-Analyzer II with detection limits of 1.43 μM for TDN, and 0.03 μM for TDP (Keefe et al., 2004).

Particulate matter on the GF/F filters from the *K. brevis* bloom encountered at Station 4 off Sanibel Island were analyzed for $\delta^{15}N$ ($n = 3$) to gain insights about potential nitrogen sources supporting the bloom (Heaton, 1986; Dawson et al., 2002). These filters were dried in a Fisher Scientific Isotemp™ oven at 60 °C for 48 h at HBOI. Analyses were completed by Isotope Services Inc., Los Alamos, NM, with a Carlo-Erba N/A Elemental Analyzer and a VG Isomass mass spectrometer using Dumas combustion. The standard used for stable nitrogen isotope analysis was N_2 in air; $\delta^{15}N$ values (as ‰) were calculated as follows:

$$\delta^{15}N = \left[\frac{R\,\text{sample}}{R\,\text{standard}} - 1\right] \times 10^3,$$

where $R = {}^{15}N/{}^{14}N$.

Fig. 1. Aerial photograph taken off the southern tip of Sanibel Island on 7 September 2005. Note the front formed from the tip of the island and extending west into the Gulf of Mexico. The white patches along the front are floating dead fish. The yellow water west of the front was caused by red tide. The dark water to the east was the plume flowing from San Carlos Bay. At the top of the image is the Sanibel Causeway. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Attenuation of incident solar irradiance through the water column (resulting from light absorption and scattering) are functions of the wavelength of light (λ). We estimated the downwelling diffuse attenuation coefficient, K_d, for photosynthetically active radiation (PAR; λ = 400–700 nm), from Secchi depth transparency (Z_d; units (m)) as follows:

$$K_d = \frac{1.5}{Z_d}.$$

2.2. Phytoplankton abundance: chlorophyll a and cell number

Specific volumes were filtered (Whatman GF/F filters, 0.7 μm pore size) for measurement of chlorophyll *a* and phaeophytin. Filters from the samples were stored in plastic Falcon 1002 Petri dishes and held frozen until analysis. Filters were extracted in 85% acetone, and the chlorophyll *a* extract was measured *in vitro* using the fluorometric method of Yentsch and Menzel (1963). The method was calibrated using a standard for chlorophyll *a* obtained from spinach (Sigma Chemical Corp.).

Allometric ataxonomy is useful in quantifying specific groups of phototrophs, such as pico-, nano-, and microplankton, in natural phytoplankton assemblages based on their pigment fluorescence (Li, 1997, 2002; Ciotti et al., 2002; Vaillancourt et al., 2004). Because of its relatively large size (cell diameter ~35 μm; Brand and Compton, 2007), *K. brevis* causes a major shift in the overall allometry of natural phytoplankton assemblages along the southwest Florida shelf, which often have abundant picoplankton (Heil et al., 2007). To allometrically assess phytoplankton abundance (total, *K. brevis*, and picoplankton), unfiltered surface water from each station was maintained in the dark at 4 °C for 4 days prior to flow cytometric analysis (due to shipment of the sample). Samples were not chemically preserved in order to minimize *K. brevis* cell disruption. Sub-samples for quantification of *K. brevis* and other abundant phototrophs were analyzed using a FACScan flow cytometer (Beckton Dickinson, San Jose, CA) equipped with a 15 mW 488 nm air-cooled argon-ion laser. Red (chlorophyll) fluorescence emissions were collected using a 650-nm long pass (LP) filter, and orange (phycoerythrin) fluorescence emissions were collected using a 575-nm band pass (BP) filter. The threshold was set on red fluorescence to identify only chlorophyll-containing particles (i.e. microalgae and detritus that contained chlorophyll or other degrading pigments). Readings were taken in logarithmic mode and analyzed using CELLQuest software (Becton Dickinson, San Jose, CA). The volume of sample analyzed was determined gravimetrically. Different regions were selected to accommodate the "signatures" of the different phytoplankton present (*K. brevis*, eukaryotic picoplankton, and cyanobacteria). Abundances were determined from the count obtained within a specified gated region and the total volume analyzed. The interpretation of flow cytometric "signatures" for ataxonomic groups was performed using methods previously described (Olson et al., 1993, 1985), based on different fluorescent signatures and cell size interpreted from forward-angle light scatter. The presence of *K. brevis* was confirmed using microscopic observation.

To determine the size of the microalgal cells analyzed by flow cytometry, the forward-angle light scatter data (indicative of cell size) were normalized to a linear scale, using particles of known size, using a calibration equation that was obtained with standard calibration beads and cultures (units, μm) as internal standards:

$$\text{size} = (0.0091 \cdot \log \text{FSC}) + 1.6518,$$

where FSC = forward scatter, i.e. the light scattered <10° as a cell passed through the laser beam.

2.3. Particle absorption

Particulate absorption sample filters were analyzed on a Hitachi U3010 dual beam spectrophotometer using a blank, wetted, GF/F filter as reference (Yentsch and Phinney, 1989). Raw optical

Table 1
Station locations, environmental data, nutrient concentrations, $\delta^{15}N$ of *Karenia brevis* bloom (Station 4), chlorophyll *a*, and spectral absorption data

	n	Station #					
		Bonita Springs 1	Lover's Key 2	Lynhall's 3	South Sanibel 4	Bowman's 5	San Carlos Bay 6
Latitude		26°330′	26°384′	26°320′	26°426′	26°440′	26°460′
Longitude		−81°847′	−81°875′	−81°958′	−82°040′	−82°140′	−82°020′
Depth (m)	1	1.5	2	1.5	1.6	1.6	1.6
Secchi depth (m)	1	1.5	1.6	1.4	0.3	1.1	1.1
K (m^{-1})	1	0.97	0.91	1.04	4.83	1.32	1.32
Temperature (°C)							
Surface	1	29.6	29.8	29.5	30.3	29.4	29.7
Bottom	1	29.8	30.0	29.9	29.8	29.4	29.5
Salinity (‰)							
Surface	1	31.9	30.0	23.2	30.1	32.1	14.5
Bottom	1	32.0	31.0	25.7	31.5	32.1	27.1
pH							
Surface	1	7.0	7.9	8.0	8.3	8.1	8.2
Bottom	1	7.6	7.9	8.0	8.2	8.1	8.0
DO ($mg\ l^{-1}$)							
Surface	1	3.4	4.9	6.2	12.6	7.0	6.8
Bottom	1	0.7	3.6	6.7	6.6	6.7	6.2
Ammonium (μM)	2	<0.2		<0.2	<0.2	<0.2	0.3 ± 0.1
Nitrate (μM)	2	0.4 ± 0.0		0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	2.4 ± 0.0
TDN (μM)	2	29.0 ± 0.0		33.7 ± 1.1	141.3 ± 34.7	23.0 ± 0.1	48.4 ± 0.2
SRP (μM)	2	0.23 ± 0.01		0.68 ± 0.01	6.44 ± 0.57	0.25 ± 0.00	1.34 ± 0.03
TDP (μM)	2	0.92 ± 0.01		1.48 ± 0.23	16.51 ± 2.47	1.02 ± 0.08	2.08 ± 0.08
$\delta^{115}N$ (‰)	3				7.83 ± 0.54		
Chlorophyll *a* ($\mu g\ l^{-1}$)							
Fluorometric	2	12.9 ± 1.4		7.3 ± 4.5	336.6 ± 140.0	12.9 ± 1.4	9.0 ± 1.3
Spectrophotometric	1	11.9	8.4	8.5	543.2	17.4	12.9
Absorption, a (m^{-1})							
Particles (a_p)	1	0.158	0.142	0.136	6.175	0.344	0.215
Chlorophyll *a* (a_p^*)	1	0.013	0.017	0.016	0.011	0.020	0.017

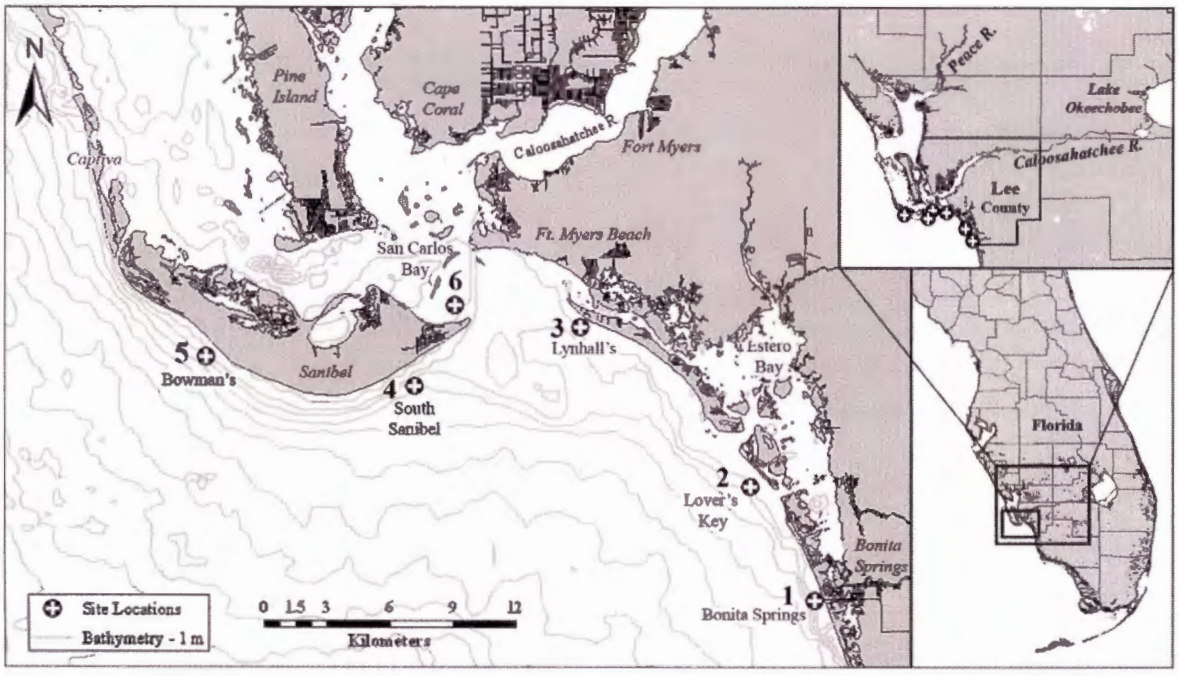

Fig. 2. Map of the study area showing sampling stations on 8 September 2005 at Bonita Springs (Station 1), Lover's Key (Station 2), Lynhall's (Station 3), South Sanibel (Station 4), Bowman's (Station 5), and San Carlos Bay (Station 6). Insets show the relation of the Lee County study area to the Caloosahatchee River, Peace River, and Lake Okeechobee.

density (OD_f) values between 350 nm and 750 nm (1.6 nm resolution) were corrected for pathlength amplification by the filter (beta correction) by

$$OD_s = 0.355\,OD_f + 0.514\,OD_f^2,$$

where OD_s is the beta-corrected optical density in suspension. The absorption coefficient for particles, a_p (m^{-1}), was then calculated from OD_s by

$$a_p(m^{-1}) = 2.3\,OD_s\left(\frac{\pi r^2}{V}\right)100.$$

where OD_s is the beta-corrected optical density in suspension, πr^2 is the area of particles on the filter, and V is the volume filtered in ml.

Chromophoric dissolved organic matter (CDOM) absorption was measured in the dual beam spectrophotometer using 10 cm quartz cuvettes after filtration through a Gelman Sterivex 0.2 μm cartridge (D'Sa and Lohrenz, 1999). Similarly filtered nanopure water was used in the reference cell. Optical density was measured between 200 nm and 750 nm (1.6 nm resolution) and the absorption coefficient for CDOM, a_y (m^{-1}), was calculated by

$$a_y(m^{-1}) = 2.3\,OD \times 10.$$

where OD is the optical density using the 10 cm sample cuvettes.

Specific absorption by phytoplankton, a^*, is calculated by normalizing cell absorption to chlorophyll *a* content. This is a measure of the cell's photosynthetic activity and varies among species. Specific absorption coefficients were estimated for samples collected from each station by

$$a_p^* = \frac{a_p 675\,(m^{-1})}{\text{extracted chl } a\,(\mu g\,l^{-1})}.$$

where a_p 675 is the value of the spectral particulate absorption coefficient at 675 nm and chl *a* is the concentration of chlorophyll *a* calculated from extracting the filter after spectrophotometric analysis.

3. Results

On 7 September, we located a *K. brevis* bloom near Point Ybel off Sanibel Island by aerial reconnaissance (Fig. 1). The sky was overcast, winds were light (10–15 km h^{-1}) and a north-to-south current was estimated at <1 knot. The bloom was observed on the northern edge of a plume of water coming from San Carlos Bay. The front between the plume and bloom is seen in the photographs as white patches that were later identified as dead fish. The extent of the plume into the open Gulf of Mexico could not be determined, but was visually apparent for 3–4 km slowly curving to the northwest. The albedo of the plume appeared to be less than that of the bloom, the bloom was yellow/brown in color, and was organized in long rows of dense color alternating with lighter color. The main portion of the plume was dark, almost black. The eastern edge of the bloom was somewhat scalloped, probably the result of active interchange across the front.

While sampling the six stations along the Lee County coast (Fig. 2) from a small boat on 8 September, we encountered the amber-colored water suggestive of dinoflagellates, as well as many dead fish, large and small, off Sanibel Island at Station 4. Onboard observers experienced throat and eye irritation characteristic of the aerosol irritants from these organisms. Summed up, in terms of the scalars measured while on station, the major differences between this station and the others were the supersaturated oxygen concentration, extremely high chlorophyll *a* concentrations and decrease in water transparency (Secchi disk) as well as a visual change in watercolor (Table 1).

Temperature (29.4–30.3 °C) and salinity (14.5–32.1 psu) at the six coastal stations were consistent with conditions reported in the long-term study of Rounsefell and Dragovich (1966) for the region and time of year (Table 1). Salinities were less than in offshore Gulf waters (35–36 psu; Ketchum and Keen, 1948), especially within the bloom at Station 4 (salinity 30 psu), because of freshening from high precipitation and land-based runoff associated with previous storm events (Fisher et al., 2006; Lapointe and Bedford, 2007; Table 1). Station 4 was also characterized by elevated DO (surface, 12.6 mg l^{-1}) and reduced Secchi depth transparency (0.3 m) in comparison to the other stations (range 3.4–7.0 mg DO l^{-1}, Secchi depth 1.1–1.6 m). Near-bottom DO indicated hypoxic conditions at Station 1, which was the station with the lowest surface pH (7.0). Water transparency was extremely low throughout the study area. The estimated K_d (PAR) for all stations except Station 4 ranged from 0.91 m^{-1} to 1.32 m^{-1}, and was 4.83 m^{-1} at Station 4 (Table 1).

Dissolved inorganic N and P were similar among the stations except for much higher SRP (6.44 ± 0.57 μM) at bloom Station 4, and higher nitrate (2.40 ± 0.0 μM) at Station 6 (Table 1). All stations were relatively high in total dissolved organic nutrients, with extremely high values at Station 4 (141 ± 35 μM TDN, 16.5 ± 2.5 μM TDP). The $\delta^{15}N$ data of the *K. brevis* bloom at Station 4 averaged +7.83 ± 0.54‰, a value similar to that found in macroalgae along this coastline in 2004 (Lapointe and Bedford, 2007).

The nutrient and chlorophyll *a* data from the six stations are shown in Table 1. The central portion of the buoyant plume is assumed to be the position of lowest observed salinity at Station 4. High values of chlorophyll *a*, DO, total dissolved nitrogen and phosphorus occurred here in the confluence of the mixed outflows of the Caloosahatchee and Peace rivers. This is consistent with what was observed from the aircraft.

Phytoplankton biomass, as chlorophyll *a* concentration, was moderate (7.3–17.4 μg l^{-1}) in the study area except for very high concentrations (337–543 μg l^{-1}) at bloom Station 4 (Table 1). The high chlorophyll concentration in surface waters of Station 4 was supported by high cell numbers of cyanobacterial (e.g. *Synechococcus* spp.) and eukaryotic picoplankton (maximum cell dimension <3 μm) and *K. brevis* (Fig. 3). As expected, given its large size (Fig. 4), *K. brevis* dominated the water optics at Station 4, despite accounting for only ~5% of total phytoplankton cells, and caused more optical forward scattering than the eukaryotic and prokaryotic picoplankton (Fig. 5). Microscopic examination of subsamples, before and after flow cytometric analysis, revealed lysis of some *K. brevis* cells, which would have underestimated abundance.

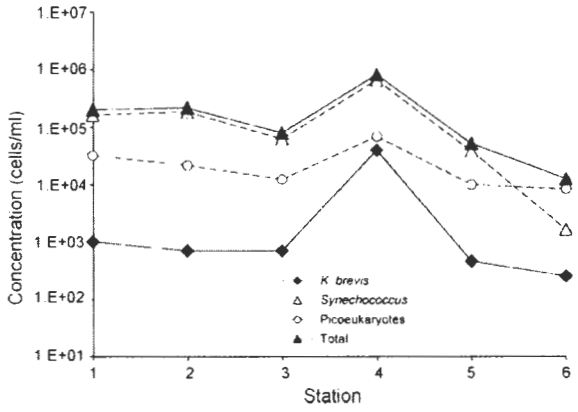

Fig. 3. Flow cytometer cell counts for cyanobacteria (*Synechococcus* sp.), picoeukaryotes, *Karenia brevis*, and total phytoplankton for Stations 1–6 sampled on 8 September 2005.

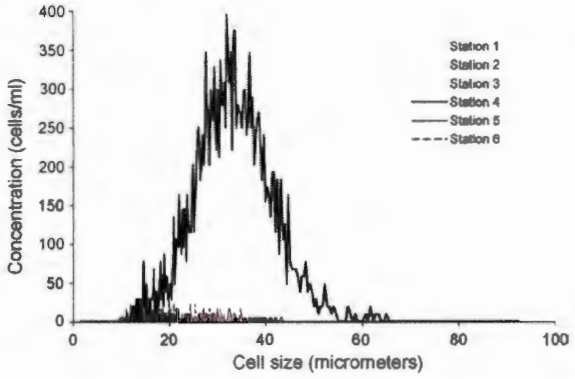

Fig. 4. Particle size distributions of phytoplankton greater than 10 μm at Stations 1–6 along the Lee County Coast. Only at Station 4 is a dramatic increase observed in cell size and quantity. The shift in the cell distribution from smaller (10–20 μm) cells to a much larger cell type (25–40 μm) correlates with the observations of the *K. brevis* bloom in the surface waters.

Spectral curves for particle absorption all showed an absorption band in the near infrared region (NIR), due to chlorophyll absorption at 675 nm (Fig. 6). As expected, the chlorophyll band from the bloom (Station 4) was more pronounced at 675 nm, with no sign of accessory chromoproteins, yet there was a much sharper rate of increase in absorbance from 570 nm to 450 nm, undoubtedly due to the conjugated–carotenoid complex of dinoflagellates

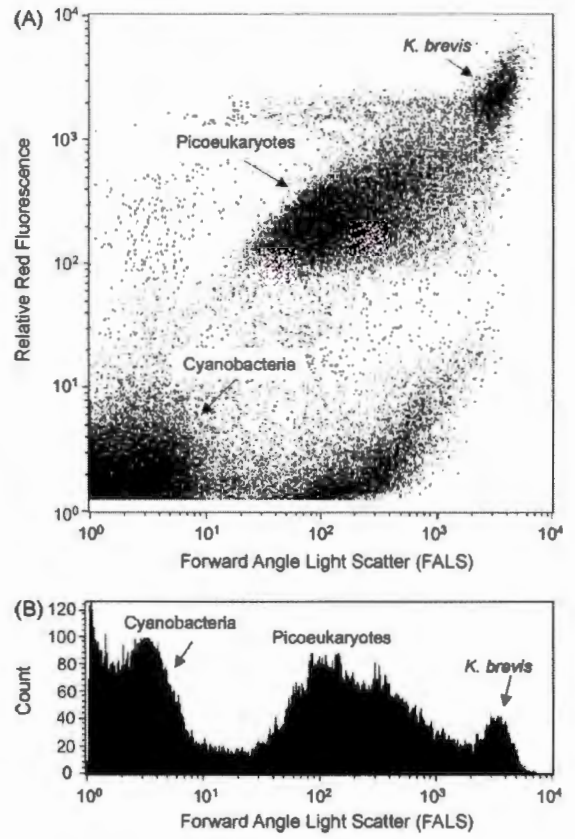

Fig. 5. Flow cytometric results for bloom Station 4. (A) Dot plot of forward angle light scatter vs. relative red fluorescence (indicative of chlorophyll), showing specific ataxonomic components of the particle population (*K. brevis*, picoeukaryotes, and cyanobacteria *Synechococcus* sp.); (B) histogram plot of frequency vs. forward angle light scattering, showing the wide phytoplankton distribution at this station, i.e. cells of two size ranges—*K. brevis* (35 μm) and *cyanobacteria* (1–2 μm).

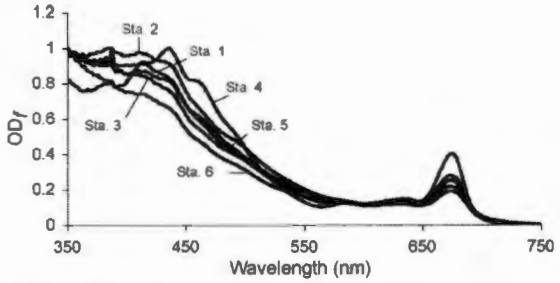

Fig. 6. Particulate spectral absorption at Stations 1–6 sampled on 8 September 2005.

(Yentsch, 1962). Particle absorption rapidly increased from 570 nm to 410 nm, likely related to the accessory pigments of *K. brevis* (Bjørnland et al., 2003). A second rapid increase in particle absorption occurred in the near-ultraviolet (UV; 350–400 nm) light region, suggesting the presence of mycosporine-like pigments (Bandaranayake, 1998; Evens et al., 2001).

CDOM was highest at Station 4, and CDOM action spectra (a_y) revealed an exponential increase in absorbance from the NIR to UV wavelengths (Fig. 7), characteristic of humic and fulvic acids in natural waters (Kirk, 1994). The CDOM brown coloration increased absorption between 500 nm and 350 nm. Station 4 also had an absorption feature centered around 300 nm that has been observed in algal exudates, and may have been caused from absorption by aromatic amino acids (Yentsch and Reichert, 1962). This feature generally is not observed in samples from open ocean waters; it may represent a non-conservative feature of CDOM (Yentsch and Reichert, 1962) that influences the slope of the conservative fraction of CDOM into the visible region.

Water transparency was extremely low throughout the study area (Table 1). K_d at Stations 1–6 ranged from 0.9 m^{-1} to 1.3 m^{-1}; at bloom station 4, K_d was 4.8 m^{-1}. To estimate the causes of high attenuation it was assumed that the fate of the radiation was absorption by particles and dissolved yellow organics (CDOM), which is nominal for coastal waters receiving considerable amounts of riverine discharge. The spectral characteristics of both were used to define their relative importance.

Comparison of spectral absorption coefficients for particles (a_p) and CDOM (a_y) indicated that CDOM dominated near-UV wavelengths at all six stations, but in the visible region (using 450 nm as a reference) CDOM absorption varied (Fig. 8). Moreover, at bloom Station 4, particle absorption was mostly related to phytoplankton cells so that major algal pigments bands, such as chlorophyll and carotenoids at blue wavelengths and chlorophyll at red wavelengths, exceeded the specific absorption of CDOM. Specific absorption by chlorophyll *a* (a_p^*) at all six stations ranged from 0.011 m^{-1} to 0.017 m^{-1}; the lowest value occurring at Station 4 (Table 1). Mean a_p^* was 0.0157 + 0.003 m^{-1}, similar to the value reported by Gallegos and Bergstrom (2005) for the potentially toxic

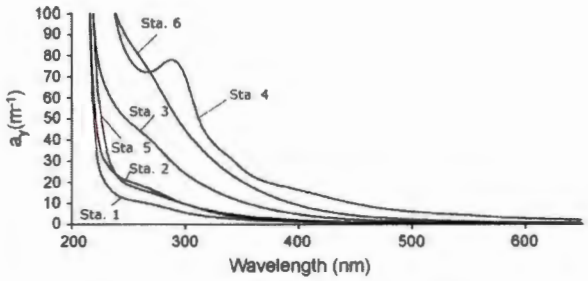

Fig. 7. UV/vis absorption spectra for CDOM at Stations 1–6 sampled on 8 September 2005.

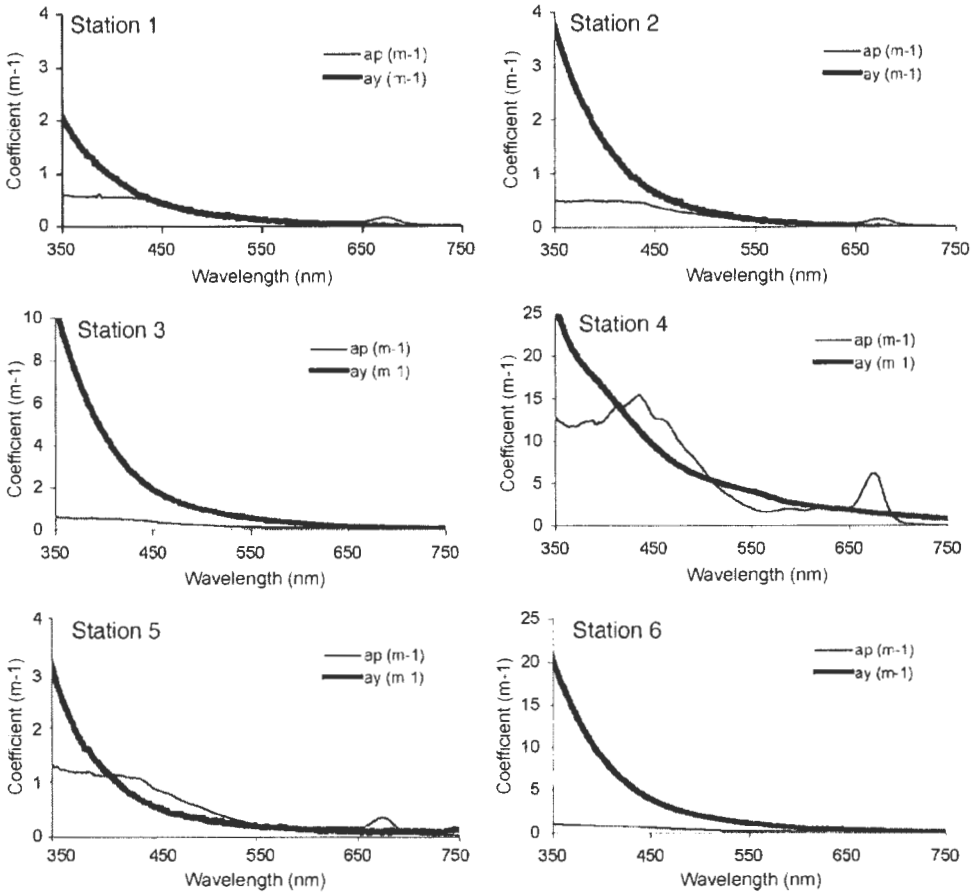

Fig. 8. Specific absorption spectra for particles (a_p) and CDOM (a_y) at Stations 1–6 sampled on 8 September 2005.

dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller, but lower than means reported for open ocean phytoplankton assemblages (0.026 m^{-1}; Yentsch and Phinney, 1989; Gallegos and Bergstrom, 2005).

4. Discussion

The *K. brevis* bloom characterized in this 2-day "snapshot" analysis occurred in lower-salinity, high-nutrient, high-CDOM surface waters. CDOM along the western Florida shelf is mostly contributed by riverine/estuarine sources (Del Castillo et al., 2000), implicating riverine transport of land-based nutrients as a major source of nutrition to the bloom. The shallow waters of the bloom area had a lower salinity surface layer and a more saline underlying layer, promoting water column stability. Such conditions have been observed in many coastal regions experiencing cultural eutrophication (e.g. Ochi, 1989; Wyatt, 1990), and were identified by Slobodkin (1953) as a necessary pre-requisite for Florida red tides. Nutrients in CDOM from land drainage have also been reported to favor some blooms (Anderson and Corbett, 1979). For example, in Swedish coastal waters, Graneli et al. (1989) found that organic N and P were major forms of nutrients in "brown water". Addition of CDOM favored growth of the dinoflagellate, *P. minimum*, possibly due to CDOM organic nutrient and heavy metal chelator content (Heil, 2005). Ingle and Martin (1971) proposed an iron/CDOM index to predict Florida *K. brevis* blooms under conditions of iron limitation.

We earlier suggested a potential commonality between the microalgal blooms observed by Ryther et al. (1958) in eutrophic waters of Long Island (enriched by duck farm runoff) and the *K. brevis* bloom sampled in this study off Sanibel Island on the west coast of Florida. The red tide we sampled in lower-salinity (30 psu) waters had very high concentrations of TDP similar to values reported over a half-century earlier by Ketchum and Keen (1948). These authors found values of 14.6–20.4 μM TP in ~32 psu water of "deep amber color" associated with red tides some 1.5 miles off Sarasota Point, Sarasota, FL. Those values bracket the mean value (16.5 ± 2.47 μM) we observed for the red tide in similar amber colored water off Sanibel Island in 2005. Considering that these studies were almost 60 years apart, the similarity in values confirms the high quality of P analyses obtained by oceanographers in that period. The SRP concentrations associated with red tide off Sanibel in 2005 were also very high, averaging 6.44 ± 0.57 μM. That is much higher than SRP concentrations in the range of 0.30–0.92 μM reported for coastal waters of Lee County in 2004 (Lapointe and Bedford, 2007). The unusually high concentrations within the red tide bloom could be due, in part, to breakage of the naked and delicate *K. brevis* cells during syringe filtration, which would release SRP and TDP from internal sources. Regardless, the high background SRP (up to 1 μM) and TDP (5 μM) concentrations observed by Lapointe and Bedford (2007) for nearshore coastal waters off Lee County in late 2004 would be considered eutrophic and capable of supporting a dense *K. brevis* bloom of $>10^6$ cells l^{-1} (Vargo et al., 2008).

Because of high background SRP and TDP concentrations in the Lee County's nearshore coastal waters, algal blooms are strongly N-limited (Lapointe and Bedford, 2007). Although there are a variety of potentially important land-based nitrogen sources to coastal waters, the mean $\delta^{15}N$ value (+7.83‰) of the *K. brevis* bloom off Sanibel closely matched the $\delta^{15}N$ values of +6–8‰ for macroalgal blooms collected on beaches and shallow reefs along this coastline

(Lapointe and Bedford, 2007). These red tide $\delta^{15}N$ values are also similar to values reported for algal tissue at the Ortona and Franklin locks along the Caloosahatchee River, which can be influenced by both ammonium-rich Lake Okeechobee releases as well as in-basin sources (Lapointe and Bedford, 2007; Fig. 2). Although various hypothetical sources of N supporting *K. brevis* blooms along the west coast of Florida have been suggested – nitrogen fixation by *Trichodesmium* sp. (Lenes et al., 2001), and shelf-break upwelling (Stumpf et al., 2008) – the $\delta^{15}N$ data for macroalgae in Lapointe and Bedford (2007), combined with the similar $\delta^{15}N$ data for *K. brevis* in this study, indicate that land-based N sources are major contributors to both of these HABs in nearshore waters. While far-field sources of nutrients might contribute to blooms in offshore waters, we suggest that the combined $\delta^{15}N$ data for macroalgae and red tides in nearshore waters represent a strong signal of cultural eutrophication (Heaton, 1986; Dawson et al., 2002) that may be linked to the increasingly intense *K. brevis* blooms (Brand and Compton, 2007). These $\delta^{15}N$ values are enriched well above values for N fixation, fertilizer N, and atmospheric deposition but are within the range of values reported for wastewater N from septic tanks, secondarily treated effluent, and cattle farms (Lapointe and Bedford, 2007). Our data and interpretations are not new to the general problem of coastal eutrophication and Florida red tide literature. Most, if not all, of the early reports from the west coast of Florida that described dead fish and discolored water associated the red tide phenomena with rainfall and runoff into coastal waters (e.g. Ketchum and Keen, 1948; Slobodkin, 1953). Our results support the analyses of Brand and Compton (2007) and Vargo et al. (2008) who also noted the importance of land-based nutrient flux to the development of dense *K. brevis* blooms in nearshore waters. That macroalgal blooms have also emerged in nearshore waters along the west coast of Florida since 2003 (Lapointe and Bedford, 2007) supports the view that nutrient enrichment has recently reached (or, surpassed) a "tipping point" for high-biomass macroalgal blooms (Lapointe, 1997). Such blooms are known to reduce light transmission, promote bottom-water anoxia, and adversely effect benthic fauna and biogeochemical cycles (Valiela et al., 1997; Lapointe and Bedford, 2007) and are considered harmful algal blooms, similar to red tides, even though the macroalgae lack direct toxicity (ECOHAB, 1995).

Recent analyses of nutrient sources have provided other insights about the role of land-based nutrient pollution in supporting nearshore *K. brevis* blooms. Estimates of nutrient fluxes from groundwater (Hu et al., 2006) and estuarine surface waters (Vargo et al., 2004) support the premise that land-based nutrients may be significant in supporting nearshore *K. brevis* blooms. Heil et al. (2001) reported that riverine inputs often were insufficient to support nearshore blooms; more recently, Vargo et al. (2008) estimated that the estuarine sources nevertheless could be important in sustaining these blooms. By their calculations, total nitrogen and total phosphorus fluxes from Tampa Bay and Charlotte Harbor could supply up to 20% of the N and up to ~90% of the P needed to support a moderate *K. brevis* bloom (3×10^5 cells l^{-1}, 0.2 divisions d^{-1}). Hu et al. (2006) noted that the hurricanes of 2004 were followed by the unusually late onset and long duration of red tides throughout 2005. The posthurricane dissolved inorganic nitrogen (DIN) inputs from Tampa Bay, alone, were estimated to have been ~35% of the inputs from all Florida rivers draining west in combination. Hu et al. (2006) suggested that DIN carried into nearshore waters from the posthurricane-related high runoff, and higher-than-normal submarine groundwater discharge, helped to initiate and fuel the persistent *K. brevis* blooms. Lapointe and Bedford (2007) also reported that the extensive red tides of 2005 followed large discharges from the Caloosahatchee and Peace rivers following the 2004 and 2005 hurricanes, which resulted in ammonium and SRP enrichment to considerable distances (>26 km) from shore.

We earlier stated our hope that this study of a *K. brevis* bloom can provide direction to assist coastal resource managers in reducing red tide outbreaks. Ryther et al. (1958) suggested, for eutrophic Great South Bay in New York, that shellfish production (inhibited by blooms) would be improved if flushing was increased. As a result, an inlet was opened, shellfish production increased, and the resource protected. Thus, benthic resources were left intact and, in fact, were improved without need of other management intervention potentially harmful to beneficial fauna (e.g. Lewis et al., 2003), or which might exacerbate bloom conditions by causing cell lysis and brevetoxin release, such as application of clay (e.g. Pierce et al., 2004; Sengco and Anderson, 2004) or heavy metal toxicants like copper sulfate (Rounsefell and Dragovich, 1966). Somewhat analogously, Slobodkin (1953) proposed a plan for estuaries along the west coast of Florida that involved reducing red tides by altering estuarine flow velocities and flushing rates. He stressed that accurate prediction of *K. brevis* blooms would require a thorough knowledge of estuarine inputs to the affected coastal waters. Because of the established linkages between land-based nutrient inputs and HABs off southwest Florida, improved management of the quality and quantity of the freshwater discharges from the watersheds, particularly the Caloosahatchee River, could help moderate blooms in the future.

The present study of a *K. brevis* bloom was also contributed with the hope of assisting coastal resource managers in efforts to continue to improve early warning systems for *K. brevis* blooms. The simple combination of bio-optical remote sensing via aerial flyovers, together with in-water sensors and flow cytometry, as used in this study, can provide critically needed information on *K. brevis* blooms during weather conditions that preclude reliance on satellite imagery and other techniques. We have suggested a minimum set of measurements that can capitalize on the significant indicators of the presence of *K. brevis* and the hydrographic conditions under which it blooms in southwest Florida. For cell size and concentrations, flow cytometric measurements of cell size distributions can be used to monitor populations of the large *K. brevis* cells. For the presence of increasing amounts of dinoflagellates, spectral absorption of the particulate and dissolved fractions of seawater can indicate the presence of cellular and released mycosporine pigments. A decrease in the specific absorption coefficient of the population (a^*_{670}) can also indicate the dominance of dinoflagellates compared to picoplankton. Temperature, salinity, and nutrient concentrations of the water column, combined with $\delta^{15}N$ signatures of *K. brevis* and various N sources within a region, can be used to determine the presence and source(s) of excess nitrogen loads that stimulate the blooms. All the sampling can be accomplished from small boats under weather conditions that limit the efficacy of satellite imagery to forecast bloom components. Sample turnaround times are short such that early warning and monitoring capabilities are possible for the specific application to *K. brevis* blooms in southwest Florida coastal waters. Similar collections of minimum datasets for specific indicators will be needed for other bloom species in other coastal regions.

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Addendum: Real-time data of this region are now available at http://recon.sccf.org. The reader is directed to the results from a new partnership of citizens, Sanibel Captiva Conservation Foundation, government and business efforts. The River Estuary and Coastal Observation Network (RECON) is a network of optical water quality sensors (Satlantic, Inc.) deployed throughout the Caloosahatchee River and estuary to provide real-time water quality data to scientists, policy makers and the general public. RECON's network of high quality, autonomous, in situ sensors can detect the presence of algal blooms and nutrient hotspots. An airborne companion to this network of activity has been proposed and is under consideration.

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