Application of 3D-Fluorescence and PARAFAC Modeling in Marine DOM Investigations

Wilson G. Mendoza
University of Miami, wilsonm2005@gmail.com

Follow this and additional works at: https://scholarlyrepository.miami.edu/oa_dissertations

Recommended Citation
https://scholarlyrepository.miami.edu/oa_dissertations/753

This Open access is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarly Repository. It has been accepted for inclusion in Open Access Dissertations by an authorized administrator of Scholarly Repository. For more information, please contact repository.library@miami.edu.
APPLICATION OF 3D-FLUORESCENCE AND PARAFAC MODELING IN MARINE DOM INVESTIGATIONS

By

Wilson G. Mendoza

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirement for
the degree of Doctor of Philosophy

May 2012
APPLICATION OF 3D-FLUORESCENCE AND PARAFAC MODELING IN MARINE DOM INVESTIGATIONS

Wilson G. Mendoza

Approved:

Rod Zika, Ph.D.
Professor of Marine and Atmospheric Chemistry

Terri A. Scandura, Ph.D.
Dean of the Graduate School

Kenneth Voss, Ph.D.
Professor/Chairman of Physics
Department of Physics

Elliot Atlas, Ph.D.
Professor of Marine and Atmospheric Chemistry

Patria Viva Banzon, Ph.D.
Scientist, National Oceanic and Atmospheric Administration (NOAA)
Ashville, North Carolina

Ralph Mead, Ph.D.
Associate Professor of Chemistry
University of North Carolina
Wilmington, North Carolina
Dissolved organic matter (DOM) plays a crucial role in the nutrient and carbon cycling in the coastal-shelf-ocean boundary. It is a major reservoir of reduced carbon and carries important information on how the sea and landscape have been modified. Due to the complexity of the biogeochemical processes in this boundary, its distribution is not well understood. This study evaluated the Excitation Emission Matrix (EEM) fluorescence method combined with Parallel Factorial Analysis (PARAFAC) modeling to resolve DOM fluorescence components, and their distribution from near-shore to off-shore. Results of this study demonstrated several advances in monitoring the temporal distribution of DOM on the continental shelf of Florida using EEM and PARAFAC, including the methods application in harmful algal bloom and oil spill studies.

This study employed PARAFAC models that were able to: 1) resolve sources of individual components of the bulk DOM from nearshore to the continental margins of southwest Florida; 2) provide insight into the biological, chemical, and physical processes that control the DOM variabilities of each of the resolved DOM components on the Shelf; 3) develop and test the hypothesis that the protein-like fluorescence component supported the October 2011 *K. brevis* bloom; 4) observe relationship of brevetoxin incorporation in marine sediments enriched with terrestrial DOM; and, 5) simultaneously discriminate residues of the Macondo crude oil components, the dispersants, and
dissociate signal related to CDOM in the water column after the Deepwater Horizon oil spill in the Gulf of Mexico.
This work is dedicated to my family for their support throughout this academic endeavor.
Acknowledgements

The author gratefully acknowledges
NOAA-AOML (Miami)-South Florida Hydrographic Survey Project
National Aeronautics Space Administration
University of Miami Oceans and Human Health
2009 NSF-NIEHS Oceans and Human Health Summer Experience (REU/G)
FIO-BP Funding
Mary Roche Fellowship 2009
# TABLE OF CONTENTS

LIST OF FIGURES........................................................................................................... viii
LIST OF TABLES................................................................................................................. xii

Chapter
1 INTRODUCTION ...........................................................................................................1
   1.1. Research Goals .......................................................................................................4
   1.2. Review of dissolved organic matter research .........................................................5
       1.2.1. CDOM Photochemistry ...................................................................................8
       1.2.2. Excitation-Emission Matrix fluorescence of DOM .......................................13
       1.2.3. Parallel Factorial Analysis Modeling ...............................................................15

2 ON THE TEMPORAL VARIATION OF PARAFAC MODELED DOM FLUORESCENCE FRACTIONS ON THE SW FLORIDA SHELF
   2.1. Rationale .............................................................................................................20
   2.2. Materials and Methods .........................................................................................23
       2.2.1. Description of sampling site ..........................................................................23
       2.2.2. Temporal and spatial sampling .....................................................................23
       2.2.3. EEM spectral measurements .........................................................................24
       2.2.4. EEM fluorescence and PARAFAC modeling .................................................26
       2.2.5. DOC and DON analysis ................................................................................27
       2.2.6. Pearson’s product moment correlation analysis .............................................28
   2.3. Results and Discussion .........................................................................................28
       2.3.1. PARAFAC modeled DOM components .......................................................28
       2.3.2. Temporal and spatial variability of fluorophores across the SW Florida Shelf .........................................................................................................................29
       2.3.3. Relationship between DOM fluorescence components and salinity ............32
       2.3.4. DOC analysis ................................................................................................37
       2.3.5. DON analysis ................................................................................................38
       2.3.5. SW Florida Shelf DOM fluorescence source and production .....................40
       2.3.6. SW Florida Shelf DOM fluorescence decrease .............................................43
5 APPLICATION OF FLUORESCENCE AND PARAFAC TO ASSESS VERTICAL DISTRIBUTION OF SUBSURFACE HYDROCARBONS AND DISPERSANT DURING THE DEEPWATER HORIZON OIL SPILL

5.1 Rationale ...........................................................................................................114
5.2 Materials and Methods ....................................................................................116
   5.2.1. Fluorescence scans of samples .................................................................116
   5.2.2. EEM spectra of reference compounds .....................................................117
   5.2.3. GC-MS analysis .......................................................................................118
   5.2.3. PARAFAC modeling ................................................................................119
5.3 Results and Discussion .....................................................................................120
   5.3.1. PARAFAC crude oil related components and validation of these components ........................................................................................................120
   5.3.2. Subsurface distribution of derived fluorescence components ..........122
   5.3.3. PAH fluorescence distribution in subsurface oil plume .......................124
   5.3.4. Dispersant-associated fluorescent component and its effect on crude oil dispersion ........................................................................................................125
   5.3.5. Humic-like associated PARAFAC modeled component .................127
   5.3.6. Commercial sensors vs. EEM fluorescence analysis .........................128
   5.3.7 Advantages of using EEM fluorescence and PARAFAC for oil spill monitoring .......................................................................................................130
5.4 Summary .........................................................................................................131

Figures .................................................................................................................133
Tables .....................................................................................................................142

6 CONCLUSION AND RECOMMENDATION .........................................................148
REFERENCES .......................................................................................................152

APPENDIX A THE PREDICTION OF PHOTOLYTIC CARBON LOSS USING DISSOLVED ORGANIC MATTER FLUORESCENCE AND MULTIPLE LINEAR REGRESSION .......170

APPENDIX B SPECTRAL CHARACTERIZATION OF DOM POINT SOURCES USING EXCITATION AND EMISSION FLUORESCENCE AND PARAFAC ......................212
LIST OF FIGURES

Figure 1.1. Idealized structure of humic acid (Modified from Stevenson, 1994)........... 6

Figure 1.2. Diagram of colored dissolved organic matter sources and sinks in the marine environment (Modified from Coble 2007).............................................................. 8

Figure 1.3. The Jablonski Diagram (patterned after Lacowicz 2006)......................... 12

Figure 1.4. A sample of an a. uncorrected EEM and b. corrected EEM fluorescence spectra. Bar in QSU units.....................................................................................................14

Figure 1.5. Schematic diagram of the PARAFAC modeling process (modified from Stedmon and Bro, 2008............................ 16

Figure 2.1. Fixed sampling stations on the southwest Florida Shelf. Stations were based on the NOAA-AOML South Florida Quarterly Hydrographic Survey Program (SFQHQS). See Table 2.1 for transect labels.........................................................48

Figure 2.2. Scores, loadings and leverage of the DOM fluorescence PARAFAC modeled components from 272 EEMS of seawater samples collected from the SW Florida Shelf. ........................................................................................................49

Figure 2.3. A sample of a. measured and b. modeled PARAFAC modeled EEM and its corresponding c. residual,  d) excitation plots (a-c) shown diagonally, and e) the derived fluorescence intensity from a five component PARAFAC model....................50

Figure 2.4. Validated split halves of the five component PARAFAC model. Each halves of the excitation and emission wavelengths show similar spectral loadings.............51

Figure 2.5. 3D fluorescence and spectral loadings of a, f) component 1, b,g) Component 2, c,h) component 3, d,i) component 4 and e, j) component 5 of the PARAFAC modeled DOM fluorescence, in seawater samples collected at different seasons across the SW Florida Shelf. ..................................................................................52

Figure 2.6. Temporal and spatial distribution of the PARAFAC modeled DOM fluorophore components across the SW Florida Shelf during these sampling periods: a) May 6-10, 2008, b) October 17-22, 2008, c) January 21-26, 2009 and d) April 20-23, 2009..................................................................................................................53

Figure 2.7. a) Monthly discharge rates (cu ft s\(^{-1}\)) of the Caloosahatchee (station 79, SFWMD DBHydro), Shark (S12A+S12B+S12C+S12D+S333, SFWMD DBHydro) and Peace (station in Arcadia, USGS-NWIS Web) Rivers from January 2008-December 2009 (SFWMD DBHydro. b) Precipitation (inches) rates in Caloosahatchee (station 79) and
Shark (S12D) Rivers (SFWMD-South Florida Water Management District, USGS-NWIS-U.S. Geological Survey National Water Information Systems)…………………………54

Figure 2.8. Contour plots of temperature (°C), salinity (psu), DOC (µM C), DON (µM), and Chl a (mg/L) in SW Florida Shelf during the NOAA hydrographic cruise on a) May 6-10, 2008, b) October 17-22, 2008, c) January 21-26, 2009 and d) April 20-23, 2009…………………………………………………………………………………………..55


Figure 2.10. Temporal distribution of Component 1 (Comp1) and Component 4 (Comp4) on multiple transects proximate to the Shark and Caloosahatchee River mouths…………57

Figure 2.11. Relationship of DOC with salinity for the month of a. May 2008, b. January 2009 and c) April 2009. …………………………………………………………………………58

Figure 2.12. Relationship of DOC (µM C) with component 1 (Comp1=HLC1) and component 2 (Comp2=HLC2) for the month of a-b) May 2008 and c-d) January 2009………………………………………………………………………………………………59

Figure 2.13. Relationship of DON with salinity for the month of a. May 2008, b. January 2009 and c) April 2009…………………………………………………………………….60

Figure 2.14. Relationship of DON (µM) with component 4(Comp4=TLC4) and component 2 (Comp5=TLC5) for the month of a-b) May 2008 and c-d) January 2009……………………………………………………………………………………………61

Figure 2.15. Relationship of chlorophyll a with salinity for the month of a. May 2008, b. October 2008, c. January 2009 and d) April 2009……………………………………………….62

Figure 3.1. Loadings of five PARAFAC modeled DOM fluorescence fractions collected on the southwest Florida Shelf during the October 2009 University of Miami Oceans and Human Health Cruise. …………………………………………………………………………86

Figure 3.2. a-e) Surface points of the individual PARAFAC modeled components (Component 1-Component 5) on the southwest Florida Shelf during the October 2009 cruise (n = 30). Bar scale in quinine sulfate units (QSU). Dash lines indicate region *K. brevis* bloom was found…………………………………………………………………………………………………87

Figure 3.3. a) Monthly discharge rates (cu ft s⁻¹) of Caloosahatchee (station 79, SFWMD DBHydro), Shark (S12A+S12B+S12C+S12D+S333, SFWMD DBHydro) and Peace (station in Arcadia, USGS-NWIS Web) Rivers from January 2009-December 2009 (SFWMD DBHydro. b) Precipitation (inches) rates in Caloosahatchee (station 79) and
Figure 3.4. a-e) Linear relationship between *K. brevis* counts and PARAFAC modeled DOM fluorescent components from Florida estuary and shelf.

Figure 3.5. *Karenis brevis* counts (cells l⁻¹) off of Sanibel Island (southwest of Charlotte Harbor) during the UM-OHH October 2009 cruise.

Figure 3.6. Surface plots of a) temperature (°C), b) salinity (psu), and chlorophyll-a estimate (RFU) on the southwest Florida Shelf.

Figure 3.7. Relationship of *K. brevis* counts (cells l⁻¹) with: a) temperature (°C) and b. salinity (psu).

Figure 3.8a. Relationship of components 1) component 1 (Comp1), 2) component 2 (Comp2), 3) component 3 (Comp3) and salinity. Right hand plots correspond to the component/salinity (n=7, from Table 3.2) relationship in the *K. brevis* bloom patch.

Figure 3.8b. Relationship of 4) components 4 (Comp4) and 5) component 5 (Comp 5) and salinity. Right hand plots correspond to the component/salinity (n=7, from Table 3.2) relationship in the *K. brevis* bloom patch.

Figure 3.9. Conceptual diagram of humic-like (component 1) and protein-like (component 5) fluorescence increase with increase salinity in a *Karenia brevis* bloom patch ~25 miles away from Charlotte Harbor. Evaporation and coastal upwelling (driven by wind) that brings materials from the sea bottom to sea surface, are two proposed mechanisms that increased fluorescence with increase salinity.

Figure 4.1. Structures of the two types of brevetoxin and their respective analogs.

Figure 4.2. Sources of sediment samples from the southwest coast of Florida were at 1) Fort Myers Beach (FMB), 2) Big Carlos Pass (BCP), and 4) Big Hickory Pass (BHP).

Figure 4.3. Percentage of PbTx-9 recovered after brevetoxin was spiked into BHP sediment. Sonication times employed were 5, 10 and 30 min (n = 2).

Figure 4.4. Reconstructed ion chromatograms of PbTx-2 and PbTx-3 from LC/MS/MS analysis of sediments and cultured cells.

Figure 4.5. Relationship of FDOM fluorescence (ex/em: 350/450 nm, n=2±SD) against brevetoxin concentration detected using HPLC-ESI-MS/MS from sediments collected in
FMB (Fort Myers Beach), BHP (Big Hickory Pass) and BCP (Big Carlos Pass) on the SW Florida coast .................................................................112

Figure 5.1. Sampling station (X) during the NOAA Gordon Gunter cruise, from May 27-June 4, 2010, near the Deepwater Horizon Oil Spill site (O) (Map scale: 2.54 cm: 9.4 km).................................................................................................133

Figure 5.2. Seven unique fluorescent components distinguished from the Deepwater Horizon oil spill contaminated seawater. See, Table 5.1 for peak excitation-emission wavelengths.................................................................134

Figure 5.3. The three components showing similar peak maxima with naphthalene and benzene..................................................................................135

Figure 5.4. Benzene and Naphthalene excitation/emission maximum fluorescence....136

Figure 5.5. Similarity of the excitation and emission peak maximum of the a) Macondo source oil and b) component 4 occurring at 225 nm (Exλ) and 335 nm (Em λ) and the estimated c) Macondo source reference crude oil concentration and d) component 4 fluorescence vertical profile in Station 30 during the NOAA Gunter cruise (June-May 2010). The estimated concentration of the BP Crude Oil was based on a regression model developed for measuring oil in seawater using BP reference oil as the reference standard. The root mean square error (RMSE) of the model prediction was 0.008 with an uncertainty of 0.92% from the mean calculated BP concentration mean. The adjusted R² of the regression coefficient was 0.993 at a confidence interval of 99%..................................................................................................................................137

Figure 5.6. Vertical profiles of the PARAFAC modeled components. Color bars in RFU. Yellow bar indicates depth at which high concentrations of the poly-aromatic hydrocarbons and the dispersants were previously observed (Camili et al., 2010; Kujawinski et al., 2010)...............................................................................................................................138

Figure 5.7. Conceptual diagram of the components’ subsurface distribution during the Deepwater Oil spill from May-June 2010.........................................................139

Figure 5.8. Total ion chromatogram of CorexitEC9500A (1.0 µg/ml). Conjugated compounds were located at retention times 9.6 and 9.9 min. Mass spectra (GC/MS/SIM) of peaks a and b and the possible fluorescent compounds present in CorexitEC9500A. Structure assignments of spectra a and b employed NIST/EPA/NIH mass spectral library.............................................................140

Figure 5.9. Macondo source crude oil (1.0 µg/ml) PAH Gas-Chromatogram. See Table 4 for the identity and concentration of the corresponding PAH compound of each peak.141
LIST OF TABLES

Table 1.1 The excitation and emission fluorescence maxima of the PARAFAC modeled DOM fluorescence and their designation .................................................................15

Table 2.1. Transect names of stations across SW Florida Shelf.........................63

Table 2.2. Variation explained and root mean squared error (RMSE) of the five PARAFAC DOM fluorescence component model..................................................66

Table 2.3. Excitation and emission fluorescence maximum of PARAFAC modeled DOM fluorescent components across SW Florida Shelf from four quarterly survey, their respective region, and designation. .........................................................67

Table 2.4. Percentage of humic-like and protein-like fluorescent component contribution to the marine DOM fluorescent pool on the SW Florida Shelf.........................68

Table 2.5. The Pearson’s product moment correlation coefficient matrix of modeled components with river discharge (DischSR-Shark River; DischCR- Caloosahatchee River) across the SW Florida Shelf. (C1-component 1; C2-component 2; C3-component 3; C4-component 4; C5-component 5)..............................................................................69

Table 3.1. Excitation and emission loading maximum wavelengths, type and possible sources of the five modeled PARAFAC DOM fluorophores during a *K. brevis* bloom event on SW Florida Shelf. Peak name, FDOM type and possible sources assignments of FDOM........................................................................................................96

Table 3.2. Slope, y-intercept and correlation coefficient of PARAFAC modeled components against temperature and salinity along the *K. brevis* bloom patch...........97

Table 4.1. Concentration from duplicate injections of PbTx-2 and PbTx-3 in sediments and cultured *K. brevis* as determined by LC/MS/MS. Station codes are the same as in Figure 4.1..................................................................................................................113

Table 5.1. Location of the NOAA Gordon Gunter cruise sampling sites and their distance from the Deepwater Horizon wellhead (DH= Deepwater Horizon Spill Site)..........142

Table 5.2. Excitation and emission fluorescence maxima of the seven PARAFAC modeled components..........................................................................................143

Table 5.3. Selected first three intense peaks on the excitation emission matrix of PAH compounds (n=3). The first peak has the highest fluorescence intensity of the PAH compound, partially dissolved in the Florida Straits surface seawater (salinity= 36.21)........................................................................................................144
Table 5.4. Macondo Source Crude Oil (1.0 µg/ml) GC/MS/SIM Selected PAH relative composition and concentration………………………………………………………………………………145

Table 5.5. Percentage of each integrated peak area relative to total peak area of CorexitEC9500 (See chromatogram in Figure 5.8)…………………………………………………………146

Table 5.6. Some of the available commercial in-situ sensors that estimates CDOM and crude oil………………………………………………………………………………………….147
Chapter 1

Introduction

Elucidating changes of elements in the biogeochemical cycles are important in determining Earth’s response to natural and human perturbations. Sequential human perturbations on Earth began during the discovery of fire, and later, the introduction of agriculture. These two factors led to land-use changes with the application of fertilizer to increase food production rate to accommodate increasing population (Sabine et al., 2004).

Likewise, with technological advancement, humans became more efficient in the utilization of products and services (transportation), exploration for oil and in the burning of fossil fuels. Utilization of the natural resources and altering landscapes to build infrastructures (e.g., houses, agricultural areas, roads, etc.) became efficient during the rise of the industrial revolution. These historical events between human activities and biosphere interactions resulted in an alteration of the biogeochemical cycling of important elements in the world’s ocean (Gruber et al., 2004).

Dissolved organic matter (DOM), a major reservoir of reduced carbon, carries important information on how the sea and landscape have been modified from land use and land use changes (i.e., agriculture) (Liu et al., 2010). Dissolved organic matter (DOM) has been used as a natural indicator of terrestrial material in the marine environment (Blough and Del Vecchio 2002; Coble 2007). However, the inherent chemistry of the DOM composition and mechanism of transport of this material in the marine environment remains complex. Because of this, sources, transport, and transformation of DOM in estuarine and coastal shelf waters, in relation to global DOM distribution, are not well understood (Jaffe et al., 2004; Liu et al., 2010). Monitoring
DOM, on the other hand, may provide information on how natural or anthropogenic activities modified or perturbed marine ecosystems.

Fluorescence measurement was employed in this study for colored dissolved organic matter (CDOM) measurement [(humic substances, the extractable colored component of DOM, comprises 40-80 wt% of dissolved organic carbon (Thurman et al., 1981)] . The advantages of fluorescence spectroscopy for the characterization of natural inherent DOM properties are its improved resolution, simplicity, sensitivity and its non-destructiveness, which requires minimal sample handling compared to other spectroscopic techniques (Coble, 1996). Isolation techniques of DOM from seawater pose possible changes in chemical composition. Fluorescence techniques have the sensitivity and spectral resolution to examine subtle changes in fluorescent dissolved organic matter (FDOM) that provide information into alteration of CDOM pool composition (Coble 1998). The scanning fluorescence mode (excitation-emission matrix-EEM) has been used in several studies (Coble 1998; Clark et al., 2002; Boehme et al., 2004; Conmy et al., 2009). However, this measure only detects strong fluorescence maxima and neglects other fluorescing components. Likewise, quantitative analysis is not also possible for this technique and becomes tedious in dealing with hundreds of samples.

Parallel factorial analysis (PARAFAC), which is a qualitative and quantitative approach to characterize DOM fluorophores, was used in this study to determine DOM sources, and the relative distribution of each of the fluorophores identified. PARAFAC technique is a three-way method used to deconvolute the complex nature of EEM. Several experiments have been successfully applied to PARAFAC in order determine
complex components in marine DOM (Yamashita and Tanoue, 2008; Yamashita and Tanoue, 2009; Yamashita et al., 2010). However, there is still a lack of studies that demonstrates use and application of the coupled EEM and PARAFAC techniques from nearshore to offshore waters on the continental shelf. **CHAPTER 2** determined the utility of EEM and PARAFAC in resolving bulk DOM on the SW Florida Shelf that included offshore DOM samples. It is the objective also of the second chapter to determine the environmental controls that mediate DOM distribution on the Shelf. Results obtained could potentially serve as a baseline for optical observation and prediction studies.

New avenues of possible EEM and PARAFAC application were explored. Since DOM had been implicated in several studies as source of nutrient to the formation of harmful algal bloom (Walsh et al., 2006; Vargo et al., 2008), EEM and PARAFAC techniques were used to determine and obtain insights on the role of DOM during a bloom event (**CHAPTER 3**). By understanding the DOM dynamics, we may be able to obtain key information to determining controls in the DOM distribution on the shelf that may have impact on environmental issues like *Karenia brevis* bloom (Walsh et al., 2003), a harmful algal bloom reported to occur regularly in both coastal and offshore shelf region of Florida (Fish and Wildlife Research Institute, 2012: http://myfwc.com/research/).

Similarly, distribution of the brevetoxins, the toxins produced by *K. brevis*, was evaluated. Brevetoxins have been implicated to have caused health and ecological problems (Kirkpatrick et al., 2004). In order to evaluate the toxins’ ecological damage and risks, factors that control its distribution have to be identified. At present, however,
there is lack of information on the fate of this toxin in the marine environment, particularly in the marine sediments, where most of organic materials accumulate. In CHAPTER 4, a single terrestrial fluorophore determined how land materials affect incorporation of the toxins in the recent marine sediments. Results suggest that an increase in terrestrial component water input to the sea will impact incorporation of toxin in marine sediments. In addition, application of PARAFAC analysis was evaluated during an oil spill event. The problem typically encountered using fluorescence on oil spills is that the fluorescence detection of crude oil components becomes less robust with areas containing high concentrations of dissolved humic materials, such as those found along the Gulf Coast states. The fluorescence of these humic materials in coastal environments can significantly interfere with the low level signals from the dispersed hydrocarbon plumes. However, this problem may be overcome by statistically treating the three-dimensional EEMS spectra using PARAFAC analysis (Stedmon and Bro, 2008) to improve detection of the crude oil and distinguish it from dissolved humic materials. Hence, another objective of this study (CHAPTER 5) is to apply and demonstrate that the PARAFAC technique can be a viable tool for identifying the hydrocarbon plumes and other complex materials from the Deepwater Horizon Oil Spill.

1.1. Research Goals

Overall: Evaluation and application of the PARAFAC model to resolve bulk DOM fluorescence components from near-coast to offshore waters.
Specific objectives

1. To evaluate the ability of EEM and PARAFAC to resolved DOM fluorescence components.

2. Determine DOM fluorescence sources and temporal variability from near-coast to offshore waters on the SW Florida continental shelf.

3. To apply EEM and PARAFAC methods in resolving DOM fluorescence components and determine the components’ roles in the October 2009 offshore *K. brevis* bloom.

4. To determine the relationship between an identified terrestrial DOM fluorophore and the incorporation of *K. brevis* brevetoxins in recent marine sediments.

5. To demonstrate application of EEM and PARAFAC to resolve hydrocarbons, CDOM and dispersants in deep water (i.e., crude oil and the dispersant residues from the Deepwater Horizon Oil Spill).

1.2. Review of dissolved organic matter (DOM) research

DOM is ubiquitous in all natural environments (Hedges et al., 2000; Ertel et al., 1984). On a global basis, DOM occurs in high concentration in soil, humus, as newly deposited marine sediment; however, seawater contains more DOM than is stored in all land plants and marine organisms combined (Hedges et al., 2000). Considered to constitute the largest pool of reduced carbon in the biosphere, the oceanic DOM export constitutes 20% total organic flux to the deep ocean, where this level controls the carbon dioxide in the atmosphere (Carlson et al., 1994; Hansell 2002). The connection of atmospheric CO₂ to global warming and climate change also makes it important to study DOM -- an important entity in the global carbon cycle (Coble, 2007).
There are two forms of carbon occurring in the ocean. Primarily, 95% occurs as bicarbonate and carbonate ions, and the remainder as organic forms (e.g., organic matter, dissolved and particulate organic carbon) (Druffel et al., 1992). Organic matter is synthesized via photosynthesis \( (2H_2O + CO_2 + \text{light} \rightarrow 2O_2 + (CH_2O) + H_2O) \). \( CH_2O \) stands for organic matter in the form of carbohydrates, such as the sugar glucose. Polysaccharides are the main form in which photosynthesized organic matter is stored in living cells. They represent the biosynthetic starting material for all reactions that yield other organic compounds. Respiration is the reverse of this reaction (Peters and Moldowan, 1993).

DOM is a mixture of materials composed primarily of carbon, oxygen, hydrogen and nitrogen (including hydrocarbons) and can have multiple functional groups that can exhibit different reactivities (Figure 1.1). It can be produced from exudates of microorganisms. On land, DOM can come from land plants rich in cellulosic materials. Materials from land can be carried to the marine ecosystem through rivers, aerosols, wind and rain. Different sources of DOM render such material extremely heterogeneous.

![Figure 1.1. Idealized structure of humic acid (modified from Stevenson, 1994)](image)
Numerous efforts have been conducted to elucidate the structure of DOM. $^{13}\text{C}$-NMR studies determined that DOM consists of carbon- and nitrogen-containing compounds. Despite research efforts of more than a decade, DOM remains a complex undefined material, and is still uncharacterized at the molecular level in soils, sediments, and seawater (Hedges et al., 2000; Kujawinski and Behn, 2006; Ohno et al., 2010). Its dynamic character is affected by various physical, chemical and biological factors. Despite the recognized role of riverine transport of organic matter from land to the ocean, little is known as to how much is permanently sequestered as export to the deep ocean, and into the marine sediments.

DOM was believed to be a residue formed from heteropolycondensation reactions from the breakdown of biomolecules by enzymes (Hedges, 1988), to form a brownish end-product. Such humification processes are the result of a condensation mechanism that includes a Maillard reaction -- a reaction between carbohydrates and amino acids that form aromatic melanoidins (Evershed et al., 1997; Hedges et al., 2000). With the availability of sophisticated instruments (i.e., $^{13}\text{C}$ and $^{15}\text{N}$ NMR) it has been determined that another fraction of the organic nitrogen in modern organic matter from sediments, soils and seawater is in amide form (Thorn et al., 1992; Aluwihare et al., 1997; McCarthy et al., 1997; Knicker, 2000; Kujawinski and Behn, 2006).

Despite the progress in DOM studies, there is still the lack of understanding of the distribution, composition and ecological functions of DOM, resulting in uncertainties in our understanding on the origins and reactivity of the uncharacterized component of DOM (Hedges et al., 2000).
1.2.1. CDOM photochemistry

CDOM is the optically active component of the bulk DOM. Extractable components of CDOM are termed humic substances (humic and fulvic acids), which are also known as Gelbstoff, a yellow acidic portion of the organic matter in seawater (Ertel et al., 1984; Harvey and Boran, 1985). Structural characterization of humic substances employed (1) reduction using metals, hydrogen, and metal hydrides; (2) oxidation with copper oxide, permanganate, and chlorine; and (3) alkaline hydrolysis methods (Sonnenberg et al., 1989). Present views of humic substances using recent nuclear magnetic resonance spectroscopy, X-ray absorption near-edge structure spectroscopy, electrospray ionization-mass spectrometry, and pyrolysis studies (Sutton and Sposito, 2005) showed that humic substances are a collection of diverse, relatively low molecular
mass components. They form a dynamic association, stabilized by hydrophobic interactions and hydrogen bonds that are capable of organizing into micellar structures in suitable aqueous environments (Sutton and Sposito, 2005).

Distribution of CDOM can vary depending on many factors (Figure 1.2). These include production source, transformations during transport, and removal mechanisms (Miller and Zepp, 1995; Zepp et al., 1998; Blough and Del Vecchio, 2002; Huang and Chen, 2009). The CDOM chemical composition and distribution dynamics can influence changes associated with water quality, biological processes, and the distribution of trace elements (Miller and Moran, 1997; Bisett et al., 2005).

Characterization of CDOM, in the past, required large volumes of water to extract material needed for chemical analysis. Such procedures alone required too much effort, and limited sampling size, and most importantly, often resulted in alteration of the inherent properties of CDOM. Technological advancement of profiling CDOM chemical composition and its dynamics renewed interest on CDOM research (Coble, 2007). However, despite reviews on the CDOM, there are still uncertainties with regards to CDOM distribution associated with seasonality, sources and sinks (Blough and Green, 1995; Clark and Zika, 2000; Blough and Del Vecchio, 2002; Coble, 2007).

**Two major types of colored dissolved organic matter CDOM**

The two major types of colored DOM are fluorescent dissolved organic matter and chromophoric dissolved organic matter. The CDOM chromophores are typically measured based on their absorption spectra in the UV-Vis region (λ=300-600 nm, Blough and Del Vecchio, 2002). The resulting absorption properties of the organic material can
be represented by the Beer-Lambert Law (Equation 1.1; Skoog et al., 1998), where absorbance (A), is related to the base-10 logarithm of the incident light intensity ($I_o$) and the transmitted light intensity ($I$) ratio. Absorbance is also equivalent to the molar absorptivity ($e$, L mol$^{-1}$ cm$^{-1}$), the path length of the sample (b, cm) and the concentration of the compound in solution (mol L$^{-1}$)

$$A = \log_{10} \frac{I_o}{I} = ebc$$  \hspace{1cm} \text{Equation 1.1}

Typical organic compounds that absorb in the UV-Vis region in the solar spectrum have double bonds involving carbon, hydrogen and oxygen. These chromophoric structures include alkenes, aromatic and heterocyclic compounds, aldehydes, ketones, nitro compounds, azo dye and many others (Larson and Weber, 1994).

Most solution-phase spectra are associated with broad absorption bands. The main reason of such broadening, according to Coyle (1986), is the large number of vibrational and rotational energy levels associated with polyatomic molecules, and the absorption of a photon can result in conversion of a portion of its energy into vibrational or rotational energy. He further added that for absorption to occur, the energy of the photon does not need to match precisely the energy required to simply change the electronic configuration. Hence, any fine structure is lost because the solvent molecules caused the lines to broaden as a result of their influence on the energy levels of the molecule.

Absorption of CDOM has been used to determine sources of organic matter, productivity, and water masses in coastal and deep ocean investigations, based on ratios of absorbances or the slope parameter, $S$ (Bricaud et al., 1981; Nelson et al., 1998;
Information from these parameters, however, is limited, and needs to be compared to other sophisticated spectroscopic techniques (e.g., Nuclear Magnetic Resonance (NMR) (Hedges et. al., 1992), size-exclusion chromatography (SEC) (Hur et al., 2006), HPLC (Lombardi and Jardim, 1999), and Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICRMS) (Kujawinski and Behn, 2006, Kujawinski et al., 2004)) to obtain more information on CDOM. Aside from that, the absorption band tends to approach undetectable limits in oligotrophic waters, and normally requires 5- to 10-cm optical cell lengths to obtain useful information (Bricaud, 1981).

The fluorescence technique has various modes that can be used to obtain more information on CDOM composition and its properties compared to absorption measurement. These modes include single wavelength scanning (Jaffe et al., 2004), a time resolved method (Clark et al., 2002), and three-dimensional EEMs (Coble et al., 1990; Coble 1996; Parlanti et al., 2000; Stedmon et al., 2003; Stedmon and Markager, 2005; Murphy et al., 2008). Although fluorescence does not provide a direct determination of the chemical structure, combining it with other methods (e.g., FT-ICRMS) provides a more in-depth molecular level determination of the optically active DOM (Ohno et al., 2010).

**Fluorescence Analysis**

Fluorescence is a more sensitive method compared to UV-Vis techniques. It can be used to measure quantitatively, extremely small amounts of material (Coyle, 1986). The absorption and emission of light typically is demonstrated by the Jablonski Diagram (Figure 1.3).
Figure 1.3. Jablonski Diagram (patterned after Lacowicz, 2006).

In this diagram, explained in detail by Lacowicz (2006), Larson and Weber (1994), and Coyle et al. (1986), S₀, S₁ and S₂ correspond to the singlet, first and second electronic states. The electronic energy levels of these fluorophores can exist at a number of vibrational levels (i.e., 0, 1, 2, as shown in Figure 1.3). Absorption of light by fluorophores results in excitation to higher vibrational levels, either S₁ or S₂. In some cases, internal conversion occurs where molecules relax to S₁ or lower vibrational energy at about 10⁻¹² s or less. Internal conversion is normally complete before emission occurs; fluorescence lifetime is typically 10⁻⁸ s. Thus, fluorescence emission results from a thermally equilibrated excited state, exiting as the lowest energy vibrational state of S₁.

The S₁ electronic state of a fluorophore can undergo a variety of possible deactivation mechanisms. First, they can return to the vibrational ground state, eventually arriving at thermal equilibrium. Consequently, the vibrational structures in both absorption and emission spectra are similar. Secondly, S₁ molecules proceed to the first triplet T₁ via intersystem crossing. This process shifts to longer wavelengths
fluorescence- and this process is called phosphorescence. There are different methods for fluorescence analysis. There is the single scan, the time-resolved, and the excitation-emission matrix fluorescence analysis. The single scan method is fast and heavily used for in-situ measurements, where a single excitation-emission wavelength is used for fluorescence measurement. Time-resolved is another heavily used method, which measures the life-time of the DOM (Clark et al., 2002). These fluorescent compounds are commonly called fluorophores. The third mode is the excitation-emission matrix (EEM) fluorescence scan, which is used in this study and will be described next.

1.2.2. Excitation and Emission Matrix (EEM) fluorescence of DOM

The EEM spectra are a collection of scanned intensities measured at various emission and excitation wavelengths collected by scanning (Figure 1.4a, b). As a result, it provides a wide range of wavelength dependent data points per sample (~5000 fluorescence data). This technique has been used in various applications and has recently been utilized in the DOM fluorescence (FDOM) measurements (FDOM – is a photometric measurement of dissolved organic matter (Coble, 1996; Stedmon and Markager, 2005; Murphy et al., 2006, 2008; Yamashita et al., 2008). Collection of EEMs were developed (Coble 1998) for different water masses to determine spectral signatures and arrive at different assignments (Figure 1.4b; Table 1.1). This technique has been cited in many recent papers. Application of EEMs has been dramatic and has gained in popularity due to its simplicity, fast data acquisition, and requirement of less sample volume.
Figure 1.4. Sample of a) uncorrected EEM, and b) corrected EEM fluorescence spectra. Bar in QSU units. Letters represent peak name assignments (Coble 1996) as, A = UVA humic acid; C = UVC humic-like; M = marine humic-like; T = tryptophan-like, and; B = tyrosine-like components. QSU –quinine sulfate unit (standard use to calibrate fluorescence intensity of DOM fluorescence, Coble 1996) Correction of raw (uncorrected) EEM involves scatter removal of light scattering by blank correction, normalized intensity using Raman peak of water, fluorescence intensity calibration using QSU, and inner filter effect (results from the absorption of the incoming light and absorption of the emission light) correction (Lacowicz, 2006).
Table 1.1. The excitation and emission fluorescence maxima of the PARAFAC modeled DOM fluorescence and its designation (Coble, 1996, 2007).

<table>
<thead>
<tr>
<th>Ex</th>
<th>Em</th>
<th>Peak name</th>
<th>Component</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>260</td>
<td>400-460</td>
<td>A</td>
<td>UVC humic-like</td>
<td>Humic, terrestrial, autochthonous</td>
</tr>
<tr>
<td>320-360</td>
<td>420-460</td>
<td>C</td>
<td>UVA humic-like</td>
<td>Terrestrial, anthropogenic, agriculture</td>
</tr>
<tr>
<td>290-310</td>
<td>370-410</td>
<td>M</td>
<td>UVA marine humic-like</td>
<td>Microbial/biological in origin</td>
</tr>
<tr>
<td>250(385)</td>
<td>504</td>
<td>M</td>
<td>UVA humic-like</td>
<td>Fulvic, terrestrial autochthonous</td>
</tr>
<tr>
<td>275</td>
<td>305</td>
<td>B</td>
<td>Tyrosine-like, protein-like</td>
<td>Autochthonous</td>
</tr>
<tr>
<td>275</td>
<td>340</td>
<td>T</td>
<td>Tryptophan-like, protein-like</td>
<td>Autochthonous</td>
</tr>
<tr>
<td>398</td>
<td>660</td>
<td>P</td>
<td>Pigment-like</td>
<td></td>
</tr>
</tbody>
</table>

1.2.3. PARAFAC modeling

PARAFAC (parallel factorial analysis) is a multi-decomposition technique, which decomposes the three-dimensional array into sets of scores and loadings, compared to bilinear principal component analysis (PCA). PCA involves abstract functions of the data to look at patterns while factorial analysis uses information (i.e., spectral data) that can be related to chemistry (Brereton, 2003). The approach for PARAFAC modeling (see Figure 1.5 for schematic flow) is a multi-step process that requires preliminary treatment to correct instrument biases from fluorescence intensity and spectral shape. Details of PARAFAC modeling will be discussed in the succeeding chapters.
Multi-step process

Data collection and pre-processing
- Measurement
- Spectral correction
- Inner filter correction
- Calibration
- Removal of scatter

Outlier Analysis
- Identify and remove possible outliers
- Identify number of components to model the data

Model Validation
- Residual analysis
- Split-half analysis
- Random initialization

Interpret model results

\[
X_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}
\]

\[
i = 1, \quad j = 1, \ldots, J; \quad k = 1, \ldots, K.
\]

Figure 1.5. Schematic diagram of the PARAFAC modeling process (modified from Stedmon and Bro, 2008).
The term multi-way describes datasets with more than two dimensions. The excitation-emission matrix (EEM) fluorescence data are multi-way. The fluorescence of the sample varies depending on the excitation wavelength and the wavelength of the emitted fluorescence (Figure 1.5a). PARAFAC is a multi-way data analysis fitted to minimize the sum of squares of the residuals (see Bro, 1997 and Stedmon and Bro, 2008)

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}, \ i=1, \ldots, I; \ j=1, \ldots, J; \ k=1, \ldots, K.$$ (equation 1.2)

where \(x_{ijk}\) refers to the fluorescence intensity of the \(i^{th}\) sample at the \(j^{th}\) variable (emission wavelengths), and at the \(k^{th}\) (excitation wavelengths) in the PARAFAC FDOM component model. \(a_{if}\) is directly proportional to the concentration of the \(f^{th}\) analyte of the \(i^{th}\) sample, \(b_{jf}\) and \(c_{kf}\) are scaled estimates of the emission and excitation spectra at wavelengths \(j\) and \(k\), respectively, for the \(f^{th}\) analyte, and \(e_{ijk}\) is the residual noise that represents the noise not explained by the model.

The application of equation 1.2 can be explained in another way. Consider one EEM with \(J\) and \(K\) wavelengths. This data corresponds to a matrix \(X\) of fluorescence intensities of size \(J \times K\). If a single fluorophore is present in the sample, then it means that the EEM is the product of the emission (the vector \(b_1\)) and excitation (the vector \(c_1\)) spectra. The resulting fluorophore then becomes the product of its excitation and emission spectra \((Z_1 = b_1 c_1)\) at unit concentration (Figure 1.5b).

The PARAFAC method conceptually is similar to bilinear PCA. PARAFAC decomposes data into trilinear, instead of bilinear components. The trilinear components
include one score and two loading vectors, while in bilinear PCA each component is composed of one score and one loading vector (Bro, 1997).

The advantage of PARAFAC is that it introduces more unique information compared to PCA (principal component analysis). The PARAFAC model of the datasets are not merely abstract orthogonal emission profiles, like in PCA (i.e., there is no rotation problem in PARAFAC). The PARAFAC loadings derived are actual estimates of the real emission spectra of the real fluorophores (Bro, 1997).

There are three main ways to determine the correct number of modeled components as detailed in Bro (1997) and Stedmon and Bro (2008). These are the split-half analysis, residual inspection, and random initialization. In the split-half analysis (Harshman, 1984), the data are divided into two sets, and apply PARAFAC model on each half. Due to the unique characteristic of the PARAFAC model, independently modeled datasets should result to similar or identical loadings, if the selected number of components is correct. In the inspection of the residuals, this process determines the systematic pattern in the residuals. If more systematic pattern is left, then more components can still be resolved. However, if the sum of squares of the residuals relative to the number of components chosen levels or becomes flat, the right number of modeled components is achieved. The third method to evaluate modeled components is random initialization. For validation using this technique, models use random numbers as initial estimates to fit the series of models. The goal is to ensure that the result is in fact a least squares and not a local minimum. If the least square solution is not attained, the chosen number of components must be validated.
PARAFAC modeling of DOM EEMS has recently been used in various coastal and oceanic studies. Some of these studies have included determination of DOM fluorescence sources in sea ice (Stedmon et al., 2007), its interannual variability (Kowalczuk et al., 2009), composition in estuarine soils and sediments (Santin et al., 2009), its interaction with trace metals (Yamashita and Jaffe, 2008), photodegradation and ammonium photochemical production (Stedmon et al., 2007), its redox reactivity (Cory and McKnight, 2005), origin and fate (Murphy et al., 2008), and sources in estuary (Stedmon et al., 2003), and production and degradation of autochthonous fractions (Stedmon and Markager, 2005; Jorgensen et al., 2011).

Application of PARAFAC has been important due the ability of this technique to distinguish different fractions of DOM pool using spectral fluorescence measurements in aquatic environments (Stedmon and Bro, 2008), and provide more chemical clues on the complexity of DOM dynamics, especially in coastal-shelf regions (Coble, 2007).
Chapter 2

On the temporal variation of PARAFAC modeled DOM fluorescence fractions on the SW Florida Shelf

2.1 Rationale

Colored dissolved organic matter (CDOM) is a component of DOM which plays a major role in optical properties in the coastal environment (Blough and Del Vecchio, 2002; Zanardi-Lamardo et al., 2002). Its optical absorption and fluorescence spectra cover a broad range of wavelengths. The extractable form of CDOM is associated with humic substances (humic and fulvic acids), also known as Gelbstoff, a yellow acidic portion of organic matter in seawater (Harvey and Boran, 1985). Chemical and optical properties of CDOM can be affected by source, rate of production, transformation, and removal mechanisms such as biological and photochemical degradation (Clark and Zika, 2000). These variations are further affected by climatic, hydrological and anthropogenic factors (Miller and Zepp, 1995; Zepp et al., 1998; Blough and Del Vecchio, 2000; Huang and Chen, 2009). The distribution and dynamics of CDOM plays a crucial role in aquatic environments in regulating water properties, biological processes, and the distribution of trace elements (Blough and Green, 1995; Miller and Moran, 1997; Clark and Zika, 2000; Blough and Del Vecchio, 2002; Bissett et. al., 2005).

The importance of DOM in the biogeochemical cycling of elements has been recognized, however, its geographic distribution and variation remains poorly defined, particularly on the coastal shelf environments (Liu et al., 2010). Measurements of DOM used to study its distribution and fate have relied on optical measurement that can use
absorption and fluorescence characteristics of the organic matter. Due to the inherent sensitivity of fluorescence over absorption in DOM measurements (Vodacek et al., 1995, Chen and Gardner, 2004), the fluorescence technique has gained wide use in optical studies in estuaries (Boyd and Osburn, 2004; Maie et al., 2007), the coastal shelf (Coble et al., 1998; Clark et al., 2002; Boehme et al., 2004; Conmy et al., 2009; Mendoza et al., 2012) and in deep oceans (Yamashita et al., 2007; Yamashita and Tanoue, 2008; Yamashita and Tanoue, 2009; Yamashita et al., 2010).

The distribution of terrestrial DOM is typically measured using photometry to measure the bulk DOM fluorescence (Clark et al., 2004; Jaffe et al., 2004). Monitoring a single fluorophore (DOM fluorescent component) limits the information obtained to characterize the DOM source. A more comprehensive DOM measurement using EEM fluorescence was previously introduced where a “peak-picking” method was used to determine and differentiate sources of DOM (Coble, 1996). This is a simple approach and multiple components can be resolved other than the terrestrial DOM component. However, quantification of the fluorescent components using the peak-picking technique can be difficult, particularly in dealing with large datasets. In another approach, principal component analysis (PCA) modeling was combined with EEM analysis to resolve large datasets (Boehme et al., 2004). However, factors obtained in PCA have purely abstract meaning (qualitative) while PARAFAC can lead to results that are physically interpretable (Brereton, 2003). Thus, this study used a technique that can determine both quantitative and qualitative model of the dataset, and extract individual DOM components characterized with specific excitation and emission wavelength from the measured three-dimensional spectra. The PARAFAC statistical modeling technique
(Stedmon and Bro, 2008) was employed to resolve and classify DOM fluorescence components and to determine factors; this information is then used to infer factors that control the component’s distribution on the continental shelf.

The continental shelf is an environment that mediates fate and transport of terrigenous materials from land to the ocean. The areal distribution of distinct DOM components was determined using their fluorescent properties, using EEM and PARAFAC methods (Stedmon et al., 2006; Alvarez et al., 2008; Boyd et al., 2010a; Boyd et al., 2010b; Guo et al., 2011). This thesis extends the application of EEM and PARAFAC to differentiate the various DOM fluorescence components and determine controls of the fluorescent DOM components temporal and spatial variability on the southwest (SW) continental shelf of Florida, during wet months (May 2008 and October 2008) and dry months (January 2009 and April 2009). The influence of the known hypersaline seawater, caused by reduced water inputs and weak basin renewal rates (Lee et al., 2006; Price et al., 2007), reverses the typical inverse relationship of DOM fluorescence and salinity (Zanardi-Lamardo et al., 2002). Mechanisms that control the reversal of the DOM fluorescence and salinity on the shelf will also be investigated. The resolved PARAFAC components establish multiple organic matter fluorescence tracers, potentially serving as baseline information for long-term monitoring of DOM fluorescence variability on the shelf.
2.2. Materials and Methods

2.2.1. Description of sampling site

The SW Florida Shelf is a broad, gently sloping region generally parallel to the coastline (Weisberg et al., 1996). It is located in a subtropical region, with the climate characterized by a relatively wet season from May to October and a dry season from November to April. The majority of river runoff comes from Shark River, the Caloosahatchee River, and the Peace River (Figure 2.1). Conversion of the Everglades system to water conservation, drainage systems and agricultural areas, dramatically changed the natural flow of these river systems (Reedy and DeLaune, 2008). Method used in this study can potentially be a tool for monitoring how future land use changes (e.g., Everglades) could affect water quality on the West Florida Shelf.

The circulation pattern of the shallow Florida Shelf is seasonally modulated by variations from winds, wave currents and water mixing (Weisberg et al., 1996). These variations can be influenced by the Gulf of Mexico Loop Current that enters through the Yucatan Strait and exits at the Gulf Stream, through the Straits of Florida, on the eastern side of the Gulf of Mexico (He and Weisberg, 2003). Monthly mean currents at mid-shelf were suggested to occur at a seasonal cycle with along-shore flows either to the southeast in spring, or to the northwest in late summer to early autumn (Weisberg, 1996).

2.2.2. Temporal and spatial sampling

Sampling cruises were conducted during April and October (2008), and January and April (2009) on the SW Florida Shelf region, aboard the R/V Walton Smith
(UM/RSMAS). Temperature, salinity, and oxygen at 1 m were measured continuously using a SBE 21 Sea-Bird thermosalinograph (Sea-Bird Electronics, Inc., WA, USA) flow-through system on the ship. A SBE 911 Sea-Bird Conductivity-Temperature-Depth (CTD) package, equipped with a Sea-Bird 911 Plus underwater unit, with dual conductivity and temperature sensors, and, an additional A/D channels, was used for measuring photosynthetically active radiation (PAR), dissolved oxygen (2 channels), and depth and for recording fluorometer and transmissometer readings. Water samples were collected using a NISKIN sampler for ≥ 1 m depth and the flow-through system for ~ 1 m depth. Samples were immediately filtered using 0.2 µm nylon filters. Vials were filled with Milli-Q water every after 5 CTD cast to monitor any contamination in the sampling. Blanks were also prepared/collection to monitor for any contamination from storage (blanks we collected before and after storage). Samples for DOM fluorescence analysis were placed in pre-combusted (450°C) scintillation vials, each covered with aluminum foil, and stored at 5°C prior to analysis (spectral measurement were conducted right after the research cruise). Samples for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analyses were placed in 60 ml Nalgene® bottles and immediately frozen at -20°C. There were a total of 71 sampling stations (Figure 2.1; Table 2.1) across the shelf. All sampling was concurrent with the South Florida Quarterly Hydrographic Survey, as part of the NOAA-AOML (Miami) South Florida Program (http://www.aoml.noaa.gov/sfros/database/Fl%20Bay.html).

2.2.3. EEM spectral measurements

For the spectral analysis, water samples were first allowed to reach room temperature. EEM was measured on a fluorescence spectrophotometer (Hitachi F-4500),
configured to scan from 200-500 nm for excitation and 200-700 nm for emission at 5 nm intervals (with both 5 nm emission and excitation slit width), and at a scan speed of 1200 nm/min with the photo-multiplier tube at 700 V. The instrument response on the excitation side was calibrated using Rhodamine B, while the emission side was calibrated using a diffuser (Hitachi High Technologies Corporation, 2001). All EEMs were corrected by subtraction with EEM of Milli-Q water (EEM of Milli-Q water for every 5 samples was used as blank for each batch). Fluorescence intensities were calibrated against quinine sulfate monohydrate in 0.1 N H$_2$SO$_4$ at Ex/Em = 350/450 nm. The inner filter effect was corrected from the measured absorbance (McKnight et al., 2001; Lacowicz, 2006). Absorbance was measured using a UV-Vis spectrophotometer (Agilent 8453). The fluorescence intensities of the PARAFAC components were reported in quinine sulfate units (QSU) (Stedmon and Bro, 2008). Data were all normalized to the Raman peak of the Milli-Q water. The detection limit of the instrument was 0.2 QSU. The signal-to-noise ratio of the instrument was observed to be ~150, and a drift of ~0.875 %/10 min was within the Hitachi F-4500 spectrofluorometer instrument sensitivity specification. S/N was determined based on the height of a Raman light peak (S) of Milli-Q water to the noise under certain instrument conditions. Calculation of the noise level (N) is based on the peak to peak definition. The time variation spectrum data in the time range of 1 to 601 s was divided into blocks of every 10 s ($N_i$). The differential Ni between the maximum and the minimum in each block was calculated. The average over the $N_i$s’ was used to calculate (N). The stability drift was calculated based on the percentage of the difference of the maximum and minimum mean value of N, divided by the maximum mean value of N. Hitachi fluorescence spectrophotometer S/N and drift
specification are >100 and within 1.5%/10 min, respectively (Hitachi High-Technologies, Corp., 2001).

**2.2.4. EEM fluorescence and PARAFAC modeling**

EEM spectra of DOM obtained are multi-way (three-way), where the fluorescence measured varies with recorded emission and excitation wavelengths. PARAFAC is modeled using a multi-way data analysis fitted to minimize the sum of squares of the residuals (see Bro, 1997 and Stedmon and Bro, 2008 for details of principles and approaches in PARAFAC modeling, employing equation 2.1).

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}, i=1, \ldots, I; j = 1, \ldots, J; k = 1, \ldots, K. \quad \text{(equation 2.1)}$$

where $x_{ijk}$ refers to the fluorescence intensity of the $i^{th}$ sample at the $j^{th}$ variable (emission wavelengths), and at the $k^{th}$ variable (excitation wavelengths) in the PARAFAC FDOM component model. $a_{if}$ is directly proportional to the intensity of the fluorescence signal due to the $f^{th}$ analyte of the $i^{th}$ sample, $b_{jf}$ and $c_{kf}$ are scaled estimates of the emission and excitation spectra at wavelengths $j$ and $k$, respectively, for the $f^{th}$ analyte, and $e_{ijk}$ is the residual noise that represents the noise not explained by the model. Corrected EEM datasets were decomposed into sets of spectral loadings (components), identified with different scaled estimates of excitation and emission wavelengths. The PARAFAC model used here employed the DOMFluor toolbox (Stedmon and Bro, 2008) and was run in the R2008 Student Version of MATLAB. For the PARAFAC analysis, the EEM range of excitation and emission wavelengths were reduced to 250-450 and 300-520 nm, both at 5 nm intervals, respectively, to minimize random noise outside the spectral range of interest. Excitation wavelengths below 250 nm were removed due to high residuals,
possibly influenced by the xenon source. Light and data emission wavelengths > 520 were removed due to Raleigh and Raman scatter and secondary protein-like fluorescence (Smilde et al., 2004; Murphy et al., 2008). The data array consisted of 272 samples with 47 emission wavelengths and 41 excitation wavelengths. The first step involved fitting of the data starting from one to eight components. The number of components was increased a reasonable fit has been obtained (fit above 99%). The estimated number of DOM detectable fluorophores was validated by the split-half technique, and was further validated by random initialization steps, which ensures that the results obtained observes least squares and not a local minimum (Stedmon and Bro, 2008).

2.2.5. **DOC and DON analysis**

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) of water samples were analyzed simultaneously using a Shimadzu TOC-V/TN system, employing a combustion catalytic oxidation method (catalytically aided with Pt) that generated carbon dioxide and nitrogen monoxide, respectively. Carbon dioxide and nitrogen monoxide were measured using non-dispersive infrared (NDIR) and chemiluminescence detectors, respectively (Shimadzu TOC-V Series Manual). Standardization (using potassium biphthalate) was performed prior to analysis each day, including running seawater reference standards produced by the Dennis Hansell Laboratory’s certified reference material program (UM/RSMAS). The precision in the DOC measurements was approximately 1-2 µM C. DON was calculated from the difference of TDN and dissolved inorganic nitrogen [DIN: NO$_3^-$+NO$_2^-$ (detection limit: 0.02 ppm N)+NH$_4^+$ (detection limit: 0.04 ppm N) measured using Alpkem Flow Solution System
Autoanalyzer]. The DIN nutrients and chlorophyll-a (Chl-a) data were provided by the South Florida Project of the NOAA-AOML (Miami).

2.2.6. Pearson’s product moment correlation analysis

Pearson’s product moment correlation (Montgomery, 2009) was used to determine the correlation of the different components to physical, biological and chemical environmental variables. The mean for each of sampling period was used in the correlation analysis against other parameters. The fluorescence mean from four sampling periods were correlated against other parameters at \( p = 0.05 \). Pearson’s product moment correlation coefficients were analyzed using Sigma Stat version 3.5 (Systat Software, http://www.systat.com/).

2.3. Results and Discussion

2.3.1. PARAFAC modeled DOM components

The PARAFAC model, derived from spectral deconvolution of 272 EEM spectra, determined 5 fluorophores, as shown in Figure 2.2. The number of components corresponds to the number of unique spectra of individual components in the mixture, characterized by scores, loadings (the EEM fluorescence spectra), and leverage (the weight of the variance it exerts compared to the rest of the samples) (Figure 2.2). The one-component model contains 93.88% of the total variance and loading emission, occurring at a longer wavelength, and an excitation at a shorter wavelength loading. The two, three and four component captured 96.40%, 98.56%, 99.21% of the variance, respectively. The optimum five-component model captured 99.53% of the total dataset variation (Table 2.2). The root mean squared for each of these modeled components in
non-negativity mode were 0.060, 0.032, 0.021, 0.016 and 0.013, respectively. The non-negative mode was used to constrain the model to help obtain parameters that do not contradict a priori knowledge, obtain unique solution, speed up algorithm implementation, and enable quantitative analysis (Bro, 1997).

A representative measured and modeled EEM and its residual is shown in Figure 2.3, including a diagonal cross section. The strong similarity of the measured and modeled EEMs and the low residual difference suggests that the five components explain the maximum variance across the bulk EEM samples. The five-component model obtained was verified using split-half and the random initialization validation (Bro, 1997; Stedmon and Bro, 2008). The split-half analysis showed a positive validation, indicating that the five-component model shows similarity to the split-modeled excitation and emission loadings (Figure 2.4). The five-component model also showed a positive result during the random initialization fitting (Bro, 1997; Stedmon and Bro, 2008), which confirmed that the selected number of components exhibits a true least square fit.

2.3.2. Temporal and spatial variability of fluorophores across the SW Florida Shelf

The resolved excitation and emission fluorescence loadings of the PARAFAC model output are shown in Figure 2.5 and Table 2.3. Based on these fluorescence loadings, spatial distributions and temporal variability of the derived fluorescence components were generated (Figure 2.6). These plots provide information on the shelf distribution and sources of the fluorophores. Strong fluorescence of components 1 and 2 were found to have spectral properties assigned to terrestrial UVA (wavelengths from 315-400 nm) humic-like material and anthropogenic UVC (wavelengths from 100-280
nm) terrestrial, respectively (Table 2.3). On the West Florida Shelf, component 1 (humic-like component 1= HLC1) and component 2 (humic-like component 2= HLC2), associated with humic-like components, tended to exhibit maximum fluorescence (Figure 2.6-a,b,m,n) in regions proximate to the West Florida Bay area (located within stations 65-71; Figure 2.1; Table 2.1) during May 2008 and April 2009.

The West Florida Bay receives freshwater input largely from Shark River. The October (2008) sampling was affected by a high precipitation (0.2 inches) and high river discharge from Shark River (6,000 cfs) (Figure 2.7). At this period, the fluorescence of HLC2 doubled in intensity (~28 QSU, Figure 2.6-f) along the coasts of the Ten Thousand Islands and in the Florida Bay transects (within stations 39-68, Figure 2.1). The observed HLC2 fluorescence (~14 QSU) showed offshore transport of associated land-derived materials on the mid-shelf (Figure 2.6f; Table 2.3). In the Caloosahatchee transect (stations CAL1-CAL5, Figure 2.1), HLC1 and HLC2 exhibited DOM fluorescence between 4-6 QSU (Figure 2.6b) during Oct 2008. At this transect, the freshwater discharge from Caloosahatchee River (Figure 2.1) reached up to ~6,000 cfs (Figure 2.7a). Despite this amount of river discharge, the HLC1 and HLC2 DOM fluorescence in the Caloosahatchee transects were less fluorescent compared to the DOM fluorescence of HLC1 and HLC2 near Florida Bay area (Figure 2.6-e,f). Studies estimated water residence time of water in Florida Bay to be ~6-12 months (Kelble et al., 2007; Lee et al., 2006) while ~ < 4 months in Charlotte Harbor (Weisberg and Zheng, 2006; Miller, 1991).

The percentage distribution to total fluorescence of these humic-like components (HLC1 and HLC2) decreased from ~51% to ~43% between Oct 2008 to Jan 2009,
respectively (Table 2.4). The contribution of these components during May 2008 and Apr 2009 increased from ~35% to ~40% (Table 2.4). The decrease in fluorescence of HLC1 and HLC2 from Oct 2008 to Jan 2009 relates to the decrease in river flow (from ~6,000 to 140 cfs: both Shark and Caloosahatchee Rivers) and precipitation rates (from 0.2 to 0.02 inches). While in May 2008 to Apr 2009, the increase in fluorescence in HLC1 and HLC2 relates to increase river flow from ~260 to ~440 cfs and precipitation from ~0.18 to ~0.3 inches in Caloosahatchee River and ~0.15 to ~0.14 in Shark River. These results suggest that the relative distribution of HLC1 and HLC2 to total fluorescence on the West Florida Shelf is influenced by river runoffs and precipitation events (Figure 2.7).

The excitation/emission of component 3 occurs at <260/290 nm (Table 2.3). There is no available DOM fluorescence component assignment in the literature for this component. At these excitation/emission wavelengths, the fluorescence could be an instrument artifact strongly influenced by xenon lamp and the physical environment, resulting to low S/N (Smiled et al., 2004). It was also previously noted that this component could be an instrument artifact or an unidentified component in other studies (Murphy et al., 2006; Murphy et al., 2008). Hence, component 3 will not be considered here.

The other two derived components were components 4 (TLC4) and 5 (TLC5), which were associated with tryptophan-like and tyrosine-like components (Table 2.3), respectively. The tryptophan-like component near the Florida Bay region (Figure 2.6-c,j,k,o: stations 65-71, Figure 2.1) exhibits a maximum fluorescence ranging from 6-8 QSU. An interesting feature of TLC4 (Figure 2.6-g) is the plume west of the Ten Thousand Islands and near mouth of Shark River during Oct 2008. These regions exhibit
low salinity areas (Figure 2.8g), indicative of freshwater discharges. TLC4 fluorescence was also elevated (Figure 2.6-g) along the Charlotte Harbor (stations CH1-CH5) and in Caloosahatchee River (stations CAL1-CAL5) transects (Figure 2.1). This elevated fluorescence coincided with high precipitation rate (0.4 inches) and high water discharge from Caloosahatchee (6,000 cfs) and Peace (~2,000 cfs) Rivers (Figure 2.7). It is interesting to note that the TLC4 elevated fluorescence normally occurs near river sources (Figure 2.6-g). Even during reduced river flow, albeit lower fluorescence during high river flow, TLC4 still exhibit high fluorescence near river sources (Figure 2.6,o). These results suggest two possible mechanisms, which will be discussed in the next section.

On the other hand, the TLC5 fluorescence tended to exhibit maximum fluorescence near the middle Florida Keys (Figure 2.6-h,p). This implies that TLC5 fluorescence is produced more in an area far from Shark River. Similarly, mechanisms of the production of TLC5, along with TLC4, will be also discussed in the next section.

2.3.3. Relationship between DOM fluorescence components and salinity

The trends of four components against salinity (except component 3) at different months are shown (Figure 2.9). Factors that influence temporal and spatial variation of the resolved DOM fluorescence components and its relationship with salinity will be discussed in this section.

Components 1 and 2.- In May (2008) and April (2009), the fluorescence of HLC1 and HLC2 showed a positive relationship with salinity, while in October (2008) and January (2009), the relationship was negative on the West Florida Shelf (Figure 2.9). This
relationship is not expected if one only considers high levels of terrestrial DOM fluorescence component from relatively freshwater sources mix with saline seawater, typically lower in DOM fluorescence. Thus other factors must account for the positive correlation.

The maximum fluorescence was ~17 QSU for HLC1 and HLC2 (Figure 2.9-a,e). During May 2008, salinity at 40 was evident near Florida Bay (Figure 2.8-b). The positive relationship of HLC1 and HLC2 with salinity relates to the hypersaline water conditions in Florida Bay. Similar positive correlation of DOM fluorescence components with salinity was observed by Coble et al (2007), and this observation was attributed to the hypersaline water condition on West Florida Bay. In order to explain that hypersalinity (influenced by evaporation effects) as one of the factors that promoted the increase in DOM fluorescence with salinity, a salinity-DOM fluorescence end-member mixing in Figure 2.9a was used. The change in salinity is from 36.3-40.3. The change in salt/volume of seawater is roughly 10%. Based on the increase in fluorescence of component 1 from 3.4 QSU to 17 QSU, there is a ~5 times increase in the fluorescence of HLC1 (Figure 2.9a). The increase in salinity can only be produced by evaporation or salt addition. Since the change in salt/volume of seawater account to only 10% of HLC1 fluorescence change, this suggest that the unaccounted increase in fluorescence is a result of autochthonous production, and not derived from a freshwater source (Milbrandt et al., 2010). Results obtained suggest that evaporation alone is not solely responsible for the high component fluorescence in high salinity since the change in DOM fluorescence component is much higher than can be explained by evaporation. Since HLC1 was assigned as a terrestrial component (Table 2.3), a mechanism involving re-suspension of
DOM fluorescence from Florida Bay sediments is considered another possibility. We noted that HLC1 and HLC2 fluorescence was elevated (Figure 2.6-a,b) near the west Florida Bay transects at ~3-4 m bottom depth (stations 64-71; Figure 2.1). The shallow nature of the Bay may have caused re-suspension of sediment materials induced by wind generated mixing, based on the observed high levels of particulate material in 0.7µ GF/A filters during water sampling at the West Florida Bay transects (station 64-71). Therefore, the positive correlation of HLC1 and HLC2 with salinity during dry months is partly due to the hypersalinity condition (influenced by evaporation) on the shelf (i.e., particularly near the West Florida Bay transects (stations 64-71) and re-suspension of sediment materials induced by wind driven mixing that released enriched DOM materials in high salinity solution, particularly in shallow waters on the West Florida Bay.

On the other hand, the negative relationship of the HLC1 and HLC2 with salinity exhibited a slope of -4 (Figure 2.9-b,f) during Oct 2008. This relationship can be related to the high discharge of freshwater (8000 cfs) and high precipitation rate (0.7 inch) from July to September 2008 (Figure 2.7). The highest DOM fluorescence of HLC1 and HLC2 occur at salinity 33 with fluorescence of ~30 QSU on Oct 2008 (Figure 2.9 b, f). In Jan 2009, the slope increased to -7.0 (Figure 2.9,c-g), and the salinity with the highest fluorescence (~40 QSU) occur approximately 31. The inverse relationship of HLC1 and HLC2 during October 2008 and Jan 2009 corresponded to the elevated HLC1 and HLC2 fluorescence at lower salinity, which could be attributed to freshwater input from Shark and Caloosahatchee Rivers run-offs. The outlier at salinity 36.5-37 (Figure 2.9, b.f) indicates DOM sources other than the riverine discharge. This is indicative of in-situ production, similar with humic-like production during May 2008 (Figure 2.9, a, e). The
difference is that the HLC1 and HLC2 fluorescence during Oct 2008 nearly doubled. The arrows represent two sources of DOM that caused DOM distribution variability on the shelf.

In order to distinguish the DOM fluorescence contributions from the Shark (stations 54-71) and Caloosahatchee Rivers (CAL-CAL5 and CH1-CH5, Figure 2.1) transects near river mouths were selected (Figure 2.10). Plots of HLC1 showed elevated fluorescence (20-30 QSU) from the Shark River transects on Oct 2008 and Jan 2009 at salinity ~ 32. HLC1 in the Caloosahatchee River transect maintains fluorescence of ~ 5-10 QSU between 36 and 38 psu. Results obtained suggest that the variation of HLC1 and HLC2 with salinity on the West Florida Shelf related to river runoff (from the negative relationship), hyper-salinity water condition on the West Florida Bay (stations 54-71, Figure 2.1) and re-suspension of sediments in shallow areas (positive relationship).

In areas where there was high river input from the Caloosahatchee River, despite high river discharge (Figure 2.7a), it was observed that DOM exhibited lower fluorescence (Figure 2.10) as compared to the region receiving DOM inputs at the Shark River transects. Previous studies attribute this difference in fluorescence intensity to rapid mixing near the coastal area that received DOM from the Caloosahatchee River region compared to the transects near Florida Bay (Figure 2.1) (Zanardi-Lamardo et al., 2004).

**Components 4 and 5.** -- Component 4 (tryptophan-like fluorescence: TLC4) fluorescence showed weak relationship ($R^2 = -0.3$) with salinity in all months (Figure 2.9-i,j,k,l). For the month of May (2008), TLC4 exhibited a positive relationship with salinity (Figure 2.9-i). This suggests that protein-like fluorescence was in-situ derived at
different salinities. The same explanation may be invoked for the relationship of TLC4 and salinity for April (2009) (Figure 2.9-l). The positive relationship of TLC4 with salinity during May 2008 and April 2009 (Figure 2.9-i,l) could be influenced by hypersaline water mixing, just like HLC1 and HLC2. Evaporation can account 10% loss in the change of salt/volume of seawater, but there is a 13 times increase in the TLC4 fluorescence. The unaccounted TLC4 fluorescence could be a result of autochthonous production at different salinities (~36-40). Production of TLC4 is consistent with the source assignment as in-situ biologically-derived material (Table 2.3).

The high riverine input during Oct 2008 and Jan 2009 did not improve the regression coefficient between TLC4 and salinity (Figure 2.9-j,k). Both relationships indicated no and very weak relationship [(R²=0.08, Oct 2008: R²=0.23, Jan 2009)] between TLC4 and salinity during these months. The TLC4 fluorescence was at ~8 QSU at salinity 32 (Figure 2.9j). However, TLC4 fluorescence is also elevated at salinity 37, which suggests autochthonous production of component 4 at this salinity. This implies that during Oct 2009, TLC4 can be related to low salinity water from river run-off and also in high salinity water. Therefore, TLC4 distribution on the shelf was influenced not only by river run-off but also autochthonous production at different salinities (~33-38).

Since it has been observed that the fluorescence maximum of TLC4 occurs near river sources, in Figure 2.10e, we also observed that the elevated fluorescence in the inverse line in Figure 2.9 relates to the low salinity (Figure 2.8j) near the West Florida Bay transect (station 65-68). The TLC4 fluorescence on the shelf in low salinity then can be related to Shark River water discharge near Florida Bay. Similarly, reduced river flow in Apr 2009, resulted to a positive relationship in the same area. Therefore, water
discharge from Shark River implicates the production of TLC4 on the West of Florida Bay. On the other hand, near the Caloosahatchee River transects, TLC4 fluorescence was maintained nearly at 8 QSU between salinity 36-38 (Figure 2.10, b,d,f,h). No salinity as low as 32 was evident during high Caloosahatchee river discharge during Oct 2008.

On the other hand, TLC5 exhibits no correlation with salinity in all sampling months (R²=0.01; Figure 2.9-m,n,o,p). This tyrosine-like component had elevated fluorescence at salinity between 36 and 38 during Apr 2008 and May 2009. During Oct 2008 and Jan 2009, component 5 exhibited low fluorescence (~5-15 QSU) at salinity below 35. At salinity ~37, TLC5 fluoresces from ~40 QSU. With closer inspection of Figure 2.9-n,o, a similar pattern of TLC5 with TLC4 could be compared. It would appear that TLC5 abundance can be related also to the freshwater river discharge (fluorescence of TLC5 in low salinity) and autochthonous production (fluorescence at salinity 37), similar to TLC4.

An upward and right arrow indicate production and evaporation results in relating TLC4 and TLC5 fluorescence with salinity during May 2008 and Apr 2009 (Figure 2.9). This relationship is in contrast during the month of Oct 2008 and Jan 2009, where the relationship indicates biological production is a strong source of these components in relation to salinity (Figure 2.9)

2.3.4 DOC analysis

The DOC content exhibited a negative relationship with salinity during the month of Jan 2009 (Figure 2.11). The DOC axis (Figure 2.12c) with HLC1, exhibits a positive intercept, implying that the low salinity end-member contains predominantly of non-
absorbing DOC and less HLC1. The large intercept (52 µM) and steep slope (+11) and high correlation (R²=0.9) indicate similarity of HLC1 and DOC in the low and high salinity end-members. This observation is then consistent that the observed spatial variability of HLC1 could be also due to mixing or dilution. We noticed that the slope and intercept of the HLC1 and HLC2 correlation with salinity are similar. This similarity suggests that a similar process affects both components. Alteration of the relationship between DOC and DOM fluorescence component (according to Blough and Del Vecchio, 2002) could result to photochemical and biological mediated alteration of the DOM consumption and production. However, on the shelf where rapid mixing of DOM fluorescence occurs, monitoring of photochemical and biological degradation would be difficult. Further study is recommended to characterize and quantify any photodegradation and microbial processes of the HLC1 and HLC2 components on the shelf.

2.3.5 DON analysis

The weak relationship of TLC4 and salinity was attributed to possible biological production and mixing, as previously discussed. Relating TLC4 and TLC5 with DON provides more supporting evidence of the spatial variations of the protein-like fluorescent DOM components on the shelf.

The DON content exhibited a negative relationship (R²= 0.5) with salinity (salinity from 31-37) during Jan 2009 (Figure 2.13). Correspondingly, the TLC4 with DON relationship obtained a low intercept (8.4 µM) and very weak slope (0.4) at salinity 36.3-39 during this month (Figure 2.14). This weak relationship represents variability of
component 4 in the total DON. The similarity of the TLC4 (Figure 2.9k) and Chl-a (Figure 2.15c) scatter at salinity from 34-36.5 provide evident relationship of TLC4 to biological production. The protein-like fluorescence (Figure 2.6-k) tended to correspond to the maximum fluorescence increase in DON during Jan 2009 (Figure 2.8-l) (stations 64-68; Figure 2.1). This indicates that phytoplankton can be related to the TLC4 production (i.e., degraded materials of the phytoplankton), which is consistent with the protein-like component assignments as biologically derived materials (Table 2.3). Similarly, the low correlation of TLC4 and DON indicates that there are other unknown covariations in their rates of in-situ production and consumption that needs further investigation.

A positive relationship of DON and TLC4 is shown in Figure 2.14 during May 2008 and Jan 2009. Similar to Jan 2009, the relationship between DON and TLC4 during May 2008 was poorly correlated (R^2=0.3). Biological production at salinity (~38.5) during May 2008 and Apr 2009, coincides with the elevated Chl-a (Figure 2.13-a,c) and TLC4 (2.9-d,p). This is consistent with the assignment of TLC4 as biologically-derived material (Table 2.3). During May 2008 and April 2009 in-situ biological production at high salinity, can be related to the high TLC4 fluorescence at salinity 36-40 (Figure 2.9-i,l). The reversal of the relationship of component 4 with salinity on the shelf (i.e., particularly near the west Florida Bay) suggests also that freshwater discharge and precipitation rates can be related to the distribution of TLC4 on the shelf.

On the other hand, TLC5 exhibited no correlation (R^2= 0.04; slope 0.4) with DON (Figure 2.14) during May 2008 and Jan 2009, implying that the low salinity end-member contains low amounts of non-absorbing DON and more of TLC5. No correlation is
observed between TLC5 and DON in salinity. The maximum fluorescence of TLC5 observed near the Middle Keys (Florida) (Figure 2.6- d,p) observed supports the assumption that TLC5 is produced from biological production. And this observation is consistent with the TLC5 potential DOM source assignment, as an autochthonous, biologically-derived material (Table 2.3).

It is observed that there are high fluorescence data points of component 5 at salinity ~36.5 (Figure 2.9-o). This fluorescence was related to high biological productivity (high Chl-a at salinity 36.5; Figure 2.15c). These results further suggest that the sources of TLC4 and TLC5 are similar and may be reasonably grouped together as protein-like components. This also follows that component 5, associated as a tyrosine-like component (Table 2.3), can be also related to freshwater discharge on the shelf. Since TLC5 fluorescence intensity at 36.5 (2.6-d,h,p) caused spatial variation of this component with TLC4 fluorescence on the shelf, we hypothesized that TLC5 is produced more in offshore waters compared to TLC4. However, the cause of this variation needs investigation.

2.3.5. SW Florida Shelf DOM fluorescence source and production

In other studies of continental shelves, high riverine inputs during wet months have been reported to contain terrestrial-derived materials (Zanardi-Lamardo et al., 2002; Boehme et al., 2004; Chen and Gardner, 2004). The water inputs from the Caloosahatchee River on the shelf contain DOM materials coming from Lake Okeechobee, enriched with DOM materials from sewage and runoff from agricultural areas (Brand, 2002; Kramer and Herndl, 2004; Brand and Compton, 2007), and the
Everglades Agricultural Area (Qualls and Richardson, 2003). This flow-through of water from the Everglades exports excess dissolved organic matter into the Shark River and in significant amounts through spillways directed toward the Florida Bay (McPherson and Halley, 1996; Qualls and Richardson, 2003). Shark River input probably contains significant quantities of DOM from mangrove litter and marsh plants in the Florida Everglades (Twilley, 1985; Maie et al., 2006a; Maie et al., 2006b; Romigh et al., 2006). Microbial degradation of labile DOM materials is a slow process in these regions according to Qualls and Richardson (2003), but solar degradation can enhance terrestrial DOM degradation. Polyphenol structures of plant-derived DOM are particularly sensitive to photolysis (Scully et al., 2004). They further observed that humic-materials tend to be refractory and inhibit these degradation processes. It was also suggested that the microbial and physical processes could initiate the formation of recalcitrant, highly-colored, high molecular weight polymeric structures observed from mangrove-derived DOM (Scully et al., 2004). Strong signals of the terrestrial DOM fluorescence in Florida Bay, enriched with DOM materials from mangrove and marsh land-plants, indicated that the DOM materials are relatively refractory and possibly enriched with high molecular weight and conjugated compounds (Coble, 1996).

The high DOM fluorescence in high salinity waters near Florida Bay was previously observed by Coble at al. (2007) and Conmy et al. (2009) and they attributed it to two likely sources of DOM fluorescence: one is the hypersaline water of Florida Bay (influenced by evaporation), and second is coastal upwelling of near-bottom waters along the shelf (i.e., Charlotte Harbor). Suspension of bottom sediments is another source of elevated DOM fluorescence in Florida Bay (Conmy, 2009). It was observed that
stations with bottom depth of ~3 m (Table 2.1) along transects near Florida Bay were susceptible to wind events that can suspend DOM materials (Lawrence et al., 2004; Green et. al., 2008). This mechanism was similarly proposed to inject and suspend into the water column, materials containing previously deposited terrestrial refractory materials. Lawrence et al. (2004) suggest that this mechanism can also stimulate bacterioplankton and phytoplankton blooms, which can eventually serve as a source material for the protein-like components in high salinity water.

Several studies have documented that the increase in tryptophan- and tyrosine-like fluorescence coincides with surface water phytoplankton blooms (Stedmon et al., 2005; Suksomjit et al., 2009; Yamashita et al., 2010). The high molecular weight protein-like structures are degraded primarily through physical-chemical and microbial processes in DOM plant derived leachates (Scully et al., 2004). This observation is consistent with the assignment of these protein-like fluorescence components, as possibly coming from remineralized materials from in-situ biological sources (Stedmon and Markager, 2005; Coble, 2007). Since protein components are labile (Biers et al., 2007; Keil and Kirchman, 1994), their distribution from the estuary or nearshore could be limited by biological and photochemical degradation. Offshore protein-like fluorescence may be derived primarily from phytoplankton die-off materials (Jolliff et al., 2003; Walsh et al., 2003; Mendoza et al., 2012). The die-off of seagrasses from hypersaline conditions is suspected to be another source of DOM protein-like components (Stabenau et al., 2004).

On the other hand, the positive correlation between river discharge and both humic-like and protein-like fluorescence components (+0.80 to +0.99; Table 2.5)
suggests that nutrients (Boyer et al., 2006) delivered through river input may also promote biological productivity (Qualls and Richardson, 2003). This process is speculated to promote protein-like fluorescence enrichment in Florida Bay and nearshore of Charlotte Harbor, after phytoplankton die-off.

The Shark River DOM is primarily derived from natural land-plant organic compounds from the Everglades (Maie et al., 2006b; Aiken et al., 2011) and mangrove litter (Twilley 1985; Romigh et al., 2006; Shank et al., 2010) while the Caloosahatchee River is enriched with DOM from farming activities and sewage (Zanardi-Lamardo et al., 2002). The observed differences in DOM fluorescence properties from these transects (Figure 2.10) indicate that DOM may have exhibited differences in the molar mass, diffusion coefficients, and its reactivity to photochemical degradation as described by Zanardi-Lamardo et al., (2002).

2.3.6. SW Florida Shelf DOM fluorescence decrease

The lack of surface water flows, and long residence time of water, promotes evaporation that could elevate DOM fluorescence on the west Florida Bay (Price et al., 2007; Lubben et al., 2009; Das et al., 2011). This net evaporation effect can be ameliorated by the tidally driven low DOM fluorescence Gulf of Mexico seawater (Nuttle et al., 2000; Price et al., 2007), and result to a seawater dilution curve (de Souza Sierra et al., 1997). On the other hand, river runoff could reverse seawater dilution curve to a freshwater dilution curve (de Souza Sierra et al., 1995). And dilution of river water with high salinity marine water accounts for the observed changes in the optical properties (Del Castillo et al., 2000).
The gradual decrease of DOM fluorescence in the five fluorescence components with distance from the shore suggests other possible DOM mechanisms that decreased DOM fluorescence, which was speculated to occur as minor processes apart from mixing or dilution. Several processes that decrease the fluorescence signal have been proposed by several authors. One is flocculation (Sholkovitz et al., 1978; Chen and Gardner, 2004), where salinity changes may have promoted flocculation from forming a stable network assembly (Chin et al., 1998; Baalousha et al., 2006) but this information cannot be accounted in this study. On the other hand, there are complex dynamics between microbial and photodegradation in the marine environment. Both microbial and photodegradation of labile DOM may result in the eventual production of low molecular weight organic compounds and other gaseous products (Zika, 1981; Moran et al., 2000; Osburn et al., 2001; Vahatalo and Wetzel, 2004; Vahatalo and Zepp, 2005), but, may not necessarily produce refractory DOM fluorescence materials (Lønborg et al., 2009). It appears that the microbial degradation of a photodegraded material may completely convert the DOM into a refractory component (Miller and Moran, 1997; Scully et al., 2004). This suggests that DOM fluorescence decrease, through solar and microbial degradation may occur at different rates and is dependent on the composition and the rate of DOM supply on the shelf.

2.3.7. Advances of using EEM fluorescence coupled with PARAFAC modeling

A simple way of monitoring optical properties of water that can characterize DOM sources across a large sub-domain is presented here. The EEM and PARAFAC modeling of the DOM fluorescence was able to resolve the source fluorescence of individual components of the bulk DOM, the components’ respective spatial distribution,
and were able to provide insight on the biological, chemical, and physical processes that control the variability of each of the resolved DOM components on the shelf.

Several of the monitoring studies of the Everglades have been limited to the Florida Bay, discounting the possible spread and offshore distribution of DOM from the Shark River. This study demonstrated that DOM from different sources exhibit distinctive behaviors in salinity gradients that can be used to trace the different sources of DOM. Using EEM and PARAFAC anthropogenic distribution can be monitored on a larger scale on the shelf. Data generated from this study can also serve as baseline information for future long-term optical field observations and predictions.

2.4. Summary

This study evaluated the Excitation Emission Matrix (EEM) fluorescence method combined with Parallel Factorial Analysis (PARAFAC) modeling to resolve DOM fluorescence components and their distribution on the southwest (SW) Florida Shelf. The PARAFAC model identified five unique DOM components from 272 Excitation and Emission Matrix(s) (EEM) samples measured across the Shelf on the May 2008, October 2008, and January and April 2009 cruises. These five different fluorophores consisted of two humic-like components that were attributed to natural (component 1: HLC1) and anthropogenic (component 2: HLC2) sources; an unidentified component (component 3); two autochthonous protein components, identified as tryptophan-like (component 4:TLC4); and tyrosine-like (component 5:TLC5) fluorophores. The increased flow rates of the Shark and Caloosahatchee Rivers input on the shelf were the main sources of the fresh terrestrial and anthropogenic humic-like DOM fluorescent
materials. This was observed during October 2008 and January 2009 and resulted in an inverse relationship between these components and salinity. The areal distribution of the resolved terrestrial DOM fluorescence on the mid-shelf indicated evidence of offshore transport of land-derived fluorophore. We also found that with reduced river input results in the reversal of the relationship of salinity with the humic-like and protein-like components. Mechanisms are proposed that produce the maximum fluorescence of the humic-like components and protein-like at high salinity. The first mechanism is the sediment suspension from wind-driven mixing which promotes increase of the humic-like components in high salinity water. This observation was evident near the west Florida Bay transects, where bottom depth is ~3-4 m. The maximum protein-like fluorescence with salinity was related to predominance of biological production in high salinity environment. High biological productivity occurs in the region where there is high fluorescence of the humic-like components. This observation suggests that the increase in humic materials, induced by suspension of materials in shallow environment in the west Florida Bay, could be also related to the increase biological production.

Results suggest that the humic-like (HLC1 and HLC2) and the protein-like components (TLC4 and TLC5) spatial distribution and temporal variation on the shelf can be influenced by precipitation and river inputs and mixing/dilution. The in-situ production of the humic-like components in high salinity water could be driven by other mechanisms and other factors [i.e., sediments suspension, in-situ production, evaporation resulting to hypersalinity condition, bottom depth]. However, the in-situ production of protein-like components is weakly correlated in high salinity water, indicates that this component is not necessarily produced in high salinity water.
Results from this study demonstrated advances in several aspects of monitoring the temporal distribution of DOM on the continental shelf of Florida using EEM and PARAFAC methods. The methods employed were able to: 1) resolved sources of the individual DOM fluorescence components of the bulk DOM, and generate spatial and temporal distribution maps of each of the components on the Shelf; and 2) provided insight into biological, chemical, and physical processes that control the DOM variability of each of the resolved DOM components.

Therefore, the coupled EEM and PARAFAC method was demonstrated to resolve DOM fluorescence that could be employed as a natural indicator in monitoring the biological, physical and biogeochemical processes that mediate DOM variability on the southwest Florida Shelf. The DOM fluorescence components excitation/emission wavelengths resolved and defined in this study could serve as reference (signature) for future fluorescent optical studies and monitoring land use change (e.g., urban development, Everglades restoration efforts) using fluorescence methods. Results generated in this study can serve also as baseline information for optical (fluorescence-based) water quality studies on the southwest Florida Shelf sub-domain.
Figure 2.1. Fixed sampling stations on the southwest Florida Shelf. Stations were based on the NOAA-AOML South Florida Quarterly Hydrographic Survey Program (SFQHS). See Table 2.1 for transect labels. Keys are situated to the south of the shelf, and Florida Bay is located on the northeastern side of the Keys.
Figure 2.2. Scores, loadings and leverage of the DOM fluorescence PARAFAC modeled components from 272 EEMS of seawater samples collected from the SW Florida Shelf.
Figure 2.3. A sample of a. measured and b. modeled PARAFAC modeled EEM and its corresponding c. residual, d) excitation plots (a-c) shown diagonally, and e) the derived fluorescence intensity from a five component PARAFAC model.
Figure 2.4. Validated split halves of the five component PARAFAC model. Each halves of the excitation and emission wavelengths show similar spectral loadings.
Figure 2.5. 3D fluorescence and spectral loadings of a, f) Component 1, b, g) Component 2, c, h) Component 3, d, i) Component 4 and e, j) Component 5 of the PARAFAC modeled DOM fluorescence, in seawater samples collected at different times across the SW Florida Shelf.
Figure 2.6. Temporal and spatial distribution of the PARAFAC modeled DOM fluorophore components across the SW Florida Shelf during these sampling periods: 1) May 6-10, 2008, 2) October 17-22, 2008, 3) January 21-26, 2009 and 4) April 20-23, 2009. [component 1 (HLC1), component 2 (HLC2), component 4 (TLC4), component 5 (TLC5)]
Figure 2.7.  a) Monthly discharge rates (cu ft s\(^{-1}\)) of the Caloosahatchee (station 79, SFWMD DBHydro), Shark (S12A+S12B+S12C+S12D+S333, SFWMD DBHydro) and Peace (station in Arcadia, USGS-NWIS Web) Rivers from January 2008-December 2009 (SFWMD DBHydro. b) Precipitation (inches) rates in Caloosahatchee (station 79) and Shark (S12D) Rivers (SFWMD-South Florida Water Management District, USGS-NWIS-U.S. Geological Survey National Water Information Systems).
Figure 2.8. Contour plots of temperature (°C), salinity (psu), DOC (µM C), DON (µM), and Chl-a (mg/L) in SW Florida Shelf during the NOAA hydrographic cruise on a) May 6-10, 2008, b) October 17-22, 2008, c) January 21-26, 2009 and d) April 20-23, 2009.
Figure 2.10. Temporal distribution of component 1 (Comp1=HLC1) and component 4 (Comp4=TLC4) on multiple transects proximate to the Shark and Caloosahatchee River mouths.
Figure 2.11. Relationship of DOC with salinity for the month of a. May 2008, b. January 2009 and c) April 2009.

a. May 2008
\[ y = 37.628x - 1293.1 \]
\[ R^2 = 0.6349 \]

b. Jan 2009
\[ y = -56.661x + 2188.9 \]
\[ R^2 = 0.7224 \]

c. Apr 2009
\[ y = 57.952x - 2028.3 \]
\[ R^2 = 0.6764 \]
a. May 2008 (Comp1)

\[ y = 9.275x + 73.14 \]
\[ R^2 = 0.5327 \]

b. May 2008 (Comp2)

\[ y = 12.008x + 70.46 \]
\[ R^2 = 0.7043 \]

c. Jan 2009 (Comp1)

\[ y = 10.758x + 52.488 \]
\[ R^2 = 0.8688 \]

d. Jan 2009 (Comp2)

\[ y = 9.0432x + 64.304 \]
\[ R^2 = 0.8933 \]

Figure 2.12. Relationship of DOC (µM C) with component 1 (Comp1=HLC1) and component 5 (Comp2=HLC2) for the month of a-b) May 2008 and c-d) January 2009
Figure 2.14. Relationship of DON (µM) with component 4(Comp4=TLC4) and component 2 (Comp5=TLC5) for the month of a-b) May 2008 and c-d) January 2009
Table 2.1. Transect names of stations across SW Florida Shelf.

<table>
<thead>
<tr>
<th>Station</th>
<th>Lat</th>
<th>Long</th>
<th>Depth (m)</th>
<th>Transect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.64500</td>
<td>-80.12667</td>
<td>10.0</td>
<td>Fowey</td>
</tr>
<tr>
<td>2</td>
<td>25.64167</td>
<td>-80.10500</td>
<td>8.1</td>
<td>Fowey</td>
</tr>
<tr>
<td>3</td>
<td>25.64500</td>
<td>-80.08333</td>
<td>50.8</td>
<td>Fowey</td>
</tr>
<tr>
<td>4</td>
<td>25.10833</td>
<td>-80.38000</td>
<td>5.0</td>
<td>Dixie Shoal</td>
</tr>
<tr>
<td>5</td>
<td>25.09333</td>
<td>-80.35500</td>
<td>7.5</td>
<td>Dixie Shoal</td>
</tr>
<tr>
<td>5.5</td>
<td>25.07967</td>
<td>-80.33417</td>
<td>5.0</td>
<td>Dixie Shoal</td>
</tr>
<tr>
<td>6</td>
<td>25.06500</td>
<td>-80.31500</td>
<td>38.0</td>
<td>Dixie Shoal</td>
</tr>
<tr>
<td>6.5</td>
<td>25.04833</td>
<td>-80.28767</td>
<td>74.0</td>
<td>Dixie Shoal</td>
</tr>
<tr>
<td>7</td>
<td>24.82083</td>
<td>-80.75667</td>
<td>4.6</td>
<td>Chan 5</td>
</tr>
<tr>
<td>8</td>
<td>24.79250</td>
<td>-80.74167</td>
<td>5.0</td>
<td>Chan 5</td>
</tr>
<tr>
<td>9</td>
<td>24.76333</td>
<td>-80.72333</td>
<td>33.0</td>
<td>Chan 5</td>
</tr>
<tr>
<td>9.5</td>
<td>24.71667</td>
<td>-80.69000</td>
<td>125.0</td>
<td>Chan 5</td>
</tr>
<tr>
<td>10</td>
<td>24.78833</td>
<td>-80.86000</td>
<td>6.0</td>
<td>Long Key</td>
</tr>
<tr>
<td>11</td>
<td>24.75333</td>
<td>-80.84667</td>
<td>8.4</td>
<td>Long Key</td>
</tr>
<tr>
<td>12</td>
<td>24.71333</td>
<td>-80.83167</td>
<td>34.0</td>
<td>Long Key</td>
</tr>
<tr>
<td>13</td>
<td>24.70167</td>
<td>-81.03000</td>
<td>6.0</td>
<td>Marathon</td>
</tr>
<tr>
<td>14</td>
<td>24.67500</td>
<td>-81.02333</td>
<td>12.7</td>
<td>Marathon</td>
</tr>
<tr>
<td>15</td>
<td>24.64333</td>
<td>-81.01667</td>
<td>33.0</td>
<td>Marathon</td>
</tr>
<tr>
<td>15.5</td>
<td>24.59167</td>
<td>-80.99167</td>
<td>130.0</td>
<td>Marathon</td>
</tr>
<tr>
<td>16</td>
<td>24.67500</td>
<td>-81.20500</td>
<td>5.6</td>
<td>7mi Bridge</td>
</tr>
<tr>
<td>17</td>
<td>24.63500</td>
<td>-81.19333</td>
<td>12.0</td>
<td>7mi Bridge</td>
</tr>
<tr>
<td>18</td>
<td>24.59833</td>
<td>-81.18333</td>
<td>32.0</td>
<td>7mi Bridge</td>
</tr>
<tr>
<td>19</td>
<td>24.61000</td>
<td>-81.42167</td>
<td>6.0</td>
<td>Looe Reef</td>
</tr>
<tr>
<td>20</td>
<td>24.57500</td>
<td>-81.41833</td>
<td>15.0</td>
<td>Looe Reef</td>
</tr>
<tr>
<td>21</td>
<td>24.53833</td>
<td>-81.41333</td>
<td>35.0</td>
<td>Looe Reef</td>
</tr>
<tr>
<td>21.5</td>
<td>24.48333</td>
<td>-81.39167</td>
<td>140.0</td>
<td>Looe Reef</td>
</tr>
<tr>
<td>22.5</td>
<td>24.39667</td>
<td>-81.82833</td>
<td>136.0</td>
<td>Key West</td>
</tr>
<tr>
<td>22</td>
<td>24.45500</td>
<td>-81.82833</td>
<td>33.0</td>
<td>Key West</td>
</tr>
<tr>
<td>23</td>
<td>24.48667</td>
<td>-81.82833</td>
<td>12.0</td>
<td>Key West</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>24</td>
<td>24.53667</td>
<td>-81.82833</td>
<td>10.0</td>
<td>Key West</td>
</tr>
<tr>
<td>25</td>
<td>24.39333</td>
<td>-82.76667</td>
<td>55.0</td>
<td>Tortugas</td>
</tr>
<tr>
<td>25.5</td>
<td>24.28667</td>
<td>-82.76667</td>
<td>360.0</td>
<td>Tortugas</td>
</tr>
<tr>
<td>26</td>
<td>24.49333</td>
<td>-82.76833</td>
<td>23.0</td>
<td>Tortugas</td>
</tr>
<tr>
<td>27</td>
<td>24.59000</td>
<td>-82.76833</td>
<td>30.0</td>
<td>Tortugas</td>
</tr>
<tr>
<td>DT9</td>
<td>24.46767</td>
<td>-83.04233</td>
<td>51.0</td>
<td>Tortugas</td>
</tr>
<tr>
<td>DT10</td>
<td>24.55000</td>
<td>-82.97167</td>
<td>24.5</td>
<td>Tortugas</td>
</tr>
<tr>
<td>DT11</td>
<td>24.61967</td>
<td>-82.91000</td>
<td>21.5</td>
<td>Tortugas</td>
</tr>
<tr>
<td>28</td>
<td>24.72833</td>
<td>-82.74833</td>
<td>30.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>28.5</td>
<td>24.90000</td>
<td>-82.61667</td>
<td>34.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>29</td>
<td>25.06333</td>
<td>-82.47833</td>
<td>34.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>29.5</td>
<td>25.22667</td>
<td>-82.35000</td>
<td>31.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>30</td>
<td>25.40167</td>
<td>-82.21000</td>
<td>26.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>30.5</td>
<td>25.57167</td>
<td>-82.07500</td>
<td>21.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>31</td>
<td>25.74167</td>
<td>-81.94333</td>
<td>16.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>32</td>
<td>25.87333</td>
<td>-81.83333</td>
<td>11.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>33</td>
<td>25.91167</td>
<td>-81.80000</td>
<td>10.0</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>34</td>
<td>25.95000</td>
<td>-81.76667</td>
<td>5.5</td>
<td>Cape Romano</td>
</tr>
<tr>
<td>CAL1</td>
<td>26.42667</td>
<td>-81.96000</td>
<td>4.7</td>
<td>Caloosahatchee</td>
</tr>
<tr>
<td>CAL2</td>
<td>26.38333</td>
<td>-82.00000</td>
<td>8.0</td>
<td>Caloosahatchee</td>
</tr>
<tr>
<td>CAL3</td>
<td>26.34167</td>
<td>-82.04333</td>
<td>10.0</td>
<td>Caloosahatchee</td>
</tr>
<tr>
<td>CAL4</td>
<td>26.25833</td>
<td>-82.13333</td>
<td>14.0</td>
<td>Caloosahatchee</td>
</tr>
<tr>
<td>CAL5</td>
<td>26.18333</td>
<td>-82.21667</td>
<td>18.5</td>
<td>Caloosahatchee</td>
</tr>
<tr>
<td>CH1</td>
<td>26.71833</td>
<td>-82.25000</td>
<td>10.0</td>
<td>Charlotte Harbor</td>
</tr>
<tr>
<td>CH2</td>
<td>26.66167</td>
<td>-82.33167</td>
<td>11.0</td>
<td>Charlotte Harbor</td>
</tr>
<tr>
<td>CH2</td>
<td>26.66167</td>
<td>-82.33167</td>
<td>11.0</td>
<td>Charlotte Harbor</td>
</tr>
<tr>
<td>CH3</td>
<td>26.55333</td>
<td>-82.46667</td>
<td>17.0</td>
<td>Charlotte Harbor</td>
</tr>
<tr>
<td>CH4</td>
<td>26.47167</td>
<td>-82.61667</td>
<td>23.0</td>
<td>Charlotte Harbor</td>
</tr>
<tr>
<td>CH5</td>
<td>26.38333</td>
<td>-82.76667</td>
<td>29.0</td>
<td>Charlotte Harbor</td>
</tr>
<tr>
<td>39</td>
<td>25.78333</td>
<td>-81.48167</td>
<td>3.4</td>
<td>Indian Key</td>
</tr>
<tr>
<td>40</td>
<td>25.76000</td>
<td>-81.52333</td>
<td>4.6</td>
<td>Indian Key</td>
</tr>
<tr>
<td>41</td>
<td>25.73333</td>
<td>-81.57500</td>
<td>5.3</td>
<td>Indian Key</td>
</tr>
<tr>
<td>42</td>
<td>25.65500</td>
<td>-81.71833</td>
<td>9.1</td>
<td>Indian Key</td>
</tr>
<tr>
<td></td>
<td>Lon</td>
<td>Lat</td>
<td>Dist</td>
<td>River</td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>-------</td>
<td>------</td>
<td>---------------</td>
</tr>
<tr>
<td>45</td>
<td>25.58350</td>
<td>-81.47067</td>
<td>7.0</td>
<td>Lostmans River</td>
</tr>
<tr>
<td>46</td>
<td>25.58333</td>
<td>-81.40833</td>
<td>6.4</td>
<td>Lostmans River</td>
</tr>
<tr>
<td>47</td>
<td>25.58333</td>
<td>-81.36667</td>
<td>5.5</td>
<td>Lostmans River</td>
</tr>
<tr>
<td>48</td>
<td>25.58333</td>
<td>-81.33000</td>
<td>5.2</td>
<td>Lostmans River</td>
</tr>
<tr>
<td>49</td>
<td>25.58333</td>
<td>-81.29167</td>
<td>4.0</td>
<td>Lostmans River</td>
</tr>
<tr>
<td>50</td>
<td>25.45333</td>
<td>-81.35000</td>
<td>6.4</td>
<td>Broad River</td>
</tr>
<tr>
<td>51</td>
<td>25.45333</td>
<td>-81.30833</td>
<td>5.5</td>
<td>Broad River</td>
</tr>
<tr>
<td>52</td>
<td>25.45333</td>
<td>-81.26000</td>
<td>4.6</td>
<td>Broad River</td>
</tr>
<tr>
<td>53</td>
<td>25.45333</td>
<td>-81.22500</td>
<td>3.7</td>
<td>Broad River</td>
</tr>
<tr>
<td>54</td>
<td>25.34533</td>
<td>-81.15283</td>
<td>4.0</td>
<td>Shark River</td>
</tr>
<tr>
<td>55</td>
<td>25.35017</td>
<td>-81.19333</td>
<td>3.7</td>
<td>Shark River</td>
</tr>
<tr>
<td>56</td>
<td>25.35000</td>
<td>-81.22733</td>
<td>4.0</td>
<td>Shark River</td>
</tr>
<tr>
<td>57</td>
<td>25.35167</td>
<td>-81.26500</td>
<td>4.6</td>
<td>Shark River</td>
</tr>
<tr>
<td>58</td>
<td>25.16667</td>
<td>-81.65333</td>
<td>17.5</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>59</td>
<td>25.16667</td>
<td>-81.49000</td>
<td>9.1</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>60</td>
<td>25.16667</td>
<td>-81.33600</td>
<td>6.7</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>61</td>
<td>25.16667</td>
<td>-81.27500</td>
<td>6.1</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>62</td>
<td>25.16667</td>
<td>-81.23500</td>
<td>4.9</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>63</td>
<td>25.16667</td>
<td>-81.20000</td>
<td>4.3</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>64</td>
<td>25.16667</td>
<td>-81.15667</td>
<td>4.0</td>
<td>Middle Cape</td>
</tr>
<tr>
<td>65</td>
<td>25.10167</td>
<td>-81.09333</td>
<td>4.0</td>
<td>East Cape</td>
</tr>
<tr>
<td>66</td>
<td>25.06833</td>
<td>-81.10833</td>
<td>4.6</td>
<td>East Cape</td>
</tr>
<tr>
<td>67</td>
<td>25.03000</td>
<td>-81.12667</td>
<td>4.6</td>
<td>East Cape</td>
</tr>
<tr>
<td>68</td>
<td>24.93000</td>
<td>-81.16667</td>
<td>4.9</td>
<td>East Cape</td>
</tr>
<tr>
<td>69</td>
<td>24.93000</td>
<td>-81.09500</td>
<td>4.6</td>
<td>West Fla Bay</td>
</tr>
<tr>
<td>70</td>
<td>24.93000</td>
<td>-81.02500</td>
<td>3.7</td>
<td>West Fla Bay</td>
</tr>
<tr>
<td>71</td>
<td>24.93000</td>
<td>-80.96333</td>
<td>3.0</td>
<td>West Fla Bay</td>
</tr>
</tbody>
</table>
Table 2.2. Variation explained and root mean squared error (RMSE) of the five PARAFAC DOM fluorescence component model iterations.

<table>
<thead>
<tr>
<th>Component Model</th>
<th>Variation explained</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93.88</td>
<td>0.060</td>
</tr>
<tr>
<td>2</td>
<td>96.40</td>
<td>0.032</td>
</tr>
<tr>
<td>3</td>
<td>98.56</td>
<td>0.021</td>
</tr>
<tr>
<td>4</td>
<td>99.21</td>
<td>0.016</td>
</tr>
<tr>
<td>5</td>
<td>99.52</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Table 2.3. Excitation and emission fluorescence maximum of PARAFAC modeled DOM fluorescent components across SW Florida Shelf from four quarterly survey, their respective region, and designation.

<table>
<thead>
<tr>
<th>Component</th>
<th>Ex</th>
<th>Em</th>
<th>Region</th>
<th>Fluorophore type</th>
<th>Tentative fluorophore type and source assignments of this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>255</td>
<td>470</td>
<td>A</td>
<td>Q2, a</td>
<td>UVA Humic-like component; Terrestrial</td>
</tr>
<tr>
<td>2</td>
<td>250(310)</td>
<td>410</td>
<td>A,M</td>
<td>Q2, C2, P1, a</td>
<td>UVC Terrestrial and Marine humic-like component, microbial, anthropogenic</td>
</tr>
<tr>
<td>3</td>
<td>(&lt;260)</td>
<td>290</td>
<td>-</td>
<td>P7</td>
<td>UVA Marine humic-like (this study), uncertain, fluorometer artifact Biological/microbial, possible instrument artifact</td>
</tr>
<tr>
<td>4</td>
<td>280</td>
<td>345</td>
<td>T</td>
<td>P7, Trp-like, δ</td>
<td>UBV Protein like, Tryptophan-like, autochthonous; biological, microbial</td>
</tr>
<tr>
<td>5</td>
<td>270</td>
<td>305</td>
<td>B</td>
<td>Tyr</td>
<td>UBV Protein like, Tyrosine-like, autochthonous; biological, microbial</td>
</tr>
</tbody>
</table>
Table 2.4. Percentage of humic-like and protein-like fluorescent component contribution to the marine DOM fluorescent pool on the SW Florida Shelf

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n (sample size)</td>
<td>64</td>
<td>39</td>
<td>77</td>
<td>79</td>
</tr>
<tr>
<td>%Humic-like FDOM</td>
<td>35%</td>
<td>51%</td>
<td>43%</td>
<td>40%</td>
</tr>
<tr>
<td>%Protein-like FDOM</td>
<td>22%</td>
<td>29%</td>
<td>27%</td>
<td>30%</td>
</tr>
</tbody>
</table>
Table 2.5. The Pearson’s product moment correlation coefficient matrix of modeled components with river discharge (DischSR-Shark River; DischCR- Caloosahatchee River) across the SW Florida Shelf. (C1-component1(HLC1); C2-component 2 (HLC2); C3-component 3; C4-component 4 (TLC4); C5-component 5(TLC5).

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>DischSR</th>
<th>DischCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.999</td>
<td>0.503</td>
<td>0.922</td>
<td>0.994</td>
<td>0.965</td>
<td>0.952</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.497</td>
<td>0.078</td>
<td>0.006</td>
<td>0.035</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0.477</td>
<td>0.908</td>
<td>0.991</td>
<td>0.975</td>
<td>0.938</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.523</td>
<td>0.092</td>
<td>0.009</td>
<td>0.025</td>
<td>0.0619</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>0.498</td>
<td>0.47</td>
<td>0.354</td>
<td>0.631</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.502</td>
<td>0.53</td>
<td>0.646</td>
<td>0.369</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.940</td>
<td>0.970</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.060</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.840</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>DischSR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DischCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3

Resolving DOM fluorescence fractions during a Karenia brevis bloom patch on the West Florida Shelf

3.1. Rationale

Harmful algal bloom (HAB) occurrences have caused tremendous problems in the economy, marine life and human health in many parts of the world (Sellner et al., 2003; Kirkpatrick et al., 2004). On the southwest Florida Shelf, Karenia brevis harmful algal bloom occurrences nearshore and offshore in the Gulf of Mexico have been documented since 1844 (Steidinger, 1983; Magaña et al., 2003). Potential sources of nutrients for K. brevis include dead fish, remineralization, estuarine flux, benthic flux, zooplankton excretion, N2-fixation (Walsh et al., 2006; Vargo et al., 2008) and enrichment from agricultural runoff, sewage and groundwater (Brand, 2002; Brand and Compton, 2007). Although there is a scientific consensus that there is a link between eutrophication and some harmful algal blooms (Anderson et. al, 2002, 2008; Heisler et al., 2008), nutrients that support the K. brevis bloom appear to come from multiple sources (Vargo et al., 2008).

The ability of K. brevis to assimilate organic carbon, nitrogen and phosphorus in addition to the inorganic available forms has been documented (Lenes et al., 2001; Walsh and Steidinger, 2001). This strategy can outcompete diatoms in a low inorganic nutrient environment, particularly in offshore waters and allows for the possible prevalence of K. brevis blooms in offshore waters of Florida, where organic nutrients were reported to be

---

1 Continental Shelf Research (2012) 23: 231-254
supplied from nitrogen fixation of \textit{Trichodesmium} species that co-occurs with high Saharan dust regimes (Walsh et al., 2006; Lenes et al., 2001, 2008). On the other hand, their abundance nearshore may also be attributed to current transport mechanisms aided by wind regimes (Steidinger and Haddad, 1981; Tester et al., 1991; Stumpf et al., 2008), and organic supplement in this region was hypothesized to be land derived organic components (Steidinger and Haddad, 1981; Walsh et al., 2006).

On the southwest Florida Shelf, runoff comes from various river sources; thus, it is complicated to discern the different sources of fluorescent dissolved organic matter (FDOM) associated with river inputs. The three major sources of river input are the Caloosahatchee and Peace Rivers, which drain into the Charlotte Harbor, and Shark River. The conversion of the Everglades to drainage systems, water conservation and agricultural areas increased Caloosahatchee River outflow into the shelf via a gated flow from Lake Okeechobee (Reedy and DeLaune, 2008). Nutrients from agriculture, urban development and sewage loading, delivered from these rivers may have increased HAB incidence on the southwest Florida Shelf (Brand and Compton, 2007). However, the contributions of these sources to the harmful algal bloom are unknown. In particular, determining the organic nutrient sources during the bloom is important in understanding the occurrence of \textit{K. brevis} blooms in shelf waters.

The fluorescent fraction of DOM (FDOM) has been widely used to trace terrestrial organic matter input from rivers to the coasts (Clark and Zika, 2000; Clark et al., 2002; Baker and Spencer, 2004) and \textit{in-situ} produced organic materials at sea. This fraction absorbs light in the UV and visible region and emits light at a longer wavelength. Analysis using the excitation and emission matrix spectroscopy (EEMs) results in a
matrix with different emitting centers, which correspond to different fluorophores from multiple sources, and from the chemical composition of the samples (Coble, 1996). Recently, parallel factorial analysis (PARAFAC), a multi-way analysis fitting of EEM was introduced to separate individual unique components from the EEM datasets, providing an added quantitative estimate of the different fluorophores that are identified by the model (Stedmon and Markager, 2005; Stedmon and Bro, 2008). As such, this method can serve as a proxy to quantify relative proportions of the organic materials originating from natural and anthropogenic sources as well as in-situ produced organic materials as described in previous studies (Stedmon et al., 2003; Stedmon and Markager, 2005; Yamashita et al., 2008; Murphy et al., 2006, 2008): (1) Humic-like components fluoresced at an excitation of 260 and emission from 400-460 nm and were possible DOM contributions from freshwater sources (e.g., river); (2) The anthropogenic humic-like materials were observed at an excitation wavelength of 320-360 nm and emission wavelength of 420-460 nm; and may originate from agricultural and sewage areas; (3) The tyrosine and tryptophan-like fluorescent components were observed at an excitation-emission wavelengths of 275/305 nm and 275/340 nm, respectively. These protein-like components were observed to increase during phytoplankton bloom events (Suksomjit et al., 2009). Some research suggests that the humic-like components (the extractable component of CDOM) act as chelators needed for cellular processes, and the nitrogen-containing humics may serve as sources of nitrogen (Vargo et al., 2008).

The goals of this study are to demonstrate the utility of a multi-wavelength fluorescence technique and PARAFAC modeling in resolving DOM sources during the October 2009 *K. brevis* bloom off of Sanibel Island on the southwest Florida Shelf, and
to present a case scenario that explains phytoplankton bloom occurrence in offshore waters.

3.2. Materials and Methods

3.2.1. Field sampling

Sampling was conducted across the southwest Florida Shelf (domain: 24°-28°N; 80.5°-83°W) in October 2009 for 12 days. Temperature and salinity were monitored using a SEABIRD thermosalinograph attached to the flow-through system on the ship. Water samples for FDOM measurement were obtained through a flow-through system collecting water at approximately 1 m depth. Water samples were immediately filtered using a 0.2 µ nylon capsule filter, placed in pre-combusted (520 °C) aluminum foil covered scintillation vials. The samples were stored for week in the refrigerator (~5°C) until analysis. Samples from rivers and estuaries were collected by grab sampling using a 1 l Teflon closed-mouth container. Samples were filtered and stored following the procedure mentioned above. Likewise, a Turner Design C6 fluorometer, equipped with a chlorophyll-a Cyclops-7 sensor, was used to estimate chlorophyll-a (λ.ex= 460 nm; λ.em=670-715 nm) in real time.

3.2.2. Karenia brevis counts

One liter of seawater was collected from each site using Niskin samplers for surface and bottom samples. Collections were made every hour during transit between stations using a customized surface polyethylene water sampler. Lugol’s solution was immediately added as a preservative to all 1 l seawater samples, which were stored in the
dark prior to analysis. Cells of *K. brevis* were allowed to settle for 48 hours after which the upper layer (800 ml) of seawater was decanted. The latter procedure was repeated until the remaining solution was about 10 ml. This volume was transferred into a capped conical tube. From this final sample, 0.1 ml was mounted on a glass slide. The *K. brevis* cells were counted under a light microscope (Olympus BH-2) and were recorded as cells per liter.

There were 22 samples collected during the cruise for the DOM analysis. Sampling for *K. brevis* counts did not necessarily coincide with the DOM sampling. Only those samples that coincided with DOM sampling and with the positive *K. brevis* counts were included in the analysis.

### 3.2.3. Spectral measurements

Spectral measurements of the EEM of water samples were analyzed using a fluorescence spectrophotometer (Hitachi F-4500). Fluorescence scan ranged from 200 to 500 nm for excitation and from 220 to 700 nm for emission. Scan intervals were configured at 5 nm. Slit width was set at 5 nm emission and excitation and scan speed were set at 1200 nm/min with photo-multiplier tube at 700 V. Correction of the instrument was made using the manufacturer’s settings (Hitachi High Technologies, 2001). Excitation and emission matrices obtained were corrected for Raleigh and Raman scatter from Milli-Q water fluorescence subtraction while the inner filter effect was corrected from the measured absorbance (Lacowicz, 1999). Absorbance was measured using the UV-Vis Spectrophotometer (Agilent 8453). All fluorescence intensities were calibrated against quinine sulfate monohydrate in 0.1 N H$_2$SO$_4$ measured at 350/450 nm.
Derived fluorescence intensities of the PARAFAC components were reported in quinine sulfate units (QSU).

3.2.4. Excitation and Emission spectral analysis and PARAFAC

PARAFAC modeling involves multi-way fitting analysis that minimizes the residual of the sum of squares of the datasets using equation 3.1. The three dimensions correspond to the excitation and emission wavelengths and fluorescence intensity. Discrimination of analyte fluorescence signal from the other spectral interferences was accomplished using parallel factorial analysis.

\[
x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}, \quad i=1, \ldots, I; \quad j=1, \ldots, J; \quad k=1, \ldots, K. \quad \text{(equation 3.1)}
\]

The \(x_{ijk}\) in the equation represents the fluorescence intensity of the \(i\)th sample at the emission mode (\(j\)th variable), and its corresponding excitation mode (\(k\)th variable) in the PARAFAC multi-way model. Meanwhile, \(a_{if}\), \(b_{jk}\), and \(c_{kf}\) represent the scaled parameters that describe the sample and variable for each individual component. The unexplained variance is represented by \(e_{ijk}\). Modeling of the corrected EEM datasets by PARAFAC was accomplished using the DOMFluor toolbox implemented in MATLAB (R2008 Student Version) as a platform (Stedmon and Bro, 2008). The EEM excitation and emission wavelengths used in the PARAFAC modeling were reduced to 250-450 nm and 300-520 nm to minimize effect of random noise outside the spectral range (Smilde et al., 2004; Murphy et al., 2008). The final data matrix array consisted of 48 EEM samples with fluorescence scans made at 47 emission wavelengths and 41 excitation wavelengths per sample. The PARAFAC model was fitted to the array split into random halves, consisting of a calibration and a validation array. The four-component model which was split-half validated, was able to explain 99.6 to 99.8% of the dataset variation. This
number of components was further validated by a random initialization analysis step to ensure observation of least squares. See Stedmon and Bro (2008) for details of DOM fluorescence analysis using PARAFAC.

3.3. Results

3.3.1. Relating PARAFAC fluorophores distribution to K. brevis count

Five DOM fluorescent components were derived from 48 EEMs using PARAFAC analysis (Figure 3.1, a-e) that exhibit different excitation and emission wavelength maxima. Spectral loadings (Figure 3.1, f-j) of these components correspond to the excitation and emission peaks identified in the literature (Coble, 1996; Stedmon and Markager, 2005; Coble, 2007). Component 1 absorbs at 250 nm and emits at a peak maximum of 425 nm. This component is identified as a humic-like material, and with a peak maximum similar to peak A (refer to Table 3.1). Component 2 is identified as peak D and fluoresces at 495 nm, which exhibits a similar spectral property with fulvic acid. Components 3 and 4 both absorb in the UVA regions and are identified as peaks C and M, respectively. Peaks C and M appear to be related to UVA humic-like and marine humic-like components. Emission peak maximum for component 5 was shifted 60 nm from component 4. This component exhibited a secondary excitation maximum at 275 nm with primary excitation absorption occurring at 250 nm.

The resulting peaks of the DOM fluorescence excitation and emission from the five identified components provide qualitative information on the identity of these components (Figure 3.1,a-e). Aside from the qualitative data, the corresponding fluorescence intensity of the five-component PARAFAC loadings provided quantitative measure of the relative fluorescence of the different EEMs from southwest Florida Shelf.
water samples. A surface map of the distribution of each of the components across the Florida Shelf was generated from this quantitative measure (Figure 3.2, a-e). The fluorescence intensity of these five components exhibits a strong signal southwest of Charlotte Harbor. The humic-like components may be influenced by the high FDOM concentration water discharged from the Peace and Caloosahatchee Rivers (Figure 3.3, a) and increase in the rate of precipitation (Figure 3.3, b). The river flow started to increase in June in three major rivers in southwest of Florida. The flow in the Caloosahatchee in October was ~ 6,000 cu. ft. s⁻¹ (Figure 3.3, a) and about 2,000 cu. ft. s⁻¹ in the Peace River (Figure 3.3). These increases in river flow correspond to the magnitude of precipitation, as evident in the recorded river flow and precipitation rate in the Caloosahatchee River (Figure 3.3, b). Thus, the river outflow and precipitation events during these periods may increase river discharge to the shelf in the month of October.

In tandem with DOM fluorescence analysis, *K. brevis* cell density was also monitored. There were a total of 22 *K. brevis* cell counts and 48 EEM measured DOM fluorescence data, but only 7 measurements from both counts and fluorescence data coincide. The *K. brevis* concentrations showed a linear relationship with the five components (Figure 3.4). Correlation coefficient of Component 2 (R²=0.89) and 5 (R²=0.81) with *K. brevis*, were relatively higher compared to other components. I also observed that component 1 and 2 also showed strong relationship with *K. brevis* (Figure 3.4, a-c) but a weak relationship with component 3. Component 4 on the other hand, exhibits a spectral property similar to a marine-humic component reported to be biologically/microbially derived (Table 3.1).
The distribution of component 5 on the shelf (Figure 3.2, e) showed a similarity to the distribution of *K. brevis* abundance (Figure 3.5) suggesting that component 5 influenced the occurrence of the bloom (or vice-versa) found ~25 miles away from Charlotte Harbor. This further supports our suggestion that the increase in the fluorescence intensity of component 5 could be related to the *K. brevis* density increase. The *K. brevis* bloom patch (<100,000 cells l⁻¹, Figure 3.5) found was verified by the counts made by the Fish and Wildlife Research Institute (October 9-16, 2009 HAB plot: Florida Fish and Wildlife Commission, 2011) in the same region.

The plume of chlorophyll-a (Figure 3.6) observed off Charlotte Harbor is indicative of biological production. This plume was in close proximity with the *K. brevis* bloom patch (Figure 3.6). The increase in chlorophyll-a (Figure 3.6) was also observed inside Charlotte Harbor, and corresponded also to an increased in protein-like fluorescence and other humic-like components (components 1-4). The presence of the chlorophyll-a plume off and inside Charlotte Harbor was consistent with high fluorescence of the protein-like component (Figure 3.2, e).

### 3.3.2. Influence of water quality parameters and physical forcing

We observe that the fluorescence intensities of the five components relating the 48 samples with increase in salinity (Figure 3.8a; Figure 3.8b) were clustered at salinity ~36. However, we decided to re-plot these datasets since the bloom patch occurred in a small region; salinity and temperature trends were re-plotted based on the available *K. brevis* cell counts (right hand plots: Figure 3.8a,b; Figure 3.8b). Re-plotting salinity and temperature against the five components resulted in a direct relationship (Table 3.2).
A positive relationship was observed with component 1-3 (humic-like associated components; Table 3.1 and Figure 3.8a,1-3) with salinity (from 35.6-36.1). Similarly, the marine humic-like component showed a low positive relationship ($R^2= 0.4$) with salinity (Figure 3.8b, 4; right hand plot). On the other hand, component 5 exhibits a strong positive relationship ($R^2= 0.94$) followed by component 2 ($R^2= 0.54$) with salinity. The positive relationship between component 5 and salinity correspond to salinity’s direct relationship with *K. brevis* (Figure 3.7, b). The strong relationship of the other components (1 and 4) with *K. brevis* is also observed but can only explain ~40% of the variability of these components with salinity (Table 3.2). This implies that this labile, protein-like component originating from a high salinity environment could be linked to the *K. brevis* bloom and that the bloom could have been initiated in a high salinity environment enriched with *in-situ* and land-derived components.

### 3.4. Discussion

#### 3.4.1. DOM fluorophore sources and distribution

Five components were identified from EEM measurements of rivers (i.e., Caloosahatchee and Shark River), estuary, coastal and shelf water from southwest Florida, and which exhibited spectral properties similar to that of the terrestrial humic materials (Stedmon and Markager, 2005). Component 1 absorbed in the UVC region and emitted at shorter wavelengths than components 2 and 3. Component 1 was found to be present in high concentrations in forest streams and wetlands (Stedmon et al., 2003) and could be exported from agriculture-dominated catchments (Stedmon and Markager, 2005). Components 2 and 3 were related to a fulvic acid fluorophore (Lochmuller and Saavedra, 1986; Stedmon and Markager, 2005) found to be ubiquitous in a wide range of
freshwater environments. These three components could be transported to the Florida coastal shelf from the Caloosahatchee and Shark River where discharge is predominantly high during the month of October (Kelble et al., 2007). This was also confirmed by the low salinity plume protruding out of Charlotte Harbor (Figure 3.7). The high discharge of river water from the Peace and Caloosahatchee Rivers was indicative of the large input of land-derived inorganic and organic nutrients to the coastal shelf of Florida (Brand and Compton, 2007). These rivers are known to contain not only DOM from natural sources, but also anthropogenic DOM from agriculture and wastewater (Clark et al., 2002).

Component 4 indicated that DOM input to the shelf contained anthropogenic DOM along with other terrestrial humic materials. This component was observed to dominate in wastewater environments and agriculture-dominated catchments (Stedmon and Markager, 2005). On the other hand, component 5 was found to exhibit a mixture of peaks N and T. Peak N is associated with labile components resulting from biological production in the water column while peak T, a tryptophan protein-like fluorescence, has been associated with surface water biological production (Stedmon et. al., 2003; Coble et. al., 1998; Determann et. al., 1996, Thurman and Malcolm, 1983).

3.4.2. Relationship of nutrients, fluorophores and K. brevis counts

The increase of *K. brevis* near the coastal waters may be influenced by the increase of nutrient inputs from terrestrial sources (Brand and Compton, 2007). The low salinity water from Caloosahatchee and Peace River are known to discharge water from anthropogenic sources (wastewater, agriculture and industrial wastes) into the Florida coastal shelf (Brand, 2002; Clark et al., 2002), which contained high concentrations of remineralized nutrients and dissolved organic matter. For the October 2009 bloom,
contributions of low salinity water enriched with nutrients from land could not be estimated since no measurements were obtained. Thus, land-based nutrient sources could not be invoked to play a role in the initiation of this bloom. However, it was proposed that nutrients and organic nutrients from estuaries could be considered supplementary sources after the *K. brevis* bloom had been initiated in the deep sea (Walsh et al., 2003, 2006; Vargo et al., 2008;).

Typically, DOM distribution is inversely related with salinity on the southwest Florida Shelf (Zanardi-Lamardo et al., 2004). However, the relationship between terrestrial components with salinity within the bloom region in this study appears to have a positive relationship (Table 3.2). This relationship between fluorescent dissolved organic matter and salinity was also observed near Caloosahatchee River and was attributed to the high rate of evaporation (Zanardi-Lamardo et al., 2004). In order to account evaporation as a factor that promoted the increase in the humic-like components, a salinity end-member mixing in Figure 3.8a,1 (right hand figure) was used. The change in salinity from 35.6 to 36.1 was calculated to account 1.4% only of the increase of the component 1 with increase in salinity. The increase in fluorescence of component 1 from 9 QSU to 136 QSU, corresponds to a 15 times increase with change in salinity. This means that evaporation cannot account solely in the fluorescence increase of component 1. It is speculated that autochthonous production of the humic-like material could increase the fluorescence in high salinity.

A conceptual diagram is presented (Figure 3.9) to explain a likely mechanism that increased both the humic-like and protein-like components in high salinity water. Component 1,2,3 are components assigned as terrestrial components (Table 3.1). Since
evaporation (evaporation of low end-member salinity water) cannot account in the increase of the terrestrial humic-like components alone with increase in salinity, another possible source of these materials is the resuspension of sediments enriched with terrestrial materials in solution from previously deposited materials from river inputs (i.e., Caloosahatchee and Peace Rivers). Wind driven mixing promote coastal upwelling (Coble et al., 2007), that brings bottom water to sea surface (~1 m). Similar mechanisms were proposed by Milbrandt et al., (2010) off of Charlotte Harbor. Since \textit{K. brevis} favors a low light environment, the terrestrial material may have served as a protection from sunlight rather than as a nutrient source (Walsh et al., 2003, 2006).

On the other hand, component 5 (Comp5; Figure 3.9), associated with \textit{K. brevis} bloom during the October 2009 cruise on the southwest Florida Shelf, showed a spectrum that was characteristic of a protein-like component. This component was associated with \textit{in-situ} biological productivity along with component 4- a marine humic-like component with microbial origin (Stedmon and Markager, 2005; Murphy et al., 2006; Coble, 1996). Such similarity could indicate production of labile organic compounds, either coming from the bloom itself, from the phytoplankton or from the diazotrophs bloom (Walsh et al., 2006). I speculate that organic nitrogen production coming from these sources could have supplied the algal bloom and allowed it to persist since \textit{K. brevis} has been observed to have the ability to assimilate amino acids (Baden and Mende, 1979) and other organic nitrogen compounds (Havens, 2004). The preference of \textit{K. brevis} to assimilate organic nutrients has been demonstrated as a mechanism to possibly gain advantage over the presence of ubiquitous diatoms that thrive in an inorganic enriched environment (Havens, 2004). The high correlation of the protein-like fluorescence component with salinity
indicates that these organic nutrients are produced \textit{in-situ} and could have supported the October 2009 \textit{K. brevis} bloom patch.

The observed seeding of \textit{Trichodesmium} population at the shelf break by the Loop Current (Walsh et al., 2006) and the collected \textit{Trichodesmium} species during the October 2009 cruise, indicate two additional possible sources of organic protein fluorescence in the bloom region. First, the high phosphate stocks from agriculture and fossil pool in coastal areas draw \textit{Trichodesmium} to advance near the coast (Lenes et. al., 2001; Walsh et al., 2001). Such movement facilitates organic nitrogen production through nitrogen fixation by \textit{Trichodesmium}, from the diazotroph’s population growth (Prufert-Bebout et al., 1993) and remineralization of its organic materials as a result of population collapse (Devassy et al., 1978). Second, DON can be produced from the diatom population biomass (Heil et al., 2001; Bronk et al., 2006), which can be fueled by the intrusion of cold, nutrient enriched water, through the 75 and 200 m isobath on the shelf from offshore (He and Weisberg, 2003) that is associated with the Loop current (Walsh et al., 2003; Walsh et al., 2006). This cross-shelf conveyance of materials toward Charlotte Harbor (Weisberg et al., 2001; He and Weisberg, 2003) facilitated by the Ekman layer transport (Weisberg et al., 2001) can be influenced by local winds (He and Weisberg, 2003). Evidence of such possible transport was confirmed by the high chlorophyll-a estimate along 26.5 °N (Figure 3.6c), with associated high salinity (~36.5 psu °C) (Figure 3.6b) and low temperature (~29°C) (Figure 3.6, a) bands. Similarly, a salinity band was also observed in the Loop Current associated oceanic water (Steidinger and Haddad, 1981) that was linked to the 1980-81 \textit{K. brevis} bloom (Haddad and Carder, 1979).
The relatively lower fluorescence intensity in the Shark River region has a lower discharge than the Caloosahatchee River (Figure 3.3), thus lowering organic material input into the shelf during this month. However, historical records of sporadic blooms of *K. brevis* above the Middle and Lower Florida Keys could potentially be driven by transport of the materials coming from Shark River. The Shark River is known to receive large input of DOM from the Everglades system and discharges enriched organic nutrient water into the Florida Bay (Childers et al., 2006; Boyer et al., 1997; Boyer et al., 1999, Rudnick et al., 1999). The carbon and nitrogen variation, at least in Florida Bay, was 99% attributed to the freshwater input from the Everglades during the rainy season (Sutula et al., 2003). Since sampling was limited and no bloom was observed in this region, land-derived DOM influence to the sporadic *K. brevis* bloom (Brand and Compton, 2007) using EEMS and PARAFAC analyses in this region cannot be established.

3.5. Summary

Four different fluorescent fractions of DOM components containing humic-like and protein-like components were identified during the October 2009 *K. brevis* bloom patch. The presence of terrestrial humic-like materials is indicative of land-based materials which were present during the bloom, while the tryptophan-like component indicates fluorescent materials from biological production. Deductions were made as to the possible organic nutrient sources of the *K. brevis* bloom patch. Based on the direct relationship of the protein-fluorescence component with *K. brevis* cell density, it is hypothesized that the source of organic materials fueling the bloom was produced *in-situ*. These results could support long-term studies of *K. brevis* on the SW Florida Shelf. The
positive relationship of the terrestrial DOM fluorescence materials with *K. brevis* suggests that the terrestrial organic materials could be related to the bloom. The presence of a humic-like component during the bloom was speculated to have been used as shade since *K. brevis* is sensitive to light. A direct salinity-humic-like DOM fluorescence relationship in the bloom region suggests that the observed humic-like component increase with salinity could be related to evaporation and resuspension of organic matter-enriched bottom sediments from wind driven mixing.

The ability of the technique used in this study to resolve the components of DOM in a dynamic coastal shelf system in southwest Florida and to trace its multiple sources makes this method a promising tool to unravel sources of mechanisms and environmental processes that supports harmful algal blooms.
Figure 3.1. Loadings of five PARAFAC modeled DOM fluorescence fractions collected on the southwest Florida Shelf during the October 2009 University of Miami Oceans and Human Health Cruise.
Figure 3.2. a-e) Surface points of the individual PARAFAC modeled components (Component 1-Component 5) on the southwest Florida Shelf during the October 2009 cruise (n = 30). Bar scale in quinine sulfate units (QSU). Dash lines indicate region *K. brevis* bloom was found.
Figure 3.3.  a) Monthly discharge rates (cu ft s\(^{-1}\)) of Caloosahatchee (station 79, SFWMD DBHydro), Shark (S12A+S12B+S12C+S12D+S333, SFWMD DBHydro) and Peace (station in Arcadia, USGS-NWIS Web) Rivers from January 2009-December 2009 (SFWMD DBHydro. b) Precipitation (inches) rates in Caloosahatchee (station 79) and Shark (S12D) Rivers. (SFWMD-South Florida Water Management District, USGS-NWIS-U.S. Geological Survey National Water Information Systems).
Figure 3.4. a-e) Relationship between *K. brevis* counts and PARAFAC modeled DOM fluorescent components.
Figure 3.5. *Karenia brevis* counts (cells l$^{-1}$) off of Sanibel Island (southwest of Charlotte Harbor) during the UM-OHH October 2009 cruise.
Figure 3.6. Surface plots of a) temperature (°C), b) salinity (psu), and chlorophyll-a estimate (RFU) on the southwest Florida Shelf.
Figure 3.7. Relationship of *K. brevis* counts (cells l⁻¹) with: a) temperature (°C) and b) salinity (psu).
Figure 3.8a. Relationship of components 1) component 1 (Comp1), 2) component 2 (Comp2), 3) component 3 (Comp3) and salinity. Right hand plots correspond to the component/salinity (n=7, from Table 3.2) relationship in the K. brevis bloom patch.
Figure 3.8b. Relationship of 4) components 4 (Comp4) and 5) component 5 (Comp 5) and salinity. Right hand plots correspond to the component/salinity (n=7, from Table 3.2) relationship in the *K. brevis* bloom patch.
Figure 3.9. Conceptual diagram of humic-like (component 1) and protein-like (component 5) fluorescence increase with increase salinity during a *Karenia brevis* bloom patch ~25 miles away from Charlotte Harbor. Evaporation and coastal upwelling (driven by wind) that brings organic matter enriched materials from the sea bottom to the sea surface, are two proposed mechanisms that increased terrestrial humic-like DOM fluorescence with increase in salinity.
Table 3.1. Excitation and emission loading maximum wavelengths, type and possible sources of the five modeled PARAFAC DOM fluorophores during a *K. brevis* bloom event on SW Florida Shelf. Peak name, FDOM type and possible sources assignments of FDOM were referred to the following references (Blough and Del Vecchio 2002; Stedmon and Markager, 2005; Murphy et al., 2006; Coble 1996; Coble 2007).

<table>
<thead>
<tr>
<th>Comp</th>
<th>λ_{ex}</th>
<th>λ_{em}</th>
<th>Peak name</th>
<th>FDOM type</th>
<th>Possible sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>425</td>
<td>A</td>
<td>UVC Humic-like</td>
<td>Terrestrial, Allochthonous</td>
</tr>
<tr>
<td>2</td>
<td>255</td>
<td>495</td>
<td>D</td>
<td>Fulvic Acid</td>
<td>Terrestrial Autochthonous</td>
</tr>
<tr>
<td>3</td>
<td>340</td>
<td>440</td>
<td>C</td>
<td>UVA Humic-like</td>
<td>Terrestrial, Anthropogenic, Agriculture</td>
</tr>
<tr>
<td>4</td>
<td>330</td>
<td>400</td>
<td>M</td>
<td>Marine Humic-like</td>
<td>Microbial, Biological, Autochthonous</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>340</td>
<td>N,T</td>
<td>Tryptophan</td>
<td>Protein-like, Labile, Autochthonous</td>
</tr>
</tbody>
</table>

(275)
Table 3.2 Slope, y-intercept and correlation coefficient of PARAFAC modeled components against temperature and salinity along the \textit{K. brevis} bloom patch.

<table>
<thead>
<tr>
<th></th>
<th>Temperature (°C)</th>
<th>Salinity (psu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope, y-intercept, (R^2, n)</td>
<td>Slope, y-intercept, (R^2, n)</td>
</tr>
<tr>
<td>Comp1</td>
<td>73.80, -2187.2, (0.051, 7)</td>
<td>201.36, -7148.8, (0.40, 7)</td>
</tr>
<tr>
<td>Comp2</td>
<td>105.39, -3164.6, (0.15, 7)</td>
<td>197.40, -7022.9, (0.54, 7)</td>
</tr>
<tr>
<td>Comp3</td>
<td>41.60, -1224.4, (0.04, 7)</td>
<td>122.84, -4355.9, (0.36, 7)</td>
</tr>
<tr>
<td>Comp4</td>
<td>9.86, -280.92, (0.38, 7)</td>
<td>54.19, -1922.6, (0.40, 7)</td>
</tr>
<tr>
<td>Comp5</td>
<td>63.65, -1924.0, (0.50, 7)</td>
<td>84.49, -3006, (0.94, 7)</td>
</tr>
</tbody>
</table>
Chapter 4

Determination of brevetoxin in recent marine sediments

4.1. Rationale

Harmful algal blooms (HAB) are a worldwide phenomenon caused by a dominating alga at extremely high concentrations. Highly dense algal blooms discolor surface waters that sometimes appear as red, green, yellow, and occasionally colorless depending on the algal species (Okaichi, 2004). Natural toxins from these blooms cause economic, environmental, and human health problems, and there is evidence that these blooms are increasing (Van Dolah, 2000).

The toxic photosynthetic dinoflagellate *Karenia brevis* (*K. brevis*) occasionally generates large blooms that kill fish and other animals (Landsberg, 2002) and cause human health problems (Van Dolah et al., 2001; Kirkpatrick et al., 2004). The blooms occur in many parts of the Gulf of Mexico but are most prevalent along the west coast of Florida (Geesey and Tester, 1993; Steidinger et al., 1998; Kusek et al., 1999). The overall distribution and abundance of *K. brevis* in space and time is not well known, both because the blooms are sporadic and the sampling has been erratic. Fish kills described by early European explorers in the fifteenth century (Kusek et al., 1999; Magaña et al., 2003) provide evidence that blooms of *K. brevis* have been occurring for many hundreds of years in the Gulf of Mexico. Brand and Compton (2007), however, have examined the last 50 years of quantitative data on *K. brevis* and concluded it has increased roughly 15-fold along the west Florida coast. The sampling however, was quite sporadic in both space and time. There are no quantitative data on *K. brevis* before around 50 years ago.

---

because *K. brevis* was not known to be the causative agent of the observed fish kills at that time.

The toxins that are associated with *K. brevis* blooms are broadly defined as belonging to a lipid soluble group of cyclic polyether compounds with a molecular weight around 900 amu (Figure 4.1). Many analogs of brevetoxin have been identified in cultures of *K. brevis*, in sea water blooms and also as metabolites in shellfish and other marine life (e.g. Abraham et al., 2006). Most of these analogs were reputed to be biosynthetic products of either PbTx-1 or PbTx-2 as precursors (Baden et al., 2005). Typically in marine waters the dominant brevetoxin is PbTx-2 with lesser amounts of PbTx-1 and PbTx-3, whereas in marine aerosols PbTx-3 dominates (Pierce et al., 2005). Recent work by Tester et al. (2008) has shown as a *K. brevis* bloom ages, there is a relative increase in the PbTx-3 compared to the PbTx-2 homologues. The mechanism is not entirely understood but is believed to be enzymatic (Poli et al., 1986).

It is unknown if the major brevetoxin analogs (PbTx-2 and 3) become incorporated into underlying sediments and may be a long term sink for these potent toxins. Pierce et al. (2004) showed that brevetoxins do flocculate with clays which suggests that absorption onto sediments may take place. Therefore, the hypothesis we wish to test is whether brevetoxins produced by large *K. brevis* blooms are incorporated in underlying sediments. Accumulation of brevetoxin in marine sediments may serve as a potential mechanism of transfer of the toxin up the food chain to higher organisms such as manatees (Trainer and Baden, 1999). Furthermore, brevetoxin may also be a potential marker for paleo-reconstruction, if brevetoxin does not significantly degrade over time.
4.2. Materials and Methods

4.2.1. Sediment collection

During December 2006 surficial sediment samples from Big Carlos Pass (BCP), Big Hickory Pass (BHP) and Fort Myers Beach (FMB) were collected by grab sampling the upper 10 cm (Figure 4.2). These sites were chosen because a red tide bloom had been in the collection area 5 months prior to sampling. The sediment samples were placed in solvent rinsed glass jars and transported to the laboratory on ice. Samples were frozen immediately upon returning to the University of Miami and were freeze-dried before analysis.

4.2.2. Extraction

The extraction of brevetoxin from sediment samples was done by sonication with a 1:1 mixture of dichloromethane and acetone (e.g. Plakas et al., 2002). The sediment weight varied between 1.5 to 2.0 grams and the total volume of solvent was 50 mL. Once completed, the solvent and sediment were separated by centrifugation at 1000 rpm for 10 minutes. The upper dichloromethane:acetone supernatant layer was transferred to solvent pre-rinsed glass vials using a Pasteur pipette. This process was repeated for a total of three times and the supernatants were pooled into one sample. The sample was evaporated to dryness under a gentle stream of ultra-high purity N₂ gas. Samples were stored at -20 °C until ready for analysis. Before HPLC analysis, the dried residue was re-solubilized in 500 μl of methanol:water (85:15 v v⁻¹) by sonicating for 30 s. Dichloromethane, methanol and acetone GC² grade solvents were purchased from
Burdick and Jackson, Corp. (Muskegon, MI) while the ultrapure HPLC grade water was purchased from Alfa Aesar (Ward Hill, MA).

In order to test the sonication times, a series of experiments that manipulated the sonication time were employed. The sonication extraction times were tested by weighing 2.0 g of freeze dried sediment sample from BHP. Before extraction 10 µL of PbTx-9 (100 µg ml⁻¹) was added to the sediment and thoroughly mixed. Analysis was performed by HPLC-DAD using the method of Hua et al. (1996).

**4.2.3. HPLC and LC-ESI/MS/MS analysis**

*K. brevis* and sediment extracts were sent to Dr. Damian Shea’s Laboratory for the LC/MS/MS analysis (Department of Environmental and Molecular Toxicology Laboratory, North Caroline State University).

The HPLC-DAD analysis was performed using an Agilent 1100 LC series following the method of Hua et al. (1995). A narrow bore reversed phase (C₁₈) Eclipse XDB (Agilent Technologies, CA, USA) 3.5 µm pore size, 2.1 x 150 mm LC column was employed to separate the PbTx mixture. The mobile phase reservoirs were (A) HPLC grade water and (B) methanol run with 15 % and 85 % ratio, respectively. Injected volume was 5 µl using a high pressure injection valve (Rheodyne 7520, Cotati, CA, USA) with a 5 µl loop. Total acquired run time was 20 minutes. The flow rate was set at 0.1 ml min⁻¹ and the column temperature was at ambient room temperature (25 °C). Absorbance of the brevetoxins was read at 215 nm using a diode array detector. The peak identified as PbTx-9 from the extraction experiment was determined against external standard calibration curves with five calibration points from 0.05 - 0.5 µg/mL. (Plot not
shown; PbTx-9, $r^2 = 0.999$). The limit of detection was 3.3 ng. These brevetoxin standards were purchased from CalBioChem (La Jolla, CA, USA). Procedural and instrumental blanks were routinely analyzed and no contamination was found.

The LC-MS-MS analyses conditions were modified after Cheng et al. (2005). The samples were separated by an Agilent 1100 LC series using a reverse phase (C$_{18}$) Phenomenex, LUNA, 3 µm pore size, 2.0 x 50 mm column. The mobile phase was 50:50 acetonitrile:water (0.3 % Acetic acid) for the first 40 minutes, then changed to 5:95 acetonitrile:water for 2 minutes and finally back to 50:50 acetonitrile : water for 8 minutes. The flow rate was set at 0.2 ml/min. Structural identification, detection and quantification was carried out using a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer, equipped with the electrospray ionization source (Thermo Fisher Scientific, Inc, USA). Nitrogen gas was used as both drying gas and nebulizing gas. Structural information was determined in selective reaction monitoring (SRM) mode matching product ions and parent mass from standards to sedimentary brevetoxins. Optimized collision induced decomposition MS/MS spectra for each of the PbTx transitions were acquired at 4 V (PbTx-2), 18 V (PbTx-3) using argon as the collision gas at a pressure of 1.0 mTorr. The SRM transitions of the two type of toxins were set at 895.5 m/z to 877.5 m/z (PbTx-2) and 897.5 m/z to 725.3 m/z (PbTx-3). Scan events of product ions were set at scan width of 1.0 s with scan time of 0.25 s. Q1 and Q3 peak width were set at 0.70 s. Total acquired segment time ran for 40 minutes. An external calibration curve was used to quantify the concentration of brevetoxins in the sediment and cell extracts with a limit of detection of 3 ng/mL (0.75 ng/g). Extracts were spiked with PbTx-9 (monitored at 899.1 m/z to 896.4 m/z at 30 V) as an internal standard. The
sediment and cell culture extracts (see section 4.2.4) were analyzed in duplicate and the concentrations are reported as mean values.

4.2.4. Cultures

Laboratory maintained *K. brevis* cultures were analyzed as well from locations that were near the sediment sampling locations. These cultures are maintained by Dr. Larry Brand at the University of Miami. The 200 ml of the culture was directly filtered on preconditioned (with methanol, 20 ml) C\textsubscript{18} SEPAK cartridge (Waters Co., Milford, MA). The cartridge was treated with HPLC grade distilled water; to lyse the cells and to remove residual salts (Pierce et al., 1992). The brevetoxin was immediately eluted with 2 ml methanol three times. The extract was evaporated to dryness with ultra-high purity N\textsubscript{2} gas, then kept frozen (-20 °C) until analysis.

4.3. Results and Discussion

4.3.1 Extraction recovery

The extraction recovery of PbTx-9 brevetoxin analog from sediment previously exposed to *K. brevis* did not vary from the set sonication time of 5, 10, 30 minutes since each sonication time recovered ~40% of the spiked brevetoxin (Figure 4.3). The standard deviation and the controls for each sonication time suggest that the time element is not a function of the extraction recovery of the brevetoxin from the sediment using the extraction method. The extent of binding of brevetoxins in marine sediments maybe a factor implicated for the modest recovery of spiked PbTx-9 in the sediment even with increase increment in sonication time.
The sonication method of extraction was employed, since it is a widely used sample preparation for sedimentary organic studies (e.g. Mead and Goni, 2006). Other than being inexpensive, it also requires short time for extraction compared to the Soxhlet extraction with relatively higher recovery efficiency (Guerin, 1999). In our experiment, we used ambient room temperature and chose the 10 min sonication rather than the 5 minutes to increase contact time with the sediment and potentially could enhance the recovery in the subsequent sediment sample extraction. The 30 minutes sonication was not employed to decrease or eliminate unwanted side reactions (e.g. Guerin, 1999).

Due to limited amount of PbTx-2 and PbTx-3 standards, PbTx-9 was used as a surrogate for PbTx-2 and PbTx3 in measuring the extraction efficiency of the toxins in the sediments. PbTx-9 (MW: 899.1), along with PbTx-2 (MW: 895.1) and PbTx-3 (MW: 897.1), belong in the same brevetoxin (B-type) backbone type (a lipid soluble group of polyether with molecular weight around 900) produced by Karenia brevis. Based on this assumption, we calculated the true concentration $C_{\text{true conc}}$, considering that using PbTx-9 extraction efficiency as a surrogate for PbTx-2 and PbTx-3 in the sediments. True concentration of brevetoxin was calculated as $C_{\text{true conc}} = \frac{C_{\text{calculated conc}}}{EF}$, where $0 < EF \leq 1$.

4.3.2 Culture and sedimentary concentrations

The brevetoxin extract from cultured K. brevis collected from the same area as where the sediment was collected, was shown to contain 25 and 3 µg / mL of PbTx-2 and PbTx-3 respectively (Figure 7.4; Table 4.1). PbTx-2 was detected in sediments collected at BCP and BHP with values of 3.6 (±0.01) and 0.8 (±0.08) ng / g dry sediment (mean
from duplicate injections), respectively, while PbTx-3 was detected from sediments collected at BCP, BHP and FMB with mean values 9.7, 3.2 and 2.7 ng / g sediment, respectively (Figure 4.4 and Table 4.1). This variability in concentration may be attributed to the hydrodynamic variation affecting the harmful algal bloom dynamics at those three separate sites (Donaghay and Osborn, 1997; Steidinger and Haddad, 1981). Another variation is the timing of *K. brevis* bloom at these sites. The sheltered condition in the passes may also introduce concentration variation due to the observed concentration of the PbTx3 extract at BCP and BHP. The frequent reworking and disturbance on the beach is another source of the toxin concentration variation. This has been observed for other molecular markers in highly energetic environments (e.g. Gordon and Goni, 2004). No clear pattern is observed on the role of terrestrial organic material (using terrestrial fluorophore) in the PbTx2 or PbTx3 deposition in sediments (Figure 4.5).

The stability and subsequent diagenetic fate of the brevetoxin analogs once deposited in the sediment remains poorly understood. There have been studies under extreme conditions which suggest the brevetoxins break down. For example, Baden et al. (1989) found brevetoxins to be stable up to 300 °C and only degraded when the pH was either below 2 or above 10. In addition, a degradation experiment was conducted by Schneider et al., (2003) of brevetoxins utilizing ozonated seawater and found that despite large doses of ozone treatment of brevetoxins some of the original brevetoxins were still recovered. These two studies point to the harsh conditions required to degrade brevetoxins and are unlikely in typical recent surficial sediments. More recently, the fate of the brevetoxins under more environmentally relevant conditions has been reported.
Abraham et al. (2006) has identified ring A opened PbTx-2 and 3 in aerosols and the water column during *K. brevis* blooms while Myers et al. (2008) reported phytoplankton competitors able to degrade PbTx-2 in the water column. Given this information, the present study suggests incorporation into the underlying sediment is possible.

The relative increase in brevetoxin concentrations on surficial sediments seems to correspond to DOM fluorescence increase, e.g., compare stations FMB and BCP (Figure 4.5). However, BHP sediment samples exhibited high DOM fluorescence but contains toxin concentration similar with FMB. We observed inconsistency in our results with regards to the preference of toxin deposition in organic material-enriched environment. The diagenetic fate of brevetoxin in marine sediments, and its interaction with organic matter remains unknown. Thus, investigation of DOM’s role in the toxin deposition or incorporation is needed, to determine its stability, degradation and diagenesis. Such mechanisms are necessary to understand how brevetoxin incorporated into the sediments enter the food web- which could have an implication in food safety, fisheries, and human health, since *Karenia brevis* bloom incidence has been prevalent and recurring annually in the SW Florida Shelf (Brand and Compton, 2007; FWRI).

### 4.4. Summary

Since it appears the brevetoxins are incorporated into underlying sediments there is potential utility of the PbTx-2 and PbTx-3 to be used as a sediment molecular marker for *K. brevis* blooms in the major areas affected in the Gulf of Mexico. The detection of brevetoxins in surficial sediments has to be approached with caution as molecular marker since underlying environmental and biological factors responsible for its deposition,
could play a role in its preservation. Even with this caveat, using brevetoxin as a molecular marker of *K. brevis* blooms would provide a more continuous and long-term record of the organisms presence and abundance. Other proxies have been used as an indicator of dinoflagellate presence. For example, the sterol dinosterol has been used in sediments but does not indicate individual species (e.g. Boon et al., 1979; Mouradian et al., 2007). Fossilized cysts in sediment can also provide a long term record of the presence and abundance of an individual species (Marret and Zonneveld, 2003). *K. brevis* is a naked dinoflagellate however, thus has no thick cell wall to fossilize therefore brevetoxins may be a good biomarker candidate to chemically fingerprint the occurrence of the organism. In order to validate this idea controlled degradation experiments need to be carried out under differing regimes (e.g. sedimentary oxic and anoxic conditions, photochemical degradation in the water column) to characterize the brevetoxin breakdown products.
Type-B Brevetoxins

PbTx-2 : \( R = CH_2C(=CH_2)CHO \)
PbTx-3 : \( R = CH_2C(=CH_2)CH_2OH \)
PbTx-8: \( R = CH_2COCH_2Cl \)
PbTx-9: \( R = CH_2CH(CH_3)CH_2OH \)
PbTx-5: \( R = \) the K-ring acetate of PbTx-2
PbTx-6: \( R = \) the H-ring epoxide of PbTx-2

Type- A Brevetoxins:

PbTx-1 : \( R = CH_2C(=CH_2)CHO \)
PbTx-7 : \( R = CH_2C(=CH_2)CH_2OH \)
PbTx-10 : \( R = CH_2CH(CH_3)CH_2OH \)

Figure 4.1. Structures of the two types of brevetoxin and their respective analogs.
Figure 4.2. Sources of sediment samples from the southwest coast of Florida were at 1) Fort Myers Beach (FMB), 2) Big Carlos Pass (BCP), and 3) Big Hickory Pass (BHP).
Figure 4.3. Percentage of PbTx-9 recovered after brevetoxin was spiked into BHP sediment. Sonication times employed were 5, 10 and 30 min (n = 2).
Figure 4.4. Reconstructed ion chromatograms of PbTx-2 and PbTx-3 from LC/MS/MS analysis of sediments and cultured cells. The instrument conditions are detailed in the Section 2. Determination of PbTx-2 and PbTx-3 used a selected reaction monitoring mode, where quantitation involves a single product ion to monitor each toxin. In these panels (a-f), PbTx2 and PbTx3 were monitored using 895 m/z and 897 m/z, respectively. Products ion 895 m/z was detected in a) *K. brevis* extracts and c) BCP sediment extract. On the other hand, the product ion 897 m/z was detected in b) *K. brevis* extract and extracts from c) BCP, e) FMB and f) BHP sediments.
Figure 4.5. Relationship of FDOM fluorescence (ex/em: 350/450 nm, n=2±SD) against brevetoxin concentration detected using HPLC-ESI-MS/MS from sediments collected in FMB (Fort Myers Beach), BHP (Big Hickory Pass) and BCP (Big Carlos Pass) on the SW Florida coast.
Table 4.1. Concentration from duplicate injections of PbTx-2 and PbTx-3 in sediments and cultured *K. brevis* as determined by LC/MS/MS. Station codes are the same as in Figure 5.1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PbTx-2</th>
<th>PbTx-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCP (ng g⁻¹)</td>
<td>3.6±0.01</td>
<td>9.7±2.0</td>
</tr>
<tr>
<td>BHP (ng g⁻¹)</td>
<td>0.81±0.08</td>
<td>3.15±0.21</td>
</tr>
<tr>
<td>FMB (ng g⁻¹)</td>
<td>N/D</td>
<td>2.7±0.28</td>
</tr>
<tr>
<td>Cells (µg mL⁻¹)</td>
<td>11.9±0.07</td>
<td>8.2±0.07</td>
</tr>
</tbody>
</table>

Station codes are the same as in Figure 4.2 (mean± SD, n=2)
Chapter 5

Application of fluorescence and PARAFAC to assess vertical distribution of subsurface hydrocarbons and dispersant during the Deepwater Horizon oil spill

5.1. Rationale

The explosion of the Deepwater Horizon drilling rig resulted in an oil spill that emanated from a seafloor wellhead gusher. The estimated amount of crude oil spilled into the Gulf was 636 million liters (Camili et al., 2010). Immense underwater plumes of oil not visible at the surface have been confirmed in the water column, (Hulguero, 2010; Camili et al., 2010). The dispersion of crude oil into the water column was promoted by the subsurface addition of Corexit 9500A near the wellhead (EPA, 2010). The dispersant added was approximately 7.95 million liters (Kujawinski et al., 2010). Dispersants are generally used to break crude oil slicks into smaller droplets to promote microbial assimilation (NRC, 1985; Cormack, 1999). However, effective dispersants tend to be more toxic and the risk more severe than the oil by itself (Zahed et al., 2010; Judson et al., 2010; Hemmer et al., 2011). Scientists have argued that derivatives of oil, together with the dispersant, may pose a higher risk than the parent compounds (NRC, 1989). Several studies demonstrated higher toxicity in marine biota when using a dispersant in spilled crude oil (Epstein et al., 2000; Anderson et al., 2009). Also, some dispersants can inhibit biodegradation activity of indigenous marine bacteria (Hamdan and Fulmer, 2011). Studies involving subsurface monitoring of large-scale oil spills, combined with dispersants in deep water, are lacking.
In order to trace the multitude of components present in dispersed crude oil, it is important to understand the fate and distribution of dispersed crude oil components in shallow and deep water environments. Of primary interest are the more soluble and readily dispersed oil components, which are also typically more toxic. These components separate from the bulk oil shortly after the oil mixes with seawater. Because of differences in solubility and density, these components will disperse differently than the higher molecular weight fractions of the crude, and hence display altered surface and water column vertical spatial distributions (Kennicutt et. al., 1987). In addition to these dispersal differences, the more soluble components typically contain multiple functional groups and extended conjugation, which makes them more chemically and photo-chemically reactive. These same characteristics offer a method for identification via fluorescence analysis. Since they are optically sensitive, these compounds can be detected and characterized at low concentrations with the use of three-dimensional fluorescence spectra or excitation-emission matrix (EEM) fluorescence analysis (Li et al., 2004; Mason and Kerley, 1988). Because of the high sensitivity of the method, this is potentially a powerful tool for identifying these dispersed plumes at great distances from their source. However, the method is less robust in areas containing high concentrations of dissolved humic materials, such as those found along the Gulf Coast states. The fluorescence of humic materials in coastal environments can interfere with the low level signals from the dispersed oil plumes. This problem can be overcome by statistically treating the three-dimensional excitation and emission (EEM) spectra using parallel factorial analysis (PARAFAC) (Stedmon and Bro, 2008). PARAFAC statistical modeling is a supervised, multi-dimensional technique that deconvolutes the spectral
array into sets of scores and loadings that describe the data in a more condensed form. The output of these models provides components with scaled estimates of the excitation and emission maximum wavelengths that correspond to unique components in the spectral data. The identity of these components can be assigned by comparing them to the fluorescence of known poly-aromatic hydrocarbon standards, source crude oil, dispersant, and CDOM spectra. The calibration of the fluorescence intensities provides a quantification process of each of the different component sources.

This study demonstrates the use of excitation-emission fluorescence spectroscopy and PARAFAC modeling to resolve components associated with crude oil residues, dispersant and colored humic-like dissolved organic matter (CDOM).

5.2. Materials and Methods

5.2.1. Fluorescence scans of samples

Deepwater Horizon water samples were collected (Figure 5.1; Table 5.1) from CTD casts during an eight-day research cruise of NOAA Ship Gordon Gunter GU-10-02 (May 27-June 4, 2010). Samples were stored in scintillation vials (20 mL) and scanned using a fluorescence spectrophotometer (Hitachi F-4500) in excitation-emission matrix (EEM) fluorescence mode. The EEM spectra of water samples were analyzed using a fluorescence spectrophotometer (Hitachi F-4500). The scan intervals were configured at 5 nm for both excitation and emission. The slit width was set at 5 nm emission and excitation, while the scan speed was set at 1200 nm/min, with photo-multiplier tube at 700 V. The fluorescence scan wavelengths ranged from 200 to 500 nm for excitation and from 250 to 550 nm for emission. The instrument response on the excitation side was
calibrated using Rhodamine B, while the emission side was calibrated using a diffuser (Hitachi High Technologies Corporation, 2001). Excitation and emission matrices obtained were corrected for Raleigh and Raman scatter from Milli-Q water fluorescence subtraction, while the inner filter effect was corrected from the measured absorbance (Lacowicz, 1999). Fluorescence intensity of all samples was normalized using Raman peak scatter of the Milli-Q water. Absorbance was measured using a UV-Vis Spectrophotometer (Agilent 8453). All fluorescence intensities were calibrated against a standard solution of quinine sulfate monohydrate in 0.1 N H₂SO₄ measured at 350/450 nm (excitation/emission wavelength). Derived fluorescence intensities of the PARAFAC components were reported in quinine sulfate units (QSU) (Stedmon and Markager, 2005).

5.2.2. EEM spectra of reference compounds

EEM spectra of the Macondo source crude oil (Description: MS Canyon 252; Source Oil; DEP Sample ID 1280038; Lot# GU2988-A0521-09805; source: Deepwater Horizon) and the dispersant (CorexitEC9500A; SL0F1240A0, provided by Nalco Co.) were obtained. EEM of benzene, toluene and PAH standards (i.e., naphthalene, anthracene, phenanthrene, chrysene and perylene) were also determined. Macondo crude oil (0.002 mg/L) and CorexitEC9500A (23 mg/L) were initially dissolved in isopropanol (IPA) to facilitate their dissolution in seawater. IPA did not exhibit any fluorescence in seawater. All standard EEM scans obtained were blank subtracted using 0.2 nylon-filtered surface Florida Straits seawater (salinity = 36.2 psu) scans. The three main excitation-emission maxima, of benzene, toluene and the standard PAHs, were selected and are listed in Table 5.3. The relative fluorescence of benzene to naphthalene is ~ 20x.
5.2.3. GC-MS analysis

The PAH composition in the Macondo source crude oil was further determined using an Agilent 6890 Gas Chromatograph with an Agilent 5973 mass spectrometer in splitless mode (Column: Restek Rxi-5SIL MS, 30 m, 0.25 mm ID, 0.25 µm, cat# 13623). Key quantifying target ions (m/z) in combination with authentic standards were used to determine and analyze PAH concentrations in crude oil (Douglas et al., 2007). On the other hand, compounds in the Corexit EC9500A mass spectra were identified with the NIST/EPA/NIH mass spectral library rather than with authentic reference compounds. Although several PAH measurements were obtained for the GC/MS analysis, only few suspected high PAH components that were present in the GC/MS profile of the Macondo Oil were analyzed to serve as spectral reference (i.e., benzene, toluene, naphthalene, anthracene, phenanthrene, chrysene and perylene).

The GC/MS analysis followed the procedure as detailed in Douglas et al., 2007. The GC/MS was calibrated with perfluorotributylamine (PFTBA) at the beginning of each analysis. Using selected PAH groups as standards (SV Calibration Mix#5 610 PAH mix, Restek Corporation), a minimum of five point calibration was established. The analyte concentrations range from 0.1 ng/uL to 10 ng/uL.

Data was acquired using a select ion monitoring (SIM). Quantification of target compounds was performed using internal standard method using the relative response factor of the parent compound. The target compounds include only the PAHs. Since many of the alkyl PAH isomers were commercially unavailable, response factors for such compounds were based upon their parent PAH (Federal Register, 1994; Table 8.1 in Douglas et al., 2007). The internal standards used were Acenaphthylene-D10,
Phenanthrene-D10, Chrysene-D12, Perylene-D12 (Semivolatile Internal Standard Mix, Supelco, Analytical).

PAHs concentrations were calculated using the following:

\[ C_a = \frac{[(A_a/A_i) \times (Amt_i/RF_i) \times D]/V_a}{ } \]

where,

- \( C_a \) = concentration of the analyte
- \( A_a \) = area of quantification ion for target analyte
- \( A_i \) = area of quantification ion for RIS (reference internal standard)
- \( Amt_i \) = amount of RIS added to sample
- \( RF_i \) = average RF(response factor) for analyte determined from initial 5-point calibration
- \( D \) = dilution factor
- \( V_a \) = sample size

5.2.4. PARAFAC modeling

PARAFAC modeling is a multi-way fitting analysis technique that minimizes the residual of the sum of squares of the datasets employing equation 5.1. PARAFAC modeling was employed using the method detailed in Stedmon and Bro (2008).

\[ x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}, i=1, \ldots, I; j = 1, \ldots, J; k = 1, \ldots, K. \quad \text{(equation 5.1)} \]

In the equation, \( x_{ijk} \) corresponds to the fluorescence intensity of the \( i \)th sample at the emission mode (\( j \)th variable) and excitation mode (\( k \)th variable), in the PARAFAC multi-way model. The \( a_{if}, b_{jf} \) and \( c_{kf} \) are the scaled parameters that describe the sample and variable for each individual component. The residual or the variance, unexplained by the model, is represented by \( e_{ijk} \). Corrected and calibrated EEM was PARAFAC modeled using the DOMFluor toolbox, implemented in MATLAB (R2008 Student Version).
(Stedmon and Bro, 2008). The PARAFAC model was validated using the split-half validation of the seven component model that explains 99.2% of the dataset variation. A random initialization analysis step was used to further validate that the number of components identified were derived as least squares and not a local minimum. See, Stedmon and Bro (2008) for details of DOM fluorescence analysis using PARAFAC.

5.3. Results and Discussion

5.3.1. PARAFAC crude oil related components and validation of these components

There were seven components (Figure 5.2) identified by the PARAFAC model. Loadings derived from the model were compared to the known excitation-emission maximum of the PAH references (Table 5.2; Table 5.3). Fluorescence components were associated with the PAH compounds based on the modeled excitation-emission maxima of the loadings similarity from the PAH standards. Similarity of the derived loadings with the PAH standard used the term “–like” (Table 5.2) after the PAH compound name, to represent close similarity of their fluorescence peak maxima.

From the model output, components 2, 4 and 6 (Figure 5.3) were associated with the arene (i.e., substituted aromatic hydrocarbons) and naphthalene-like mixtures due to similarity in the excitation and emission maxima to that of the naphthalene and arene PAH standards (Figure 5.4; Table 5.2). Component 4 is observed to exhibit similar spectral peak of naphthalene excitation and emission at 225/330 (Figure 5.4b). We also observed that component 4 exhibits higher fluorescence loading of component 4 at 225/330 nm (Macondo source oil fluorescence maximum, Figure 5.5a). Component 4 association with the Macondo source oil was further tested by comparing its vertical
profile at station 30 (Figure 5.5d) with the vertical profile of Macondo source oil maxima at the same station (Figure 5.5c). Only the vertical distribution of component 4 exhibits a similar profile with the vertical fluorescence distribution using peak maxima of the source oil.

The fluorescence peak of components 2 showed similarity with benzene excitation/emission peak at 225/335 nm (Figure 5.4a) while component 6 is speculated to be a mixture of the benzene and naphthalene components, of unknown proportion, since it also exhibits similar spectral peaks with naphthalene and benzene (Table 5.3). The proposed assignments of components 2,4,6 indicate differences in the relative abundance of naphthalene-like and benzene-like components (Table 5.2) but does not indicate absence in any of these two fractions in the fluorescent components derived.

Another identified component was component 7, which was associated with phenanthrene (Figure 5.2; Table 5.2). This component has an excitation at 240 nm and emission at 365 nm. Phenanthrene is the third most abundant crude oil fraction after the anthracene and naphthalene groups, as identified in the Macondo source oil (Table 5.4).

The assignments of PAH compounds corresponding with each fluorescence component were further validated of their relative abundance using GC-MS. Results obtained showed naphthalene as the highest fraction in the Macondo source oil followed by anthracenes, then the phenanthrenes (Table 5.4). Since we analyzed PAH standards only, we did not confirm the presence of arene (benzene) compounds using GC-MS. However, previous work determined the presence of substituted aromatic hydrocarbon such as phenol, xylene, and ethylbenzene in the water column near the blow out site (Camili et al., 2010). In this study, aromatic hydrocarbon assignment was compared to
the fluorescence of the PAH standard instead, and collectively termed arene compounds (Table 5.3). From these comparisons, we propose the assignment of PAH compound/s for each of the fluorescence components derived in Table 5.2.

5.3.2. Subsurface distributions of derived fluorescence components

We observed that component 4 had a maximum fluorescence at the sea surface (200 QSU) in station 4 (Figure 5.6c1). Subsurface fluorescence of component 4 in the same station was observed at 100 QSU at ~400 m depth but decreased at station 5 to 50 QSU (Figure 5.6d), nearly at the same depth (~350). At 1650 m depth, component 4 fluorescence was ~120 QSU. Near to station 4, station 6, component 4 fluorescence was ~70 QSU nearly at the same depth (~1600 m). The decrease in component 4 fluorescence from station 4 to nearby stations indicates dilution of the naphthalene-like enriched component 4. The same pattern is observed for component 2 (arene-like/benzene-like enriched) and component 7 (phenanthrene-like) in station 4, but the fluorescence lowered by more than half (Figure 5.6c2). Component 2 also exhibit elevated fluorescence at the surface (~45 QSU), at 400 m (~30QSU) in station 5 (Figure 5.6d), and at 1600 m (~40QSU) (Figure 5.6c2). Component 7 (phenanthrene-like component), the third most fluorescent material after component 2 in station 4 (Figure 5.6,c1). Component 7 fluorescence (~20) is nearly the same from station 4 to station 5. Components 3 and 6 exhibited lower fluorescence at stations 4, 5 and 6, located northeast from the well.

Note that at stations 4, 5 and 6, we cannot determine the fluorescence of the previously observed plume (~1100 m) since we don’t have samples collected at this
At station 3 (north of the well), the maximum fluorescence (~78 QSU) was at ~400 m depth (Figure 5.6b), a little lower than the fluorescence of component 4 in station 4, then followed by component 2 (arene-like/benzene-enriched component) at ~20 QSU (Figure 5.6b). This suggest that the component 2 and 4 in station 3 was diluted compared to the same components in station 4. This suggests that the relative abundance of component 2 and 4 fluorescence tend to be higher southeast of the well than in the northern region stations.

At station 2, northwest from the well, component 2 and 6 showed maximum fluorescence at 200 m and 300 m, respectively. It is interesting to note that component 4 is less abundant in this station compared to stations 3, 4, 5 and 6.

At stations south of the well, maximum fluorescence of component 2 was observed at ~1100 m, particularly in stations closer to the DWH well (i.e., stations 9, 19, 29 and 30; Figure 5.6). Similarity of spectra of component 2 suggests abundance of benzene-like enriched component south and southwest of the spill. It is interesting to note that at these stations, there is a substantial decrease of component 4 relative to component 2 (>10 QSU). Component 6, on the other hand, exhibit higher fluorescence than component 2, but less fluorescent than component 2 at the ~1100 m at stations 9, 19, 29 and 30 (Figure 5.6). For component 6, we don’t know the relative abundance of the benzene-like or the naphthalene-like component (Table 5.2). Hence, we treat component 6 as a mixture of these two components.
Comparison of the component 2,4,6 and 7 across stations is difficult due to variability in the sampling depth. But based on their maximum fluorescence with depth of stations 4 and station 29, component 4 (surface: 200 QSU; 400 m: 100 QSU; 1600 m: 120 QSU) is higher in station 4 but low in station 29 (>10 QSU). Whereas, component 2 maximum fluorescence at station 4 (surface: 45 QSU; 400 m: 30 QSU; 1600 m: 40QSU) exhibited nearly similar fluorescence in station 29 (surface: 30 QSU; 580 m: 28 QSU; 1200 m: 35 QSU). In addition, since there no station northeast from the spill area, it would be impossible to determine the components fractions between the gap from the well and station 6 (plume at ~1100 as black box (Figure 5.7).

It is interesting that we observed enrichment of the naphthalene-like component southwest of the oil spill, but the benzene-like component tended to exhibit nearly the same fluorescence across stations. Although dilution may be a factor that decreased the fluorescence in the northeast stations, the gap (black box at 1100 m, Figure 5.7) from the well to these stations is unknown, but may be critical in explaining processes that cause component 4 distribution to vary across stations.

5.3.3. PAH fluorescence distribution in subsurface oil plume

We derived PARAFAC-modeled fluorescence components to monitor oil subsurface distribution. A recent study associated this plume with a deep subsurface horizontal intrusion mechanism (Zhang et al., 2011). The occurrence of this plume at ~1200 m depth was attributed to multiple subsurface intrusions that contained dissolved gas and oil along with small droplets of liquid oil (Socolofsky et al., 2011). This horizontal intrusion process may have led to the horizontal transport (Thibodeaux et al.,
This subsurface oil plume was previously reported by Camili et al., (2010). At this depth, we also observed component 2, associated with a benzene-like/arene-like enriched component. Component 2 was observed to have higher fluorescence at ~1100 m depth at stations 9, 19, 20 and 30 (Figure 5.6) which were all located south and southwest from the well. Our results showed maximum fluorescence at depth observed by other studies (Camili et al., 2010).

The fluorescence technique used here explores fluorescence technique to monitor abundance of certain groups of PAH fluorescent compounds, however, mechanisms that govern variations in the fluorescence intensities of the different fractions in the subsurface deepwater in the Gulf is unknown and that the subsequent fate and transport of these components relative to the true oil needs further study.

5.3.4. Dispersant-associated fluorescent component and its effect on crude oil dispersion

Fluorescence peak excitation and emission maxima of CorexitEC9500A in seawater showed consistent similar fluorescence maxima with component 5 (Table 5.2). This component showed a stronger contribution of the 235/415 fluorescence maximum than the 235/304 maximum. In order to further validate our assumption, composition of Coexist EC9500A was evaluated using GC-MS (Figure 5.8). Results showed that the compounds with retention time from 5.0 to 7.5 min were predominantly compound mixtures of alcohol and alkenes, while the two peaks resolved at 9.6 and 9.9 min were identified as bus(2-ethylhexyl) maleate (CAS# 142-16-5) and bus(2-ethylhexyl) fumarate (CAS# 141-02-6) (Figure 5.7). The percentage of the peaks associated with the maleate
(3%) and the fumarate (22%) fractions relative to the total area of all the peaks was considerable (Table 5.5). These compounds were ingredients of Corexit9500 as identified by the NIST/NIH/EPA/ mass spectral library. The double bond functionality of these compounds was most likely responsible for the weak fluorescence observed with the dispersant. According to Coyle (1986), double bond compounds tend to have a weak fluorescence due to the large change in the geometry between the ground state and the relaxed single state, and in the less radiation released from the decay of the single excited state. This likely explains the observed lesser fluorescence of Corexit EC9500A compared to the poly-aromatic-enriched Macondo crude oil. Based on these results, Corexit9500A fluorescent material is most associated with the 235/304 nm peak fluorescence. The other peaks may be contributions from hydrocarbon artifacts not completely separated by the model. Nonetheless, we assigned component 5 to the dispersant since it is the only component that resolved the peak fluorescence maximum of CorexitEC9500A reference in seawater.

In the water column, the dispersant-associated component tended to correspond with a lower fluorescence of component 4 and a higher fluorescence of components 2 and 6, particularly at ~1100 m depth. Component 5, attributed to fluorescence of CorexitEC9500A, also showed a high concentration at ~1100 m depth (Figure 5.6), which is consistent with recent work (Kujawinski et al., 2010). Using LC/MS/MS, Kujawinski et al. (2010) observed a high concentration of the diocetyl sodium sulfosuccinate (DOSS), with concentrations of ~12 µg/L at 1100 m depth and 4.0-8.2 µg/L at the surface. DOSS is one of the ingredients of CorexitEC9500A (Nalco Co.). As component 5 shows a vertical profile similar to the DOSS concentrations in the water
column, there is potential use of this fluorescent component as a tracer of the CorexitEC9500A dispersant. The maleate and fumarate compounds identified in the dispersant may be used as possible standards in the quantitative fluorescence-based analysis of CorexitEC9500A distribution.

At stations 9, 19, and 30, an intense fluorescence signal attributed to component 5 was observed (Figure 5.5; Table 5.2). These particular stations were proximate to the spill site. Since the dispersant was added close to the gushing oil (Figure 5.7), this could mean that component 4 decrease in fluorescence could be related to the dispersant addition. However this assumption needs further validation, to determine if indeed the dispersant played a role in the decrease of component 4 near the wellhead and the increase of component 4 northeast of the station.

**5.3.5. Humic-like associated PARAFAC modeled component**

Component 3 was observed to exhibit an excitation-emission spectral maximum at 250/410-460 nm, which is within the fluorescence range assigned for the UVA humic-like fluorophore (Coble, 2007). This fluorophore has an excitation of 250 nm and emission at 440 nm (Table 5.2), which was associated with a fluorophore derived from humic-like material (Coble, 2007). The humic-like related component (Figure 5.6) exhibits an almost similar depth fluorescence profile with the crude oil (component 2,6) and component the dispersant-associated component (component 5). The humic-like component was observed to exhibit a fluorescence maximum at a depth previously observed in other studies (Camili et al., 2010). The observed humic-like associated plume along with the crude oil may have occurred as a consequence of DOM (related to
humic substances) microbial degradation, which caused DOM fluorescence to intensify (Yamashita and Tanoue 2008; Nelson et al., 1998). Previous studies have suggested a strong link between CDOM and microbial degradation of oil. The observed increase in the microbial activity and diversity by Hazen et al. (2010) in the Deepwater Horizon plume promoted oil degradation, as indicated by the decrease of the oxygen levels (Valentine et al., 2010) and increase CDOM fluorescence in the same subsurface plume observed between 1100-1200 m (Camili et al., 2010). Results suggest that there is a relationship between the humic-like component (component 3) fluorescence increase with component 2 (associated with the benzene-like enriched component) and component 5 (associated with the dispersant-like fluorescence (Figure 5.6; Figure 5.7). More work is needed to understand how the dispersant promotes microbial degradation of the oil and the humic-like components, in order to understand the variations of these fluorescent components in the deepwater.

5.3.6. Commercial sensors vs. EEM fluorescence analysis

During the Deepwater Horizon oil spill, *in-situ* CDOM sensors were generally used for oil spill monitoring. CDOM sensors are among the sensors commonly installed in research vessels for hydrographic surveys. These sensors are sensitive to conjugated and aromatic compounds, where crude oils are enriched with these compounds. The difference of the vertical profiles of CDOM and the PAHs (Figure 5.5) showed fluorescence could distinguish these two components. Distinguishing PAHs and CDOM may cause uncertainty using commercial sensors due to the fixed wavelengths monitoring the PAH almost in the same range (Table 5.6): Wetlab CDOM sensor = 370/460, while
the crude oil sensor have an emission from 400-600 (broad range of wavelength). This was due possibly to the broad fluorescence wavelength settings of these sensors which causes overlap in chemo-sensing the individual PAH in crude oil and CDOM. This means that the presence of CDOM in environments containing PAH could give false positive fluorescence in estimating crude oil.

Commercial sensors have different ranges of fluorescence wavelengths used to detect crude oil (Table 5.6). Crude oils are composed of extremely complex numbers of hydrocarbons that have different composition depending on their sources. If the wavelengths are not optimized to target a specific crude oil (e.g., Macondo Crude oil) component, the wavelengths used to detect the distribution of the oil using commercial fluorescence-based sensors may cause significant uncertainties as previously mentioned. The Macondo crude oil tended to have a fluorescence fingerprint at an emission maxima < 400 nm, but, for example, the in-situ sensor for Turner Cyclops-7 for hydrocarbon detection operates only within the range of 400-600 nm (Table 5.6). Note that CDOM components tend to fluoresce at 400-460 nm, which represent the fluorescence of UVA humic-like component of DOM (Coble, 2007). Hence, an optimized fluorescence wavelength may be needed to target a particular crude oil and to discriminate it against other crude oil (i.e., natural oil seeps) and other interfering components, such as CDOM.

Although, a combination of all crude oil sensors had been proposed to cover a wider range of wavelengths for oil detection of the different components in seawater (Goodman, 1994), acquisition of fluorescence sensor measurements pose more uncertainty compared to the information that an excitation-emission matrix fluorescence data can generate. Due to the inherent variability of the crude oil and CDOM,
optimization based on the inherent optical property of the Macondo oil and other components using EEM and PARAFAC could substantially improve the certainty of resolving these components in the water column. The optimization method employed in this study illustrates a simpler technique that could be used to improve certainty in detecting simultaneously different crude oil components during an oil spill event, and dissociate noise signals from CDOM, especially if the oil spill extent reached areas with high river inputs.

5.3.7. Advantages of using EEM fluorescence and PARAFAC for oil spill monitoring

In terms of the methodological aspects, direct measurement of seawater without having to extract the PAH in seawater is advantageous. Exclusion of extraction minimizes volumes needed. Solvent-solvent extraction typically requires at least 1000 ml of seawater while fluorescence measurement requires a minimum of 10 ml.

In the long-term, the method applied here is important for monitoring fates of the oil spill components. This is because use of EEM combined with PARAFAC modeling can resolve and monitor simultaneously the inherent fluorescence properties of the oil, dispersant, and even interferences from terrestrial materials (i.e., CDOM contribution from river inputs and in-situ produced). Because of this methodological efficacy, it is easier to determine the oil and dispersant distribution and manage the risks posed to vulnerable marine communities. Likewise, techniques presented here would be helpful to scientists who are studying ecological effects of the spill. This fluorescence technique coupled with PARAFAC modeling is a viable and sensitive tool for simultaneously monitoring the fate of the subsurface crude oil and the dispersant residues.
5.4. Summary

The use of excitation and emission matrix (EEM) fluorescence and parallel factorial analysis (PARAFAC) modeling techniques for monitoring crude oil components in the water column were evaluated. Four of the seven derived PARAFAC loadings were associated with the Macondo crude oil components. The other three components were associated with the dispersant, an instrument artifact and colored dissolved organic matter (CDOM).

Fluorescence measurement observed abundance of naphthalene-like enriched fluorescent component northeast from the wellhead. The benzene-like/arene-like component was more abundant south and southwest near the blowout well compared to the naphthalene-like component. Mechanisms that govern this spatial variability are unknown and needs further investigation.

On the other hand, the fluorescence of the associated benzene-like component exhibited a maximum at ~1100 m at the south and southwest stations. The maximum fluorescence of the component associated with the dispersant (i.e., Corexit EC9500A) was also observed at the same depth. The plume observed at this depth was attributed to the dispersed crude oil from the Deepwater Horizon oil spill. Results demonstrate that EEM and PARAFAC can simultaneously resolve and monitor crude oil and dispersant components in the water column. Hence, the method used should be applicable for studying other oil spills.

This study only focused on the Macondo crude source oil (as reference) in developing a fluorescence-based monitoring tool for PAH after the deepwater Horizon Oil spill in the Gulf. The next step of this work is to determine the efficacy of EEM and
PARAFAC analysis in distinguishing the Macondo crude oil from other crude oils (e.g., Arabian Crude, North Slope Crude, South Louisiana Crude).
Figure 5.1. Sampling stations (X) during the NOAA Gordon Gunter cruise, from May 27-June 4, 2010, near the Deepwater Horizon Oil Spill site (O) (Map scale: 2.54 cm: 9.4 km)
Figure 5.2. Seven unique fluorescent components distinguished from the Deepwater Horizon oil spill contaminated seawater. See Table 5.1 for peak excitation-emission wavelengths.
Figure 5.3. The three components showing similar peak maxima with naphthalene and benzene.
Figure 5.4. Benzene and Naphthalene excitation/emission maximum fluorescence.
Figure 5.5. Similarity of the excitation and emission peak maximum of the a) Macondo source oil and b) component 4 occurring at 225 nm (Ex\(\lambda\)) and 330 nm (Em \(\lambda\)) and the estimated c) Macondo source reference crude oil concentration and d) component 4 fluorescence vertical profile in Station 30 during the NOAA Gunter cruise (June-May 2010). The estimated concentration of the BP Crude Oil was based on a regression model developed for measuring oil in seawater using BP reference oil as the reference standard. The root mean square error (RMSE) of the model prediction was 0.008 with an uncertainty of 0.92% from the mean calculated BP concentration mean. The adjusted R\(^2\) of the regression coefficient was 0.993 at a confidence interval of 99%.
Figure 5.6. Vertical profiles of the PARAFAC modeled components. Color bars in RFU. Yellow bar indicates depth at which high concentrations of the poly-aromatic hydrocarbons and the dispersants were previously observed (Camili et al., 2010; Kujawinski et al., 2010).
Figure 5.7. Conceptual diagram of the modeled components’ subsurface distribution during the Deepwater Oil spill (May-June 2010). The thickness of the lines corresponds to the relative fluorescence of the components at selected stations during the May-June 2010 cruise. The oil plume at ~1100 is based on previous observations (Camili et al., 2010). Black boxes and gray dashed lines correspond to the unknown fluorescence of the different components.
Figure 5.8. Total ion chromatogram of Corexit EC9500A (1.0 µg/ml). Conjugated compounds were located at retention times 9.7 and 10.0 min. Mass spectra of the primary fluorescent compounds in Corexit EC9500A are shown (peaks a and b). Structure assignments of compounds a and b were done with the NIST/EPA/NIH mass spectral library.
Figure 5.9. Macondo source crude oil (1.0 µg/ml) PAH GC/MS (SIM) Analysis. See Table 4 for the identity and concentration of the corresponding PAH compound of each peak.
Table 5.1. Location of the NOAA Gordon Gunter cruise sampling sites and their distance from the Deepwater Horizon wellhead (DH= Deepwater Horizon Spill Site).

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude</th>
<th>Longitude (W)</th>
<th>Distance from the wellhead</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH</td>
<td>28° 44.697’, N</td>
<td>88° 22.617’ W</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>28° 55.914’ N</td>
<td>88° 44.760’ W</td>
<td>40.90 km</td>
</tr>
<tr>
<td>3</td>
<td>29° 00.297’ N</td>
<td>87° 56.918’ W</td>
<td>39.80 km</td>
</tr>
<tr>
<td>4</td>
<td>28° 53.759’ N</td>
<td>88° 01.034’ W</td>
<td>38.48 km</td>
</tr>
<tr>
<td>5</td>
<td>29° 05.870’ N</td>
<td>88° 23.860’ W</td>
<td>49.74 km</td>
</tr>
<tr>
<td>6</td>
<td>28° 54.380’N</td>
<td>87° 58.900’W</td>
<td>41.45 km</td>
</tr>
<tr>
<td>9</td>
<td>28° 39.230’N</td>
<td>88° 20.740’W</td>
<td>11.69 km</td>
</tr>
<tr>
<td>17</td>
<td>28° 40.830’N</td>
<td>88° 27.220’W</td>
<td>10.00 km</td>
</tr>
<tr>
<td>19</td>
<td>28° 40.030’N</td>
<td>88° 28.01’ W</td>
<td>12.94 km</td>
</tr>
<tr>
<td>29</td>
<td>28° 41.61’ N</td>
<td>88° 26.11’ W</td>
<td>8.67 km</td>
</tr>
<tr>
<td>30</td>
<td>28° 40.702’ N</td>
<td>88° 25.506’W</td>
<td>9.42 km</td>
</tr>
</tbody>
</table>
Table 5.2. Excitation and emission fluorescence maxima of the seven PARAFAC modeled components.

<table>
<thead>
<tr>
<th>Comp</th>
<th>Ex λ</th>
<th>Em λ</th>
<th>Similar material</th>
<th>Macondo Crude/Corexit 9500 Ex/Em λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>220</td>
<td>380</td>
<td>-</td>
<td>Instrument artifact; uncertain</td>
</tr>
<tr>
<td>2</td>
<td>220, 255, 270</td>
<td>330</td>
<td>Benzene/Arene-like enriched</td>
<td>225/330-335</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Naphthalene-like</td>
<td>225/330; 270/335</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>440</td>
<td>Humic-like</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>225, 270, 280</td>
<td>340</td>
<td>Naphthalene-like enriched</td>
<td>225/335; 270/335</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Benze/Arene-like</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>235, 310</td>
<td>304, 415</td>
<td>-</td>
<td>Corexit EC9500A : 230/304</td>
</tr>
<tr>
<td>6</td>
<td>225, 280</td>
<td>340</td>
<td>Benzene-like</td>
<td>225/335; 270/335</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Arene-like</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>240</td>
<td>365</td>
<td>Phenanthrene-like</td>
<td>250/365-370</td>
</tr>
</tbody>
</table>
Table 5.3. Selected first three intense peaks on the excitation emission matrix of PAH compounds (n=3). The first peak has the highest fluorescence intensity of the PAH compound, partially dissolved in the Florida Straits surface seawater (salinity= 36.21).

<table>
<thead>
<tr>
<th>PAH compound</th>
<th>Peak</th>
<th>Excitation Peak (±5)</th>
<th>Emission Peak (±5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1</td>
<td>225</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>250</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>250</td>
<td>550</td>
</tr>
<tr>
<td>Toluene</td>
<td>1</td>
<td>260</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>260</td>
<td>560</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>225</td>
<td>415</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1</td>
<td>275</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>225</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>230</td>
<td>495</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1</td>
<td>250</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>250</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>250</td>
<td>380</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>1</td>
<td>250</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>250</td>
<td>345</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>290</td>
<td>360</td>
</tr>
<tr>
<td>Chrysene</td>
<td>1</td>
<td>245</td>
<td>385</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>230</td>
<td>690</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>255</td>
<td>385</td>
</tr>
<tr>
<td>Perylene</td>
<td>1</td>
<td>330</td>
<td>560</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>225</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>235</td>
<td>380</td>
</tr>
</tbody>
</table>
Table 5.4. Macondo source crude oil GC/MS/SIM selected PAH composition and concentration.

<table>
<thead>
<tr>
<th></th>
<th>Response</th>
<th>RT</th>
<th>ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene-D8</td>
<td>3103687</td>
<td>5.295</td>
<td>1000</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2577656</td>
<td>5.314</td>
<td>295.84</td>
</tr>
<tr>
<td>2-Methyl Naphthalene</td>
<td>2632445</td>
<td>5.972</td>
<td>628.06</td>
</tr>
<tr>
<td>1-Methyl Naphthalene</td>
<td>1661649</td>
<td>6.069</td>
<td>410.41</td>
</tr>
<tr>
<td>C2-Naphthalenes</td>
<td>1040583</td>
<td>6.682</td>
<td>119.48</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>132087</td>
<td>6.681</td>
<td>18.93</td>
</tr>
<tr>
<td>Acenaphthene-D10</td>
<td>1382646</td>
<td>6.936</td>
<td>410.84</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>63390</td>
<td>6.936</td>
<td>77.23</td>
</tr>
<tr>
<td>C3-Naphthalenes</td>
<td>463180</td>
<td>7.305</td>
<td>119.22</td>
</tr>
<tr>
<td>Fluorene</td>
<td>274350</td>
<td>7.528</td>
<td>139.86</td>
</tr>
<tr>
<td>C4-Naphthalenes</td>
<td>71348</td>
<td>8.099</td>
<td>142.58</td>
</tr>
<tr>
<td>C1-Fluorene</td>
<td>279702</td>
<td>8.455</td>
<td>1000</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>149477</td>
<td>8.477</td>
<td>245.34</td>
</tr>
<tr>
<td>Phenanthrene-D10</td>
<td>1382646</td>
<td>8.477</td>
<td>290.23</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>739711</td>
<td>8.477</td>
<td>18.38</td>
</tr>
<tr>
<td>Anthracene</td>
<td>739711</td>
<td>8.477</td>
<td>18.38</td>
</tr>
<tr>
<td>C2-Fluorene</td>
<td>203577</td>
<td>8.623</td>
<td>58.99</td>
</tr>
<tr>
<td>C1-Dibenzothiophenes</td>
<td>100748</td>
<td>8.867</td>
<td>20.22</td>
</tr>
<tr>
<td>C1-Phenanthernes/Anthracenes</td>
<td>507504</td>
<td>9.059</td>
<td>204.72</td>
</tr>
<tr>
<td>C3-Fluorene</td>
<td>560227</td>
<td>9.069</td>
<td>162.83</td>
</tr>
<tr>
<td>C2-Dibenzothiophenes</td>
<td>154857</td>
<td>9.279</td>
<td>31.08</td>
</tr>
<tr>
<td>C2-Phenanthernes/Anthracenes</td>
<td>1402839</td>
<td>9.502</td>
<td>565.9</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>34831</td>
<td>9.663</td>
<td>12.17</td>
</tr>
<tr>
<td>C3-Dibenzothiophenes</td>
<td>41166</td>
<td>9.821</td>
<td>8.26</td>
</tr>
<tr>
<td>Pyrene</td>
<td>41019</td>
<td>9.885</td>
<td>14.16</td>
</tr>
<tr>
<td>C3-Phenantherene/Anthracene</td>
<td>222782</td>
<td>9.952</td>
<td>91.89</td>
</tr>
<tr>
<td>C4-Dibenzothiophenes</td>
<td>221809</td>
<td>10.215</td>
<td>44.52</td>
</tr>
<tr>
<td>C1-Fluoranthenes/Pyrenes</td>
<td>36043</td>
<td>10.413</td>
<td>12.57</td>
</tr>
<tr>
<td>C4-Phenantherenes/Anthracenes</td>
<td>58646</td>
<td>10.665</td>
<td>29.11</td>
</tr>
<tr>
<td>C2-Fluoranthenes/Pyrenes</td>
<td>83474</td>
<td>10.665</td>
<td>29.11</td>
</tr>
<tr>
<td>Naphthobenzothiophene</td>
<td>49431</td>
<td>10.876</td>
<td>9.92</td>
</tr>
<tr>
<td>C1-Dibenzothiophenes</td>
<td>152848</td>
<td>11.200</td>
<td>51.73</td>
</tr>
<tr>
<td>C3-Phenantherene/Anthracene</td>
<td>152913</td>
<td>11.199</td>
<td>55.2</td>
</tr>
<tr>
<td>Chrysene-D12</td>
<td>2646775</td>
<td>11.172</td>
<td>1000</td>
</tr>
<tr>
<td>Chrysene</td>
<td>152913</td>
<td>11.199</td>
<td>55.2</td>
</tr>
<tr>
<td>C3-Fluoranthenes/Pyrenes</td>
<td>397884</td>
<td>11.246</td>
<td>127.41</td>
</tr>
<tr>
<td>C1-Naphthobenzothioiophenes</td>
<td>38137</td>
<td>11.286</td>
<td>7.03</td>
</tr>
<tr>
<td>C4-Fluoranthenes/Pyrenes</td>
<td>56861</td>
<td>11.688</td>
<td>18.21</td>
</tr>
<tr>
<td>C1-Chryenes</td>
<td>72172</td>
<td>11.731</td>
<td>25.97</td>
</tr>
<tr>
<td>C2-Naphthobenzothioiophenes</td>
<td>31680</td>
<td>11.967</td>
<td>5.84</td>
</tr>
<tr>
<td>C2-Chryenes</td>
<td>85445</td>
<td>12.311</td>
<td>30.75</td>
</tr>
<tr>
<td>C3-Naphthobenzothioiophenes</td>
<td>35290</td>
<td>12.587</td>
<td>6.5</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>19833</td>
<td>12.627</td>
<td>6.18</td>
</tr>
<tr>
<td>Benzo[j,k]fluoranthene</td>
<td>30134</td>
<td>12.627</td>
<td>9.32</td>
</tr>
<tr>
<td>Perylene</td>
<td>47151</td>
<td>13.056</td>
<td>16.79</td>
</tr>
<tr>
<td>C3-Chryenes</td>
<td>217459</td>
<td>12.951</td>
<td>78.25</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>46947</td>
<td>13.056</td>
<td>15.11</td>
</tr>
<tr>
<td>C4-Naphthobenzothioiophenes</td>
<td>20850</td>
<td>13.042</td>
<td>3.84</td>
</tr>
<tr>
<td>Perylene-D12</td>
<td>3015373</td>
<td>13.232</td>
<td>1000</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>47714</td>
<td>13.056</td>
<td>15.85</td>
</tr>
<tr>
<td>C4-Chryenes</td>
<td>86473</td>
<td>13.598</td>
<td>29.30</td>
</tr>
<tr>
<td>Indeno[1,2,3-c,d]pyrene</td>
<td>16410</td>
<td>15.091</td>
<td>4.52</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>15922</td>
<td>15.121</td>
<td>4.77</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>15599</td>
<td>15.579</td>
<td>4.58</td>
</tr>
</tbody>
</table>
Table 5.5. Percentage of each integrated peak area relative to total peak area of Corexit EC9500A (See chromatogram in Figure 5.8.)

<table>
<thead>
<tr>
<th>Peak #</th>
<th>R.T. Min</th>
<th>Peak Height</th>
<th>Corr Area</th>
<th>Corr % Max</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.306</td>
<td>120333</td>
<td>833457</td>
<td>1.09</td>
<td>0.237</td>
</tr>
<tr>
<td>2</td>
<td>5.409</td>
<td>215204</td>
<td>2826837</td>
<td>3.7</td>
<td>0.804</td>
</tr>
<tr>
<td>3</td>
<td>5.426</td>
<td>157767</td>
<td>1880433</td>
<td>2.46</td>
<td>0.535</td>
</tr>
<tr>
<td>4</td>
<td>5.511</td>
<td>4221789</td>
<td>65256101</td>
<td>85.47</td>
<td>18.571</td>
</tr>
<tr>
<td>5</td>
<td>5.54</td>
<td>4498150</td>
<td>62016127</td>
<td>81.23</td>
<td>17.649</td>
</tr>
<tr>
<td>6</td>
<td>5.631</td>
<td>804951</td>
<td>12302920</td>
<td>16.11</td>
<td>3.501</td>
</tr>
<tr>
<td>7</td>
<td>5.666</td>
<td>400710</td>
<td>3553028</td>
<td>4.65</td>
<td>1.011</td>
</tr>
<tr>
<td>8</td>
<td>5.689</td>
<td>628486</td>
<td>6308102</td>
<td>8.26</td>
<td>1.795</td>
</tr>
<tr>
<td>9</td>
<td>5.752</td>
<td>369362</td>
<td>3485972</td>
<td>4.57</td>
<td>0.992</td>
</tr>
<tr>
<td>10</td>
<td>5.78</td>
<td>416325</td>
<td>4141457</td>
<td>5.42</td>
<td>1.179</td>
</tr>
<tr>
<td>11</td>
<td>5.803</td>
<td>208676</td>
<td>3032714</td>
<td>3.97</td>
<td>0.863</td>
</tr>
<tr>
<td>12</td>
<td>5.854</td>
<td>210743</td>
<td>2002290</td>
<td>2.62</td>
<td>0.57</td>
</tr>
<tr>
<td>13</td>
<td>5.877</td>
<td>197027</td>
<td>5912496</td>
<td>7.74</td>
<td>1.683</td>
</tr>
<tr>
<td>14</td>
<td>5.98</td>
<td>412220</td>
<td>8676865</td>
<td>11.36</td>
<td>2.469</td>
</tr>
<tr>
<td>15</td>
<td>6.06</td>
<td>437467</td>
<td>4668637</td>
<td>6.11</td>
<td>1.329</td>
</tr>
<tr>
<td>16</td>
<td>6.089</td>
<td>1506712</td>
<td>13876567</td>
<td>18.17</td>
<td>3.949</td>
</tr>
<tr>
<td>17</td>
<td>6.123</td>
<td>326608</td>
<td>3340144</td>
<td>4.37</td>
<td>0.951</td>
</tr>
<tr>
<td>18</td>
<td>6.152</td>
<td>552797</td>
<td>9448634</td>
<td>12.38</td>
<td>2.689</td>
</tr>
<tr>
<td>19</td>
<td>6.192</td>
<td>488441</td>
<td>7110865</td>
<td>9.31</td>
<td>2.024</td>
</tr>
<tr>
<td>20</td>
<td>6.214</td>
<td>587089</td>
<td>7791493</td>
<td>10.2</td>
<td>2.217</td>
</tr>
<tr>
<td>21</td>
<td>6.237</td>
<td>408584</td>
<td>3756508</td>
<td>4.92</td>
<td>1.069</td>
</tr>
<tr>
<td>22</td>
<td>6.272</td>
<td>547006</td>
<td>9781360</td>
<td>12.81</td>
<td>2.784</td>
</tr>
<tr>
<td>23</td>
<td>6.317</td>
<td>602137</td>
<td>6246220</td>
<td>8.18</td>
<td>1.778</td>
</tr>
<tr>
<td>24</td>
<td>6.352</td>
<td>353920</td>
<td>4984346</td>
<td>6.53</td>
<td>1.418</td>
</tr>
<tr>
<td>25</td>
<td>6.375</td>
<td>321062</td>
<td>5573310</td>
<td>7.3</td>
<td>1.586</td>
</tr>
<tr>
<td>26</td>
<td>6.46</td>
<td>259972</td>
<td>5042463</td>
<td>6.6</td>
<td>1.435</td>
</tr>
<tr>
<td>27</td>
<td>6.655</td>
<td>123586</td>
<td>1096807</td>
<td>1.44</td>
<td>0.312</td>
</tr>
<tr>
<td>28</td>
<td>9.701</td>
<td>1120991</td>
<td>10093639</td>
<td>13.22</td>
<td>2.872</td>
</tr>
<tr>
<td>29</td>
<td>10.004</td>
<td>588244</td>
<td>76350167</td>
<td>100</td>
<td>21.728</td>
</tr>
</tbody>
</table>
Table 5.6. Some of the available commercial *in-situ* sensors that estimate CDOM and crude oil.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Application</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelsea AQUA-Track</td>
<td>Hydrocarbon detection</td>
<td>EX $\lambda = 239$ nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM $\lambda = 360$ nm</td>
</tr>
<tr>
<td>Turner Cyclops-7 Crude Oil sensor</td>
<td>Hydrocarbon detection</td>
<td>EX $\lambda = 300$ to $400$ nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM $\lambda = 400$ to $600$ nm</td>
</tr>
<tr>
<td>WetLabs WetStar CDOM sensor</td>
<td>CDOM detection</td>
<td>EX $\lambda = 370$ nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM $\lambda = 460$ nm</td>
</tr>
<tr>
<td>Seapoint UV Fluorometer</td>
<td>CDOM detection</td>
<td>EX $\lambda = 239$ nm,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM $\lambda = 360$ nm</td>
</tr>
</tbody>
</table>
Chapter 6

Conclusion and Recommendation

Results from this study using EEM and PARAFAC methods demonstrated advances in several aspects of monitoring the distribution of DOM on the continental shelf of Florida. Methods resolved individual components of the bulk DOM nearshore and offshore using a derived 5-component PARAFAC model. Each component’s variability could be related to the biological, chemical, and physical processes that control the DOM variability on the shelf during May 2008, October 2009, January 2009 and May 2009. We found that the variation of the humic-like (HLC1 and HLC2) and protein-like (TLC4 and TLC5) components on the West Florida shelf were related to precipitation, river inputs and mixing. The observed elevated humic-like fluorescence of these components in high salinity water on the shelf were related to other mechanisms such as sediment suspension, in-situ production and evaporation. In high salinity water, a corresponding elevated protein-like fluorescence component was observed.

EEM and PARAFAC methods were also applied further evaluated in harmful algal bloom and oil spill studies. The EEM and PARAFAC model used to study the October 2009 K. brevis bloom resolved five DOM fluorescence components. We observed a positive relationship between K. brevis cell counts and the humic-like and the protein-like components derived from the PARAFAC model. We speculate that protein-like associated component may have served as the organic nitrogen source for the bloom formation, while the terrestrial refractory DOM may serve to reduce light intensity, since K. brevis thrive in light-sheltered environments. Thus, application of EEM and
PARAFAC, for the first time in HAB studies, provided information on how the DOM may influence harmful algal bloom formation.

In another study, I used a single terrestrial fluorophore component to define the relationship between brevetoxin concentration and amount of terrestrial DOM. We observed that the brevetoxins (PbTx-2 and PbTx-3) concentration in sediments were variable. Further study is needed to understand the role of organic matter enrichment of sediments on the fate and deposition mechanisms of these toxins.

The application of EEM and PARAFAC also demonstrate applicability to oil spill research. Four of the seven components derived PARAFAC model was associated with the Macondo crude oil components based on the derived 7-component PARAFAC model. The other three components were associated with the dispersant (i.e., Corexit9500), an instrument artifact and colored dissolved organic matter (CDOM). Measurement of vertical distribution of fluorescence of the associated benzene and naphthalene-like components of crude oil in the Gulf of Mexico located a maximum at ~1200 m. The maximum fluorescence of the component associated with the dispersant (i.e., Corexit EC9500A) was observed at the same depth. The plume observed at this depth was attributed to the dispersed crude oil from the Deepwater Horizon oil spill. Results demonstrate that EEM and PARAFAC can resolve and monitor fluorophores that can be associated with selected crude oil (i.e., benzene-like, naphthalene-like and phenanthrene-like) and dispersant components in the water column. While there are still uncertainties involve in this work due to the inherent complexity of PAHs in crude oils, further study is recommended to determine the efficacy of the method in distinguishing the Macondo crude oil fluorescent components relative to other crude oils.
In conclusion, the application of EEM and PARAFAC is a viable technique to resolve DOM fluorescence components. The technique can be used to obtain more chemical clues in the aquatic environments on the complexity of DOM and crude oil components from near-shore to offshore waters since these methods can simplify distribution of DOM and crude oil fluorescence.

Other relevant work

Research also is needed to identify factors that contribute to DOM variability on the shelf, including refining sources and degradation products of DOM with application of EEM and PARAFAC methods. Of particular interest is the fate of the DOM in the marine environment, to further understand the DOM distribution from nearshore to the SW Florida continental shelf. Two initial studies had been done to further account for other factors that could influence DOM variation on the shelf. One is photodegradation and the other one is the different sources of DOM.

In Appendix A, this study used the EEM and PARAFAC to resolve DOM fluorescence components affected by photodegradation on the continental shelf of Florida. However, results obtained on the DOM fluorescence degradation of water collected from four sites on the Shelf did not exhibit a clear trend, including the DOC relationship with degradation. It cannot be determined if the cause of this variation is the length of the photodegradation experiment (i.e., 30 days), the uncontrolled solar light, or the exposure of the sample to the atmosphere during sampling. The photolysis research
needs further work. It is recommended that the photolysis experiment should last for 24 hours of solar exposure, with replicates, to minimize effects of light intensity variation.

In, Appendix B, this study used EEM and PARAFAC to develop and refine specific DOM sources signatures by resolving spectral wavelengths of the fluorophores derived from point DOM sources on the Florida Shelf. The study presented in Appendix B is a trial experiment to characterize DOM from known sources and determine if EEM and PARAFAC can resolve specific DOM point sources from marine, terrestrial and atmosphere. Initial results indicate that multiple wavelengths (i.e., three to four fluorophores) may have to be used to discriminate different point sources. This work is still in progress and need significant improvement, in terms of data organization and interpretation.
References


Hemmer, M.J., Barron, M.G., Greene, R.M., 2011. Comparative toxicity of eight oil dispersants, Louisiana sweet crude oil (Lsc), and chemically dispersed Lsc to two aquatic test species. Environmental Toxicology and Chemistry 30(10), 2244-2252.


Judson, R.S. et al., 2010. Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. Environmental Science and Technology 44(15), 5979-5985.


Appendix A

The prediction of photolytic carbon loss using dissolved organic matter fluorescence and multiple linear regression

A1. Rationale

Colored dissolved organic matter (CDOM) is a fraction of the bulk DOM that absorbs or fluoresces over a broad range of ultraviolet and visible wavelengths. The major fraction of this material is associated with humic substances (humic and fulvic acids), also known as Gelbstoff, a yellow acidic portion of organic matter in seawater (Zafiriou et al., 1984), typically leached from terrestrial soils and transported to the marine environment through rivers; thereby making it an effective riverine input tracer (Coble 1996). The optical properties of CDOM can vary depending on its production source, transformation during transport from freshwater to marine transition waters, and removal mechanisms through biological and photochemical degradation. Distribution and dynamics of CDOM distribution plays a crucial role in the biogeochemical cycling in aquatic environments in regulating water properties, biological processes and trace elements distributions (Miller and Moran, 1997; Zafiriou et. al., 1984).

The photodegradation of DOM from solar exposure in aquatic environment generates products important to biological and aquatic processes (Mopper and Kieber, 2002). The decrease in CDOM from solar exposure results in molecular weight reduction (Lou and Xie, 2006), photochemical production of inorganic nutrients, biologically labile organic substrates (Miller and Zepp, 1995; Miller et al., 2002; Vähätalo et al., 2004;
Vähätalo and Zepp, 2005) and other volatile organic carbon and reactive species (Clark and Zika, 2000). As such, photodegradation could influence terrestrial DOM degradation and removal rates in coastal-shelf environments and could have important consequences for biological productivity in marine ecosystems (Moran and Zepp, 1997) and in marine carbon cycling.

In order to quantify and monitor degradation rates of DOM fluorescence components from solar exposure, excitation-emission matrix (EEM) was coupled with parallel factorial analysis (PARAFAC). PARAFAC is a statistical modeling technique recently used to decompose three-dimensional array datasets that provide both qualitative and quantitative model outputs which separate complex signals into their individual fluorescent components (Stedmon and Bro, 2008; Brereton, 2003). PARAFAC decomposition can resolve overlapping fluorophores in a DOM fluorescence spectrum (Stedmon et al., 2003). Combination of EEM and PARAFAC can provide a more comprehensive picture of DOM fluorescence spectral properties, which could correspond to terrestrial, marine and anthropogenic sources based on their excitation-emission maxima. Moreover, the EEM method can be used to monitor DOM fluorescence changes due to photochemical, biological and physical processes (Coble, 2007). This technique has been used in assessing DOM dynamics in freshwater (Hudson et al., 2007), estuaries (Stedmon and Markeger 2005), and coastal environments (Yamashita et al., 2010; Yamashita et al., 2008; Boehme et al., 2004; Stedmon et. al., 2003; Sierra et al., 2005; Parlanti et al., 2000).

Estimation of the corresponding carbon (DOC) loss after photochemical degradation can be associated with a decrease of the DOM fluorescence components
(Moran et al., 2000). However, the weak relationship between DOC and DOM fluorescence in coastal and shelf environments showed a low (Vodacek et al., 1997) and oftentimes non-linear (Chen et al., 2002) correlation due to variations caused by DOM sources, seasonality, photobleaching and bacterial degradation. Improving the estimation of DOC is necessary before photolytic loss of carbon can be estimated using an optical method.

Thus, this study investigates the effect of solar light on the degradation of various DOM colored components using (EEM) fluorescence analysis and PARAFAC. This optical approach provides a better estimate of the DOC removed through an equation that improves the DOC-DOM fluorescence linearity.

A2. Methods

A2.1 Description of sampling area

Southwest (SW) Florida Shelf (Figure A1) is a region with a broad and a gentle slope with 100-m isobaths located 150-200 km offshore (He and Weisberg, 2003). The circulation pattern of the shallow environment of the Florida Shelf makes it susceptible to variations coming from winds, wave currents and water mixing. These variations can be influenced by the Gulf of Mexico Loop Current that enters through the Yucatan Strait, and exits at the Gulf Stream, through the Straits of Florida (He and Weisberg, 2003). On the western side is the Gulf of Mexico, while on the south, the Florida Keys are situated, and the Florida Bay is located on the north-eastern side.

The SW Florida Shelf is located in a subtropical region, with the climate characterized by a relatively wet season from May to October and dry season from
November to April. The majority of river runoff comes from the Shark River, Ten thousand Islands, Caloosahatchee River, and Peace River. Projects that converted the Everglades to conserve water, drainage systems and agricultural development areas affected the natural flow of these river systems (Reedy and DeLaune, 2008). These activities resulted in increase nutrient transport unto the shelf (Brand and Compton, 2007). The net result of this has been major environmental impacts, such as harmful algal blooms (Vargo et al., 2008; Tester and Steidinger, 1997) that have caused major health (Fleming et al., 2005; Kirkpatrick et al., 2004) and environmental problems (Walsh et al., 2006). Thus, four sites (Figure A1) were selected for sample collection of DOM materials across the shelf. Two sampling areas were close to river mouths with high DOM load: Station CH2 (near Charlotte Harbor and Caloosahatchee River-higher contribution from anthropogenic DOM sources), and Station 59 (water outflow coming from Shark River). The third one is in Station 30 (mid-shelf, oligotrophic, shallow water) and the fourth one in Station DT9 (outer-shelf, oligotrophic, deep water). These sites provided information on the reactivities of the DOM reaching these distances from land.

A2.2. Sampling and Photo-degradation experiment

Sampling was conducted during the South Florida Quarterly Hydrographic Cruise across the Florida Shelf (Figure A1) conducted by NOAA/AOML/South Florida Program (21-25 January 2009) aboard the R/V Walton Smith (RSMAS-University of Miami). The ship utilized an underway flow-through system equipped with a SEABIRD thermosalinograph, monitoring temperature and salinity (Table A1). This system was used to collect 20 L of seawater in acid washed glass (~ 500 oC, 20 h) containers at four sites (Table A1). Samples were filtered immediately with 47 mm, 0.2 µm Nylon (Gelman
Sciences) filters using a glass Millipore filtration system to eliminate microbial effects on the photodegradation experiments. Filtered samples were maintained at 5°C in the dark prior to photo-degradation experiments. Two hundred (200) ml was placed in a stoppered quartz flask with three replicates per station. The quartz containers were submerged in a flowing water bath, just below the surface to prevent temperature effects from direct heating of the container during solar exposure. The water temperature (at 24 ± 1.1°C) was monitored daily for thirty days. A set of controls were covered with a black plastic material to prevent light penetration to the water samples. Before the experiment commenced, water samples were preconditioned (dark) in the water bath for a day. Collected samples (20 ml) for FDOM analyses were placed in pre-combusted vials (500°C, 20 h), and stored at 5°C. Subsamples were collected on the fourth day after each prior sampling. The collected 60 ml samples for DOC analysis, were placed in acid washed Nalgene bottles, and stored until analysis. The solar exposure experiments were conducted on the RSMAS-University of Miami SLAB rooftop. Solar intensity for PAR and UV was measured using sensors, for collected photosynthetic active radiation (PAR) and UV data. The UV and solar radiation sensors have ranges of 290-390 nm UV range and 300-1100 nm, respectively, which were both included in the weather station package (Davis Model, Vantage Pro2). Solar radiation was integrated for the sample exposure period (Figure A2). There were six sampling periods during the 30 day photodegradation experiment. Each sampling consists of four solar degraded samples and four dark-unexposed samples, with a total of 48 samples from the entire photodegradation experiment. These 48 samples were analyzed and used in the modelling experiment and in the multiple linear regression analysis of fluorescence analysis for DOC estimation.
**A2.3. EEM Spectral measurements**

Samples were acclimated to room temperature before measurement. From the three replicated samples per station during exposure, one replicate was randomly selected for the EEM measurement. The measurement of the (EEM) was carried out using a fluorescence spectrophotometer (Hitachi F-4500). The instrument configuration was calibrated using the manufacturer’s settings (Hitachi F-4500 Manual). The EEMs were obtained by scanning from 200-500 nm for excitation and 200-700 nm for emission at 5 nm intervals (with both 5 nm emission and excitation slit widths) and a scan speed of 1200 nm/min with a PMT at 700 V. Correction were made for inner filter effects that could result in fluorescence intensity attenuation due to absorption of the incident light or absorption of the emitted light (McKnight et al., 2001; Lacowicz, 2006). Absorbance was measured using the UV-Vis Spectrophotometer (Agilent 8453). Then, EEMs were corrected by subtraction with Milli-Q water spectral response. Fluorescence intensities were calibrated against the quinine sulfate monohydrate in 0.1 N H$_2$SO$_4$, with a 0.9996 regression coefficient at an Ex/Em wavelength =350/450 nm.

**A2.4. PARAFAC modeling**

EEMs of DOM obtained are multi-way (three way) where fluorescence measured varies with recorded emission and the excitation wavelength. PARAFAC is modeled using a multi-way data analysis fitting to minimize the sum of squares of the residuals (Stedmon and Bro, 2008) using equation A1.

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}, \ i=1, \ldots, I; \ j=1, \ldots, J; \ k=1, \ldots, K. \quad \text{(equation A1)}$$
where $x_{ijk}$ refers to the fluorescence intensity of the $i$th sample at the $j$th variable (emission mode) and at the $k$th variable (excitation mode) in the PARAFAC DOM-fluorescent component model. The $a_{ijf}$, $b_{jif}$ and $c_{jif}$ are the scaled parameters that describe the samples and variables for each individual components while $e_{ijk}$ is the sum of the square of the residual, representing the unexplained variance not captured by the model. Corrected EEM datasets were modeled by PARAFAC using the DOMFluor toolbox run in the R2008 Student Version of MATLAB (Stedmon and Bro 2008). The EEM excitation and emission wavelengths used were reduced to 250-450 nm and 300-520 nm (both at 5 nm intervals), respectively, to minimize random noise outside the spectral range of interest: Excitation wavelengths below 250 nm were removed due to high residuals influenced possibly by the xenon light source. Data emission wavelengths > 520 nm were removed due to the Rayleigh and Raman scatter and secondary protein fluorescence (Smilde et al., 2004; Murphy et al., 2008). The data array used consisted of 48 samples with 47 emission wavelengths and 41 excitation wavelengths. The PARAFAC model was fitted to the array split into halves (consisting of a calibration and a validation array) sequentially from three to ten components. The model ranked components identified were facilitated by comparison of the calibration and validation arrays to the excitation and emission spectra of the components. The optimized number of DOM fluorophore components was validated by the split-half technique and were further validated by random initialization steps, which ensures results are based on least squares and not a local minimum (Stedmon and Bro, 2008). The fluorescence intensities
of the PARAFAC components were reported in quinine sulfate units (QSU) (Coble, 1996).

**A2.5. DOC analysis**

Dissolved organic carbon (DOC) was measured as triplicates per sampling period during photodegradation from each collection site. Photodegraded water samples were analyzed for DOC using a Shimadzu TOC-V/TN system employing a high temperature catalytic oxidation method (catalytically aided with Pt) generating carbon dioxide and nitrogen monoxide. Carbon dioxide was measured using a non-dispersive infra-red detector (Shimadzu TOC-V Series Manual). Standardization with potassium acid phthalate was verified using a consensus reference material seawater from Dennis Hansell’s Lab (RSMAS-University of Miami). Also, in every sixth analysis, the system was systematically checked with both deep and surface water reference waters. The precision in the DOC measurements was ~1-2 µmol C L⁻¹, similarly detailed in the work of Mathis et al. (2008).

**A2.6. Estimation of DOC-FDOM relationship using multiple linear regression (MLR)**

We observed that the change in DOM fluorescence was not entirely dependent on the DOC content of the water samples in this experiment. In order to account molecular variation from photochemical effects, we developed multiple linear regression statistics (Brereton, 2003) that improved estimating dependency of DOC concentration with DOM fluorescence. This method pre-selects only the fluorescent component that provides high correlation with DOC and was identified as a humic material (Figure A4). In this case, we used components 1 and 2 with correlation coefficient of $R^2 = 0.74$ and $R^2 = 0.82$. 
respectively. Component 3 was not included since it displayed a negative effect on DOC concentration in the 3 components-MLR model. The MLR analysis was performed using SIGMASTAT version 3.5 (Systat Software, Inc, 2006).

\[
[\text{DOC}] = 35.582 + 2.188 \times F.I.\text{maxComp1} + 3.736 \times F.I.\text{maxComp2} \quad \text{(equation 2)}
\]

where \(F.I.\text{maxcomp1}\) and \(F.I.\text{maxcomp2}\) refers to the fluorescence intensity of component 1 and component 2, respectively. Prediction error of the model was calculated by determining the sum of the residuals between the true and predicted concentrations; thus, the larger the error, the worse the prediction (Figure 5).

A3. Results and Discussion

The CDOM composition in the SW Florida Shelf has been described to be dominated by terrestrial DOM components influenced by river inputs from Caloosahatchee and Shark Rivers (Stanenau and Zika 2004; Zanardi-Lamardo et al., 2004). Its optical properties and carbon content on the southwest Florida shelf could vary with DOM source, hydrology, geomorphology (Jaffe et al., 2004), molar mass distributions (Zanardi-Lamardo et al., 2004), and photochemical and biological transformations (Clark et al., 2002). Photodegradation of CDOM collected near the river mouth, mid-shelf and near the continental margin resulted in the four different DOM fluorescence components having different photochemical reactivities.

A3.1. Qualitative identification of DOM fluorescent components

Output of the four component PARAFAC model (Figure A3) was verified by the split-half and random initialization analyses and explained 99.7% of the variance from 48
EEMs on non-negativity mode. Four fluorescent PARAFAC modeled components were distinctly identified from EEMs of photo-degraded water samples collected from stations 56 (near Shark River mouth), station CH2 (near Charlotte Harbor), station 30 (mid-shelf) and station DT9 (near Dry Tortugas). These samples were exposed to diurnal solar radiation, with day and night periodicity (Figure A2). Spectral loadings of the PARAFAC DOM fluorescence (Figure A3, a-d) were similar to reported components (Stedmon et al., 2003; Cory and McKnight, 2005; Yamashita et al. 2008). Fluorescence excitation and emission maximum loadings for each component are listed in Table A2, with fluorophore type and possible sources as described from other studies. The four components (Figure A3, Table A2) were identified as a UVA humic like component (A) for component 1, occurring at peak excitation and emission pair at <250 and 460 nm; UVA humic-like (A) for component 2, with a weak maximum peak band occurring at peak excitation and emission pair of <250 and 400 nm excitation; a combined humic and marine humic (A, M), for component 3, occurring at peak excitation and emission pair at <250 and 405 nm, with secondary excitation at 310 nm; and a tryptophan-like (T) component occurring at peak excitation and emission pair 275 and 340 nm.

The corresponding initial fluorescence intensity of the terrestrial components (mean fluorescence of component 1 and 2 is highest at station 56, a location close to Shark River water discharge, while station DT9 (near Dry Tortugas) has the least fluorescence (Table A3). A general decrease in DOM fluorescence in component 1 and 2 after photo-irradiation is apparent in most sites (Table A3). It also appears that there is a degradation trend of DOM collected from near-shore DOM fluorescence samples to the outer-shelf. Photo-degraded sample fluorescence intensity from station 56, exhibit a
similar initial fluorescence from sample collected in station CH2. Similarly, the same
trend is exhibited on the fluorescence intensity of DOM collected from other sites.

In general, the fluorescence intensities of four fluorescent components decreased
after solar radiation. The photobleaching magnitudes were different among samples as
well as the DOM components (Table A3). However, there are cases that with solar
exposure, apparent positive increase in fluorescence of DOM is observed. This is
exhibited by component 4 fluorophore (+3.04), a protein-like component (Table A2),
from station 56 (near Shark River) and to a lesser magnitude (+1.09) for component 2
collected from station 30. Positive production of component 4 which exhibit similar
fluorescence property with a tryptophan-like component (Table A2), indicate
photochemical production of labile LMW organic -nitrogen-containing materials near
Shark River. LMW nitrogen production, including inorganic by-products, has been
demonstrated to be produced from solar degradation (Zika 1981; Bushaw et al., 1996).
The organic nitrogen could have been produced or transported from the water draining
from the Everglades marshes, described to have high concentrations of dissolved of DOC
and DON (Qualls and Richardson 2003). On the other hand, the photoproduction in the
mid-shelf of comp 2 has similar properties to one of the photochemical by-products
(Murphy et al., 2006) of a terrestrial DOM component. This component could be one of
the photo-products of low molecular labile components (Moran and Zepp 1997; Osburn
et al., 2001; Vahatalo et al., 2003).
A3.2. Behavior of PARAFAC modeled fluorescent DOM components during photodegradation

The four components derived have different degradation rates across the shelf. Component 1 derived from terrestrial sources appears to have low degradation rate from samples near Shark River mouth and near the continental margin. Degradation tends to be significant in the mid-shelf region and less significant near Charlotte Harbor. Component 2 is derived from anthropogenic sources. This highly reactive component is highest in samples from Charlotte harbor followed by the samples from the Dry Tortugas and Shark River. In the mid-shelf region, however, there seems to be a production of component 2. Component 3 is a mixture of both terrestrial and marine components. It exhibits a similar reactivity relative to component 1 but to a lesser degree. Component 4 appears to be a protein material resulting from production in Shark River samples after photodegradation. Samples from Caloosahatchee River and Dry Tortugas are relatively unreactive. In the mid-shelf region however, the protein-like component exhibits a significant loss of fluorescence.

Terrestrial DOM that is distant from a land source is chemically transformed and shows a reduction in fluorescence intensity that appears unrelated to DOC content. Effect of UV radiation was responsible for 75% and 44% in the decomposition of CDOM at the surface and in the whole water column (optical model based on quantum yield), respectively (Vahatalo and Wetzel, 2004).

It appears that the DOM on the SW Florida Shelf is a mixture of fluorescent compounds susceptible to different photodegradation rates. These terrestrial materials
contain not only refractory but also relatively labile components. This change in the extent of labile properties of the DOM on the SW Florida Shelf could be related to the increase in the supply of DOM from anthropogenic sources, to microbial sources (Kramer and Herndl 2004; Lønborg et al., 2009) and to photodegradation processes (Kieber et al., 1989; Kieber et al., 1990; Kouassi and Zika, 1990; Miller and Moran 1997). The supply of anthropogenic materials in SW Florida waters could have been increased by re-routing the flow of the Lake Okeechobee to Caloosahatchee River, which is enriched with nutrients from Everglades agricultural region (McPherson and Halley 1996; Steinman et al., 2002; Zanardi-Lamardo et al., 2002; Brand 2002; Brand et al., 2007); while the discharged water from Shark River into Florida Bay contains natural and urban and agricultural non-point run-off DOM materials from the Everglades Park (LaPointe and Clark, 1992; Clark et al., 2002).

On the other hand, fluorescent protein material (component 4) is associated as a DOM by-product from biological productivity (Table A2). The net export of DON quantities from the Everglades Agricultural Area (Qualls and Richardson, 2003) could potentially export excess dissolved organic nitrogen unto Shark River in significant amounts through the spillways (McPherson and Halley, 1996; Qualls and Richardson, 2003). Component 4 was described to consist of biological labile material derived from autochthonous processes (Stedmon et al., 2003; Yamashita et al., 2008). This study showed some fractions of protein-like components are photodegradable; however, other fractions can be relatively refractory after a long-term light exposure (Bushaw et al., 2006). The susceptibility of nitrogen organic groups to photo-bleaching can be connected to the structural relationship of nitrogen in the humic structure. Nitrogen compounds can
be associated with humic materials as a free-amino group, in an open chain, and as part of a heterocyclic ring, a bridge constituent linking quinine groups together, or an amino group attached to aromatic rings. The manner in which these protein components are complexed with organic constituents including humic materials and polyvalent cations, determines their stability (Stevenson, 1994). Little is known however, about the N-bound characterization of this component as they exist in the marine systems. Studies however documented that the associated N labile component in humic substances could lead to photo-production of \( \text{NH}_4^+ \) after light exposure (Reche et al., 1998; Bushaw et al., 1996; Stedmon et al., 2005) an indication that photo-degradation can be a sink and a source of available nitrogen (Bronk 2002).

**A3.3. Estimate of DOC removed using combined multiple linear regression and PARAFAC modeled fluorophores**

Fluorescence of DOM depends on the fluorophore’s structure as well as its surrounding environment, while DOC is a measure of bulk carbon. There can be a high correlation of FDOM and DOC in near freshwater systems, due to the highly conjugated materials and high carbon content from terrestrial environments (Clark et al., 2002). Measurement using a linear regression approach even with single excitation-emission wavelength has been shown to provide a good agreement between FDOM and DOC, particularly during high river discharge (Clark et. al., 2002). However, this relationship dramatically weakens with distance from the terrestrial DOM source (Vodacek et al., 1997; Chen and Gardner, 2004). Non-linearity is even observed in regional scale sampling using high resolution measurements (Chen and Gardner, 2004). One primary reason is the restriction of the wavelength used in a linear regression method, which is
focused on a single component. This strategy disregards other potential fluorescent components, which could also constitute a major fraction of the bulk DOC. The study presented here has overcome that problem by applying MLR using DOM PARAFAC modeled fluorescence components. Application of MLR uses an equation that can be adjusted by including components that improve the regression coefficient for the DOC-DOM fluorescence relationship, and hence, make it possible to estimate DOC removal using optical method via degradation.

During photo-irradiation experiments, DOM fluorescent components 1 and 2 have a high co-linearity with DOC across stations and photo-irradiation (Figure A4), but this is not true for component 3 and 4. This suggests that removal mechanisms are involved in the transport of FDOM across station 56 and station CH2 to station 30 and station DT9. The magnitude of decrease in components 1 and 2 co-varied with the DOC, indicating the feasibility of improving FDOM and improve its correlation with the DOC concentration.

The linear regression with one of the PARAFAC modeled components provided a robust correlation (Figure A5), however, it must be taken into account that there are other fluorescing components which have similar identity. In order to estimate photo-degraded DOC concentration using the plural PARAFAC components, a multiple regression was applied. An MLR is a statistical parameter that calibrates component responses in a mixture, and is known to improve the correlation against complementary information (Brereton, 2003). Since a minus sign was obtained for component 3 in 3 components-MLR model (components 1, 2, and 3), 2 components-MLR model using components 1 and 2 were applied (equation 2). This suggests that carbon amount of component 3 might be quite smaller than those of components 1 and 2, even though a negative linear
relationship was found between component 3 and DOC. Component 4 was also excluded since it was determined to decrease the linear fit significantly. After MLR implementation using two modeled PARAFAC DOM components, the estimate of DOC improved by about 27% compared to a linear fitting using a single-scan mode excitation emission fluorescence approach.

The DOC-DOM fluorescence relationship was evaluated to determine an optical method to estimate DOC, in the assumption that photo-oxidation may be a confounding factor in the non-correlation between DOC and FDOM (Chen and Gardner, 2004). Some of the confounding factors can be attributed to the inherent chemical composition of the DOM properties. For instance, unlike soil humics, marine humics exhibit a regular and consistent structure in all marine environments (Harvey and Boran, 1985). They further added that the difference between these two humics occur in the relative proportion of their proton on paraffinic, aromatic and oxygenated carbons, and in their degree of interaction with transition metals. Another confounding factor is the presence of strong oxidants. Peroxides are among the oxidants that had been found in surface waters, where these oxidants can cause CDOM to bleach without necessarily changing the DOC concentration (Cooper and Zika 1983; Zika et al., 1985; Petasne and Zika et al., 1987; Cooper et al., 1988). Although these confounding factors cannot be directly identified, variations from these factors are captured in the PARAFAC fitting of the model. In this process of determining the number of components, the amount of unexplainable variables in the residual can be reduced.

In this study, regression between DOC and fluorescence is enhanced by employing two of the identified terrestrial components as compared to using a single
component. Using equation 2, assuming that the photodegradable DOC is largely attributed to components 1 and 2, loss of DOC was estimated from changes of components 1 and 2 on the SW Florida Shelf water during the 30 days of solar exposure (Table A3). The range of DOM fluorescent intensities of components 1 and 2 in those four sites were used to estimate photo-degraded DOC concentration. The estimated carbon loss using PARAFAC-MLR ranged from 14 µM C to 115 µM C (Table A5). The measured DOC ranged from 7 µM C to 60 µM C. The measured DOC gain in stations CH2 and 30, indicate possible contamination of the samples. Hence, the discrepancy between the DOC measured and the PARAFAC-MLR predicted DOC (Table A5).

The terrestrial FDOM components are short-lived in the marine environment and photo-degradation is considered one of the sinks (Clark and Zika, 2000). The DOM fluorescence intensity found after 30 days of incubation in stations 30 and DT9 could be indicative of an incomplete mineralization of DOM after prolonged solar exposure (Vahatalo and Wetzel, 2004). This could probably explain the estimated 50-70% loss of carbon from DOM photo-degradation. This percentage of DOC loss obtained in this study, is slightly above the magnitude in the estimated photo-degradation loss (31%) in the estuary (Moran et al., 2000), and is within the range (~50%) previously reviewed (Blough and Del Vecchio, 2002). The remaining DOC could either be refractory, or is still labile, and could still be susceptible to further photodegradation.

Furthermore, we have shown that DOM fluorescent components collected across the SW Florida Shelf is susceptible to photodegradation but can exhibit variable photochemical reactivity. From this result, solar photodegradation is likely a key mechanism that the terrestrial organic matter is removed on the shelf, and a mechanism
that perhaps further primes DOM labile components to a more refractory material (Zika, 1981). The rest of the solar un-degraded fraction of the organic may be removed by other means (e.g., biological mechanisms, physical transport to deeper water and flocculation and other mechanisms) as reported in several studies (Mopper and Stahovec, 1986; Clark and Zika, 2000; Piccini et al., 2009). Also, this study demonstrated a significant improvement of DOC concentration estimation using a MLR formula using derived PARAFAC modeled DOM fluorescent components. Through this method, carbon (DOC) loss was estimated from a 30-day solar exposure of SW Florida Shelf collected DOM samples.

A3.4. Application of derived MLR-PARAFAC in estuarine, near-shore and in an open ocean

The derived equation was developed to monitor terrestrial intrusion unto the shelf (Liu et al., 2010). It follows therefore, that the derived equation can used also in the estuary, nearshore, and coastal environments. These environments are typically shallow and well-mixed with high DOM influence from river inputs. In the deep ocean, the major sources of DOM are microbially derived (Nelson et al., 2004; Ogawa et al., 2001; Jorgensen et al., in press), and the DOC concentration and DOM fluorescence relationship is typically opposite (Coble, 2007; Yamashita and Tanoue, 2008). Hence, the equation developed here is applicable only to continental shelves, and a new equation needs to be derived for optical estimates of DOC loss in the deep ocean.
A4. Conclusion

DOC is a measure of bulk carbon while EEM measurement of fluorophores distinguishes different fluorescing compounds from both terrestrial and in-situ produced materials. DOM remains a heterogeneous mixture of compounds that is susceptible to solar degradation in a dynamic shelf environment. Separating the bulk DOM into its unique components using fluorescence methods and PARAFAC makes it easier to monitor the behavior of different compounds, and select relevant components that improve FDOM-DOC relationship.

In using the multi-dimension fluorescence and PARAFAC analysis, five distinct fluorophores were derived during the photodegradation experiment. Results showed that the bulk DOM on the SW Florida shelf contains a mixture of four different components. The behavior of these components collected near the Shark River mouth, Charlotte harbor, and the mid-shelf were highly variable. Component 1 exhibits a low degradation rate relative to the samples collected near the Shark River mouth and on the continental margin. Degradation tends to be significant at the mid-shelf region and less significant near the Charlotte harbor. Component 2 exhibits a significant photodegradation in Charlotte harbor samples followed to a lesser extent by the samples from the Dry Tortugas and Shark River. In the mid-shelf, there is a component 2 production while component 3 exhibits a similar reactivity with component 1 but to a lesser degree. Component 4, a protein component, result in the production of the Shark River samples after photodegradation. Samples from Caloosahatchee River and Dry Tortugas were relatively unreactive. In the mid-shelf region, however, the protein-like component tends to exhibit a significant loss of fluorescence.
It appears that the nearshore terrestrial DOM fluorescence near river mouths was relatively reactive while mid-shelf and continental margin DOM tended to be relatively refractory. Use of the terrestrially derived component 1 and 2 improved significantly the DOC estimation. The 30 days DOM photodegradation using the developed MLR-PARAFAC equation corresponded to an estimated DOC removal of 14-115 µM C. However, measured DOC indicates a much lower value, and further indicates that contamination may have occurred. Hence, the experiment conducted may not show effective use of the MLR-PARAFAC model in estimating DOC from photodegradation of DOM. It is recommended to conduct a shorter period of photodegradation experiment to minimize contamination.

However, this study may have demonstrated that using EEM and PARAFAC techniques, DOM across the Florida shelf has been demonstrated to have different photoreactivities, and that photodegradation of DOM components near river mouth and mid-shelf can result in the production of other photochemical by-products. And using relevant components of the DOM fluorescence in the marine DOM pool, correlation with DOC may be improved, making it possible to improve optical estimate of DOC removal via solar light degradation. Quantifying removal rates of DOC on continental shelves is important in understanding carbon biogeochemical cycling in the coastal ocean.
A: References


Brezonik PL. Chemical kinetics and process dynamics in aquatic systems. CRC; 1994.


Figure A1. Sampling sites across southwest Florida Shelf. Station codes used was based on the South Florida Hydrographic Survey (NOAA-AOML). Location of these stations have the following coordinates: station 56 (25.350°N; 81.227°W), station CH2 (26.664°N; 82.332°W), station 30 (25.403°N; 82.212°W), and station DT9 (24.267°N; 83.042°W).
Figure A2. a) A cumulative profile of 24-h solar radiation profile  b) Integrated daily solar radiation (W m\(^{-2}\)) during the solar experiment exposure of SW Florida collected seawater from 1 March to 30 March 2009.
Figure A3. Split-Half Validation of the 4-component PARAFAC model.
Figure A4. a-d) Identified modeled components and e-h) corresponding excitation and emission loadings. Peaks of modeled components resemble a) humic-like material c) humic/marine-humic like and d) tryptophan-like material compared to identify PARAFAC modeled EEMS.
Figure A5. Monitored fluorescence intensity (QSU) of the components (Fig.3; a-d) in solar exposed water collected in Stn. 56.

\[ y = -0.4564x + 25.913 \quad R^2 = 0.2706 \]  

\[ y = -0.5438x + 28.687 \quad R^2 = 0.3733 \]  

\[ y = -0.1488x + 11.302 \quad R^2 = 0.0969 \]  

\[ y = -0.1295x + 6.9019 \quad R^2 = 0.3876 \]
Figure A6. Monitored fluorescence intensity (QSU) of the components (Fig.3; a-d) in solar exposed water collected in Stn. CH2.
Figure A7. Monitored fluorescence intensity (QSU) of the components (Fig.3; a-d) in solar exposed water collected in Stn. 30.
Figure A8. Monitored fluorescence intensity (QSU) of the components (Fig.3; a-d) in solar exposed water collected in Stn. DT9.
Figure A9. DOC concentrations in seawater during a month long solar exposed samples. Samples were collected from a) Stn. 56, b) Stn. CH2, c) Stn. 30 and d) Stn. DT9 (see Fig.1 for the sites location).
Figure A10. Predicted a) DOC values (µmol C L⁻¹) calculated based on multiple linear regression of the PARAFAC modeled fluorescent components [Comp1=(250:46), Comp2=(250:400) and observed DOC values. b) Direct linear regression of single scan mode excitation: emission fluorescence (350/450) and observed DOC. Error of prediction a) $E_\% = 13\%$ (adj $R^2=0.873$) b) $E_\% = 24\%$ (adj $R^2=0.628$).
Table A1. Salinity and temperature during sampling across the southwest Florida Shelf stations below 1 meter. Salinity. Data provided by the NOAA-AOML/South Florida Program.

<table>
<thead>
<tr>
<th>Station</th>
<th>Lat</th>
<th>Long</th>
<th>Salinity</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>25.350°N</td>
<td>81.227°W</td>
<td>36.014</td>
<td>15.961</td>
</tr>
<tr>
<td>CH2</td>
<td>26.664°N</td>
<td>82.332°W</td>
<td>35.776</td>
<td>16.075</td>
</tr>
<tr>
<td>30</td>
<td>25.403°N</td>
<td>82.212°W</td>
<td>36.699</td>
<td>19.404</td>
</tr>
<tr>
<td>DT9</td>
<td>24.267°N</td>
<td>83.042°W</td>
<td>36.587</td>
<td>20.791</td>
</tr>
</tbody>
</table>
Table A2. Variation explained in 4 component model.

<table>
<thead>
<tr>
<th>Component number</th>
<th>% variation</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>98.75%</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>99.2%</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>99.5%</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table A3. Excitation and emission fluorescence maximum of PARAFAC modeled DOM fluorescence components of dark and solar exposed incubated samples and their respective region and designation.

<table>
<thead>
<tr>
<th>Comp</th>
<th>λ\textsubscript{ex}</th>
<th>λ\textsubscript{em}</th>
<th>Region</th>
<th>Fluorophore type</th>
<th>Tentative fluorophore type and source assignments of this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>460</td>
<td>A</td>
<td>Q2</td>
<td>UVA Humic-like component; Terrestrial</td>
</tr>
<tr>
<td>2</td>
<td>(&lt;260)</td>
<td>400</td>
<td>A</td>
<td>C3, C9, P4, Q2</td>
<td>UVA humic-like, anthropogenic, Biological/microbial, possible instrument artifact; photochemical by-product</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>405</td>
<td>A, M</td>
<td>SQ1, C2, P1</td>
<td>UVA Terrestrial and Marine humic-like component, microbial, anthropogenic</td>
</tr>
<tr>
<td>4</td>
<td>275</td>
<td>340</td>
<td>T</td>
<td>P7, Trp-like</td>
<td>UVB Protein like, Tryptophan-like, autochthonous; biological, microbial</td>
</tr>
</tbody>
</table>
Table A4. Total change in DOM fluorescence (QSU) at different sites after thirty (30) days exposure with 7.008 kW m\(^{-2}\) integrated solar radiation. Negative corresponds to fluorescence loss while positive indicates gain in fluorescence.

<table>
<thead>
<tr>
<th>STATION</th>
<th>Initial Fl.</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>23.06 ± 3.25</td>
<td>-1.66</td>
<td>-5.24</td>
<td>-0.13</td>
<td>+3.04</td>
<td>-6.46</td>
</tr>
<tr>
<td>CH2</td>
<td>16.30 ± 6.84</td>
<td>-4.43</td>
<td>-6.73</td>
<td>-2.81</td>
<td>-1.68</td>
<td>-0.38</td>
</tr>
<tr>
<td>30</td>
<td>11.69 ± 2.92</td>
<td>-9.61</td>
<td>+1.09</td>
<td>-9.50</td>
<td>-11.04</td>
<td>+24.05</td>
</tr>
<tr>
<td>DT9</td>
<td>3.87 ± 1.58</td>
<td>-2.22</td>
<td>-5.99</td>
<td>-0.70</td>
<td>-1.6</td>
<td>-6.50</td>
</tr>
<tr>
<td>Range (FL loss)</td>
<td>-1.66 to -9.61</td>
<td>-5.24 to -6.73</td>
<td>-0.13 to -9.50</td>
<td>-1.68 to -11.04</td>
<td>-0.38 to -6.50</td>
<td></td>
</tr>
</tbody>
</table>
Table A5. Estimated measured (meas) and PARAFAC-MLR predicted (pred) DOC loss/gain of solar exposed DOM collected in stations 56, CH2, 30 and DT9. Negative corresponds to carbon loss while positive indicates gain in carbon.

<table>
<thead>
<tr>
<th>Station</th>
<th>DOC meas ini</th>
<th>DOC meas final</th>
<th>DOC pred ini</th>
<th>DOC pred final</th>
<th>DOC loss/gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>216.9</td>
<td>210.4</td>
<td>185.6</td>
<td>70.58</td>
<td>-6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-115.02</td>
</tr>
<tr>
<td>CH2</td>
<td>127</td>
<td>144.27</td>
<td>126.62</td>
<td>94.9</td>
<td>+17.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-31.72</td>
</tr>
<tr>
<td>30</td>
<td>86.75</td>
<td>110.8</td>
<td>90.63</td>
<td>76.26</td>
<td>+24.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-14.37</td>
</tr>
<tr>
<td>DT9</td>
<td>91.21</td>
<td>31.51</td>
<td>84.71</td>
<td>41.6</td>
<td>-59.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-43.11</td>
</tr>
</tbody>
</table>
Appendix B

Spectral characterization of DOM point sources using excitation and emission fluorescence and PARAFAC

B1. Rationale

The production of DOM is ubiquitous in the terrestrial and marine environment. DOM production has an important role in the organic carbon cycle and in the biogeochemical cycling of other important elements and compounds that control marine biological processes, influence atmospheric composition and affect oceanic processes (Zepp, 1997; Clark and Zika, 2000). In the marine environment, the heterogeneous and complex compositions of DOM have their origin from both terrestrial sources and marine-humic substances from phytoplankton (Zika and Cooper, 1987). Evidence shows that biological processes play a big role in the production of refractory materials of DOM. The production of this refractory material mechanistically can be facilitated by the polymerization of degraded plant residues, and the formation of marine DOM by free-radical cross-linking of unsaturated lipids produced from both algal (Carlson and Mayer 1983; Carder et al. 1989; Hulatt et al., 2009) and microbial processes (Harvey and Moran, 1985; McKnight and Aiken, 1998; Lønborg et al., 2009). As such, apparent differences should be observed in the chemical properties of DOM produced in both terrestrial and marine environments (Clark and Zika, 2000).

Despite decades of research conducted on DOM, its heterogeneity and complexity remain variable in various spatial and temporal scales. Thus, it makes it difficult to trace DOM sources, especially in high mixing zones. Determinations of the DOM sources often use stoichiometric ratios of DOC, DON and DOP (Qualls and Richardson, 2003).
Also, isotopic measurements are widely used to validate particulate DOM sources (Wang et al., 2002; Ziegler and Fogel, 2003; Stern et al., 2007). However, isotopic analyses often encounter uncertainties due to the wide range of isotopic values that correspond to a particular DOM source. Approaches in isolating and characterizing humic substances (McDonald et al., 2004), a major component of DOM, have used chromatography techniques, mass spectrometry and $^{13}$C and $^1$HNMR, FT-IR spectroscopy (Stevenson, 1994) to determine fine chemical structure of DOM. Despite sophistication of these suites of molecular analyses, the DOM structure is still unambiguously elucidated (Clark and Zika, 2000) and remains a complex and heterogeneous compound (Hedges et al., 2000; Hedges et al., 1997).

In this study, the optical fluorescence properties of DOM will be used as a primary tool to trace multiple sources of DOM. The optical measurement of DOM has gained wide use because of its simplicity and sensitivity of analyses: needs less sample volume throughput, relatively fast analysis, and can trace multiple sources from both chemical and biological by-products (Coble, 1996). The use of fluorescence over absorption measurements has increased; since, it provides additional information in the chemical composition of the different sources of DOM, based on the excitation and emission peaks (Coble, 1996; Coble et al., 1998; Stedmon and Markager, 2005) and fluorescence intensity ratio at specific wavelengths (Del Castillo et al., 2000; Conmy et al., 2004a; Sierra et al., 2005).

The work of Coble (1996) revolutionized the use of the EEM to analyze DOM fluorescence, and had been coupled with PARAFAC, a statistical modeling technique introduced by Stedmon and Bro (2003), purposely used to quantitatively measure DOM
fluorescence between and among samples. This analysis involves fluorescence measurement in a wide range of excitation and emission wavelengths to generate a matrix fluorogram. The emitting peak centers are used to locate sources by their ratios of the humic-like components. This modeling technique provided an improved comparison of tracing changes of the FDOM from the different sources. Determinations of the fate of DOM from different sources provide a better understanding of how the mechanisms of colored organic elements are recycled in the freshwater and marine environments (Yamashita et al., 2008; Stedmon and Markager, 2005) and at different temporal scales (Kowalczuk et al., 2009). Determination of DOM sources is critical in estimating the rates of FDOM sources and productions, which can be correlated to carbon transfer in coastal ocean (Jaffé et al., 2004). To ascertain specific sources, this study will determine excitation and emission signatures of DOM from the marine, terrestrial and atmospheric sources. This includes accounting of DOM production from different sources to the pool of FDOM on the shelf, including contributions from anthropogenic sources input in the estuarine to the shelf area.

B2. Methods

B2.1. Sampling of DOM sources

Samples from different sources were collected from various sites across south Florida (Figure B1). Sites numbered correspond to the location and type of samples collected (Figure B4): a) Aldrich Humic acid was diluted in 0.1 N H₂SO₄ to prepare 0.07 mg/L sample. From the same stock solution, 20 EEM spectra were scanned to determine spectral signature of a standard humic acid; b) Lake and River samples - Lake Okeechobee, the Caloosahatchee River and the Shark River water samples were grab-
sampled, placed in a glass container, and were immediately stored at 5°C, prior to analysis. Samples were diluted to 1/20 from its original solution to facilitate readout of fluorescence within the standard QSU dynamic range. The Caloosahatchee River and the Shark River water samples were sampled on a small boat, during the University of Miami’s Oceans and Human Health Cruise (UM-OHH, October 2010), on the Southwest Florida Shelf; c) Atmospheric and rain samples - Bulk aerosol samples were gathered on the roof of the SLAB building (~20 m above ground/sea level), at the Rosenstiel School of Marine and Atmospheric Science (RSMAS), located on Virginia Key (25°N, 83°W). The sampling site was at the water’s edge, about 4 km from mainland Florida, on the island of Virginia Key. These samples were collected from May 11, 2007 to July 31, 2009. Miami aerosol samples were often collected over multiple days because aerosol filters were not exchanged on weekends, during rain/lightning events, or during high winds. The bulk aerosol samples were initially collected on pre-combusted (500°C) Pall A/E glass fiber filters, and subsequently on Whatman QMA quartz fiber filters. Dust samples, collected on filter paper, were placed in 50 ml pre-combusted glass vials, to which 25 ml of de-ionized water was added. Contact time was estimated to be > 5 hrs before analysis and was stored at 5°C. The rain samples were captured using automatic samplers that opened during rain events; the samplers were similar to the apparatuses used in the National Atmospheric Deposition Network (http://nadp.sws.uiuc.edu/NADP/). Rainwater samples were retrieved after a rain event and always within 24 hours after collector deployment. Samples were immediately filtered using 0.2 μm Supor® filters and were stored at 5°C. These samples were randomly sampled during the month of June, July and August of 2008 and 2009.
d) Marine biota samples/incubation- Samples were collected during the UM-OHH Cruise. Approximately 0.30 grams of seagrass (Thalassia sp.), brown algae (Sargassum sp.), and withered leaves of mangroves (Rhizophora mangle) were dried for 24 hrs at 60°C, and weighed. Fifty (50) milliliters of water were used to incubate the samples for 30 days, in glass, aluminum-covered bottles and stored at room temperature (~25°C). Due to the high concentration of fluorescent DOM, extracts were diluted and placed in scintillation vials. Samples were stored at 5°C prior to analysis. Samples from each species were prepared in three replicates. Samples were diluted so that fluorescence intensity was within the standard QSU fluorescence range (0-100 QSU);
e) Karenia brevis and diatom samples- Samples were obtained from Larry Brand’s Phytoplankton Laboratory at the University of Miami’s Rosenstiel School of Marine and Atmospheric Science.

All samples, except the Florida Strait samples, were filtered using a glass syringe with a 0.2 μ nylon membrane Gelman filter, prior to EEM fluorescence scan in order to normalize particle size of the DOM.

**B2.2. EEMS scanning and correction**

Spectral measurements

During the EEM spectral analysis, water samples were acclimated at room temperature (25°C). The measurement of the excitation and emission matrix (EEM) was conducted using a fluorescence spectrophotometer (Hitachi F-4500) configured to scan a wavelength range from 200-500 nm for excitation, and 200-700 nm emission at 5 nm intervals (with an emission and excitation slit width of 5 nm), and a scan speed of
1200 nm/min at PMT of 700 V. The instrument configuration was corrected using the manufacturer’s settings (Hitachi F-4500 Manual). All EEMS obtained for each grouping were corrected by subtraction with Milli-Q water. Fluorescence intensities were calibrated against the quinine sulfate monohydrate in 0.1 N H₂SO₄ with an obtained ~0.9996 regression coefficient (at 350/450 nm). Also, the inner filter effect in all EEM spectra was corrected (McKnight et al., 2001; Lacowicz, 2006) from the measured absorbance. Absorbance was measured using the UV-Vis Spectrophotometer (Agilent 8453). The fluorescence intensities of the PARAFAC components derived were reported in quinine sulfate units (QSU). Similar EEM spectral analysis was applied in all DOM sources samples.

PARAFAC modeling

A similar method was used here, as in previous studies in performing the PARAFAC modeling (Stedmon and Markager, 2005). Excitation and emission matrix fluorescence of DOM was recorded against range of emission and the excitation wavelengths. PARAFAC was modeled using a multi-way, data analysis fitting to minimize the sum of squares of the residuals. See Bro, 1997; and Stedmon and Bro, 2008 for details of the principles and approaches used in using PARAFAC modeling, employing equation B.1.

\[ x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}, \quad i = 1, \ldots, I; \quad j = 1, \ldots, J; \quad k = 1, \ldots, K. \]  

(equation B.1)

where \( x_{ijk} \) refers to the fluorescence intensity of the ith sample at the jth variable (emission wavelengths) and at the kth (excitation wavelengths) in the PARAFAC DOM-
Fl component model. $a_{if}$ is directly proportional to the concentration of the $f$th analyte of the $i$th sample, $b_{jf}$ and $c_{kf}$ are scaled estimates of the emission and excitation spectra at wavelengths $j$ and $k$, respectively, for the $f$th analyte, and $e_{ijk}$ is the residual noise that represents the noise not explained by the model. Corrected datasets of EEM were modeled using PARAFAC, employing the DOMFluor toolbox run in the R2008 Student Version of MATLAB (Stedmon and Bro, 2008). The EEM excitation and emission wavelengths used were reduced to 250-450 and 300-520 nm, respectively (both at 5 nm intervals), to minimize random noise outside the spectral range of interest. Excitation wavelengths below 250 nm were removed due to high residuals, possibly influenced by the source xenon light and data emission wavelengths. Greater than 520 nm were removed due to the Raleigh and Raman scatter and secondary protein fluorescence (Smilde et al., 2004; Murphy et al., 2008). The data array used for the PARAFAC modeling for each group was separated and modeled independently. Although this procedure is tedious, normalization of the PARAFAC model of each class is simpler. In addition, independent modeling of the different groups was employed purposely to determine uniqueness or similarity of the different components from the different sources. Then, the PARAFAC model was fitted to the array, split into random halves (consisting of a calibration and a validation array) sequentially from three to ten components. The model-ranked components identified were facilitated by comparison of the calibration and validation arrays to the excitation and emission spectra of the components. The optimized component models were able to explain an average of 99.5% of all the dataset variation; and, the estimated number of DOM fluorophore components were validated by the split-half technique and were further validated by
random initialization steps, which ensures the result observes least squares and not a local minimum (Stedmon and Bro, 2008).

**B3. Results and Discussion**

**B3.1. PARAFAC model result**

PARAFAC models for each specified source were classified per class and were modeled separately. The variables explained range in identifying the different components of the EEM DOM spectra were from 93.0%-99.9 % (Table B2). Correspondingly, the emitting fluorescence centers of the selected DOM sources in this study showed multiple components (refer to Figure B2 and Table B1). The number of samples run, in each model, has a minimum sample set of 12 to 41 scanned EEMS (See Table B1). Although, it was recommended that at least 20 scanned EEMS be included in the model (Stedmon and Bro, 2008), the 12 samples were still analyzed due to sample limitation. The resulting variation explained in separating the components was still close to 99%. The spectral composition of the excitation and emission maxima was referenced from published assignments (Table 1; Coble, 2007; Stedmon, 2008).

**B3.2. Emitting centers of the spectral fluorescence from the different DOM sources**

From the freshwater environments, the PARAFAC DOM fluorescence modeled components are relatively consistent in the first component at 250 nm excitation and 490 nm maximum wavelengths. The DOM from Lake Okechobee (LakeOke) exhibited three DOM fluorescence components, and, its river tributary, the Caloosahatchee River, exhibited a similar number of components. However, the Shark River (SharkR) contained an added component totaling to 4 components. The first component of
LakeOke, CalooR and SharkR were consistent to be composed of UVA humic-like from terrestrial source and terrestrial-autochthonous in source (Stedmon and Markager, 2005; Yamashita and Tanoue. 2008). Differences occurred in the number of components and the composition of the succeeding components (Figure 2). LakeOke and SharkR tend to have more humic-like related components, while CalooR tends to have an added protein-like component mixture. This means that water from LakeOke and SharkR contain more terrestrial-derived organic matter components than the CalooR samples (Zanardi-Lamardo et al., 2002). The high protein-like component enriched water of the CalooR water could be related to the mineralized DOM from a biological growth in a nutrient enriched water from fertilizers and sewages coming from the Everglades Agricultural Regions (Zanardi-Lamardo et al., 2002; Brand and Compton, 2007). Distinguishing factors between these three types of DOM sources can be based on the number of components, since the excitation and emission maxima tend to fluoresce at the same wavelengths. Another distinguishing property of the CalooR in particular, is the presence of the tryptophan component (Table 4.1). This DOM fluorescence component from lake and river water samples can be distinguished from the other sources merely based on the emission maximum of the first component (495 nm).

The rain and aerosol samples have three PARAFAC modeled DOM components determined (Table 4.2). Rain samples tend to have a stronger humic-like character than the aerosol samples. The aerosol samples tend to have a more protein-like component and a marine humic-like character. A secondary component of the aerosol samples also indicates that it has a similar fluorescence with a terrestrial humic-like DOM component.
Rain samples and aerosol samples can be distinguished easily using their first emission fluorescence maximum component wavelength.

Three components were derived from the EEM spectra of the DOM from the incubation of the mangrove, seagrass and sargassum dried leaves. The first component is predominately due to the variation from the humic-like component. Among the species incubated, the mangrove species (Table 3) exhibited the highest fluorescence for component, which correspond to the UVA humic-like from terrestrial, anthropogenic source (Coble 2007), while sargassum showed high fluorescence for component 1. The seagrass fluorescence for the three components was the least. Although it is hard to compare the *K. brevis* cultures and the diatoms since these are old cultures, it is apparent that both cultures exhibit high DOM fluorescence intensity from their third component, which corresponds to both UVC and UVA marine humic-like components (ref). The fluorescence intensity of the incubated species can be sequenced as follows: mangrove > sargassum > seagrass (Table B3). It seems that the DOM fluorescence character of the different marine species can be distinguished between each other using their relative fluorescence intensity. It is interesting to note, however, that no protein-like fluorescence was distinguished, since biological productivity of phytoplankton has been related to the presence of the protein-like fluorescence (Stedmon et al., 2006; Murphy et al., 2008; Yamashita and Tanoue, 2008). It is likely that the microbes are not present to facilitate degradation of these materials. The concentration could also be very low and undetectable. This protein-like component of the DOM fluorescence from the marine species could probably represent the 3% unexplained variability by the model. The *K.*
brevis HAB and diatom cultures were not compared due to the age of culture and unknown quantity of cells.

In general, the DOM fluorescence characteristics of the point sources in southwest Florida were predominantly humic-like in character. The first component shows similarity in their chemical composition followed by the second component. Based on the first component, identified DOM terrestrial sources tend to have an emission maximum at a longer wavelength, and tend to shift to a shorter wavelength than in the marine identified DOM sources, while DOM from the atmospheric samples tend to fluoresce at a shorter wavelength than the DOM from the marine samples. However, changes in the fluorescence maximum wavelength tend to occur after the first and second component. Hence, the third and succeeding excitation and emission wavelength pair of the principal components then potentially can be used as a signature for each point source.

**B3.3. Compositional heterogeneity and fluorescence intensity**

In general, PARAFAC modeled DOM components from various sources were distinguished (Figure B2a). Most sources exhibit similar spectral property of the humic acid standard, indicative of chemical transformation of DOM into humic chemical functional components. However, some sources have additional, unique spectral property not present in the humic material components. Such variation could be due to availability of more or less of the major functional groups due to differences in the molecular make up of the different sources.
Terrestrial samples

Apart from qualitative determination, the range of fluorescence from each source was also quantified. Comparison provides relative magnitude of fluorescence contribution from different sources. Highest fluorescence, at least for component 1, was exhibited by Lake Okeechobee (~670 QSU) followed by the two rivers: Shark and Caloosahatchee (~200 QSU). A single-scan excitation emission (350/450 nm) fluorescence analysis (Figure 3), showed mangrove species tend to produce high DOM fluorescence. Except for Avicennia marina species, the other species exhibit DOM fluorescence at the same magnitude as the fluorescence incubation of marine species from Florida. The similarity is indicative of a possible commonality in the degradation pathways for different sources. Studies conducted from known terrestrial sources such as lakes and rivers, exhibit the same spectral property. And fluorescence maximum at 430 nm exhibits high conjugation of compounds (Coble, 1996). In that case, incubated marine species contain highly conjugated and possibly aromatic compounds to give high fluorescence intensity (especially from sargassum and mangrove species). However, even the set of samples does not give similar fluorescence. This variability could be attributed to factors such as parts and maturity of the plant exuding high DOM fluorescence material and its susceptibility to microbial degradation. Bacterial degradation of specific components of autochthonous and allochthonous CDOM may impact the spectral characteristics observed throughout the estuary, and suggest that CDOM optical properties are partially a function of the CDOM’s origin and mixing history (Boyd and Osburn, 2004). Also, hydrological conditions can affect concentration and composition of CDOM discharged into estuaries and coastal oceans. In the South
Atlantic Bight (Kowalczuk et al., 2009), during drought conditions, the plume consisted of 60% humic-like components and 20% proteinaceous components. During high river flow regimes, significant contributions are from the humics and minor contributions are from the protein components. According to Chen and Bada (1992), the major sources of DOM fluorescence in the global ocean are from particles and in situ formation. The DOM sources can come from rivers, rain, diffusion from sediments, and can be released from microorganisms, while the major sink is photochemical bleaching (Chen and Bada 1992).

**Marine samples**

Marine species produce colored dissolved organic matter. For instance, mangroves, in surface waters of the southwest coast of Florida, provide a significant input of DOM to the estuarine region (Jaffé et al., 2004), since they are highly productive ecosystems, fringing about 60-75% of the tropical coast (Clough 1998). As such, they can export large quantities of organic matter from plant litter in coastal waters (Alongi et al., 1990; Robertson et al., 1992). Condition of the litter can also be a determinant of DOM production, since it was observed that mangroves mid-senescence litters produced higher CDOM than severely senesced leaves (Shank et al., 2010). Although, they observed that POM and DOM were exported to estuaries in similar proportion, mangrove-POM was removed rapidly in the water column, while DOM behaves conservatively. The removal rate of DOM and POM can be attributed to geochemical barrier zone factors from different estuaries (Dittmar et al., 2001). Similarly, seagrasses have been observed to produce CDOM in an in-situ experiment in the Florida Keys (Stabeneau et al., 2004).
Likewise, a parallel study in Florida Bay and Taylor Slough observed that seagrasses and phytoplankton were major sources of UDOM (ultrafiltered dissolved organic matter) based on high O-alkyl C concentrations and low aromatic carbon concentrations (Maie et al., 2005). Results showed that rate of CDOM production exhibits dependence with temperature, which was related to possible microbial interaction (Stabeneau et al., 2004).

In a similar experiment, seagrass species from the Philippines were shown to produce colored dissolved organic matter and was estimated to be about ~50 QSU (Figure 3). So, seagrasses, along with mangrove derived DOM were known to contain protein degradable components and also, microbial and physical processes that initiated the formation of recalcitrant, highly colored, high molecular weight polymeric structures (Scully et al., 2004). On the other hand, sargassum, a brown macroalgae known to have high absorption among the eleven species in the intertidal and subtidal found on UK shores (Hulatt et al., 2009) has been documented to contribute to CDOM production, but, at variable rates (Shank et al., 2010), similarly with other mangroves and seagrasses species. The coral reefs are another benthic community that produce CDOM, but, the least producer compared to other communities (Figure 4.3). This could be due to the fact that they are efficient in recycling organic materials. And while there are only a few studies of CDOM production of corals, one study was able to document that there is a difference in the coral organic matter fluorescence intensity, as compared to seawater and humic acids; but, they are not necessarily different in chemical attributes using the excitation-emission scanning fluorescence analysis (Matthews et al., 1996). The mangrove-, seagrass-, sargassum- and coral-derived organic matter could then significantly contribute in the near estuary and coastal regions; but, their contribution
diminishes with distance from the nearshore. In this case, in-situ production from phytoplankton becomes important. In Japan’s Ise Bay and Mikawa Bay, it was demonstrated that bulk marine humic-like fluorescence was not derived from a terrestrial origin, but, rather an in-situ biological production (Yamashita and Tanoue, 2004). In the same study, a degradation study validated using a natural phytoplankton stock demonstrating rapid production of the marine humic-like fluorophore. The fraction of CDOM in DOM fluorescence was higher during an algal bloom event than during non-bloom conditions (Zhao et al., 2009). Evidence shows that organic matter from marine sources has higher sulfide and nitrogen than terrestrial sources, but, both have a similar carbon and hydrogen content. Thus, marine DOM fluorescence was suggested to originate from in-situ degradation products of the plankton (Nissenbaum and Kaplan, 1972). However, there have been problems encountered as to the sources of CDOM in terrestrially dominated regions, in terms of in-situ production. There have been cases where no correlation was observed between phytoplankton and CDOM, suggesting that phytoplankton is not an immediate or significant source of CDOM (Blough et al., 1993; Vodacek et al., 1997; Rochelle-Newall, 1999). According to Blough and Del Vecchio (2002), such contrasting findings can be resolved by leveling the amount of DOM from the terrestrial sources and from that of the phytoplankton. Similarly, in coastal and offshore waters, similar contrasting observations were reported. At the Bermuda-Atlantic Time-Series station (BATS) station, it was observed that there was no correlation with CDOM and chlorophyll (Nelson et al., 1998). Other workers proposed that microbial processes transform materials exuded by phytoplankton (Tranvik, 1993). Despite several mechanisms have been proposed, the precise mechanisms remain undetermined (Blough
and del Vecchio, 2002). However, this study validates the fact that phytoplankton does produce CDOM. Such non-correlation between chlorophyll and CDOM in terrestrial dominated regions is due to the high signal in the terrestrial component, overshadowing the effect from the marine component. For instance, near the Shark River, the presence of mangroves in the terrestrial and marine margin transition causes the signal from mangrove CDOM to be dominant. In coastal areas, especially in shallow water, the contribution from seagrasses and mangroves can be significant, including near mariculture areas, like in the Philippines. In this case, the correlation between CDOM and phytoplankton cannot be observed. However, in coastal areas devoid of these benthic communities, the correlation between phytoplankton and CDOM is feasible, especially far from riverine intrusion. In offshore areas, non-correlation between phytoplankton and CDOM could occur in areas with high solar exposure (Mopper et al., 1991; Kouassi and Zika, 1992) and high precipitation, especially near equatorial regions. Photobleaching effect can decrease the fluorescence property of DOM, while rain events can bring more DOM particles in sub-surface waters. Microbial processes, however, can act in the production and consumption of DOM (Nelson et al., 1998). The correlation between phytoplankton and CDOM could happen in areas less affected by these environmental conditions.

The marine fulvic and humic acids appear to be responsible for the water coloration in the offshore regions of the Gulf of Mexico, where these materials appear to have molecular weights, consistent with the extrapolation of the soil-derived curves of their absorption coefficients (Carder et al., 1981). Numerical model experiments showed wind-driven offshore transport of the Apalachicola River plume (West Florida Shelf) is
likely a physical mechanism that connects the variation observed between the difference of the river discharge with oceanic variability over the middle and outer shelf (Morey et al., 2009). However, circulation patterns, volume of river discharge, and multiple sources of rivers are just among the complex variables that caused CDOM variability (Conmy et al., 2004b). Large-scale temporal and spatial variation was observed in the Northern Gulf of Mexico, dominated by the Mississippi and Atchafalaya Rivers. Circulation patterns, volume of river discharge, and multiple sources of rivers are complex variables for biological and photochemical study of CDOM (Conmy et al., 2004a). In the Neponset Watershed River, for instance, CDOM variation was found to depend on landuse type, discharge/precipitation and growing degree days’ temperature (Huang and Chen, 2009); while another study of fluorescence of the North Carolina River samples shows that pH alters both intensity and spectral shape, while metals only alter the fluorescence intensity (Cabaniss and Shuman, 1987). In large scale environments, use of high resolution measurement allows for the distinguishing of water masses that could indicate rapidly varying source waters, from days to weeks, in river dominated systems (Chen and Gardner 2004). However, emission wavelengths used in this case is relatively specific and limited, thus limiting the information that can be obtained.

Atmospheric samples

Atmospheric samples are contributors of FDOM in the marine environment (Miller et al., 2009; Santos et al., 2009). Atmospheric and rain samples tend to exhibit similar magnitude in the DOM fluorescence with the Florida Shelf and Straits. In rain samples, 86% of the average total recovery of the FDOM fluorescence with 50%
estimated hydrophilic component, while 14% was very hydrophobic. Rainwater contains significantly more hydrophilic components than found in aquatic provinces (Miller et al., 2009). Rainwater samples consist mostly of hydroxylated and carboxylic acids with predominantly aliphatic characteristics, containing a minor component of aromatic structures (Santos et al., 2009). HULIS (humic-like substances) fluorescence was highest during convective events of the continental margin, suggesting possible terrestrial/anthropogenic sources. In low wind speed, stagnation conditions of the atmosphere builds up local sources, especially anthropogenic sources, while during well-mixed conditions, HULIS decreases (Muller et al., 2008). On the other hand, acidic compounds were more abundant than neutral compounds of the fine aerosol water-soluble organic compounds (WSOC) in all seasons in the Po, Valley Italy. The main structural feature of the aerosol WSOC using an H-NMR, are highly oxidized species with residual aromatic nuclei and aliphatic chains (Decesari et al., 2001). The autumn sample exhibits higher aromatic content than during the summertime; and, the signals due to carbon from phenols, ketones and methoxy groups were observed. These signals were attributed to lignin breakdown products, which are likely released during the wood combustion process, thus indicative of high contribution from biomass burning (Duarte et al., 2005).

**B3.4. FDOM production in the marine environment**

Mangrove leaves produce high DOM fluorescing compounds. A schematic diagram shows the contributing sources to the pool of organic matter on the Southwest Florida Shelf, suggesting that the marine DOM fluorescence component can also have a
significant contribution and should be resolved from the fluorescent DOM terrestrial and atmospheric sources counterpart. And, this study shows that there are overlaps in many of the DOM fluorescence components, indicative that some of the DOM fluorescent marine components can mimic optical properties of terrestrial sources. If this is the case, the question arises whether DOM fluorescence can resolve between the terrestrial and marine DOM fluorescence component sources. It could be that the pathways of oxidation of the organics, either terrestrial or marine are similar. Thus, the reason the first components of the modeled DOM fluorescence, from the different sources, tend to have a similar excitation-emission maximum (Table 1). This study does not contradict the majority of the DOM fluorescent fraction near river sources, since they consistently indicate humic-like components derived from terrestrial sources. The controversy becomes significant in mixing regions further away from river inputs. The conundrum is the pre-determined marine humic-like component (Coble, 1997), where they can also be detected in non-marine environments. It can be surmised that the similarity in most components can be due to the degradation of lignin-related phenolic structure in humic substances, which showed structural elements that tend to occur in all analyzed samples. The structure and functionality of polyphenols played an important role in the determination of the extent of ring cleavage of these polyphenols that could be related to the CO$_2$ release, the formation of aliphatic fragments, the contents of carboxyl groups and the yield of humic polymers formed (Wang and Huang, 1994). And, that the absolute and relative amounts of these different degradation products, which vary with the origin of the sample, were found to be not unique for humic matter of a particular origin (Johansson et al., 1994).
B3.5. Implication to DOM fluorescence cycling in shelf regions

Despite similarities in the DOM fluorescence components from the different sources, the common pathway of DOM degradation could probably explain overlap of some of the components. Similarity in the pathway could probably explain the loss of terrestrial DOM in the coastal ocean and assume a marine property, since they proceed a similar degradation pathway. And findings of terrestrial DOM (e.g. lignins in the deep ocean) is one of the evidence that terrestrial DOM, in fact, can be transported to deeper waters (Opshal and Benner, 1997). Despite the overlapping fluorescence maxima between groupings, it appears that use of the number of components identified their order and their fluorescent intensities are factors needed to verify the DOM sources. But, one needs caution in assigning sources since the factors needed for the source determination, as mentioned previously, can be locally specific for each geographic locale. Also, it could be that despite the complexity of the vast majority of the operationally defined humic substances, composed of mixtures of microbial and plant biopolymers, and, their degradation products, they are not distinctly different chemically (Kelleher and Simpson, 2006). However, this is not to rule out the existence of a fingerprint of humic macromolecules, because it could be just a method limitation.

B4. Conclusion

DOM fluorescence from various terrestrial, marine and atmospheric sources has similarity of the peak maximum excitation and emission wavelengths, particularly in their first components of the PARAFAC modeled EEMS. The similarity indicates a possible common pathway for degradation of DOM, irrespective of source. Source of variation,
beyond the second component, differs from different sources and is possibly used to differentiate the DOM origin. This can have an implication in the assumption of bulk DOM fluorescence source in high mixing regions, in identifying sources. Either marine or terrestrial DOM fluorescence can assume properties that are similar with each other, especially in their first components. Similarity in some of the fluorescence components indicates a majority of the labile DOM degradation has a common transformation mechanism, while the source of variation, using the excitation/emission wavelength maxima of the third and fourth component from the different sources can potentially be used to differentiate DOM origin. The fluorescence excitation/emission wavelength are as follows: Lake Okechobee, 250/300 nm; Shark River, 250/305 nm and 250/380 nm; Caloosahatchee River, 260(310)/445 nm; Rain, 270/330 nm; Aerosol, 300/350 nm; and Marine, 250(315)/410 nm. Thus, identified fluorescence wavelengths can be used to identify point sources of point DOM.

Acknowledgment

The authors would like to thank the 2009 NSF-NIEHS Oceans and Human Health Summer Research Experience for Undergraduate/Graduate (REU/G), NSF and NIEHS OHH Center grants: NIEHS # 1 P50 ES12736 and NSF #OCE0432368/0911373 and the Mary Roche Fellowship for the financial support. We would also like to acknowledge the support provided by the Quarterly Cable Calibration and Hydrographic Survey at 27°N in the Florida Straits program, a project of the NOAA-AOML (Miami, USA) and the research crew of the R/V Walton Smith (RSMAS-University of Miami).
B:References


Sierra, M.M.D., M. Giovanela, E. Parlanti, and E.J. Soriano-Sierra (2005), Fluorescence fingerprint of fulvic and humic acids from varied origins as viewed by single-scan and excitation/emission matrix techniques. Chemosphere 58, 715-733.


Figure B1. Sampling sites: 1) Aerosol and rain, 2) Shark River and mangrove leaves, 3) Seagrass, 4) Sargassum, 5) *K. brevis* and Diatoms, 6) Caloosahatchee River
Figure B2. Excitation and emission maxima of the different sources. Boxes correspond to range of excitation and emission wavelengths assignments of spectral fluorescence of DOM (Coble 1996). Refer to table 1.
Figure B3. Fluorescence intensity of dissolved organic matter produced from dark incubated marine tropical Philippine species of corals: PC (Pocillopora damicornis), SH (Seriatopora hystrix), brown algae: SS(Sargassum sp.), HC (Hormophysa cuneiformis), seagrasses: TH (Thallasia hemprichii), EA (Enhalus acoroides) and, mangroves: SA (Sonneratia alba), AM (Avicennia marina), collected from Cape Bolinao, Philippines. Incubation was conducted for 45 days. Error bars indicate standard deviation, n=3. (unpublished data)
Figure B4. Schematic diagram of the different FDOM sources in the Southwest Florida Shelf. The DOM fluorescence distribution in freshwater-marine mixing zones can be influenced by multiple sources coming from marine, atmospheric and terrestrial sources and can vary across different temporal and spatial scales.
Table B1. Excitation and emission fluorescence maximum of PARAFAC modeled DOM fluorescence across Southwest Florida Shelf from four quarterly survey, their respective region, and designation (Coble 1996, 2007; Stedmon and Markager 2003; Murphy et al., 2008)

<table>
<thead>
<tr>
<th>Ex</th>
<th>Em</th>
<th>Peak name</th>
<th>Component</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>260</td>
<td>400-460</td>
<td>A</td>
<td>UVC humic-like</td>
<td>Humic, terrestrial, autochthonous</td>
</tr>
<tr>
<td>320-360</td>
<td>420-460</td>
<td>C</td>
<td>UVA humic-like</td>
<td>Terrestrial, anthropogenic, agriculture</td>
</tr>
<tr>
<td>290-310</td>
<td>370-410</td>
<td>M</td>
<td>UVA marine humic-like</td>
<td>Terrestrial, marine, anthropogenic</td>
</tr>
<tr>
<td>250(385)</td>
<td>504</td>
<td></td>
<td>UVA humic-like</td>
<td>Fulvic, terrestrial autochthonous</td>
</tr>
<tr>
<td>275</td>
<td>305</td>
<td>B</td>
<td>Tyrosine-like, protein-like</td>
<td>Autochthonous</td>
</tr>
<tr>
<td>275</td>
<td>340</td>
<td>T</td>
<td>Tryptophan-like, protein-like</td>
<td>Autochthonous</td>
</tr>
<tr>
<td>398</td>
<td>660</td>
<td>P</td>
<td>Pigment-like</td>
<td></td>
</tr>
</tbody>
</table>
Table B2. Multiple sources of DOM fluorescence samples from Humic (humic acid), LakeOke (Lake Okeechobee), SharkR (Shark River), CalooR (Caloosahatchee River), Rain (rain), Aerosol( atmospheric aerosols), MAR marine species (i.e. South Florida seagrass, mangrove and sargassum species. Assignments were based on Yamashita et al., 2008; Murphy et al., 2008; Coble et al., 2007; Stedmon et al 2007; Murphy et al., 2006; Coble 1996)

<table>
<thead>
<tr>
<th>SOURCES</th>
<th>COMPONENT</th>
<th>EX</th>
<th>EM</th>
<th>FL</th>
<th>REGION</th>
<th>COMPOSITION</th>
<th>SOURCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humic</td>
<td>Comp1</td>
<td>255(360)</td>
<td>515</td>
<td>44.11 ± 4.47</td>
<td>UN</td>
<td>UVA Humic-like</td>
<td>Fulvic, Terr, Auto</td>
</tr>
<tr>
<td></td>
<td>Comp2</td>
<td>255(325)</td>
<td>420</td>
<td>28.98 ± 2.99</td>
<td>A</td>
<td>UVC Humic-like</td>
<td>Humic, Terr, Auto</td>
</tr>
<tr>
<td></td>
<td>Comp3</td>
<td>255</td>
<td>290</td>
<td>36.63 ± 4.94</td>
<td>-</td>
<td>Artifact, Uncertain</td>
<td></td>
</tr>
<tr>
<td>LakeOke</td>
<td>Comp1</td>
<td>250(330)</td>
<td>490</td>
<td>669.70 ± 141.70</td>
<td>UN</td>
<td>UVA Humic-like</td>
<td>Fulvic, Terr, Allo</td>
</tr>
<tr>
<td></td>
<td>Comp2</td>
<td>250(325)</td>
<td>410</td>
<td>466.40 ± 82.60</td>
<td>A,C</td>
<td>UVC Humic-like</td>
<td>Humic, Terr, Allo</td>
</tr>
<tr>
<td></td>
<td>Comp3</td>
<td>250</td>
<td>300</td>
<td>307.90 ± 37.60</td>
<td>-</td>
<td>Artifact, Uncertain</td>
<td></td>
</tr>
<tr>
<td>SharkR</td>
<td>Comp1</td>
<td>250(320)</td>
<td>495</td>
<td>203.90 ± 51.60</td>
<td>UN</td>
<td>UVA Humic-like</td>
<td>Fulvic, Terr, Auto</td>
</tr>
<tr>
<td></td>
<td>Comp2</td>
<td>250(310)</td>
<td>425</td>
<td>233.10 ± 49.00</td>
<td>A,C</td>
<td>UVC Humic-like</td>
<td>Humic, Terr, Allo</td>
</tr>
<tr>
<td></td>
<td>Comp3</td>
<td>250</td>
<td>305</td>
<td>218.00 ± 20.70</td>
<td>-</td>
<td>Artifact, Uncertain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comp4</td>
<td>250</td>
<td>380</td>
<td>86.60 ±13.30</td>
<td>M</td>
<td>UVA Marine humic-like</td>
<td>Anthro, wastewater</td>
</tr>
<tr>
<td>CalooR</td>
<td>Comp1</td>
<td>250(355)</td>
<td>495</td>
<td>160.8 ±74.50</td>
<td>UN</td>
<td>UVA Humic-like</td>
<td>Fulvic, Terr, Auto</td>
</tr>
<tr>
<td></td>
<td>Comp2</td>
<td>250</td>
<td>310</td>
<td>126.4 ± 83.00</td>
<td>B</td>
<td>UVB Try-like, Protein-like</td>
<td>Auto</td>
</tr>
<tr>
<td></td>
<td>Comp3</td>
<td>260(310)</td>
<td>445</td>
<td>52.4 ± 31.20</td>
<td>A,C</td>
<td>UVC Humic-like</td>
<td>Humic, Terr, Auto</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UVA Humic-like</td>
<td>Terr, Anthro, Agri</td>
</tr>
<tr>
<td>Rain</td>
<td>Comp1</td>
<td>250(315)</td>
<td>430</td>
<td>11.29 ± 5.86</td>
<td>A,C</td>
<td>UVC Humic-like</td>
<td>Humic, terr, Auto</td>
</tr>
<tr>
<td></td>
<td>Comp2</td>
<td>250</td>
<td>310</td>
<td>13.57 ± 8.57</td>
<td>-</td>
<td>Artifact, Uncertain</td>
<td></td>
</tr>
<tr>
<td>Comp</td>
<td>T</td>
<td>M</td>
<td>A, C</td>
<td>UVA Marine Humic-like</td>
<td>UVC Humic-like</td>
<td>Terr, Anthro, Agri</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>---</td>
<td>---</td>
<td>-----</td>
<td>-----------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>Comp1</td>
<td>250(320)</td>
<td>350</td>
<td>9.11 ± 13.39</td>
<td>T, M</td>
<td>Try-like, Protein-like</td>
<td>Humic, Terr, Auto</td>
<td></td>
</tr>
<tr>
<td>Comp2</td>
<td>250(305)</td>
<td>430</td>
<td>6.17 ± 6.15</td>
<td>A, C</td>
<td>UVA Marine Humic-like</td>
<td>Terr, Anthro, Agri</td>
<td></td>
</tr>
<tr>
<td>Comp3</td>
<td>300</td>
<td>350</td>
<td>8.05 ± 4.36</td>
<td>M</td>
<td>UVA Marine Humic-like</td>
<td>Anthro, wastewater</td>
<td></td>
</tr>
<tr>
<td>Comp1</td>
<td>250</td>
<td>450(290)</td>
<td>168.65 ± 189.20</td>
<td>A, -</td>
<td>UVC Humic-like</td>
<td>Terr, Anthro, Auto</td>
<td></td>
</tr>
<tr>
<td>Comp2</td>
<td>355</td>
<td>450</td>
<td>113.21 ± 113.41</td>
<td>C</td>
<td>UVA Humic-like</td>
<td>Terr, Anthro, Agri</td>
<td></td>
</tr>
<tr>
<td>Comp3</td>
<td>250(315)</td>
<td>410</td>
<td>73.18 ± 62.82</td>
<td>A, M</td>
<td>UVC Humic-like</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table B3. Production of marine components from Florida. Comparison of fluorescence maximum after 30 days of dark incubation. (n=3±SD)

<table>
<thead>
<tr>
<th>Species</th>
<th>Comp1</th>
<th>Comp2</th>
<th>Comp3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mangrove</td>
<td>Rhizophora mangle</td>
<td>1298 ± 391.93</td>
<td>891.58 ± 1.75</td>
</tr>
<tr>
<td>Sargassum</td>
<td>Sargassum sp.</td>
<td>762.07 ± 242.90</td>
<td>259.34 ± 55.76</td>
</tr>
<tr>
<td>Seagrass</td>
<td>Thallasia testudinum</td>
<td>76.22 ± 46.44</td>
<td>14.10 ± 8.93</td>
</tr>
<tr>
<td>HAB culture</td>
<td>Karenia brevis</td>
<td>28.69 ± 1.53</td>
<td>18.57 ± 0.66</td>
</tr>
<tr>
<td>Diatoms culture</td>
<td></td>
<td>104.26 ± 1.14</td>
<td>35.53 ± 1.66</td>
</tr>
</tbody>
</table>