January 2012

Optical Detection and Classification of Phytoplankton Taxa through Spectral Analysis

Daniel Tyler Sensi
University of South Florida, dsensi@mail.usf.edu

Follow this and additional works at: http://scholarcommons.usf.edu/etd

Part of the Oceanography Commons, Optics Commons, and the Other Oceanography and Atmospheric Sciences and Meteorology Commons

Scholar Commons Citation


This Thesis is brought to you for free and open access by the Graduate School at Scholar Commons. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Scholar Commons. For more information, please contact scholarcommons@usf.edu.
Optical Detection and Classification of Phytoplankton Taxa through Spectral Analysis

by

Daniel T. Sensi

A thesis submitted in partial fulfillment of requirements for the degree of Master of Science College of Marine Science University of South Florida

Major Professor: Chuanmin Hu, Ph.D.
John Walsh, Ph.D.
Leanne Flewelling, Ph.D.

Date of Approval:
November 15, 2012

Keywords: Ocean Optics, Tampa Bay, Florida, Estuaries, Spectroscopy, Taxonomy

Copyright © 2012, Daniel T. Sensi
DEDICATION

To my wife Stephanie, thank you for everything you have done for me. Without your love and support, my life would not be the same. I would not have been able to accomplish what I have without you. I am forever grateful and will always love you.

To my parents Carl and Claudia Sensi, thank you for always being there for me. You have always encouraged me and told me to do what I love most. Thank you for your love and support you have given me all these years. I could not have asked for a better set of parents to have raised me and given me so much. I am forever grateful and will always love you.

To my brother, Andrew Sensi, you have been such an amazing brother. I couldn’t have asked for a better role model than you. You have always been a man of honor and I have tried to model myself after you. Thanks for always being there for me when I have needed a brother and a friend. I have always looked up to you and will continue to do so. Thanks for setting such a great example for me. I love you buddy.

To my teachers at Flint Hill School, Frederick Atwood and Frederick Chanania, I will always remember what I have learned from you two. You began my intrigue for the sciences. Without your passion and selflessness of molding young minds I would probably not be where I am today. Thank you for all you
have done for me and all that you have done for countless individuals over the years. You two are simply amazing.

To my advisor at Old Dominion University, Alexander Bochdansky, thank you for guiding me through my undergraduate career. Thank you for your leadership and experience that you passed onto me. I would also like to thank professor Fred Dobbs at ODU. Thank you as well for your encouragement and expertise in the field of Oceanography. Thank you for your guidance and preparing me for my graduate career.

To my friends and lab mates at USF College of Marine Science, thanks for all the wonderful times. We are such a close knit family at CMS. There has been countless times where I have received support, friendship and laughs. Thank you for everything. I would also like to thank all the other professors at USF CMS and those that I have collaborated with at the FWC Fish and Wildlife Research Institute, including all of those in the HAB (Harmful Algal Bloom) department who help set up cruise surveys to collect samples, collected samples when I was unable to, and performed cell counts for countless hours under the microscope. There are so many giving individuals from these institutes. I can’t believe I found a place with so many selfless people eager to help and collaborate with one another.
ACKNOWLEDGMENTS

To my funding at the College of Marine Science, federal grants, NASA’s Ocean Biology and Biogeochemistry program, as well as the donors of the St. Petersburg Downtown Partnership Fellowship, Dr. Peter Betzer and Mr. Kenneth Heretick, and the collaborated effort with USGS for which the fellowship exists. I would not have been able to pursue my graduate degree, or been able to have had this amazing experience without this funding. Thank you.

To my committee members, Drs. Leanne Flewelling, Chuanmin Hu and John Walsh, thank you for all your help and support during my final push of my thesis. I would not have been able to accomplish what I have without your knowledge and expertise. Thank you for guiding me along the way.

Lastly, my advisor at University of South Florida College of Marine Science, Chuanmin Hu, thank you for taking a chance on me and giving me the privilege to continue my education in graduate studies. I am forever grateful for the knowledge and experience that you have passed on to me. Thank you for giving up your time and your energy to teach me all that you have. I have grown in so many ways because of you. Thank you for everything.
TABLE OF CONTENTS

LIST OF TABLES .............................................................................................................................................. ii
LIST OF FIGURES ............................................................................................................................................ iii
ABSTRACT ......................................................................................................................................................... v
INTRODUCTION ............................................................................................................................................... 1
  A. Harmful Algal Blooms (HAB’s) in the Gulf of Mexico ................................................................. 1
  B. Methods to identify and quantify phytoplankton ................................................................. 4
OBJECTIVES ..................................................................................................................................................... 8
METHODOLOGY ............................................................................................................................................... 9
  Field & Lab Methods ............................................................................................................................. 9
    Filter Pad Processing ......................................................................................................................... 14
    Fluorometric Processing .................................................................................................................. 17
RESULTS ......................................................................................................................................................... 18
  Pigment Composition ......................................................................................................................... 18
  Phytoplankton Taxa: Tampa Bay ..................................................................................................... 22
    Method 1: Derivative Analysis ....................................................................................................... 29
    Method 2: Relative Height Analysis ............................................................................................. 31
    Method 3: Integration Analysis ..................................................................................................... 34
  Phytoplankton Taxa: Indian River Lagoon ................................................................................... 36
    Method 1: Derivative Analysis ....................................................................................................... 40
    Method 2: Relative Height Analysis ............................................................................................. 43
    Method 3: Integration Analysis ..................................................................................................... 45
DISCUSSION .................................................................................................................................................. 48
SUMMARY & CONCLUSION ...................................................................................................................... 51
REFERENCES ................................................................................................................................................... 54
LIST OF TABLES

Table 1: Tampa Bay sampling effort between May 2011 and September 2012.......................... 10

Table 2: Indian River Lagoon sampling effort from April 2011 through September 2011.. .......... 12

Table 3: Estimated pigment concentrations (mg m$^{-3}$) from the spectra-matching optimization (Fig. 9), following the approach of Bricaud et al., (2004). .................. 20

Table 4: Performance matrix for the three techniques applied to Tampa Bay data in order to differentiate *P. bahamense* blooms from other taxa. .............................................. 36

Table 5: Performance matrix for the three techniques applied to Indian River Lagoon data in order to differentiate *Pedinophyceae* blooms from other taxa. ......................... 47
LIST OF FIGURES

Figure 1: *Pyrodinium bahamense* cells (left) and bloom in Old Tampa Bay in 2011 (right). .......... 2
Figure 2: Scanning electron micrograph of *Pseudo-nitzschia australis*. 1,000x magnification and 10,000x magnification (insert). .................................................................................. 2
Figure 3: *Micromonas pusilla* a species in the *Pedinophyceae* class (left) and a photo of the discolored water from the *Pedinophyceae* bloom in the Indian River Lagoon summer 2011 (right). ........................................................................................................ 3
Figure 4: *Karenia brevis* bloom off Little Gasparilla Island during the 2006 bloom (left) and *Karenia brevis* cell (right). .......................................................... 4
Figure 5: Map of Tampa Bay and station locations. ................................................................. 10
Figure 6: Map of the Indian River Lagoon on Florida’s east coast. ...................................... 11
Figure 7: Filter pad processing set-up showing: 1) a DOS system computer to run the spx.exe program; 2) a tungsten-halogen lamp as a light source; 3) a 512-channel “Spectrix” radiometer and 4) a pad box to measure \( a_\phi \) and \( a_d \) on filter pad and samples against reference water on a blank baseline filter pad. ....................... 15
Figure 8: Measured weight-specific absorption spectra of the main phytoplankton pigments, Bidigare et al. (1989). .......................................................................................... 19
Figure 9: An example of measured \( a_\phi(\lambda) \) (purple) from the marine diatom genus *Pseudo-nitzschia*, as compared with the modeled \( a_\phi(\lambda) \) through a spectra-matching optimization using the known mass-specific absorption of individual pigments (Bidigare et al., 1989). .............................................................. 20
Figure 10: Measured [Chl\_a] (mg m\(^{-3}\)) versus modeled [Chl\_a] (mg m\(^{-3}\)). ......................... 21
Figure 11: \( a_\phi(\lambda) \) of all *P. bahamense* bloom (a) and non-bloom (b) samples from Tampa Bay. ................................................................................................................ 23
Figure 12: \( a_\phi(\lambda) \) of all *Pseudo-nitzschia spp.* bloom (a) and non-bloom (b) samples from Tampa Bay. ........................................................................................................ 24
Figure 13: Average \( a_\phi(\lambda) \) of all bloom and non-bloom samples of various phytoplankton taxa from Tampa Bay. ......................................................................................................... 26
Figure 14: (a) \( a_\phi^*(\lambda) \) spectra of *P. bahamense* bloom samples highlighting region of unique increase in \( a_\phi^*(\lambda) \) from (570-600nm) and relating this increase to the Chl\_c \( a_\phi^*(\lambda) \) spectra highlighted in (b) measured weight-specific absorption spectra of the main phytoplankton pigments (Bidigare et al., 1989). ....................... 28
Figure 15: (a) Averaged 1st order derivative of the $a_{\phi}^*(\lambda)$ spectra for all phytoplankton taxa in Tampa Bay.

Figure 16: 1st derivative $a_{\phi}^*(578-588\text{nm})$ summation for all phytoplankton taxa in Tampa Bay plotted against cell concentration.

Figure 17: Relative height method for $a_{\phi}^*(\lambda)$ of $P. bahamense$ bloom samples from Tampa Bay.

Figure 18: Relative heights of $a_{\phi}^*(\lambda)$ for all phytoplankton taxa in Tampa Bay plotted against cell concentrations.

Figure 19: Integration method for $a_{\phi}^*(\lambda)$ of $P. bahamense$ bloom samples.

Figure 20: Integration of all $a_{\phi}^*(\lambda)$ spectra from (572-600nm) for all the Tampa Bay samples.

Figure 21: (a) $a_{\phi}^*(\lambda)$ for the Pedinophyceae bloom samples collected from in the Indian River Lagoon (IRL). The spectral region of interest is outlined in black and enlarged in (b), where the spectra in the black outline represent bloom samples with concentrations of >6,000,000 cells/L.

Figure 22: Averaged $a_{\phi}^*(\lambda)$ spectra for all phytoplankton taxa from Tampa Bay and IRL.

Figure 23: Measured weight-specific absorption spectra of the main phytoplankton pigments (Bidiger et al., 1989).

Figure 24: 1st order derivative $a_{\phi}^*(\lambda)$ of Pedinophyceae bloom taxa and other taxa from the Indian River Lagoon from (450-600nm).

Figure 25: Averaged 1st order derivative of the $a_{\phi}^*(\lambda)$ spectra for all phytoplankton taxa in Tampa Bay and IRL. The circled in (a) denotes the region of interest which is enlarged in (b).

Figure 26: 1st derivative $a_{\phi}^*(\lambda)$ summation (479-483nm) for Pedinophyceae bloom taxa and all other taxa from IRL and Tampa Bay versus cell concentration.

Figure 27: Pedinophyceae bloom $a_{\phi}^*(\lambda)$ spectra showing method for relative height.

Figure 28: Relative height analysis of $a_{\phi}^*(\lambda)$ for all phytoplankton taxa in IRL and Tampa Bay. The green dots in the circled areas are the Pedinophyceae samples.

Figure 29: Integration method for $a_{\phi}^*(\lambda)$ of Pedinophyceae bloom samples.

Figure 30: Integration algorithm of $a_{\phi}^*(\lambda)$ versus cell count for the Pedinophyceae bloom samples and all of phytoplankton taxa from Tampa Bay and the IRL.
Phytoplankton serve as the bottom of the marine food web and therefore play an essential role in marine ecosystems. On the other hand, coastal phytoplankton communities can adversely affect the marine ecosystem and humans. A variety of techniques have been developed to measure and study phytoplankton, including in situ methods (e.g., flow cytometry) and laboratory methods (e.g., microscopic taxonomy). These provide accurate measurements of phytoplankton taxa and concentrations, yet they are limited in space and time, and synoptic information is difficult to obtain with these techniques.

Optical remote sensing may provide complementary information for its synoptic nature, as demonstrated by satellite estimates of major phytoplankton taxa in major ocean basins. It has remained a challenge, however, for coastal and estuarine waters due to their optical complexity. One pioneering work relied on hyperspectral absorption spectra of phytoplankton pigments (Millie et al., 1995), from which Gymnodinium breve (i.e., Karenia brevis) blooms on the West Florida shelf could be detected and quantified in situ. However, whether a similar approach can be developed for estuarine waters where toxic blooms are often found is still unknown. Thus, the objective of this study is to test and develop an approach to classify major phytoplankton taxa found in two estuaries in Florida, U.S.A., based on optical analysis of the phytoplankton absorption spectra.
In this study, over 250 surface water samples were collected on numerous cruise surveys from two Florida estuaries (Tampa Bay, ~1000 km² on the west coast; and the Indian River Lagoon, ~900 km² on the east coast). The samples were filtered and then processed using standard NASA protocols to determine 1) their spectral absorption coefficients due to phytoplankton pigments, \( a_\phi(\lambda) \) (m⁻¹), and 2) their chlorophyll \( a \) concentrations (mg m⁻³). \( a_\phi(\lambda) \) was further normalized by Chl \( a \), resulting in chlorophyll-specific absorption coefficient, \( a_\phi^*(\lambda) \) (m²mg⁻¹).

For each sample, phytoplankton cell counts were enumerated by the Florida Wildlife Conservation Commission (FWC) Fish and Wildlife Research Institute (FWRI) through microscopic taxonomy. The \( a_\phi^*(\lambda) \) data were then categorized based on the dominant phytoplankton taxa, and were separated as either bloom or non-bloom using a 100,000 cell/L threshold of the dominant taxa. Three techniques were tested for classifying phytoplankton taxa using absorption spectra; a 1st derivative summation, a relative height analysis, and an integration analysis. The integration technique proved to be the most successful of the three. This technique performed an integration of \( a_\phi^*(572-600\text{nm}) \) against a linear baseline, and yielded an 81% success rate (13 of 16 samples) and 9% false positive rate (13 of 144 samples) in separating blooms of the dinoflagellate \textit{Pyrodinium bahamense} from other bloom and non-bloom taxa found in the Tampa Bay estuary. The same integration technique, but with the wavelength range shifted to 471 nm – 490 nm, was also applied to the samples collected in the Indian River Lagoon estuary from summer 2011 to study the green flagellate of the class \textit{Pedinophyceae}. The results showed an 80% success rate (8 of 10}
samples) and a 0.5% false positive rate (1 of 156 samples) in separating the *Pedinophyceae* bloom taxa from other bloom and non-bloom taxa found in both the Indian River Lagoon and Tampa Bay.

The number of bloom samples was relatively low (16 from Tampa Bay and 10 from IRL). Thus, the results from this study are preliminary and will require more sampling in order to further develop this technique to a practical method for field use. However, the results obtained from this study are comparable to those from other techniques for classification of phytoplankton taxa, for example, BreveBuster, SIPPER, FlowCAM, and satellite ocean color remote sensing of the open ocean. Yet this technique extends to optically complex estuarine waters, and therefore may represent a step towards the ultimate goal of applying satellite remote sensing in characterizing phytoplankton taxa in estuaries. Once confirmed with more samples from the same two estuaries as well as from other estuaries, an immediate next step may be the implementation of *in situ* optical instruments on either buoys (e.g., MARVIN in Tampa Bay) or flow-through systems to provide continuous characterization of major phytoplankton taxa in the two estuaries.
INTRODUCTION

A. Harmful Algal Blooms (HAB’s) in the Gulf of Mexico

Phytoplankton play an essential role in marine ecosystems as they serve as the bottom of the food web. However, harmful algal blooms (HABs) can produce a variety of detriments to the environment and the biota (Anderson et al., 2008). There are ~70 phytoplankton species that are considered harmful or toxic (Smayda, 1997). Of these, 75% are characterized in the dinoflagellate class. HAB species can adversely affect their environment in three different ways: produce biotoxins, have unique structures that damage organisms at higher trophic levels, and/or produce large biomass accumulations (blooms).

Various HABs regularly occur and bloom in the Gulf of Mexico (GOM) and Florida coastal waters. These include, but are not limited to, *Pyrodinium bahamense, Pseduo-nitzschia, Pedinophyceae, and Karenia brevis*.

*Pyrodinium* blooms have been found in Tampa Bay, Florida’s largest open-water estuary (Phlips et al., 2006). *P. bahamense* is a HAB species with spherical cells and two flagella: one horizontal, and one vertical for locomotion (Figure 1). *P. bahamense* can produce saxitoxins (Steidinger 1975). These saxitoxins can be sequestered in puffer fish as well as benthic shellfish beds and through bioaccumulation, can cause Paralytic Shellfish Poisoning (PSP) if consumed (Hallegraeff et al., 1988).
Figure 1: *Pyrodinium bahamense* cells (left) and bloom in Old Tampa Bay in 2011 (right). Photo credit: micrographs by Y. Fukuyo & K. Matsuoka (left) and Dorian Aerial and Architectural Photographics (right).

*Pseudo-nitzschia* is another HAB genus of pennate diatoms (Figure 2).

Some species of *Pseudo-nitzschia* are known to produce the neurotoxin (Domoic acid) which can be sequestered in shellfish, and when consumed can cause Amnesic Shellfish Poisoning (ASP) (Dortch et al., 1997, Parsons et al., 2012).

Figure 2: Scanning electron micrograph of *Pseudo-nitzschia australis*. 1,000x magnification and 10,000x magnification (insert). Photo credit: Peter E. Miller UCSC.

Some HAB species have been found in the phytoplankton class *Pedinophyceae*. Species in this class are primarily green flagellates which are
very small (<3 μm in diameter) (Moestrup et al., 1991). They are known to produce extreme bloom events with concentrations of 10-100 million cells/L or higher (Daugbjerg et al., 1995). Such blooms discolor the waters, block out sunlight, deplete oxygen and distress marine organisms (Thomsen et al., 1998). An unidentified species of this class bloomed in summer 2011 in Indian River Lagoon on Florida’s East Coast (Figure 3).

Figure 3: Micromonas pusilla a species in the Pedinophyceae class (left) and a photo of the discolored water from the Pedinophyceae bloom in the Indian River Lagoon summer 2011 (right). Photo credit: Worden Lab (A. Engman, R. Welsh, & A.Z. Worden) (left) and FWC (right).

Karenia brevis, another commonly found HAB species in the Gulf of Mexico known for producing “red tides” (Kirkpatrick et al., 2004; Cannizzaro et al., 2009) (Figure 4), is a dinoflagellate that can produce neurotoxins in high concentrations during a bloom (Tester and Steidinger 1997). These red tides can cause fish kills and respiratory irritation in humans (Kirkpatrick et al., 2000; Cheng et al., 2010; Kirkpatrick et al., 2010).
Figure 4: *Karenia brevis* bloom off Little Gasparilla Island during the 2006 (left) and *Karenia brevis* cell (right). Photo credit: Paul Schmidt Charlotte Sun Herald (left) and Gert Hansen (WoRMS Photography) (right).

**B. Methods to identify and quantify phytoplankton**

There are multiple ways to identify and quantify phytoplankton in natural mixed assemblages. The gold standard method is microscopic taxonomy, where water samples are examined under a microscope, with phytoplankton cells identified and enumerated (Semia, 1979). This technique is perhaps the most reliable way to identify phytoplankton taxa but it is labor intensive. Another method is to quantify phytoplankton pigments using High Pressure Liquid Chromatography (HPLC) (Horvath et al., 1967; Gieskes et al., 1983), where pigments are separated based on their density differences. Depending on the HPLC-determined pigment, information on phytoplankton taxa can be inferred (Kennedy et al., 1972). An additional method is to characterize phytoplankton taxa through *in situ* measurements using instruments such as SIPPER (Samson et al., 2001) or FlowCAM (Seracki et al., 1999; Cucci & Sieracki et al., 2000; Brown et al., 2009). SIPPER is a planktonic image viewer that uses a line-scan
camera to identify plankton in the water passing through the instrument (Luo et al., 2004; Luo et al., 2005). Its lower detection limit is ~100μm (largest phytoplankton taxa) and is therefore more suited for zooplankton identification (Remsen et al., 2004; Culverhouse et al., 2006). FlowCAM is an in-situ flow cytometer that is designed for the microplankton size range (20-200 μm) (Rose et al., 2004). The instrument can automatically record size and fluorescence of phytoplankton cells. It is used with software that captures images of a cell and compares it to a known cell for identification. One study found that the accuracy of the image software depended on the similarity of the species found in any particular mixed assemblage, with an 80-90% success rate and a 20-50% false positive rate (Buskey & Hyatt, 2006).

The field and laboratory-based methods provide accurate information on phytoplankton taxa and concentration, yet they are limited in both space and time. On the other hand, satellite remote sensing has been used in recent years to classify phytoplankton taxa at synoptic scales (e.g., Sathyendranath et al., 2001; Alvain et al., 2005; Devred et al., 2006; Uitz et al., 2006; Hirata et al., 2008; Bailey et al., 2008; Bailey et al., 2010; Mouw and Yoder, 2010; Kostadinov et al. 2010; Pan et al., 2010). Yet they are limited to open oceans. In coastal waters and estuaries, optical properties become more complex as water constituents other than phytoplankton (e.g., colored dissolved organic matter (CDOM), or suspended sediments) often dominate the remotely-sensed signals, making classification of phytoplankton extremely challenging, not to mention quantification of their concentrations (Sathyendranath et al., 2004; Franz et al.,
Thus, it is highly desirable to develop optical means to classify and quantify phytoplankton taxa in optically complex coastal and estuarine waters, with the ultimate application in optical remote sensing.

Detection and quantification of phytoplankton taxa through optical spectroscopy (mainly through the absorption spectra) are not new, but have been attempted for decades. Phytoplankton absorption spectra \( a_\varphi(\lambda), \text{m}^{-1} \) differ in both shape and magnitude due to a variety of conditions including cell concentration and pigment composition (Prieur & Sathyendranath 1981, Hoepffner & Sathyendranath 1993). Pigment composition varies among phytoplankton groups. Some pigments, like chlorophyll \( a \) are found in all phytoplankton groups, whereas others, like peridinin are more limited (Stauber & Jeffery, 1988; Brewin et al., 2010). Because \( a_\varphi(\lambda) \) is effected by the various pigments, it can be used to infer information on phytoplankton groups and possibly species (Ciotti et al., 2002; Nair et al., 2008).

One pioneering study utilizing \( a_\varphi(\lambda) \) to classify and quantify Gymnodinium breve (i.e., \( K. \ brevis \)) was based on laboratory measurements and field studies on the west Florida shelf (WFS) (Millie et al., 1995; Millie et al., 1997; Kirkpatrick et al., 2000). The studies combined \( a_\varphi(\lambda) \) and taxonomic cell counts data to separate \( K. \ brevis \) blooms from other taxa through a 4\textsuperscript{th} derivative analysis. The results led to the implementation of the BreveBuster instrument that can be mounted on ocean gliders to measure \( K. \ brevis \ in situ \) on the WFS (Robbins et al., 2006). The BreveBuster collects and analyzes \textit{in situ} \( a_\varphi(\lambda) \) and compares it with the known \( K. \ brevis \) \( a_\varphi(\lambda) \) using a similarity index (SI). Likewise, derivative
analysis of \( a_p(\lambda) \) (particulate absorption coefficient, \( \text{m}^{-1} \)) has been used to quantify phytoplankton pigments (Bidigare et al., 1989). The *in situ* optical spectroscopy has only been applied to open-ocean or shelf waters. Whether or not it is applicable to more optically complex estuarine waters is unknown, but it largely depends on whether there are unique optical (i.e., absorption) signatures associated with the various phytoplankton taxa commonly found in estuarine waters. The study here attempts to fill this knowledge gap through field and laboratory measurements as well as spectral analyses for two large estuaries in Florida.
OBJECTIVES

Given the pioneering works from several published studies to optically classify phytoplankton taxa for open-ocean and shelf waters but the lack of information on optical classification for estuarine waters, this study takes advantage of the existing bloom-monitoring program of FWRI and combines $a_{\phi}(\lambda)$ and phytoplankton taxonomy data to develop an optical method to classify phytoplankton taxa in estuarine waters. Specifically, the objectives are to:

- Conduct spectral analysis to determine the unique spectral signatures of different phytoplankton taxa and the causes of these spectral signatures.
- Develop a method to identify phytoplankton taxa in estuaries using optical spectral analyses.
METHODOLOGY

Field & Lab Methods

FWRI in St. Petersburg, Florida, has continuously collected and measured water samples from 10 stations in the Tampa Bay Estuary starting in May 2011 (Figure 5, Table 1). Water samples were collected once a month during non-bloom periods and every other week during blooms. May and June of 2011 only had 6 stations whereas all other collection dates had 10 stations, with a total of 239 surface water samples collected. FWRI had samples collected in the Indian River Lagoon on Florida’s east coast (Figure 6, Table 2). This sampling effort was conducted from April to September 2011. One surface water sample was collected from each of the two stations, with a total of 14 samples collected from the sampling effort in this region for this study. When a representative from the Optical Oceanography Lab (OOL) at University of South Florida (USF) College of Marine Science (CMS) was unable to participate in the field sampling, FWRI collected extra water when requested. Samples were collected at surface using 1-liter brown Nalgene bottles. Although more than 250 total samples were collected, there were only 227 that passed the quality control after processing. The samples that did not pass the quality control were ones that became contaminated or had an error occur during processing.
Figure 5: Map of Tampa Bay and station locations. (Lat., Lon.): 1 (28.0200, -82.6752), 2 (27.9723, -82.6729), 3 (27.9797, -82.6265), 4 (27.9418, -82.5779), 5 (27.9572, -82.6230), 6 (27.9420, -82.6746), 7 (27.9055, -82.6047), 8 (27.9202, -82.5605), 9 (27.8452, -82.5718), 10 (27.7717, -82.5813). Water samples have been collected and analyzed from these stations between May 2011 and September 2012.

Table 1: Tampa Bay sampling effort between May 2011 and September 2012. Of the 239 samples collected by FWRI and the Optical Oceanography Lab at USF/CMS, 215 showed high quality and were therefore used in this study. Note: bold fonts denote when the Optical Oceanography Lab (OOL) joined FWRI in the sampling effort.

<table>
<thead>
<tr>
<th>Cruise Date</th>
<th>Samples Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/19/11</td>
<td>6</td>
</tr>
<tr>
<td>06/09/11</td>
<td>6</td>
</tr>
<tr>
<td>07/07/11</td>
<td>6</td>
</tr>
<tr>
<td>07/20/11</td>
<td>10</td>
</tr>
<tr>
<td>08/04/11</td>
<td>10</td>
</tr>
<tr>
<td>08/17/11</td>
<td>1</td>
</tr>
<tr>
<td>08/31/11</td>
<td>10</td>
</tr>
<tr>
<td>09/14/11</td>
<td>10</td>
</tr>
</tbody>
</table>
(Table 1 Continued)

<table>
<thead>
<tr>
<th>Date</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/26/11</td>
<td>10</td>
</tr>
<tr>
<td>11/21/11</td>
<td>10</td>
</tr>
<tr>
<td>12/16/11</td>
<td>10</td>
</tr>
<tr>
<td>01/20/12</td>
<td>10</td>
</tr>
<tr>
<td>02/22/12</td>
<td>10</td>
</tr>
<tr>
<td>03/09/12</td>
<td>10</td>
</tr>
<tr>
<td>04/03/12</td>
<td>10</td>
</tr>
<tr>
<td>04/19/12</td>
<td>10</td>
</tr>
<tr>
<td>05/04/12</td>
<td>10</td>
</tr>
<tr>
<td>05/15/12</td>
<td>10</td>
</tr>
<tr>
<td>05/31/12</td>
<td>10</td>
</tr>
<tr>
<td>06/13/12</td>
<td>10</td>
</tr>
<tr>
<td>06/28/12</td>
<td>10</td>
</tr>
<tr>
<td>07/11/12</td>
<td>10</td>
</tr>
<tr>
<td>07/26/12</td>
<td>10</td>
</tr>
<tr>
<td>08/08/12</td>
<td>10</td>
</tr>
<tr>
<td>08/24/12</td>
<td>10</td>
</tr>
<tr>
<td>09/05/12</td>
<td>10</td>
</tr>
</tbody>
</table>

239 samples

215 Quality Control

Figure 6: Map of the Indian River Lagoon on Florida’s east coast. The north station, station 1 (28.7319, -80.7172) and the south station, station 3 (28.4408, -80.6344) are where FWRI collected samples for this study.
Table 2: Indian River Lagoon sampling effort from April 2011 through September 2011. One sample was collected from each of the two stations during this period. Of the 14 samples collected by FWRI, 12 showed high quality and were used in this study.

<table>
<thead>
<tr>
<th>IRL Sampling Effort</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cruise Date</td>
<td>Samples Collected</td>
</tr>
<tr>
<td>04/18/11</td>
<td>2</td>
</tr>
<tr>
<td>05/09/11</td>
<td>2</td>
</tr>
<tr>
<td>05/23/11</td>
<td>2</td>
</tr>
<tr>
<td>06/21/11</td>
<td>2</td>
</tr>
<tr>
<td>07/18/11</td>
<td>2</td>
</tr>
<tr>
<td>08/16/11</td>
<td>2</td>
</tr>
<tr>
<td>09/21/11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>14 samples</td>
</tr>
<tr>
<td></td>
<td>12 Quality Control</td>
</tr>
</tbody>
</table>

During the cruise surveys, sample bottles were rinsed three times with estuarine waters on site to ensure that any previous water residue would be removed. Surface samples were roughly taken from 0.1-0.5 meters below the surface. Bottles were then put in an ice filled cooler with the lid closed for storage on the cruise. The dark environment of the ice-filled cooler stops photosynthesis, and slows down respiration in order to have the sample bottle as the closest representation of phytoplankton in the natural environment. Once brought to the lab, samples were processed under standard NASA protocols. Using a filtration rig, samples were filtered onto Whatman GF/F filters (25 mm diameter) under a vacuum pressure of 5-7mmHg (maximum bottle time ~6 hours from collection to filtration). Filtration stopped once there was enough pigment collected on the filter pad visible to the naked eye. Pads were placed in Fisher Histoprep tissue capsules, wrapped in aluminum foil and placed in a -80°C freezer until processed. The filtrate was used to record the volume filtered on the pad and was filtered again using Whatman 47 mm diameter 0.2μm pore size
nylon membrane or Nuclepore™ polycarbonate filter pads for CDOM analysis. The first 20-50 ml that passed through the filter and into the Erlenmeyer flask was used as a rinse and discarded. The rest of the sample was collected in 125 ml brown qorpak bottles. Bottles were then stored in a -40°C freezer until they were processed.

FWRI collected water samples using the same method as described above. In addition, they also preserved water samples using unacidified Lugol’s solution. The unacidified Lugol’s solution was prepared in advance of the cruises and was made by dissolving 20 grams of potassium iodide (KI) and 10 grams of iodine crystals in 200 ml of distilled water containing 20 ml of glacial acetic acid. In the field, after a water sample had been collected, a separate dark container was used to mix 2ml of the unacidified Lugol’s solution for every 125 ml of sample. The bottle was capped tightly and inverted several times to ensure even mixing. Once samples had returned to the lab, the samples could have been counted immediately, or if that was not possible, they were counted later as the Lugol’s solution maintained the biological characteristics of the water sample at time of collection. When the preserved samples were ready to be counted, the sample was first inverted at least 20 times. This allowed the cells that had settled to be evenly mixed throughout the sample. Then, 3ml of sample was extracted with a pipette, and was placed in a Lab-Tek™ chambered cover glass system. Using an inverted microscope with objective/ocular combination to magnify 100-200x, the surface of the sample chamber was scanned at 40 or 50x to see if any cells were floating. If all the cells had not settled to the bottom, then
the surface and the bottom of the sample had to be counted. At 150x magnification there are about 20 rows in a sample. The sample was counted by moving the field of view horizontally. When the edge of the chamber was reached after a row of counting, the field of view was moved vertically to start a new row; assuring that the new row did not overlap or leave a gap between the previous row. Once the entire sample had been counted, the number of cells for each taxa were divided by three and multiplied by $10^3$ since a 3 ml aliquot of sample was used.

**Filter Pad Processing**

The Quantitative filter technique (Mitchell, 1990) was used to determine the absorption spectra due to particulates (phytoplankton $a_p(\lambda) +$ detritus $a_d(\lambda) =$ particles $a_p(\lambda)$). Filter pads were removed from storage in a -80°C freezer and given time to thaw. The sample filter was placed with a reference filter into a custom made transmissometer box. Each of the filters sat on glass plates. One at a time, three times each, the filters were slid over-top a tungsten-halogen light source where the transmittances ($T_{\text{sample}}(\lambda)$ and $T_{\text{reference}}(\lambda)$) were recorded using an in-house custom built 512-channel spectroradiometer (~350-850nm) ("Spectrix"). For the three scans on each filter; the dark current was subtracted from each, normalized by integration time and then were averaged to obtain ($OD_p(\lambda)$) (Figure 7).
Pigment extraction (Holm-hansen & Reimann., 1978, Kishino et al., 1985) was used to extract pigments from the sample filter (just measured for \( a_p(\lambda) \)) in order to collect pigments for fluorometric analysis and to measure \( a_d(\lambda) \) using the above mentioned technique. The sample filter was placed on a filtration tower with a 125 ml qorpak bottle underneath to collect the filtrate. 10-15 ml of hot (sub-boiling) 100% methanol was poured into the filter tower and onto the filter to allow for pigment extraction. The methanol was initially drawn through the filter using one pump of a vacuum hand pump, but the pressure was then released by breaking the suction seal. Once the methanol had passed through the filter, or ~5 minutes had passed, another 10-15 ml of hot methanol was poured on the
filter. This step was repeated once more, allowing for the filter to extract for ~15-20 minutes and to have ~40-60 ml of methanol pass through the filter and into the amber qorpak bottle for fluorometric analysis. Since multiple samples were run during one session (~24 samples and only 4 methanol extraction towers) each of the towers had to be cleaned and rinsed with room temperature methanol so as to not contaminate future samples. Transmittances of the extracted filter \((T_{\text{detritus}}(\lambda))\) and \(T_{\text{reference}}(\lambda)\) were measured three times and the OD\(_d(\lambda)\) calculated.

\[
\text{OD}_p(\lambda) = -\log_{10} \left[ \frac{T_{\text{sample}}(\lambda)}{T_{\text{reference}}(\lambda)} \right] \\
\text{OD}_d(\lambda) = -\log_{10} \left[ \frac{T_{\text{detritus}}(\lambda)}{T_{\text{reference}}(\lambda)} \right]
\]

From this, the absorption coefficients of particulate matter \((a_p(\lambda))\), detritus \((a_d(\lambda))\) and phytoplankton \((a_\phi(\lambda))(m^{-1})\) and \((a_\phi^*(\lambda))(m^2mg^{-1})\) were calculated as follows.

\[
a_p(\lambda)(m^{-1}) = \frac{2.303 \times [\text{OD}_p(\lambda) - \text{OD}_p(\lambda\text{null})]}{\beta^* l_s} \\
a_d(\lambda)(m^{-1}) = \frac{2.303 \times [\text{OD}_d(\lambda) - \text{OD}_d(\lambda\text{null})]}{\beta^* l_s} \\
a_\phi(\lambda)(m^{-1}) = a_p(\lambda) - a_d(\lambda) \\
a_\phi^*(\lambda)(m^2mg^{-1}) = \frac{a_\phi(\lambda)}{[\text{Chl} \ a]} 
\]

Where \(l_s\) is the geometric pathlength of the filtered material in suspension:

\[
l_s = \frac{V_f(m^3)}{A_f(m^2)}
\]

Where, \(V_f\) is the volume of seawater filtered \((m^3)\) and \(A_f\) is the clearance area of the filter \((m^2)\). And \(\beta\) or “Beta factor” is the pathlength elongation factor that helps correct for pathlength increases due to multiple scattering inside the filter.
\[ \beta = 1.0 + 0.6^{\text{OD}(\lambda)^{0.05}} \quad (8) \]

Note that the \( \beta \) factor you choose depends on your sample. The \( \text{OD}_p \) will depend on how much “color” is filtered onto the pad. Ideally, \( \text{OD}(675) \geq 0.04 \) and \( \text{OD}(440) \leq 0.4 \).

**Fluorometric Processing**

Fluorometric chlorophyll and phaeopigment analysis was performed on the filtrate collected earlier through the hot methanol extraction (Kishino et al., 1985). Samples were processed on two Turner Designs 10-AU Field Fluorometers. One fluorometer used the technique presented by (Holm-Hansen and Riemann, 1978), the other by (Welschmeyer 1994). They will be referred to as the “acid” and “no-acid” techniques respectively. Where \( R_{\text{bcorr}} \) is the corrected ratio of chlorophyll \( a \) concentration before the addition of acid (both “acid” and “no acid” technique), and \( R_{\text{acorr}} \) is the corrected ratio of chlorophyll \( a \) concentration after the addition of acid (“acid” technique only). ‘\( r \)’ is the ratio of pure chlorophyll \( a \) standard measured before and after acid (“acid” technique only).

\[
[\text{Chl} \ a]_{\text{no acid}} = R_{\text{bcorr}} \times (\text{vol. MeOH/Vol. seawater}) \quad (9)
\]

\[
[\text{Chl} \ a]_{\text{acid}} = (r/r-1) \times (R_{\text{bcorr}} - R_{\text{acorr}}) \times (\text{vol. MeOH/Vol. seawater}) \quad (10)
\]

\[
[\text{Pheo}]_{\text{acid}} = (r/r-1) \times (R_{\text{acorr}} \times r - R_{\text{bcorr}}) \times (\text{vol. MeOH/Vol. seawater}) \quad (11)
\]

\[
R_{\text{bcorr}} = R_b \times \text{Secondary_Solid} \_\text{correction_value} \quad (12)
\]

\[
R_{\text{acorr}} = R_a \times \text{Secondary_Solid} \_\text{correction_value} \quad (13)
\]

\[
\text{Secondary_Solid} \_\text{correction_value} = \\
[((\text{Low_value}_{\text{cal./Low_value}}) + (\text{High_value}_{\text{cal./High_value}})) / 2 \quad (14)
\]
RESULTS

All samples were processed using the methods above to obtain [Chl a] (mg m\(^{-3}\)), \(a_\phi(\lambda)\) (m\(^{-1}\)), \(a_\phi^*(\lambda)\) (m\(^2\)mg\(^{-1}\)) (from OOL measurements and analyses), and concentrations of phytoplankton cells (from FWRI measurements and analyses). These formed the basis to conduct spectral analysis and to develop optical methods for classifying phytoplankton taxa in Tampa Bay and Indian River Lagoon.

Pigment Composition

The first attempt was to understand what phytoplankton pigments may have contributed to the observed \(a_\phi(\lambda)\). Because no HPLC measurement was available, a spectra-matching optimization was used to estimate the pigment composition in each water sample, following the approach developed by Bricaud et al., (2004). This approach used the mass-specific absorption spectra for individual pigments provided by Bidigare et al. 1989 (Figure 8). By altering their proportions, a best match was found between the modeled \(a_\phi(\lambda)\) and measured \(a_\phi(\lambda)\), from which the proportions of individual pigments that contributed to the measured \(a_\phi(\lambda)\) were determined. Such determined pigment concentrations are termed as “modeled” in this study.
Figure 8: Measured weight-specific absorption spectra of the main phytoplankton pigments, Bidigare et al. (1989).

Figure 9 shows an example of the measured and modeled $a_{\phi}(\lambda)$ using the individual pigment spectra in Fig. 8. The model produced a spectrum that closely mimicked the measured $a_{\phi}(\lambda)$, from which the individual pigments were determined simultaneously (Table 3). Although HPLC measurements can provide the ultimate ground truth to validate such modeled pigment composition (Korthals & Steenbergen, 1985), in the absence of such measurements a measure of validity can be obtained by comparing the modeled [Chl_a] and the measured [Chl_a], with the assumption that if they agree well the pigment composition for other pigments derived from the same spectra-matching optimization model may also be valid. Fig. 10 shows such modeled [Chl_a] as compared with measured [Chl_a] from all water samples. Clearly, the excellent
agreement suggests that the pigment composition may also be valid and can be used to explain the spectral shapes of $a_\phi(\lambda)$ found in different phytoplankton taxa.

Figure 9: An example of measured $a_\phi(\lambda)$ (purple) from the marine diatom genus *Pseudo-nitzschia*, as compared with the modeled $a_\phi(\lambda)$ through a spectra-matching optimization using the known mass-specific absorption of individual pigments (Bidigare et al., 1989). The pigment composition that yielded the modeled $a_\phi(\lambda)$ is listed in Table 3.

Table 3: Estimated pigment concentrations (mg m$^{-3}$) from the spectra-matching optimization (Fig. 9), following the approach of Bricaud et al., (2004). Such a pigment composition would produce $a_\phi(\lambda)$ that best matches the measured $a_\phi(\lambda)$ (Fig. 9).

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Concentration (mg m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a</td>
<td>4.735369778</td>
</tr>
<tr>
<td>Dv-Chla</td>
<td>0.471178862</td>
</tr>
<tr>
<td>Chl b</td>
<td>0</td>
</tr>
<tr>
<td>Dv-Chlb</td>
<td>0.255024731</td>
</tr>
<tr>
<td>Chl c 1, 2</td>
<td>1.110074012</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>0</td>
</tr>
<tr>
<td>19'-BF</td>
<td>0</td>
</tr>
<tr>
<td>19'-HF</td>
<td>0</td>
</tr>
<tr>
<td>Peridinin</td>
<td>3.38752264</td>
</tr>
<tr>
<td>Diadinoxanthin</td>
<td>0.132082929</td>
</tr>
</tbody>
</table>
(Table 3 Continued)

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeaxanthin</td>
<td>0</td>
</tr>
<tr>
<td>Alloxanthin</td>
<td>0.503935852</td>
</tr>
<tr>
<td>β-carotene</td>
<td>0.478945123</td>
</tr>
<tr>
<td>α-carotene</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 10: Measured [Chl\_a] (mg m\(^{-3}\)) versus modeled [Chl\_a] (mg m\(^{-3}\)). The model derives the pigment composition (including the proportion of [Chl\_a] from a sample through a spectra-matching optimization to find the best match between modeled and measured \(a_φ(λ)\) (Bricaud et al., 2004). (a) Tampa Bay and (b) Indian River Lagoon.
Phytoplankton Taxa: Tampa Bay

The $a_\phi^*(\lambda)$ spectra were categorized according to the cell counts of their dominant phytoplankton taxa. For the majority of the samples, the dominant cell count represented >90% of the total cell count in the sample, making the sample a proper representation of that phytoplankton taxa. Note that the terms of bloom and non-bloom come from the cell count data for that sample, where a bloom sample has >100,000 cell/L of the dominant species, and a non-bloom sample has <100,000 cells/L of the dominant species for each sample. This threshold was determined from trial and error until the best classification results were achieved. Then, $a_\phi^*(\lambda)$ of each phytoplankton class (taxa, bloom versus non-bloom) was examined to locate the unique spectral signatures associated with the class. Figure 11 shows the $a_\phi^*(\lambda)$ spectra of *P. bahamense* bloom (a) and non-bloom (b) samples from Tampa Bay. The bloom samples show much lower $a_\phi^*(\lambda)$ than the non-bloom samples, especially in the blue wavelength range (400-500nm), a typical phenomenon with packaging effect (Bricaud et al., 1981; Sathyendranath et al. 1987; Stramski & Bricaud, 1988; Nelson & Prezelin 1990). Similar to Fig. 11, Fig. 12 shows the $a_\phi^*(\lambda)$ spectra of bloom and non-bloom samples collected from Tampa Bay for the diatom *Pseudo-nitzschia spp*. Unlike those for the *P. bahamense* taxa, there is no distinguishable trend in the magnitude of $a_\phi^*(\lambda)$ between bloom and non-bloom samples. However, the focus of this study is not on the spectral magnitude but on the spectral shapes. Thus, the changes, or lack-there-of, in the magnitude of $a_\phi^*(\lambda)$ between bloom and non-bloom samples will not affect the classification results.
Figure 11: $a_{\phi}(\lambda)$ of all *P. bahamense* bloom (a) and non-bloom (b) samples from Tampa Bay. The highlighted region denotes a unique spectral signature only observed in the *P. bahamense* bloom samples.
Figure 12: $a_\phi(\lambda)$ of all *Pseudo-nitzschia* spp. bloom (a) and non-bloom (b) samples from Tampa Bay. The highlighted region denotes a unique spectral signature only observed in the *P. bahamense* bloom samples.
indicated that there was a spectral feature between 550 nm and 600 nm for \( P. \ bahamense \) bloom samples that was not observed in other samples from Tampa Bay (Figure 11 & 12). Next, the \( a_\phi^*(\lambda) \) spectra for all the Tampa Bay samples were averaged according to their dominant taxa (Figure 13). The \( P. \ bahamense \) bloom \( a_\phi^*(\lambda) \) spectra samples exhibit an obvious difference from the other averaged taxa with lowered absorption in the blue wavelength range (400-500 nm). However, these are the average \( a_\phi^*(\lambda) \) spectra. Recalling earlier figures (11 & 12 a & b) the individual \( a_\phi^*(\lambda) \) spectra of each phytoplankton taxa vary in both magnitude and shape. So even though the average \( a_\phi^*(\lambda) \) spectra for \( P. \ bahamense \) bloom samples can be well distinguished from other samples, in practice this is not useful because \textit{a priori} knowledge is required to average all samples of the same taxa. The real challenge lies in being able to differentiate \( a_\phi^*(\lambda) \) spectra for different phytoplankton taxa without any \textit{a priori} knowledge of the cell counts. That is why the focus is shifted to the range denoted by the box in Fig. 13 a, which is enlarged in Fig. 13 b. Figure 13 b distinguishes the spectral feature of the averaged \( P. \ bahamense \) bloom samples from the \( a_\phi^*(\lambda) \) spectra from other phytoplankton taxa. Now that there is a clear difference in the \( a_\phi^*(\lambda) \) spectra of \( P. \ bahamense \) bloom samples from all other taxa in Tampa Bay, on average, the next step is determine what pigment is causing this feature, and if individual \( a_\phi^*(\lambda) \) spectra can be differentiated.
Figure 13: Average $a_\phi(\lambda)$ of all bloom and non-bloom samples of various phytoplankton taxa from Tampa Bay. The arrow in (a) points to the average *P. bahamense* bloom $a_\phi(\lambda)$ spectra and the box denotes the spectral region that is enlarged in (b), where the spectral differences between the samples is highlighted in the black outline.
From comparison of the individual $a_{\phi}^*(\lambda)$ spectra of *P. bahamense* bloom samples with those of the “measured weight-specific absorption spectra of the main phytoplankton pigments” (Bidigare et al., 1989), there appears a connection between the local peak in the *P. bahamense* bloom $a_{\phi}^*(\lambda)$ and the absorption spectra of Chl_c (Figure 14 a & b). There is a precise overlap for when *P. bahamense* bloom $a_{\phi}^*(\lambda)$ spectra begins to increase at around 570 (nm) (Fig. 14 a), with a peak absorption around 585 (nm), and a second trough around 600 (nm) and the $a_{\phi}^*(\lambda)$ spectra of Chl_c (Fig. 14 b). There are other absorption peaks in the *P. bahamense* bloom $a_{\phi}^*(\lambda)$ spectra around 625 (nm) and a third at 670 (nm). These two absorption peaks are due to a combination of chl_a, chl_c, and divinyl chl_a for the first, and chl_a and divinyl chl_a for the second. However, these regions at 625 nm and 670 nm are not unique, as they are present in all of the $a_{\phi}^*(\lambda)$ spectra no matter the taxa. The $a_{\phi}^*(\lambda)$ peak at 585 nm in the *P. bahamense* bloom spectra is unique however, as evident by the individual and average $a_{\phi}^*(\lambda)$ spectral graphs (Figures, 11, 12 & 13 a & b).

Now that a unique feature has been detected in the $a_{\phi}^*(\lambda)$ spectra the next step is to quantitatively classify this feature, and be able to do so without *a priori* knowledge of cell counts. Previous studies have used 2$^{nd}$ and 4$^{th}$ derivative analysis to separate pigments in $a_{\rho}(\lambda)$ spectra (Bidigare et al., 1989) as well as optical density (OD) in Gymnodinium breve (Millie et al., 1995). This study used several methods to classify the various phytoplankton taxa, which will be described in detail below.
Figure 14: (a) $a_\phi^*(\lambda)$ spectra of *P. bahamense* bloom samples highlighting region of unique increase in $a_\phi^*(\lambda)$ from (570-600nm) and relating this increase to the Chl\_c $a_\phi^*(\lambda)$ spectra highlighted in (b) measured weight-specific absorption spectra of the main phytoplankton pigments (Bidigare et al., 1989).
**Method 1: Derivative Analysis**

A 1\textsuperscript{st} order derivative analysis was performed on all of the $a_{\varphi}^*(\lambda)$ spectra for the phytoplankton taxa in Tampa Bay and then averaged (Figure 15). Since the unique feature to be separated in the *P. bahamense* bloom spectra is an increase in absorption, this area will show up as a positive value in the 1\textsuperscript{st} derivative graph. The larger the increase in $a_{\varphi}^*(\lambda)$, the more positive the 1\textsuperscript{st} derivative value will be, and vice versa for all decreases. So, the 1\textsuperscript{st} derivative analysis has separated *P. bahamense* bloom samples in both spectral shape and magnitude, whereas the normal $a_{\varphi}^*(\lambda)$ in Fig. 13b only revealed a difference in spectral shape. The focus is specifically on the region around 585 nm as previously stated. The averaged 1\textsuperscript{st} derivative $a_{\varphi}^*(\lambda)$ spectra for *P. bahamense* bloom samples is significantly different than the other taxa in our region of interest. In fact, the averaged data show that only *P. bahamense* bloom spectra are positive in this region. So, a summation of the 1\textsuperscript{st} derivative spectra was used to separate these *P. bahamense* bloom samples from other samples. The 1\textsuperscript{st} derivative $a_{\varphi}^*(\lambda)$ spectra of all the individual phytoplankton taxa were summed from 578nm-588nm and plotted against cell concentration (Figure 16).
Figure 15: (a) Averaged 1st order derivative of the $a_\phi(\lambda)$ spectra for all phytoplankton taxa in Tampa Bay. The arrow denotes the averaged *P. bahamense* bloom samples and is enlarged in (b) to show that it is the only spectra with a positive value in the region of interest.
Although on average the 1st derivative $a_\phi^*(\lambda)$ spectra of *P. bahamense* bloom samples seem to be distinct, on individual samples the separation is not as clear. The use of higher order derivative analysis was also applied to the data with no remarkable improvements. The success rate for the 1st derivative analysis of all phytoplankton taxa in Tampa Bay is 56% (9 of 16 *P. bahamense* bloom samples yielded a positive summation), with a false positive rate of 10% (14 of 144 samples).

**Method 2: Relative Height Analysis**

After an unsuccessful attempt at the use of derivative analysis, a new approach was taken. The goal is to develop a process that can differentiate individual $a_\phi^*(\lambda)$ spectra of *P. bahamense* bloom samples from other taxa without
the use of *a priori* knowledge of cell counts. Figure 14b shows the $a_{\phi}^*(\lambda)$ spectra of *P. bahamense* bloom samples with a local peak absorption around 585 nm. Accordingly, a relative height algorithm was applied to this region. The relative height of a sample is determined by taking the $a_{\phi}^*(\lambda)$ value at a particular wavelength (585nm), and then subtracting a baseline average of two values on either ends of this peak wavelength (Figure 17). This difference is then divided by the baseline average (equation 15).

$$\text{Relative Height} = \frac{[a_{\phi}^*(585)-\text{Average}(a_{\phi}^*(570&600))]}{\text{[Average}(a_{\phi}^*(570&600))]}$$ (15)

In theory, any increase in $a_{\phi}^*(\lambda)$ spectrum around 585nm should show a positive height, and any decrease should show a negative height. The relative
heights of $a_\phi^*(\lambda)$ spectra for all the phytoplankton taxa in Tampa Bay were calculated and plotted against cell concentrations (Figure 18).

![Figure 18: Relative heights of $a_\phi^*(\lambda)$ for all phytoplankton taxa in Tampa Bay plotted against cell concentrations. The horizontal line denotes the 100,000 cell/L bloom threshold, the vertical denotes the zero mark and the circled region highlights the $P. bahamense$ bloom samples.]

This relative height analysis has an 81% success rate of successfully detecting $P. bahamense$ bloom samples (13 of 16) with a 15% false positive rate (22 of 144). Of these, there were 7 Pseudo-nitzschia spp. non-bloom, 4 of which had $P. bahamense$ cell counts of 1,000 cells/L and greater. There were 8 $P. bahamense$ non-bloom samples that were positive, 3 of these had cell counts of 60,000 cells/L or greater. There were two Rhizosolenia spp. bloom samples that had a positive relative height, these samples did not have specific cells counts, they were just labeled as Rhizosolenia spp. bloom, so it is unknown as to how large the Rhizosolenia spp. bloom was and what, if any, other phytoplankton taxa
were present in the sample. The three others were two *Pseudo-nitzschia spp.* bloom samples and a *Skeletonema spp.* bloom, none of which contained any *P. bahamense*.

**Method 3: Integration Analysis**

A third and final method for bloom detection was used on the Tampa Bay $a_\phi^*(\lambda)$ samples. The final method is an integration of the $a_\phi^*(\lambda)$ spectra and is slightly more complicated than the previous two methods. An integration of any line adds up the area beneath that line relative to a specified baseline. Since each $a_\phi^*(\lambda)$ spectrum is slightly different than any other, a normalized baseline was created for each. The baseline is the slope of the line that connects the endpoints that the integration is performed across. Then, in 1nm increments, the difference between the $a_\phi^*(\lambda)$ of the sample and the sloped baseline is recorded and summed across the integrated area (Figure 19). Like before, a larger integrated area will equate to a larger peak across that area, but will be a more precise depiction of each absorption peak than the relative height method. The integration was performed from 572nm to 600nm on all of the $a_\phi^*(\lambda)$ spectra from all the phytoplankton taxa in Tampa Bay (Figure 20). This technique shows improvement with an 81% success rate (13 of 16 samples), but with a false positive rate reduced to 9% (13 of 144 samples) as compared to the $1^{st}$ derivative summation and the relative height analysis. Five of the false positives are from *P. bahamense* non-bloom samples, 2 of which had concentrations >60,000 cell/L. There are 6 *Pseudo-nitzschia spp.* non-bloom false positives instead of 7, 4 of these still have >1,000 cells/L of *P. bahamense*. The other two are from a
*Pseudo-nitzschia* spp. bloom sample which had no cells of *P. bahamense*, and a *Rhizosolenia* spp. bloom sample with undocumented cell counts.

Figure 19: Integration method for $a_\phi^\star(\lambda)$ of *P. bahamense* bloom samples. The blue shaded area represents the summed area from the spectral integration.

Figure 20: Integration of all $a_\phi(\lambda)$ spectra from (572-600nm) for all the Tampa Bay samples. The horizontal line denotes the 100,000 cell/L bloom threshold, the vertical denotes the zero mark and the circled region highlights the *P. bahamense* bloom samples.
In summary, among the three methods tested for Tampa Bay samples, the integration analysis provided the best results in separating *P. bahamense* blooms from and other blooms, with the major results listed in Table 4.

Table 4: Performance matrix for the three techniques applied to Tampa Bay data in order to differentiate *P. bahamense* blooms from other taxa.

<table>
<thead>
<tr>
<th></th>
<th>Success Rate</th>
<th>False Positive Rate</th>
<th>False Negative Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyrodinium bahamense</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Derivative</td>
<td>56% (9 of 16)</td>
<td>10% (14 of 144)</td>
<td>44% (7 of 16)</td>
</tr>
<tr>
<td>Relative Height</td>
<td>81% (13 of 16)</td>
<td>15% (22 of 144)</td>
<td>19% (3 of 16)</td>
</tr>
<tr>
<td>Integration</td>
<td>81% (13 of 16)</td>
<td>9% (13 of 144)</td>
<td>19% (3 of 16)</td>
</tr>
</tbody>
</table>

**Phytoplankton Taxa: Indian River Lagoon**

There was a primitive green flagellate species in the *Pedinophyceae* class that bloomed in summer 2011 in the Indian River Lagoon, Florida. Their genus and species, however, were never determined. The $a_{\varphi}^*(\lambda)$ spectra of the *Pedinophyceae* bloom show a distinct shouldering in the blue-green wavelengths around 490nm (Figure 21). The circled area represents the region of interest for this phytoplankton bloom. Figure 21 b shows the same data, but from (400-600nm) in order to accentuate the shouldering feature. The circled region in this figure denotes the bloom samples with more than 6 million cells/L of this green flagellate. The other two spectra are still considered bloom samples, as per the 100,000 cell/L bloom/non-bloom thresholds in this study, but had concentrations of 104,000 and 161,000 cells/L. It is important to note the size difference between the green flagellate in the *Pedinophyceae* class and *P. bahamense*. *Pyrodinium bahamense* are spherical and have a diameter around 35μm. The
green flagellates in the *Pedinophyceae* class are also spherical, but are only 1/10\textsuperscript{th} the size with a normal diameter ~3μm or less. Next, $a_{\varphi}^\ast(\lambda)$ spectra of the *Pedinophyceae* bloom samples were averaged and compared to the other averaged $a_{\varphi}^\ast(\lambda)$ spectra from Tampa Bay and Indian River Lagoon (IRL) (Figure 22). The highlighted spectrums in the figure are from the IRL, with the orange line representing the *Pedinophyceae* bloom samples. The area that is circled is the same area of interest from the previous figures with the individual $a_{\varphi}^\ast(\lambda)$ spectra of the *Pedinophyceae* bloom samples (Figure 21). Just as with the *P. bahamense* bloom samples from Tampa Bay, this area was compared to Bricaud’s assumed weight specific absorption spectra for the main phytoplankton pigments (Figure 23). In this case, there are multiple pigments, 5 in fact, that could possibly be link to the observed spectral signature. The five pigments that show an increased absorption in the region of interest (480-500nm) are: Diadinoxanthin, Zeaxanthin, Alloxanthin, β-carotene and α-carotene.
Figure 21: (a) $a_\phi^*(\lambda)$ for the Pedinophyceae bloom samples collected from in the Indian River Lagoon (IRL). The spectral region of interest is outlined in black and enlarged in (b), where the spectra in the black outline represent bloom samples with concentrations of >6,000,000 cells/L.
Figure 22: Averaged $a_{\phi}^\star(\lambda)$ spectra for all phytoplankton taxa from Tampa Bay and IRL. The highlighted spectrums are from the IRL (a), where the orange is the *Pedinophyceae* bloom from the IRL. The circled region, which is enlarged in (b) denotes the region of interest from the previous figures with the individual $a_{\phi}^\star(\lambda)$ spectra for the *Pedinophyceae* bloom.
Figure 23: Measured weight-specific absorption spectra of the main phytoplankton pigments (Bidigare et al., 1989). The highlighted area corresponds to the area of interest for the *Pedinophyceae* bloom samples from the IRL. The highlighted spectra in the circled region denote the 5 pigments that may be the cause of the shouldering feature seen in the *Pedinophyceae* bloom samples.

**Method 1: Derivative Analysis**

Similar to Tampa Bay, samples collected from the IRL were analyzed using the 1\textsuperscript{st} derivative analysis technique (Figure 24). The highlighted region denotes the spectral region of interest for the *Pedinophyceae* bloom samples that contain more than 6 million cells/L concentration, and they are also the only spectra that show a positive 1\textsuperscript{st} derivative. Next, the 1\textsuperscript{st} derivative was performed on all the $a_\phi(\lambda)$ spectra for each taxa, and then averaged according to the taxa (Figure 25). Here, the outlined area denotes the region of interest (Figure 25 a),
which is enlarged to show that the averaged *Pedinophyceae* bloom taxa have the highest 1\textsuperscript{st} derivative value of any taxa, although it is below zero (Figure 25 b).

![Figure 24: 1\textsuperscript{st} order derivative $a_\phi^*(\lambda)$ of *Pedinophyceae* bloom taxa and other taxa from the Indian River Lagoon from (450-600nm). The black circle is highlighting the *Pedinophyceae* bloom samples that have a positive 1\textsuperscript{st} derivative value and have cell counts >6 million cells/L.](image)

In figure 25 the averaged 1\textsuperscript{st} derivative of the $a_\phi^*(\lambda)$ spectra for the *Pedinophyceae* bloom samples is negative over the area of interest (470-500nm). However, recalling figure 24 which showed the individual spectra, there are 7 *Pedinophyceae* bloom spectra with positive values across this region, and 3 with negative. So, even though the average 1\textsuperscript{st} derivative spectrum does not show a positive value and separate the *Pedinophyceae* bloom taxa, the individuals do separate themselves. The next step is to develop a summation range for the 1\textsuperscript{st} derivative $a_\phi^*(\lambda)$ spectra and apply in to all the individual taxa for the IRL and Tampa Bay and plot this versus cell count. The technique performed a simple summation from (479-483nm) (Figure 26). Using a 1 million cell/L threshold, this method has a success rate of 87% (7 of 8) and no false positives.
Figure 25: Averaged 1st order derivative of the $a_\phi(\lambda)$ spectra for all phytoplankton taxa in Tampa Bay and IRL. The circled in (a) denotes the region of interest which is enlarged in (b). The thick pale blue line is the average Pedinophyceae bloom 1st derivative $a_\phi^*(\lambda)$. Although on average they are not above zero in this region, they are larger than any other taxa.
Figure 26: 1st derivative $a_\phi(\lambda)$ summation (479-483nm) for *Pedinophyceae* bloom taxa and all other taxa from IRL and Tampa Bay versus cell concentration. The vertical bar denotes the zero mark. The circles denote the three negative *Pedinophyceae* samples.

**Method 2: Relative Height Analysis**

Next, a relative height of the $a_\phi(\lambda)$ was calculated for the individual samples of the *Pedinophyceae* bloom samples. The relative height was calculated by taking the $a_\phi(\lambda)$ at 484nm and subtracting a baseline average between (474 nm – 490 nm) then dividing by that baseline average (Figure 27). The relative height was calculated for each individual taxa and plotted against cell concentration (Figure 28). Again, there is a clear separation of the *Pedinophyceae* bloom samples (>6 million cell/L) from the rest of the taxa from IRL and Tampa Bay. This technique has a 100% success rate (8 of 8 samples) of separating *Pedinophyceae* bloom samples from other taxa, with a 4% false positive rate (6 of 156).
Figure 27: Pedinophyceae bloom $a_{\phi} (\lambda)$ spectra showing method for relative height.

Figure 28: Relative height analysis of $a_{\phi} (\lambda)$ for all phytoplankton taxa in IRL and Tampa Bay. The green dots in the circled areas are the Pedinophyceae samples. The vertical line denotes the zero mark.
Method 3: Integration Analysis

Lastly, the integration technique that was developed for the *P. bahamense* bloom taxa was applied to the *Pedinophyceae* bloom data, with slight modifications. The integration was performed from 471 nm to 490 nm and the distances from the $a_\phi(\lambda)$ spectra to the baseline were calculated for each wavelength across the integrated area and summed, giving the area under the curve (Figure 29). This technique was performed on all of the individual taxa and plotted against cell count (Figure 30).

![Integration method for a_\phi*(\lambda) of Pedinophyceae bloom samples. The green shaded area represents the summed area from the spectral integration.](image_url)
Figure 30: Integration algorithm of $a_\lambda (\lambda)$ versus cell count for the *Pedinophyceae* bloom samples and all of phytoplankton taxa from Tampa Bay and the IRL. This figure shows a log/log plot with all negative values (68) displayed as (0.0001). The two circled regions are the *Pedinophyceae* samples.

There is clear separation of the *Pedinophyceae* samples with >6 million cells/L. Using the 1 million cell/L threshold, this technique has a 100% success rate (8 of 8 samples) with a false positive rate of 0.5% (1 of 156). Of the 156 samples used in this figure, 68 are negative, which are plotted with an integration value of 0.0001 so they can be seen on the log-log plot. Of these 68 samples; 15 are *P. bahamense* non-bloom, 3 are *P. bahamense* bloom, 16 are *Pseudo-nitzschia* non-bloom, 9 are *Pseudo-nitzschia* bloom, 5 are *Chaetoceros sp.* bloom, 5 are *Skeletonema sp.* bloom, 5 are *Thallasiosira sp.* bloom, 4 are *Rhizosolenia sp.* bloom, 4 are *ceratellum sp.* bloom, 1 is *Cyclotella sp.* bloom, and the last 1 is from a Microphytoflagellate bloom.
In summary, similar to the results obtained from the Tampa Bay samples, among the three methods tested for the IRL water samples, the integration analysis provided the best results in separating *Pedinophyceae* blooms from non blooms and other blooms, with the major results listed in Table 5.

Table 5: Performance matrix for the three techniques applied to Indian River Lagoon data in order to differentiate *Pedinophyceae* blooms from other taxa.

<table>
<thead>
<tr>
<th>Pedinophyceae</th>
<th>Success Rate</th>
<th>False Positive Rate</th>
<th>False Negative Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Derivative</td>
<td>87.5% (7 of 8)</td>
<td>0% (0 of 156)</td>
<td>12.5% (1 of 8)</td>
</tr>
<tr>
<td>Relative Height</td>
<td>100% (8 of 8)</td>
<td>4% (6 of 156)</td>
<td>0% (0 of 8)</td>
</tr>
<tr>
<td>Integration</td>
<td>100% (8 of 8)</td>
<td>0.5% (1 of 156)</td>
<td>0% (0 of 8)</td>
</tr>
</tbody>
</table>
DISCUSSION

The prototype techniques for classifying blooms of two different phytoplankton taxa in two different Florida estuaries showed preliminary success, as these taxa contained unique pigments resulting in identifiable signatures in the absorption spectra. Depending on the applications, the bloom identification success rate (~80%), false positive rate and false negative rate may or may not be acceptable. Satellite-based techniques to identify *K. brevis* blooms on the WFS have success rates of about 80% (Tomlinson et al., 2010). In this regard, the results may be acceptable. In a management perspective, false positives will lead to false alarming while false negatives will lead to bloom events undetected, making early mitigation efforts impossible. In this regard, false negative classification should be reduced in future efforts. However, the technique by no means is intended to replace phytoplankton taxonomy. Rather, it will provide complementary information to phytoplankton taxonomy as phytoplankton absorption is nearly a routine measurement in bio-optical field work. Furthermore, the optical classification may lead to development of *in situ* instrumentation, similar to BreveBuster, to continuously measure the phytoplankton absorption and classify the phytoplankton taxa in the field. However, the results cannot be over interpreted mainly due to the low number of bloom samples for each identified taxa. For *P. bahamense*, there were only 16
bloom samples (>100,000 cells/L) while for Pedinophyceae taxa there were only 8 bloom samples (>1 million cells/L). Thus, future effort should include collecting more samples from the two estuaries as well as from other estuaries in the Gulf of Mexico to further test the validity of this technique and to improve its overall performance through algorithm coefficient and threshold tuning. For example, a threshold of 100,000 cell/L was used for bloom detection of P. bahamense in Tampa Bay. This threshold was rather arbitrary in order to achieve a compromise between successful bloom detection and false positives and false negatives. With a much larger dataset, the threshold may be adjusted to achieve the best compromise.

The ultimate application of such an optical technique is through remote sensing, simply because of its synoptic and frequent coverage. Currently, there are two hyperspectral sensors measuring the ocean’s reflectance. One is the Hyperion onboard the EO-1 satellite. Although preliminary studies showed its application in deriving water quality parameters (Brando et al., 2003), it may not be applicable in deriving hyperspectral phytoplankton absorption due to its low signal to noise (Hu et al., 2012; Jafari et al., 2012). The other hyperspectral sensor is HICO (Hyperspectral Imager for the Coastal Ocean) (Gao et al., 2012) onboard the International Space Station. Its signal-to-noise ratio is higher than Hyperion, but the coverage is erratic. Yet it may be possible to derive hyperspectral phytoplankton absorption once an image is acquired from Tampa Bay or Indian River Lagoon where concurrent taxonomy data are available, from which the optical classification technique developed in this study may be tested.
In the long run, hyperspectral sensors specifically designed to measure the ocean are being planned at NASA and the European Space Agency (ESA). These include the Geostationary Coastal and Air Pollution Events (GEO-CAPE) and Pre-Aerosol, Cloud, ocean Ecosystem (PACE) (National Academic Press, 2007; NASA 2010; Hu et al., 2012) that may be launched in orbit in the next decade. While algorithm development for these future sensors is still underway, the attempts in this study represent a step towards the ultimate goal of applying satellite remote sensing data in classifying and quantifying phytoplankton taxa in optically complex coastal and estuarine waters.
SUMMARY & CONCLUSION

Various techniques have been developed and used to study phytoplankton taxa in the past decades, yet only optical techniques show the potential in applications of remote sensing for synoptic assessment, even though the techniques are not mature for such applications over optically complex coastal and estuarine waters.

Over the past two years, this study built upon previously established optical techniques on measuring and characterizing phytoplankton absorption spectra but extended to two Florida estuaries to test whether optical spectroscopy could be used to differentiate major phytoplankton taxa. Specifically, this study compared phytoplankton absorption spectra, $a_\varphi(\lambda) \ (m^{-1})$, and mass-specific absorption spectra, $a_\varphi^*(\lambda) \ (m^2 mg^{-1})$, determined from about 250 water samples from Tampa Bay and Indian River Lagoon to their corresponding cell count concentrations in order to develop an optical method to detect and differentiate different phytoplankton taxa. Various spectral analyses were tested, with results showing that of the three quantitative techniques (1st derivative analysis, relative height of local spectral peaks, and integration of local spectral peaks), the integration method showed the best performance in differentiating blooms (>100,000 cells/L) of the dinoflagellate and HAB species *P. bahamense* in Tampa Bay from other phytoplankton taxa while keeping false
positive and false negative detections relatively low (Table 4). The spectral integration was based on $a_\phi^*(572 \text{ nm} - 600 \text{ nm})$ where the local absorption peak appears to originate from chlorophyll $c$ according to a spectra-matching optimization model using individual absorption spectra from all phytoplankton pigments. A similar integration technique but with wavelengths shifted to (471 nm – 490 nm) spectral region also yielded the best performance for differentiating *Pedinophyceae* blooms (>1 million cells/L) from other bloom and non-bloom taxa in Indian River Lagoon and Tampa Bay (Table 5). The local peak at (471 nm to 490 nm) appears to originate from a combination of the following phytoplankton pigments: Diadinoxanthin, Zeaxanthin, Alloxanthin, $\beta$-carotene and $\alpha$-carotene. These model-derived observations require validations using HPLC analysis in the future.

Although preliminary success was achieved, the technique at present is immature for development of *in situ* autonomous instruments (e.g., BreveBuster). For example, the limited number of bloom samples and arbitrary threshold for bloom specification, particularly between the two taxa analyzed, must be enhanced. Future sampling from these two and other Florida estuaries are required for assessment of established methodology and algorithm improvement. Further, hyperspectral data collected from remote platforms such as HICO may be tested with the method when concurrent field-based taxonomy data are available. This may ultimately lead to the development of remote sensing algorithms to obtain synoptic information on major phytoplankton taxa in optically complex coastal and estuarine waters. In any event, however, phytoplankton
taxonomy provides the ground truth for algorithm development and validation, and thus should continue to play an important role in studying coastal ocean phytoplankton dynamics and water quality changes.
REFERENCES


