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Carlton R. Hall  
Charles R. Bostater  
Robert Virnstein

**SPIE.**

# Plant pigment types, distributions, and influences on shallow water submerged aquatic vegetation mapping

Carlton R. Hall<sup>\*1a</sup>, Charles R. Bostater, Jr.<sup>b</sup> and Robert Virnstein<sup>c</sup>

<sup>a</sup>Ecological Program, Dynamac Corporation, Kennedy Space Center, Florida

<sup>b</sup>Marine Environmental Optics Laboratory & Remote Sensing Center  
College of Engineering, Florida Institute of Technology, Melbourne, Florida

<sup>c</sup>St. Johns River Water Management District, Palatka, Florida

## ABSTRACT

Development of robust protocols for use in mapping shallow water habitats using hyperspectral imagery requires knowledge of absorbing and scattering features present in the environment. These include, but are not limited to, water quality parameters, phytoplankton concentrations and species, submerged aquatic vegetation (SAV) species and densities, epiphytic growth on SAV, benthic microalgae and substrate reflectance characteristics. In the Indian River Lagoon, Fl. USA we conceptualize the system as having three possible basic layers, water column and SAV bed above the bottom. Each layer is occupied by plants with their associated light absorbing pigments that occur in varying proportions and concentrations. Phytoplankton communities are composed primarily of diatoms, dinoflagellates, and picoplanktonic cyanobacteria. SAV beds, including flowering plants and green, red, and brown macro-algae exist along density gradients ranging in coverage from 0-100%. SAV beds may be monotypic, or more typically, mixtures of the several species that may or may not be covered in epiphytes. Shallow water benthic substrates are colonized by periphyton communities that include diatoms, dinoflagellates, chlorophytes and cyanobacteria. Inflection spectra created from ASIA hyperspectral data display a combination of features related to water and select plant pigment absorption peaks.

**Keywords:** hyperspectral, pigments, absorption, inflection spectra, submerged aquatic vegetation, remote sensing

## INTRODUCTION

Utilization of hyperspectral remote sensing data in shallow water environments to accurately estimate water quality and map benthic habitat characteristics remains problematic as a result of the diversity of factors that contribute to the intensity and shape of the upwelling light fields.<sup>1, 2, 3, 4, 5</sup> These factors include abiotic components such as surface wave characteristics, depth, sediment grain size, total suspended sediment (TSS) load, substrate physical characteristics and photo-reactive dissolved organic chemicals such as tannins and lignin. Biotic factors influencing spectral shape and intensity include bacteria and protists (i.e. phytoplankton, epiphyton, and periphyton) and the major plant groups (Chlorophyta, Phaeophyta, Rhodophyta, and Tracheophyta).<sup>1, 3</sup> The biotic component has both biophysical and biochemical mechanisms that potentially alter the shape of the upwelling light field and ultimately the hyperspectral remote sensing signatures. Biophysical features in a SAV bed may include leaf area, leaf angle distributions, canopy height, and degree of canopy closure.<sup>3, 4, 5</sup> In this assessment, being conducted in the Indian River Lagoon, Florida, USA we focus on the biochemical features associated with the photosynthetic and auxiliary pigment of aquatic plants. Biophysical influences on the hyperspectral signature are not included although their importance is recognized.

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\*[hallcr@kscems.ksc.nasa.gov](mailto:hallcr@kscems.ksc.nasa.gov): phone 321 454-3846 Dyn-2 Kennedy Space Center Fl. 32899

Development of imaging spectrometers has led to an increase in investigations and utilization of biochemical based absorption features for research and management applications. Remote sensing of biochemical constituents may overcome limitations of traditional in-situ surveys that lack spatial coverage, resolution and precision required for sound management decisions.<sup>6</sup> A basic principle in remote sensing is that features of interest reflect or emit light in uniquely different ways and these differences can be detected and recorded by the remote sensing system.<sup>7,8,9</sup> In the Indian River Lagoon Florida, USA, (Figure 1) targets of interest include seagrasses (submerged vascular plants) and the green, red and brown macroalgae that make up a significant portion of lagoon primary production, standing stock, and aquatic habitat structure,<sup>10,11</sup> Seagrasses play a significant role in estuarine ecosystems. They are dominant primary producers, stabilize sediments, provide structure and nursery function, and directly and indirectly influence biogeochemical cycling.<sup>12</sup> Seagrasses are more sensitive to declining water quality because of their higher light requirements (15-25% surface irradiance) than other primary produces such as macroalgae, and benthic microalgae (periphyton).<sup>13</sup> These groups are of extreme interest to resource managers who have defined the distribution and abundance of seagrasses in the system as a metric of ecosystem health. The spatial distribution and composition of SAV has also been defined as an indicator of success or failure of pollution load reduction management strategies.<sup>14</sup>

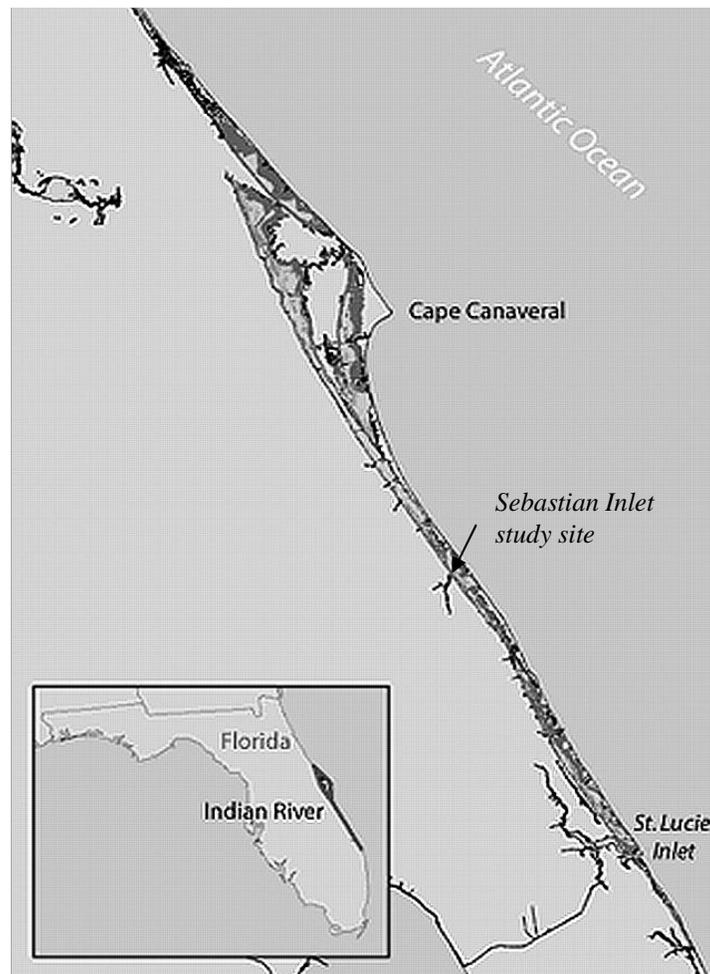


Figure 1. Location of the Indian River Lagoon on the east central Florida coast. The lagoon extends for more than 150 miles from New Smyrna Beach to Jupiter Inlet. Average depth is 1 m. The Sebastian Inlet and Sebastian River are centrally located in the lagoon (map modified from NOAA Coastal Services Center).

Figure 2 displays our conceptual model with four primary areas of photon interaction. First, the downwelling light field interacts with the water surface where waves and wavelets influence surface scattering and transmission into the water column. Light passing downward into the water column is modified spectrally by absorbing features such as phytoplankton pigments. Photons may be transmitted or

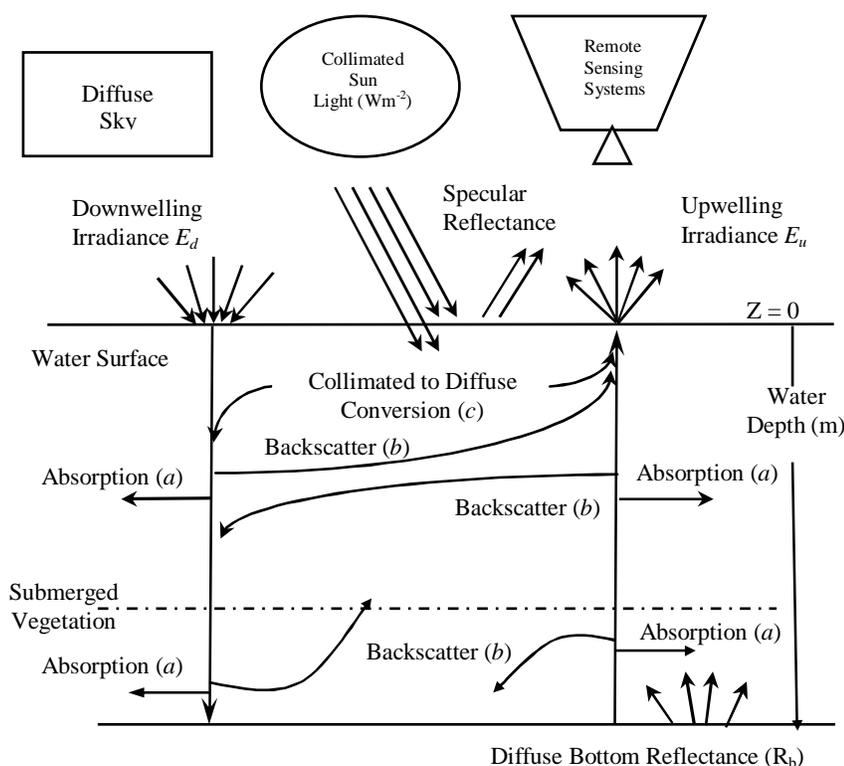


Figure 2. Conceptual model of photon interactions in the aquatic environment. Absorption is a function of the presence and relative abundance of light absorbing plant pigments. Between the water surface and the layer of submerged vegetation phytoplankton are the primary absorbers. In the submerged vegetation layer, seagrass, macroalgae, and associated epiphytes are the primary absorbers. At the bottom, absorption by photosynthetically active periphyton contributes to the spectral shape of the upwelling light field.

forward scattered continuing the downward trajectory or they may be backscattered into the upwelling light field. Photons reaching the submerged aquatic vegetation layer may be absorbed or scattered by epiphytes or SAV or, if not intercepted, they may continue their downward travel to the bottom. At the bottom, photosynthetically active periphyton may selectively absorb photons in relative proportion to their pigment concentrations, health, and biomass. Light that is not absorbed by periphyton or the bottom substrates is scattered into the upwelling light field where it may interact with epiphytes, SAV and phytoplankton on its way back to the surface. The model is expressed mathematically as a set of coupled differential equations representing the two flow irradiance fields in the upwelling and downwelling directions.

$$\frac{dE_d(z)}{dz} = -(a + b)E_d(z) + bE_u + cE_s(z), \quad (1)$$

$$\frac{dE_u(z)}{dz} = (a + b)E_u(z) - bE_d - cE_s(z), \quad (2)$$

$$\frac{dE_s(z)}{dz} = -\alpha E_s(z). \quad (3)$$

In these equations, a and b represent the absorption and backscatter coefficients for the layer of concern and are expressed as

$$a_l = a_{H^2O}^* + a_{CHLa}^* xC_{CHLa} + a_{CHLb}^* xC_{CHLb} + a_{\alpha CAR}^* xC_{\alpha CAR} + .. \quad (4)$$

$$b_l = b_{H^2O}^* + b_{CHLa}^* xC_{CHLa} + b_{CHLb}^* xC_{CHLb} + b_{\alpha CAR}^* xC_{\alpha CAR} + .. \quad (5)$$

Table 1. presents a summary of the major (most abundant) taxonomic groups commonly observed in the Indian River Lagoon with their dominate pigments and associated peak absorption wavelength regions. Seagrasses, like most vascular plants, contain chlorophyll a and b, chlorophyllidae, alpha and beta carotene, lutein and other carotenes. Chlorophyll-a, and chlorophyll-b, display bi-modal absorption with primary maxima between 420 and 460 nm and secondary peak absorption in the 640-680 nm region. Beta-carotene, a common ancillary pigment in vascular plants, can also contribute significantly to spectral shape by absorbing broadly in the region below 500 nm with a peak near 460 nm. Absorption by these three pigments is generally considered the major factor producing the spectral shape common to vascular plants with other pigments such as violaxanthin generally contributing to absorption below 480 nm.<sup>5, 9</sup>

Table 1. Major plant groups potentially contributing to spectral shape in hyperspectral remote sensing signatures of the Indian River Lagoon, FL. Listed are common genera, major pigments, and approximate wavelengths of absorption maxima. Absorption peak values are indicative of wavelength regions in the remote sensing signal that may display changes in curvature as a result of plant density and health.

Major Group	Nomenclature	Common Genera in the Indian River Lagoon	Primary Plant Pigments	Absorption peaks (nm)
Seagrass	Tracheophyta (vascular plants)	<i>Syringodium, Halodule Thalasia, Halophila,</i>	chlorophyll-a chlorophyll-b lutein beta-carotene	430, 668 448, 645 445 453
Macroalgae (attached and drift)	Chlorophyta (Green algae)	<i>Caulerpa, Enteromorpha, Ulva, Acetabularia</i>	chlorophyll-a chlorophyll-b lutein	417, 668 448, 645 445
	Rhodophyta (Red algae)	<i>Gracilaria, Hypnea, Laurenci, Acanthophora</i>	chlorophyll-a lutein phycoerythrin phycocyanin	417, 668 445 570 605

	Phaeophyta (Brown algae)	<i>Dictyota, Sargassum</i>	chlorophyll-a chlorophyll-c fucoxanthin	417, 668 515
Microalgae (phytoplankton, epiphytes, and periphyton)	Diatoms	<i>Skeletonema, Dactyliosolen, Cerataulina, Thalassionema</i>	chlorophyll-a chlorophyll-c fucoxanthin	417, 668 515
	Dinoflagellates	<i>Pheopolykriko, Pyrodinium, Pyrodinium, Akashiw</i>	chlorophyll-a peridinin	417, 668 480
Bacteria (picoplankton, epiphytes, and periphyton)	Cyanobacteria (blue-green algae)	<i>Synechococcus,</i>	chlorophyll-a phycocyanin zeaxanthin	417, 668 605 450

Source: Pigment absorption peaks modified from DHI Water and Environment Hoersholm, Denmark. Certificate of Analysis.; 26

The Chlorophyta (green macroalgae) are in general similar to the Tracheophyta with chlorophyll-a and b representing the dominate pigments. The Rhodophyta (red algae) are unique in their make up with an absence of chlorophyll –b and an abundance of water soluble biliproteins like phycocyanin and phycoerythrin. These unique pigments absorb in the 605 and 570 nm regions respectively giving this group its unique coloration. The Phaeophyta or brown algae contain the chlorophyll –c pigments in addition to fucoxanthin, a pigment that absorbs strongly in the 500 nm region. Dinoflagellates, common epiphytes and periphytes contain the pigment peridinin that absorbs in the 460 nm region.

A graphical comparison of the absorbance signatures for the major pigments is shown in Figure 3. Chlorophyll-a, and chlorophyll-b, display bi-modal absorption with primary maxima between 420 and 460 nm and secondary peak absorption in the 640-680 nm region. Beta-carotene, a common auxiliary pigment in vascular plants, can also contribute significantly to spectral shape by absorbing broadly in the region below 500 nm with a peak near 460 nm. Absorption by these three pigments is generally considered the major factor producing the spectral shape common to vascular plants (see Figure 5a).

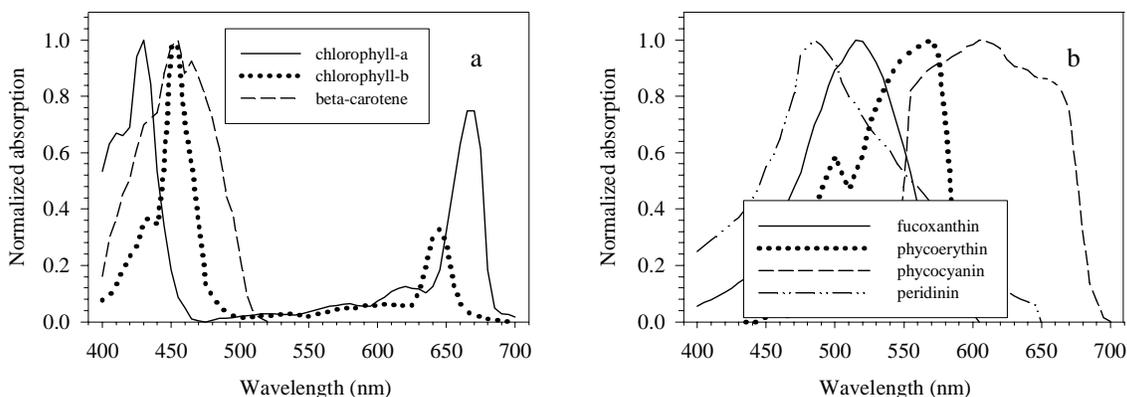


Figure 3. Relative locations and shapes of wavelength specific absorption features associated with the presence of plant pigments common to vascular plants and the green, red, and brown algae. Absorbance data were normalized to a 0-1 scale to emphasize the shape and position of the absorption features.

## Methods

Hyperspectral images of remote sensing reflectance were collected on May 29<sup>th</sup>, 2003 utilizing an AISA+ sensor on a Beech Queen Air aircraft. The target area covered the Sebastian Inlet, adjacent Indian River Lagoon and mouth of the Sebastian River at its confluence with the Lagoon Figure 4. The AISA+ sensor was flown with the spectral resolution of  $\approx 4.6$  nm, and produced 77 bands (between 399 and 755) with a swath width of  $\approx 500$  m with an  $\approx 1.2$  meter pixel size, and altitude of over  $\approx 1000$  m (3,300 ft), and FOV of 30 degrees. Details of the flight are available in Bostater et al., 2003.<sup>15</sup> Image processing and reflectance spectra extraction were conducted with ENVI. Average reflectance values were generated for six target areas, three over sand and 3 over SAV. These included bare sand substrate less than 0.25 m deep, bare sand between 0.5 and 1.0 m deep and deep sand greater than 2.0 m deep. Average reflectance values for SAV were collected in a similar fashion. Extracted reflectance spectra were normalized to 0-1 scale over a range of 400 to 700 nm. Inflection spectra were calculated according to Bostater et al.<sup>2, 16</sup>

Reflectance signatures of seagrasses (*Syringodium* and *Halodule*) and macroalgae (*Gracilaria*, *Caulerpa*, and *Acanthophora*) were collected in the laboratory using an ASD Field Spec Pro FR. SAV specimens were collected in the field, placed in plastic bags in the dark on ice and returned to the lab for processing. Holding time was less than 4 h. Tissue or leaf samples were stacked approximately 1 cm deep on a black background. The fiber optic probe was fixed in place 3 cm above the target at 0° in the center of a 7 cm diameter fiber optic light ring. A halogen lamp was used as the light source. Integration time was set at 64 ms and a Spectralon white panel was used as the reference source. The system was allowed to warm up for 1 h prior to data collection. Twenty average reflectance spectra ( $n = 30$ ) were collected from each of the five SAV targets. The targets were rotated 5° to 10° between each set of 30 scans. Target reflectance was estimated as the average of the 20 reflectance spectra (600 scans). Data were normalized to a 0-1 scale over a range of 400 to 700 nm.

## RESULTS AND DISCUSSION

Reflectance signatures (Figure 5) measured for the five SAV samples displayed varied shape as a result of pigment specific absorption. The two seagrasses (Figure 5a) and the green macroalgae, *Caulerpa*, (Figure 5b) had reflectance signatures similar in shape to terrestrial plants.<sup>17, 18</sup> For these three taxa reflectance was low between 400 and 475 nm as a result of the chlorophyll and carotene complex absorption features. Each displayed peak reflectance in the 560 nm region due to the absence or low concentrations of pigments such as fucoxanthin and biliproteins. Reflectance decreased to minima in the 670 nm region in response to the bimodal chlorophyll absorption feature before increasing rapidly in the classic red edge fashion through 700 nm. Reflectance spectra for the two red algae (Figure 5b) displayed similar trends and were notably different than *Caulerpa* and the vascular plants. The chlorophyll-a absorption features are present in the 400-420 nm and 680 nm regions but the absence of chlorophyll b appears to limit the absorption feature to narrower wavelength regions. Of special interest are the two significant absorption features associated with the biliproteins. Phycocyanin produces an absorption feature at approximately 630 nm and phycoerythrin absorption influences to the 570 nm region. These unique characteristics should allow for the discrimination and mapping of the red macroalgae in situations where it predominates relative to the spatial scale of the imagery.

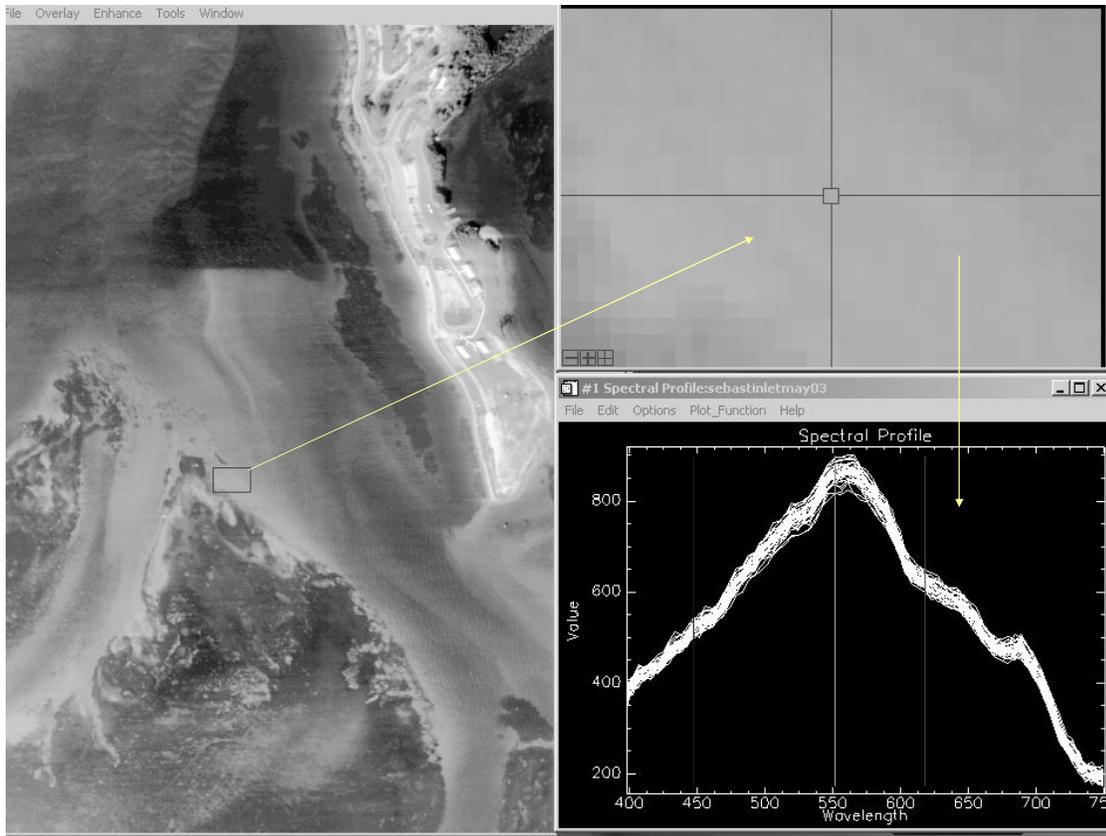


Figure 4. Example of the reflectance signature extraction process from the hyperspectral image collected at Sebastian Inlet, May 2003. In the image the roads and buildings of the Sebastian Inlet State Park and a large SAV bed are obvious. Twenty pixels were selected from the zoom area that represents bare sand between 0.5 and 1.0 m deep. Note the uniformity of the spectra.

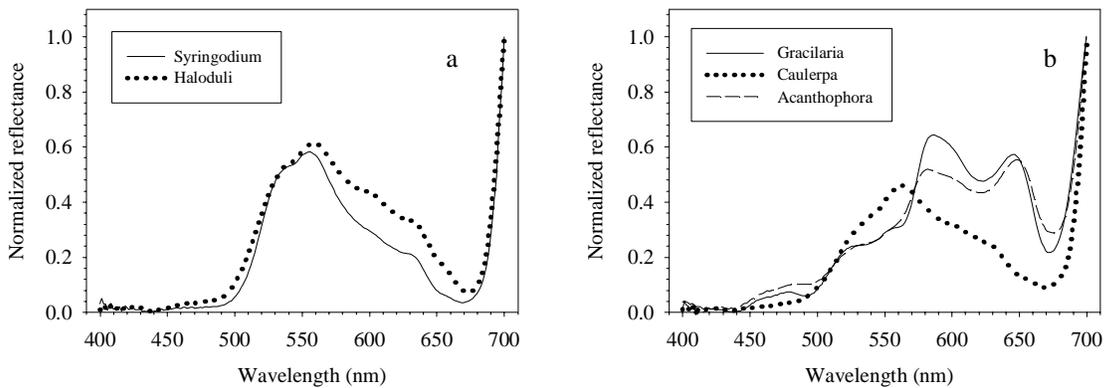


Figure 5. Laboratory reflectance signatures for the common submerged aquatic vegetation from the Indian River Lagoon, Fla. Reflectance data were normalized to a 0-1 scale to emphasize the shape and position of the reflection and absorption regions.

Normalized reflectance signatures for three depths of bare sand and SAV are given in Figure 6. Sandy soils typically display a gradual increase in reflectance from 400 to 700 nm.<sup>19</sup> Water covered soils (sediments) display a reflectance signature that is influenced by the increased attenuation in the region above 600 nm associated with water depth.<sup>19</sup> As water depth increases, attenuation of the reflectance signature increases. Imbedded in this general trend are the influences of both the phytoplankton and periphyton communities with associated diatoms, dinoflagellates and picoplankton. Badylak and Philps<sup>20</sup> present results of a two year study of phytoplankton communities of the Indian River Lagoon. They found the system dominated by dinoflagellates, diatoms and cyanobacteria.

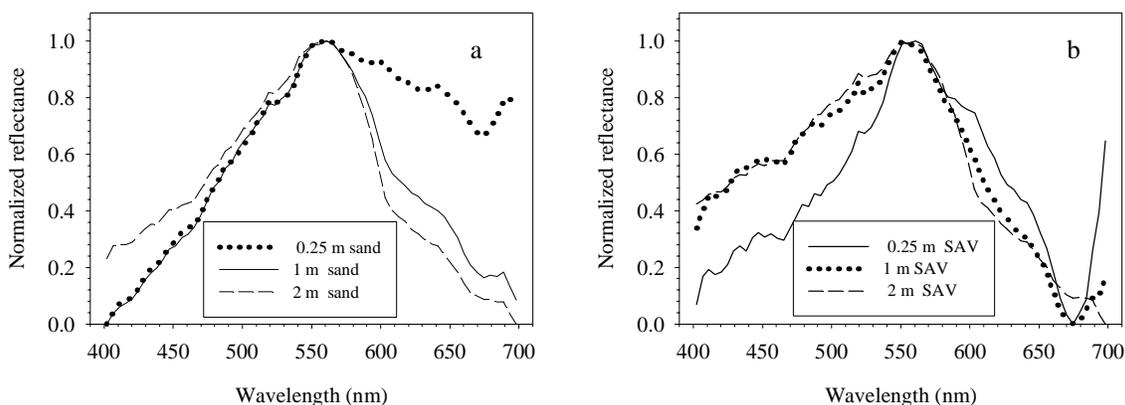


Figure 6. Extracted reflectance signatures for bare sand and SAV covered areas in the May 2003, hyperspectral image from the Sebastian Inlet area. Reflectance data were normalized to a 0-1 scale to emphasize the shape and position of the reflection and absorption regions.

Regions of the lagoon with flow-restrictions had the highest standing crops. In wet years these areas commonly had blooms of dinoflagellates and in dry years the flow-restricted areas had increases in the picoplanktonic cyanobacteria. Areas of the lagoon near the inlets were typically dominated by diatoms. Diatom blooms were also common in areas of intermediate flow and high external loading of phosphorous. The spatial and temporal patterns observed in dominant species were in part attributable to patterns in key environmental variables, including salinity, temperature and nutrient concentrations. Typical chlorophyll-a concentrations in the Indian River Lagoon range between 2-30 mg/m<sup>3</sup>.

In our conceptual model the periphyton or benthic microalgae will also potentially influence the remotely sensed reflectance signature through the bottom reflectance estimate. Two factors that influence the periphyton community include sediment grain size and the degree of coupling with overlying surface waters. Periphyton communities may be seeded by settling of organisms from the overlying water column and in shallow systems like the Indian River Lagoon resuspension is highly probable in storm events. Pigment concentrations are typically lower in fine grained sediments, possibly a result of reduced pore space and limited light penetration.<sup>20, 21</sup> Cahoon and Safi<sup>21</sup> report on a study of Nanuku Harbor, New Zealand where periphyton chlorophyll-a concentrations ranged between 11.8 and 340 mg/m<sup>2</sup> with an average of 97.5 mg/m<sup>2</sup>. Variability in concentration among replicates averaged 36% reflecting the patchiness of periphyton at cm scales. Chlorophyll-a is typically the most abundant pigment but relatively large amounts of fucoxanthin, a diatom marker pigment was often present. The fucoxanthin:chlorophyll-a ratio ranged between .49 and .86 suggesting most periphyton was comprised of diatoms.

Epiphytes growing directly on the SAV represent another potential absorption feature in the conceptual model. Examination of epiphytic growth on *Halodule*, *Syringodium* and *Thalassia* revealed primarily diatoms. However, brown algae *Myriotrichia subcorymbosa* and red algae *Acrochaetium flexuosum* increased in biomass in enriched waters.<sup>23</sup> Increase in chlorophyll-a in epiphyte growth was highly correlated (.97) to increases in fucoxanthin, zeaxanthin and violaxanthin.<sup>24</sup> In Florida Bay, epiphytic growth was dominated by diatoms with lesser amounts of dinoflagellates and traces of cyanobacteria.<sup>24</sup> On occasion the red macroalgae will dominate the epiphytic biomass and periodically large areas of SAV will be covered by green-black slim comprised of about 85% cyanobacteria and 15% diatoms.<sup>24</sup>

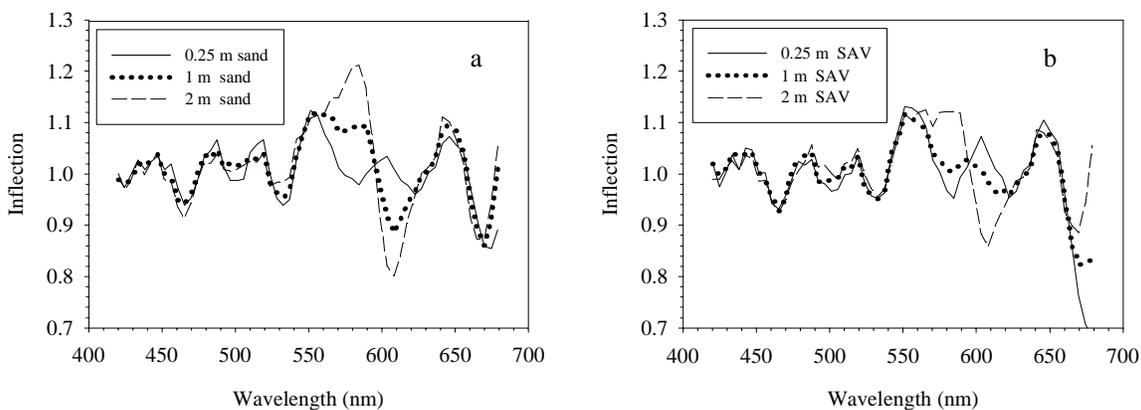


Figure 7. Estimated inflection signatures for bare sand and SAV covered areas in the May 2003, hyperspectral image from the Sebastian Inlet area. Inflection values below 1.0 indicate the presence of a potential pigment based absorption feature.

Figure 6 shows the presence of several absorption features in the data related to chlorophyll and possibly other pigments. The depth related attenuation tends to mask some features. Figure 7 presents the inflection estimate for the sand and SAV data extracted from the ASIA+ hyperspectral data cube. The inflection estimator enhances the ability to separate closely related absorption features. In the inflection spectra areas with values below 1 are indicative of absorption regions.

For the sand and SAV spectra there are 7 unique regions of interest. All six spectra display an absorption feature at 460 nm that corresponds well to chlorophylls and carotenes. A second subtle feature exists at approximately 500 nm and a third at 530 nm in line with the peak absorption region of fucoxanthin. The 500 nm feature may be related to the pure water harmonic.<sup>19</sup> For both the sand and SAV 0.25 m sites there is high correspondence between the spectra with both displaying absorption features in the 570 and 630 nm regions corresponding to phycocyanin and phycoerythrin, respectively. This strongly suggests the presence of cyanobacteria periphyton and epiphyts in the shallow high energy area. In the SAV, red algae may also be present. Sand and SAV inflection spectra for the 1 m and 2m sites display absorption features in the 610 nm region, possibly a function of water attenuation.<sup>19</sup> The presence of chlorophyll in all six location is indicated by the strong absorption features near 680 nm.

## CONCLUSIONS

Results of this preliminary evaluation indicate hyperspectral remote sensing will prove a valuable tool in shallow water ecosystem research, mapping and management. The inflection spectra developed for hyperspectral remote sensing reflectance data from different bottom types and depths show that pigment-

specific absorption features can be detected and identified, at least in areas where water is shallow and turbidity is moderate. These results are similar to finding for the clear water carbonate sediments in the Lee Stocking Island area.<sup>19</sup> Additional work needs to be conducted to quantify pigment concentrations and ratios in the different regions of the Indian River Lagoon for possible applications to resource management including the development of SAV maps that distinguish the major taxonomic groups using hyperspectral imaging techniques. These results suggest it is highly possible to discriminate pigments known to occur exclusively in the vascular plants, red algae and brown algae. It may be more difficult to distinguish green algae from the vascular plants.

## ACKNOWLEDGEMENTS

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