# Report as of FY2007 for 2006MD124B: "Salinity effects on using hyperspectral radiometry to determine leaf nitrogen of emergent wetland macrophytes"

## **Publications**

Project 2006MD124B has resulted in no reported publications as of FY2007.

## **Report Follows**

## Progress Report to Maryland Water Resources Research Center

# Salinity effects on using hyperspectral radiometry to determine leaf nitrogen of emergent wetland macrophytes

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#### **Statement of Problem**

The Clean Water Act (CWA) stipulates that States report the health and quality of all water bodies, including wetlands, in a National Water Quality Inventory Report, but only 4% of wetlands were included in the most recent edition (USEPA 2002a). By 2012 the USEPA's leniency will end and States will be required to report wetland water quality and ecological health (USEPA 2001). The lack of reporting stems from technical difficulties associated with sampling wetlands and unresolved issues in defining wetland health. In Maryland, water quality monitoring and reporting is conducted by the Maryland Department of the Environment (MDE) and the Maryland Department of Natural Resources (DNR). Recently, MDE has received an EPA grant to develop guidelines for programs to monitor wetland water quality (Denise Clearwater, MDE, pers. comm.).

The low monitoring rate of wetlands is due largely to the time and expense of intensive direct sampling in areas that are difficult to access. Presently, the U.S. EPA National Health and Environmental Research Lab is working directly with seven states to test less intensive sampling methods, such as rapid biological assessment and GIS analysis of land use (e.g., Landscape Development Intensity Index), in an effort to fully develop wetland assessment tools (Richard Sumner, USEPA, pers. comm.). However, it is clear that more tools need to be developed and tested to increase the nation's capability for assessing wetland water quality and ecological health and to meet statutory requirements of the CWA.

Nitrogen is a ubiquitous pollutant that shifts wetland plant composition, lowers diversity and increases productivity ((DiTommaso and Aarssen 1989; Morris 1991; Baldwin unpublished). Agricultural and urban runoff and atmospheric deposition are sources of excess nitrogen to wetlands and open-water systems, such as Chesapeake Bay. In general anthropogenic inputs of nutrients to the biosphere is increasingly viewed as a global threat to ecosystem integrity (Vitousek et al. 1997; Fenn et al. 1998). Although Maryland's load of total nitrogen to Chesapeake Bay decreased by 28% from 1986 to 2001 nitrogen loading remains a priority concern for achieving the 2000 Chesapeake Bay Agreement (MDNCWG 2001). Statewide, point source nitrogen loads have decreased from 14,300 MT to 7710 MT (46%) and agricultural loads have dropped from 14,600 MT to 9590 MT (34%). Urban loads, on the other hand, have grown 19%, from 5370 MT to 6390 MT.

#### **Hyperspectral Radiometry for Ecosystem Assessment**

Hyperspectral radiometry measures the electro-magnetic energy reflected from a surface in hundreds of narrow (1—10 nm) spectral bands in the ultraviolet (UV), visible (VIS), near-infrared (NIR) and shortwave-infrared (SWIR) portions of the spectrum. Hyperspectral radiometric imaging can be conducted on the ground with commercially available equipment or from above with airborne (e.g., AVIRIS) and satellite (e.g., Hyperion) systems. In ecosystem radiometry, hyperspectral reflectance is affected by the vegetation's photopigments (e.g., chlorophylls and carotenoids; Hader and Tevini 1987), other leaf biochemicals (e.g., cellulose, lignin; Curran and Kupiec, 1995), inorganic elements (e.g., water, nitrogen, metals), plant morphology, ground cover, soil properties, incident irradiance quality and other environmental

factors. When using hyperspectral radiometry to assess the nitrogen levels of leaves or whole ecosystem canopies, much of the effect is due to the strong relationship between reflectance, chlorophyll and nitrogen, although other indirectly associated nitrogen effects (e.g., higher leaf water content) may be partially responsible for the response.

Historically, radiometric remote sensing techniques have been employed to delineate wetland types and track their quantity and location, but recently the techniques have been employed to assess wetland quality and stress levels based on nutrients, metals, salinity, and invasive species (Anderson and Perry 1996; Penuelas et al. 1997; Tilley et al. 2003; Tilley et al. 2004; Wilson and Ustin 2004; Xue et al. 2004; Poynter-Jenkins et al. 2005; Tilley et al. in press). Of course, hyperspectral radiometry has been widely used to assess the condition of open-water aquatic ecosystems--classifying the trophic status of lakes (Koponen et al. 2002, Thiemann and Kaufmann 2000) and estuaries (Froidefond et al. 2002), characterizing algal and red tide blooms (Stumpf 2001, Kahru and Mitchell 1998), and identifying and classifying submerged aquatic vegetation (Williams et al. 2003). Research on applying the techniques to assess the health of emergent wetlands needs acceleration.

Our most recent wetland radiometric studies (Tilley et al. 2005b; Tilley et al. 2005c) have, for example, used partial least squares (PLS) regression (described below) to predict sub-surface water total nitrogen levels in the absence of salinity ( $R^2=70\%$ ). These findings supported our earlier efforts that found leaf reflectance indices [e.g., Photochemical Reflectance Index: (R<sub>531</sub>- $R_{570}$ )/( $R_{531}+R_{570}$ ); red-edge (wavelength of maximum slope at red—near-infrared transition); and simple ratios (e.g., R<sub>493</sub>/R<sub>678</sub>) responsive to water column ammonia in a brackish treatment marsh (Ahmed 2001; Tilley et al. 2003). The PRI was found by others (Gamon et al. 1997) to be positively related to nitrogen, phosphorus, and potassium fertilization rates for annual, deciduous and evergreen upland species. The red-edge is responsive to leaf chlorophyll concentration, which is strongly influenced by nitrogen availability (Carter and Miller 1994). Strachan et al. (2002) included PRI along with the red-edge as necessary members of a multi-index reflectance model developed for classifying nitrogen application rates in corn (Zea mays). Read et al. (2002) found a simple blue to red reflectance ratio  $(R_{415}/R_{710})$  as a strong indicator  $(R^2 = 0.70)$  of leaf nitrogen in cotton (Gossypium hirsutum). We have also found that the normalized difference vegetation index (NDVI) and floating water-band index (fWBI) were responsive to small (1 part per thousand) changes in salinity (Tilley et al., in review).

Figure 1 provides an example of the findings we made during the first MWRRC funded project. High nitrogen availability decreased reflectance in the green waveband (Figure 1a) and blue and red wavebands (data not shown), which indicated that more photosynthetically active radiation was absorbed when more nitrogen was available. Preliminary results from our newest, on-going experiment (Tilley and Baldwin 2005) revealed that heavy metal (Zn) stress in common marsh macrophytes significantly increased reflectance in the green, red and near-infrared wavebands (Figure 1b), which supports our notion that wetland hyperspectral radiometry can be used to assess heavy metal stress in wetlands.

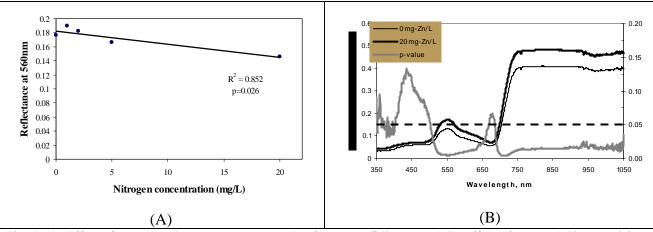


Fig. 1. (A) Effect of elevated nitrogen on greenband reflectance (560 nm) and (B) effect of elevated Zinc on visible (400-700 nm) and near-infrared (700 –1050 nm) reflectance of *Spartina* and *Typha*.

The large amount of spectral data gathered with hyperspectral radiometry presents a strong case for employing multivariate data analysis techniques that can handle multicollinearity (i.e., the correlation among spectral bands). Partial least squares (PLS) regression is a type of eigenvector analysis that can reduce full-spectrum data to a small set of independent latent factors (i.e., PLS-components) that explain the most about dependent variable response (Esbensen 2002). PLS regression is related to principal components analysis (PCA), which decomposes the independent spectral data (matrix X) to its principal components (latent factors) to ascertain which spectral bands are related and possibly important in explaining dependent variable response. Whereas PCA decomposes the X matrix while completely ignoring the Y matrix, PLS regression exploits information contained in the response matrix (Y) to decompose the spectral data (X) to latent factors that are used to build a regression model that explains the most about Y. Thus, PLS overcomes the multicollinearity problem, which is a concern with a method such as stepwise multiple linear regression (MLR) (Grossman et al. 1996) that has been used extensively in analyzing hyperspectral images. PLS has a proven history in chemometrics and is becoming a preferred method for relating hyperspectral reflectance to ecosystem properties as shown by Smith et al. (2003) and Townsend et al. (2003) who used it to develop highly predictive reflectance models of temperate forest canopy nitrogen concentration from airborne and satellite hyperspectral images, respectively. More recently Wilson and Ustin (2004) used PLS discriminant analysis (PLS-DA) of leaf hyperspectral reflectance to classify the Cu and Cd exposure levels of three salt marsh species (Frankenia spp., Salicornia spp., and Scirpus spp.), finding relatively low prediction errors (4.4 to 8.8%). Kooistra et al. (2003) used PLS to relate the reflectance of a facultative upland grass species (Lolium perenne) growing in a restored Dutch floodplain to the Zn concentration of the soil. Preliminary results from our wetland imaging (Poynter-Jenkins et al. 2005b) suggested that PLS-DA could detect the presence of invasive

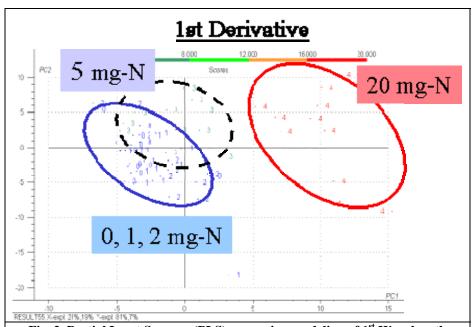


Fig. 2. Partial Least Squares (PLS) regression modeling of 1st Wavelength Derivative of Reflectance classified nitrogen treatment. The 20 mg-N/L treatment was clearly distinguished by the first PLS-component (abscissa) while the second PLS-component (ordinate) indicated some separation of the 5 mg-N treatment. The first PLS-component used 21% of spectral variation to explain 81% of nitrogen treatment. In contrast, the first PLS-component of the model that used the untransformed reflectance used 98% of the spectral variation to explain only 3% of the nitrogen treatment, while the second PLS-component used an additional 1% to explain 37% of nitrogen treatment. Thus, spectral derivative transforms improve predictive efficacy of nitrogen.

species like *Phragmites* australis (88% accuracy) and classify the cover of dominant species like *Polygonum* arifolium (62% accuracy). We have also found success using PLS to build hyperspectral models predictive of nitrogen availability based on (1) individual leaf reflectance in potstudies (Fig. 2) and (2) field-based canopy reflectance of two tidal freshwater marshes with distinctly different species compositions (Poynter-Jenkins et al. 2005a). As mentioned above, we have a funded project to assess whether we can build a PLS/hyperspectral

model predictive of leaf Zn concentration in three common coastal marsh macrophytes (*Phragmites, Typha*, and *Spartina*; Tilley and Baldwin 2005).

Therefore, there is mounting evidence that the combination of PLS and hyperspectral imaging can create an effective tool for assessing many features of wetlands including N availability, species composition and heavy metal-induced stress. One major question about applying the technique to assess nitrogen levels in coastal wetlands located at the freshwater/brackish fringe is whether salinity produces a strong counteractive effect on the nitrogen signal because salinity has been found to increase visible reflectance and reduce near-infrared (Wang et al. 2002), which is opposite of nitrogen. This is the major question we propose to address in the Md. Water Resources funded project. Clarification of this question will continue to advance wetland hyperspectral radiometry as a practical tool for assessing wetland water quality and ecological health.

#### **Long-term Goals for Wetland Radiometry Research**

Our research efforts are focused on developing wetland hyperspectral radiometry as an assessment tool complimentary to existing techniques, which will quantify nitrogen (Tilley et al. 2004) and metals (Tilley et al. 2006) in marsh plant tissue, and distinguish which marshes have high nitrogen availability (Tilley et al. 2004), whether they are freshwater or brackish systems. Development of these fundamental capabilities would eventually lead to the ability to remotely sense which wetlands were under stress from high nutrient or metal loading in coastal

environments. Thus, our work will assist the States and US EPA in meeting the statutory requirements, which will lead to improved management of the nation's wetlands.

Our proposed research project supports the program objectives of the MWRRC by exploring new ideas in wetland remote sensing for water quality monitoring, fostering the research of a junior faculty (Tilley has completed 4 y at UMCP, receiving his Ph.D. in 1999), and training students in a new technology. In general, our research develops information necessary to protect and enhance water quality and habitats supporting natural ecosystem function and to translate new research knowledge and technologies to decision makers and citizens of the Chesapeake Bay, Mid-Atlantic region and the nation.

Table 1 indicates how MWRRC funding fits into our long-term research and development efforts to develop a wetland hyperspectral radiometry as a tool that can assess the water quality and stress of wetlands. Our earliest in brackish treatment marshes demonstrated that leaf reflectance was affected by nitrogen and salinity. Our more recent work confirmed this capability and indicated species had a strong effect on reflectance, especially in response to elevated nitrogen (Tilley et al., in review). Funding from Md. Sea Grant College supported field-based experimentation that confirmed our greenhouse results (Tilley et al. 2004). Currently, we are testing whether we can detect metal stress (Zn) in common marsh macrophytes with the radiometer. We have submitted proposals to NOAA's CICEET program over the last 3 years to conduct an experiment that would be the field-scale compliment to the project proposed here. Once we obtain funding to complete field-based experimentation, we plan to test the developed algorithms on imagery gathered from satellite (Hyperion) or airborne (e.g., AVIRIS) platforms. If that is successful wetland hyperspectral radiometry should be ready for end-users to pilot-test in real applications of wetland water quality monitoring in support of National Water Quality Inventory Reports (303b).

Table 1. Tasks required to develop wetland hyperspectral radiometry as a tool for wetland assessment.

Tasks	Status	Comments	
Proof-of-Concept	Completed 2002	Tilley et al. 2003; Tilley	
-	•	et al. 2007	
Leaf-scale nitrogen modelling	2003-2005	Tilley et al. (in review)	
Field-scale nitrogen fertilization	2004-2005	Tilley et al. 2004;	
		Poynter-Jenkins et al.	
		2005a.	
Leaf-scale detection of zinc stress	2005-2006	Tilley et al. 2006	
Leaf-scale nitrogen with salinity effects	2006-2008	MWRRC (this project)	
Field-scale nitrogen w/ salinity effects	2008-2010	Planned	
Satellite/airborne testing	2008-2010	Planned	
Public application	2011	Planned	

In addition to meeting a statutory need for monitoring wetlands, wetland hyperspectral radiometry would help environmental managers screen large expanses of wetlands to identify which ones are nitrogen "hot-spots"; that is potential sites receiving excessive amounts of non-point source runoff. Identifying these major sources of NPS runoff, which has traditionally been very difficult, would allow for directed application of environmental management techniques to reduce nitrogen in runoff and groundwater. This capability will benefit society by improving science-based management of agricultural operations and urban stormwater management to

reduce impacts on coastal resources. Locally, this is important since agriculture and urban runoff are two of the predominant causes of eutrophication in the Chesapeake Bay (Jaworski et al. 1992; Chesapeake Bay Program 1995).

By 2012 States will be required by the U.S. EPA to report on the water quality conditions and ecological health of wetlands. Thus, development of wetland hyperspectral radiometry is timely and will have a significant impact on state monitoring strategies and capabilities.

The emerging field of precision agriculture, whereby satellite, airborne, and handheld spectroradiometers are employed to measure nitrogen status of crops, demonstrates the potential of employing remote sensing technologies to understand the nutrient status of wetland ecosystems. Advancing the capability of wetland remote sensing to quantify the nitrogen status of wetlands can (1) provide a tool for the large scale monitoring of water quality in difficult-to-access wetlands, (2) offer a rapid screening method for identifying nitrogen "hot-spots" in a watershed, (3) enable near real-time monitoring in areas suspected of producing significant quantities of non-point source (NPS) pollution, and (4) be used to monitor wetlands used as treatment filters.

#### **Project Objectives**

Having developed proof-of-concept that elevated availability of nitrogen changes the visible and near-infrared reflectance of common wetland macrophytes (Tilley et al. 2003; Tilley et al. 2005a), that low level salinity changes in the brackish range alter visible and near-infrared reflectance of marsh macrophytes (Tilley et al. in review), and that partial least squares (PLS) regression modeling can predict nitrogen effects on leaf reflectance of common marsh macrophytes and the availability of sub-surface nitrogen in tidal freshwater marshes (Tilley et al. 2004), an important next step is to investigate the effects of salinity and its interactive effects with nitrogen on the reflectance of emergent marsh macrophytes. This can lead to the development of PLS/hyperspectral models that are predictive of marsh nitrogen and salinity across the fresh/brackish coastal gradient. This ability will be especially useful where coastal marshes are periodically inundated with brackish water during drought years (Baldwin, personal observation) or are gradually shifting to brackish conditions due to relative sea level rise. Also, our previous experiments never evaluated a salt marsh species like *Spartina alterniflora* or *Spartina patens* so the proposed research will include these species.

Specific objectives are:

- 1. Determine whether salinity decreases near-infrared and increases visible reflectance of freshwater and salt/brackish marsh macrophytes;
- 2. Determine whether there is an interaction effect between nitrogen and salinity on near-infrared and visible reflectance of freshwater and salt/brackish marsh macrophytes;
- 3. Determine whether species has a significant effect on hyperspectral reflectance.
- 4. Determine whether PLS models that use hyperspectral reflectance can distinguish the nitrogen levels of leaf tissue across a gradient of salinity expected at the tidal freshwater/brackish interface.
- 5. Determine whether PLS models that use hyperspectral reflectance can distinguish the salinity of the water column across a gradient of salinity expected at the tidal freshwater/brackish interface.

<u>Timeline</u>. Originally, this was planned to be a one year project that started March 1, 2006 and ended February 28, 2007. However, we were not able to hire a graduate research assistant, which precluded us from completing the project by the original deadline. We were granted a nocost extension to February 28, 2008. With the hiring of Mr. Aaron Lewis from the MEES

program we are on target to complete the project by the new deadline. We will collect plant samples from local marshes in July and August of this year and transplant to the greenhouse microcosms where they will be used for the experiment. By end of August plants should be established so nitrogen and salinity treatments may begin. Treatments will be conducted during August and September. Data will be analyzed from September until January, and the final report will be written in February.

#### Methods, Procedures, and Facilities

**Procedures** 

Experimental Design. Greenhouse marsh microcosms containing one each of six common marsh species (Acorus calamus, Phragmites australis, Typha latifolia, Spartina patens and Spartina alterniflora) will be treated with four levels of salinity and four levels of N in a 5x4x4 factorial treatment arrangement in a randomized block design with four replicates of each treatment, resulting in 4 blocks of 16 microcosms (i.e., 64 microcosms in total) and 320 individual plants. A microcosm will consist of a black plastic tub with five pots each containing a different species. The randomized block design removes variation due to gradients of light, temperature, humidity, and other greenhouse variables. Target salinity levels will be 0, 1, 3, and 7 ppt while N levels will be background, 1, 5, 20 mg-N l<sup>-1</sup> (Table 2). Seedlings will be collected from marshes on the Patuxent River and propagated in plastic pots containing a peat-perlite mixture at the UMCP Research Greenhouse Complex, which offers state-of-the-art control over lighting, humidity, temperature, irrigation, and pests (UMCP 2003). Previously we have successfully collected plants for use in greenhouse studies using this method from Patuxent River (Baldwin et al. 2001; Tilley et al. in press) and Louisiana delta plain coastal marshes (Baldwin and Mendelssohn 1998). Plants will be supplied weekly with Hoagland's solution minus any N (Mendelssohn et al. 2001). Treatments will be prepared by combining Instant Ocean® synthetic sea salt (Aquarium Systems, Mentor, OH) and ammonium chloride in dechlorinated municipal water. Because ammonium is the dominant form of available N in wetlands, ammonium chloride has been used by us (Clarke and Baldwin 2002) and others (e.g., Wang 1991) in ammonia toxicity studies. Resulting chloride concentrations will be far below levels toxic to aquatic plants (230 mg L<sup>-1</sup>, USEPA 2002b). Treatments will be initiated by flushing and filling tubs with treatment solutions. Pots will be kept saturated by maintaining water levels 5-10 cm below surface. The experiment will last 4-6 weeks.

Table 2. Nitrogen and salinity treatments for marsh microcosms will be replicated four times with each microcosm containing one potted individual of each of five plant species (total microcosms = 64).

		Salinity (parts per thousand, ppt)				
		0 (S0)	1 (S1)	3 (S2)	7 (S3)	
Nitrogen (mg-N L <sup>-1</sup> )	Nominal (N0)	N0×S0	N0×S1	N0×S2	N0×S3	
	1 (N1)	N1×S0	N1×S1	N1×S2	N1×S3	
	5 (N2)	N2×S0	N2×S1	N2×S2	N2×S3	
	20 (N3)	N3×S0	N3×S1	N3×S2	N3×S3	

The levels of salinity chosen were based on ranges that we have observed at tidal freshwater marshes on the Nanticoke River (Baldwin, unpublished data). Normally, salinity levels are <0.5 ppt, but during the late summer months or drought years salinity can increase to several ppt due to decreased freshwater flow. Nitrogen levels were selected to bracket levels measured in Nanticoke tidal freshwater wetlands (1-2 mg/L, Tilley et al. 2004) to an upper bound level (20 mg-N/L) that could reasonably be expected to occur in wetlands receiving agricultural non-point source runoff or wastewater treatment discharge (some forms of agriculture such as confined

animal operations can generate even higher ammonium concentrations that are toxic to wetland plants; Clarke and Baldwin 2002).

<u>Data Collection</u>. Measurements of leaf photosynthesis, transpiration and hyperspectral reflectance will be made prior to application of treatments, near the mid-point of the experiment, and at the end of the 4 week period. Photosynthesis and transpiration (net CO<sub>2</sub> and H<sub>2</sub>O exchange, respectively) will be measured with a portable infrared gas analyzer (Analytical Development Company, Herts, England: model LCA-2). Leaves will be clamped with the LCA-2, ambient air pulled through at constant flow rate, and CO<sub>2</sub> and H<sub>2</sub>O inlet and outlet concentrations measured. Leaf reflectance will be measured with a hyperspectral radiometer (Analytical Spectral Devices, Boulder, CO: model ASD Handheld 325-1075 nm). Percent reflectance will be found by dividing leaf reflectance by the reflectance of a calibrated white panel (LabSphere, North Sutton, NH: Spectralon). The plants, located in black tubs, will be placed in full sun outside the greenhouse while photosynthesis, transpiration and reflectance are measured between 1000 to 1500 h with a 1° field-of-view foreoptic attached to the ASD. Leaf samples will be harvested from each plant at the end of the treatment period to analyze for N concentration at an independent lab.

<u>Data Analysis</u>. A full factorial mixed effects analysis of variance (ANOVA) will be conducted to examine the effects of N, salinity, and species on leaf photosynthesis, transpiration, spectral band reflectance, reflectance indices, and N concentration using SPSS for Windows 12.0 (SPSS Inc., Chicago, IL). Differences among means will be distinguished with Tukey's honestly significant difference procedure. Significant differences will be defined at the 0.05 probability level. Partial Least Squares (described below) regression will be used to develop predictive models of N and salinity treatment levels and leaf N concentrations. We will use Unscrambler 9.0 (Camo Process, Oslo, Norway) to conduct PLS. Sample data will be split into training and test sets to perform calibration and validation, respectively. The number of PLS components to include in the final model will be chosen for the model with the minimum root mean square error of prediction (RMSEP) based on the independent test set. The coefficient of determination of the final model will also indicate model efficacy. Various pre-processing transformations will be tested including normalization, first and second derivatives, and multiplicative scatter correction which can reduce scatter and non-linear effects.

#### **Facilities**

Our proposed research will be carried out using the Ecosystem Engineering Design Laboratory, Wetland Ecology and Engineering Laboratory, and University of Maryland Research Greenhouse Complex. Both laboratories are housed within the Department of Environmental Science & Technology at the University of Maryland, and are well-stocked with standard and advanced equipment and materials for ecological and environmental research including spectrophotometers, light meters, a portable photosynthesis system, a digital canopy LAI meter, hip and chest waders, soil augers, drying ovens, a muffle furnace, refrigerators, a grinding mill, balances, glassware, and safety equipment. Specific equipment used in the proposed research includes an ASD Handheld Hyperspectral Radiometer and a Spectralon Calibrated White Panel. Additionally, we have a 24-ft pontoon boat, a 17-ft single hull craft and a jon boat, all with trailers, that can be used to visit marsh sites for collecting donor plant material. The Biological Resources Engineering department also has several trucks and vans for towing the boats to collect plants.

#### **Results**

At this time we do not have any results to report. Data will be collected this summer.

#### Personnel

We have hired a Marine-Estuarine-Environmental Science (MEES) masters student as our graduate research assistant. He will begin work in July 2007. We also have employed 5 undergraduate research assistants to assist with the study during the last year. We have demonstrated the wetland radiometric assessment technique to students enrolled in Restoration Ecology (NRMT 444), which has an enrollment of 15-25 students. We also plan to demonstrate our methods to state and federal wetland managers.

Dr. David Tilley, an associate professor of ecological engineering in the Department of Environmental Science & Technology at the University of Maryland, is the lead investigator. His responsibilities include project oversight, supervision of student assistants, collection and analysis of radiometric data. Dr. Andy Baldwin, associate professor of wetland plant ecology in the Department of Environmental Science & Technology at the University of Maryl will assist with experimental design, plant identification, and inferential statistical analysis.

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