Abstract:

Coastal waters in Alabama and 28 creeks and rivers in Alabama and 15 in Mississippi are listed as impaired by high mercury levels, according to the US Environmental Protection Agency (EPA, 2006). These include Fish River, which is in the Weeks Bay, AL, NERR. However, concentrations in sediments are between 2 and 3 orders of magnitude higher than those in the (impaired) overlying water. Transfer of mercury from the sediment into the water is a critical component in the process of bioaccumulation that ultimately results in mercury-contaminated fish. Sediments contain very high numbers of microalgae and I demonstrated in a prior NERR GRF (1 year) that sediment-associated microalgae in the Weeks Bay NERR were contaminated with mercury. When these microalgae are resuspended into the water column by tides and winds, they transfer both nutrients for grazers (nitrogen, phosphorus) and mercury.

This project will examine the role that resuspension of benthic microalgae plays in making mercury available for trophic transfer by grazers. The food quality of microalgae in the sediment and in the water column will be quantified as N:P:Hg, through repeated sampling of 3 sites in the Weeks Bay NERR and 2 sites in a contrasting ecosystem, the Grand Bay, MS, NERR. The microalgae will be separated from other particulate material by centrifugation through colloidal silica. Food quality will be compared to the taxonomy and functional structure (i.e. proportion of benthic origin) of the microalgae. A direct comparison of transfer by benthic microalgae will be made by repeated sampling through 3 natural resuspension events (storms).

This project addresses the Research Focus Area of non-point source pollution and nutrient dynamics in both the Weeks Bay and Grand Bay NERRs. Because benthic microalgae and resuspension are ubiquitous, the findings will be applicable to other coastal and estuarine environments.

Background

Mercury contamination is rising throughout the United States. All Alabama Gulf Coast waters are listed as mercury impaired, according to Section 303d of the Clean Water Act (EPA 2006), as are 15 creeks and rivers in Mississippi and 27 in Alabama, including Fish River, part of the Weeks Bay, AL, NERR. The last broad survey of mercury in Mobile Bay, AL, (ADEM 1991) showed that sediment levels were 900-950 ppb in the Weeks Bay National Estuarine Research Reserve (Fig.1). Under funding from a prior one-year NERR fellowship, I demonstrated (Novoveska 2005) that there was up to 493 ppb of total mercury in benthic microalgae in Weeks Bay. Mercury is concentrated in higher trophic levels: Shelton (2005) measured up to 1,300 ppb of total mercury in largemouth bass from the Weeks Bay NERR, which exceeds the FDA approved maximum consumption level of 1,000 ppb. However, there was no correlation between mercury in periphyton and standard water-quality parameters (temperature, salinity etc.) nor was there a geographic gradient in the mercury load (Novoveska 2005), suggesting non-point source pollution with as-yet undescribed transfer mechanisms. Effects of non-point source pollution and nutrient dynamics are one of the Research Focus Areas of this panel.

Forms of mercury with relatively low toxicity can be transformed into forms with very high toxicity (methylmercury) through biological processes that take place in estuarine sediments (Heyes et al. 2004). Methylmercury is transferred more efficiently through the ecosystem and this form dominates in organisms (Mason 1996). Mercury concentration in the water is usually 2-3 orders of magnitude lower than in the sediments and biota (Absil and van Scheppingen 1996; Peres et al. 1997) and predators and herbivores receive the vast majority of mercury from their food source rather than directly from the water (Watras and Bloom 1992). The primary source of mercury in the biota is likely to be the sediment and bioaccumulation will therefore depend in part on the efficiency of transfer from the sediment to the water column. *I propose to measure the transfer between sediment and water column by resuspension of benthic microalgae.*

Benthic Resuspension and Mercury Transfer

Sediments serve as a substrate for transfer of contaminants between benthic and pelagic species (Mason and Lawrence 1999). Algae living on the sediment adsorb the contaminants directly (Genter 1996) and mercury uptake by microalgae is the largest single concentrating step in the chain of trophic transfers that leads to dangerous levels of mercury in top predators (Watras and Bloom 1992; Hill et al. 1996).

Resuspension of the benthos is critical to mercury transfer (Kim et al. 2006). While only a minor proportion of benthic microalgae are resuspended (Sloth 1986), the water-column can contain a high but temporally-variable proportion of displaced benthic species (Shaffer and Sullivan 1988). There is a vast disparity between concentrations of benthic and water-column microalgae (e.g. MacIntyre et al. 1996) and resuspension under realistic shear stresses can enhance water column chlorophyll concentrations by 3-11 $\mu g\ l^{-1}$ (MacIntyre et al. 2004). For comparison, the mean chlorophyll concentration in Weeks Bay and its tributaries is c. 20 $\mu g\ l^{-1}$ (Pennock et al. 2001), so resuspension represents a significant enhancement of food available to grazers in the water column. Resuspension is driven by winds and tides (Shaffer and Sullivan 1988; Litaker et al. 1993), as illustrated in Fig. 2. The resuspension of benthic microalgae into the water column transfers nutrients *and contaminants* while changing the physical and chemical composition of the water column. *Higher mercury concentration in the water column is a result of remobilization of mercury from sediments by benthic resuspension* (Faganeli et al. 2003).

Some microalgal taxa, notably pennate diatoms and euglenoids are *primarily* associated with sediments and are most likely to accumulate sediment-associated contaminants. I have already demonstrated that benthic diatoms in the Weeks Bay NERR, where sediment mercury is high, are themselves heavily contaminated with mercury (Novoveska 2005). Their resuspension would therefore introduce both nutrients (N, P) and mercury into the water column and make them available to grazers. Food quality for grazers has been parameterized as the N:P ratio (e.g. Rico-Villa et al. 2006) but modifying this as the N:P:Hg ratio allows the effect of mercury on food quality to be parameterized. Because of their association with high-mercury sediments, benthic microalgae are likely to differ in food quality (as N:P:Hg) from microalgae in the low-mercury water column. However, the mercury concentration in aquatic biota can vary seasonally (Herrin et al. 1998) and there can also be significant seasonal variation in the density and taxonomic structure of the benthic microalgae (MacIntyre et al. 1996). There are significant differences in the taxonomic structure of the water column microalgae over the cycle of seasonal succession (Fig. 3). *Consequently, it is important to consider seasonal variation in relative abundance and taxonomic structure of both the water column and benthic microalgae in assessing the impact of sediment-associated mercury on food quality.*

Goal and objectives

I hypothesize that benthic microalgae will have higher mercury content (as N:P:Hg) than water column microalgae. I also hypothesize that resuspension events, such as storms, will increase the benthic microalgal biomass in the water column and therefore increase the amount of mercury available for uptake (as N:P:Hg) by other organisms. These will be tested as follows:

1) By measuring sediment mercury concentrations at 3 sites in Weeks Bay and 2 sites in a contrasting NERR, Grand Bay, accounting for inter-seasonal variability and sampling through three resuspension events (storms);
2) Quantifying mercury concentration in both the benthic and water column microalgae;
3) Identifying the benthic and pelagic species present in the water column; and
4) Relating the food quality of both benthic and water column microalgae (as N:P:Hg) to the functional and taxonomic structure of each assemblage.

Site description

Weeks Bay NERR is a shallow, wind driven, microtidal estuary of 3,075 acres, located in Alabama on the eastern shore of Mobile Bay (Fig. 1). Direct freshwater discharge into the bay comes from the Fish River (73% of the flow) and Magnolia River (Schroeder 1996). Grand Bay National Estuarine Research Reserve is a marine protected area located in extreme southeastern Mississippi in Jackson County (Fig.1). The Grand Bay NERR is comprised of approximately 18,000 acres, found chiefly within the Grand Bay National Wildlife Refuge and the Grand Bay Savanna Coastal Preserve. It contains a variety of tidal and non-tidal wetland habitats, including salt marshes, saltpans, and bays and bayous. Grand Bay is well flushed with relatively nutrient-poor and clear high-salinity water from the Gulf of Mexico and is therefore a contrasting ecosystem to the relatively eutrophic, turbid and brackish waters of Weeks Bay (Fig. 4). Atmospheric deposition of mercury has recently (2005 – 2006) been measured at Weeks Bay by USGS and is currently being measured at Grand Bay by NOAA.

Methods

Sediment with associated microalgae and water column microalgae will be sampled at three stations in Weeks Bay and two stations in Grand Bay four times in Year 1 (Fig.1). Doing so will account for temporal and spatial variability in sediment mercury due to cycles of deposition and resuspension, and for variability in the abundance and taxonomic composition of the microalgae. Sediment will be sampled with a pole-corer (Onuf et al. 1994) and water samples will be collected in acid-washed polycarbonate carbuoys. Microalgae and other particulate material will be concentrated from the water samples by continuous-flow centrifugation (Hamilton et al. 2005). Microalgae will be separated from the sediment and from other particulate material in the seston by centrifugation through Ludox TM-50 colloidal silica (de Jonge 1979; Hamilton et al. 2005).

Mercury concentration in the bulk sediment, seston and microalgae will be determined by inductively-coupled plasma-mass spectrometry (ICP-MS; detection limit of 0.1 ppb of total Hg), by a commercial lab (Trace Element Laboratory, Dartmouth, NH). Microalgal biomass and taxonomic structure will be described by microscopic identification and HPLC quantification of marker pigments (Van Heukelem et al. 1992; Mackey et al. 1996) to determine changes in biomass and composition between the sites and seasons. Turbidity and total seston will be measured (Etcheber 1981), as will temperature, conductivity, salinity, pH and dissolved oxygen (YSI 556 Multiprobe System). Particulate N and P concentrations will be determined on a Costec CHN analyzer and a Scalar San+ autoanalyzer respectively (Solorzano and Sharp 1980). Each analysis will be performed on 3 independent samples for true replication (Hurlbert 1984).

ISCO automated water samplers will be deployed before and during three natural disturbance event (storms) in Weeks Bay in Year 2. The samplers will collect water every 2-3 hours during the event for total of 12 samples per event. These samples will be analyzed in the same manner as seasonal samples to quantify changes in water column microalgal abundance, taxonomic structure and food quality (as N:P:Hg).

Analysis of variance (Sokal and Rohlf 1981) will be used to determine whether mercury concentrations differ among bulk sediment, bulk seston, benthic and pelagic microalgae and between sites and over time. Regression analysis will be used to test for a simple relationship between sediment mercury concentration and the concentration in benthic microalgae. Principal components analysis (PCA) and multi-dimensional scaling (MDS) will be used to infer the relationship between the taxonomic structure of the water column microalgae (i.e. proportion of cells/biomass of benthic origin and by taxon) and food quality (N:P:Hg).

Project Significance

Fish River, part of the Weeks Bay NERR, is listed as mercury impaired (EPA 2006) as are Alabama Gulf Coast waters and 28 creeks and rivers in AL and 15 in MS. Fish from the Fish River are not safe to eat. However, prior research has shown that mercury is a non point-source pollutant, so effective monitoring and management will depend on an understanding of the mechanisms by which mercury is transferred through the food web. Mercury in Weeks Bay and other estuaries is concentrated in sediments and the dynamics of its transfer into the water column are poorly understood. This proposal addresses the primary step in sediment/water column coupling and transfer of nutrients (N, P) and mercury, through which potential bioaccumulation in the water column will proceed. Non-point source pollution and nutrient dynamics are the first of four focus areas of this program. The proposed mechanism of resuspension of benthic microalgae is general and widespread. As such, the findings of this project will be widely applicable to other estuarine systems.

Maps and Figures

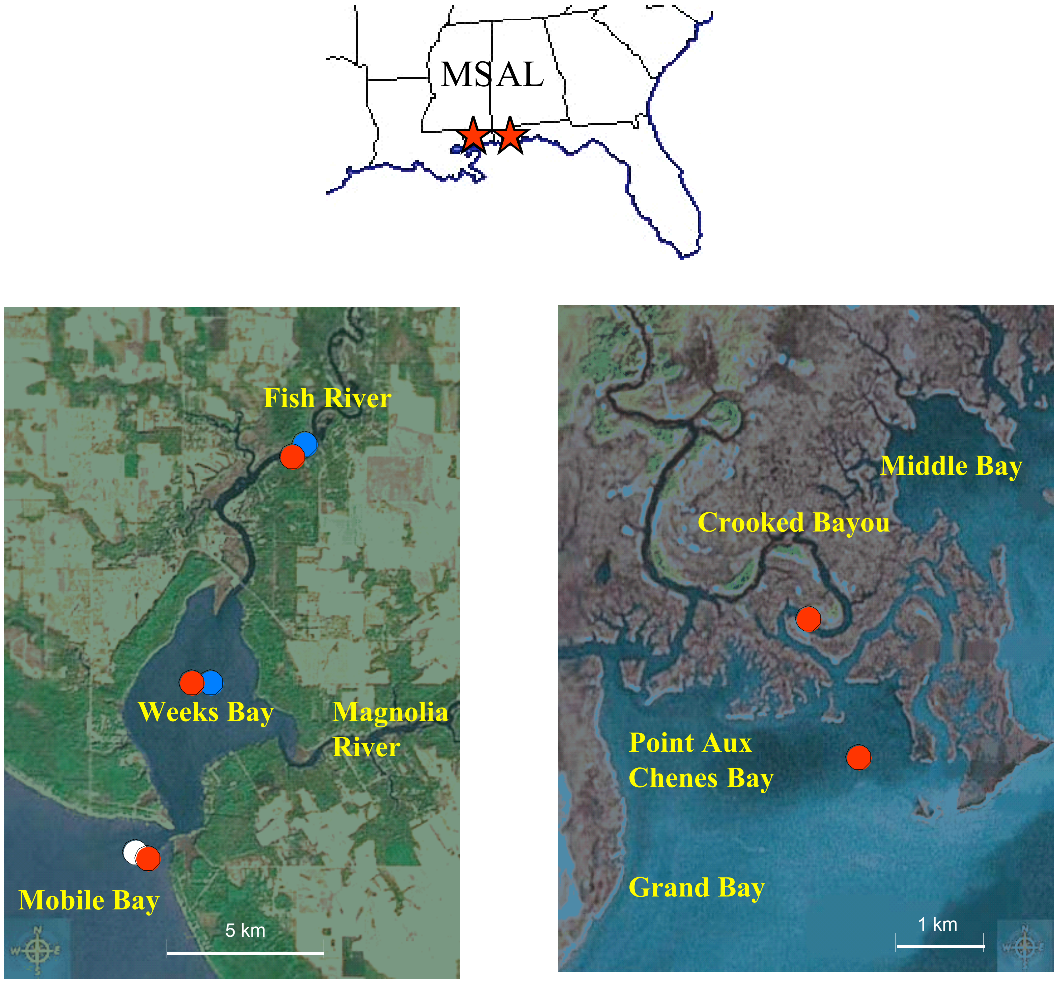

Fig.1 Weeks Bay NERR, AL (left), with proposed research sites (red). Microalgae composition was determined at the mouth of Weeks Bay (white). ADEM (1991) measured 900 ppb in the sediments in the middle of the bay and 950 ppb in southern parts of Fish River (blue).
Grand Bay NERR, MS (right), with proposed sites (red).

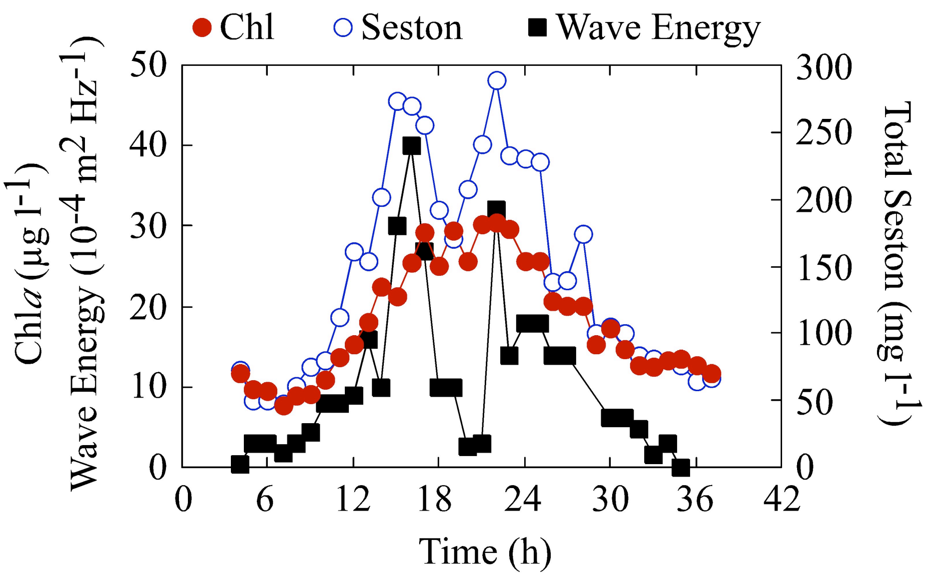

Fig. 2 Storm-driven resuspension of chlorophyll *a* and seston in the Choptank River, MD, 8/27-28/1998. Wave energy was calculated from the frequency spectra of 10-minute records of water height, collected at 5 Hz. Data are from Chiscano (2000).

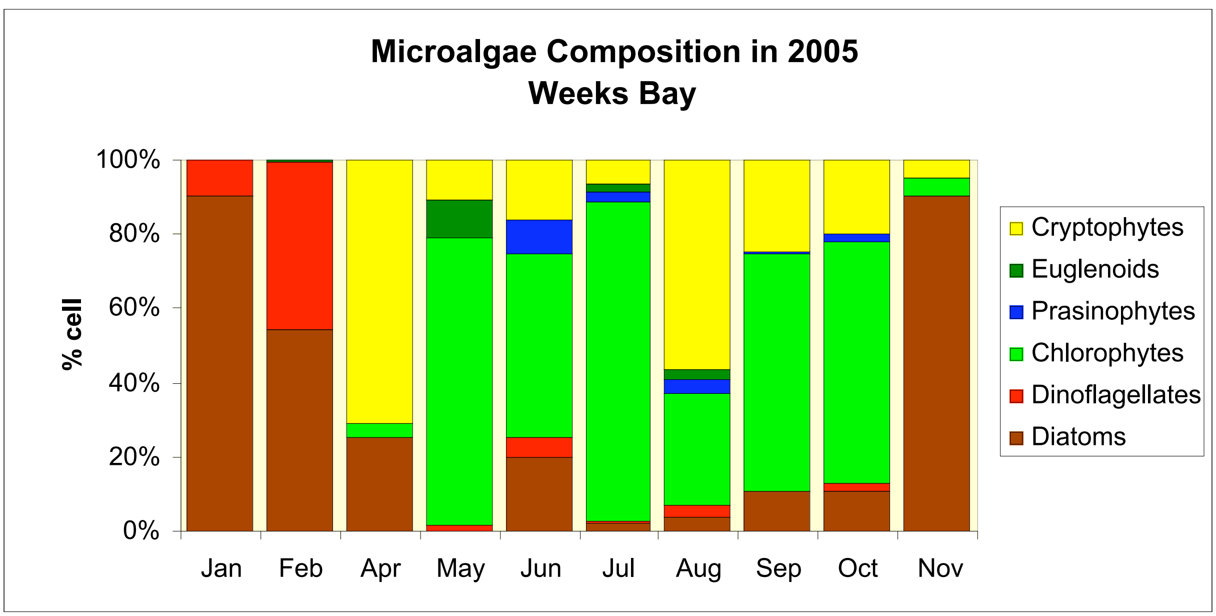

Fig. 3 Microalgae composition follows an annual cycle with diatoms dominating in the winter and high abundance of chlorophytes during the summer. Taxonomic data are based on the cell counts outside the mouth of Weeks Bay (Data: MacIntyre, unpubl.).

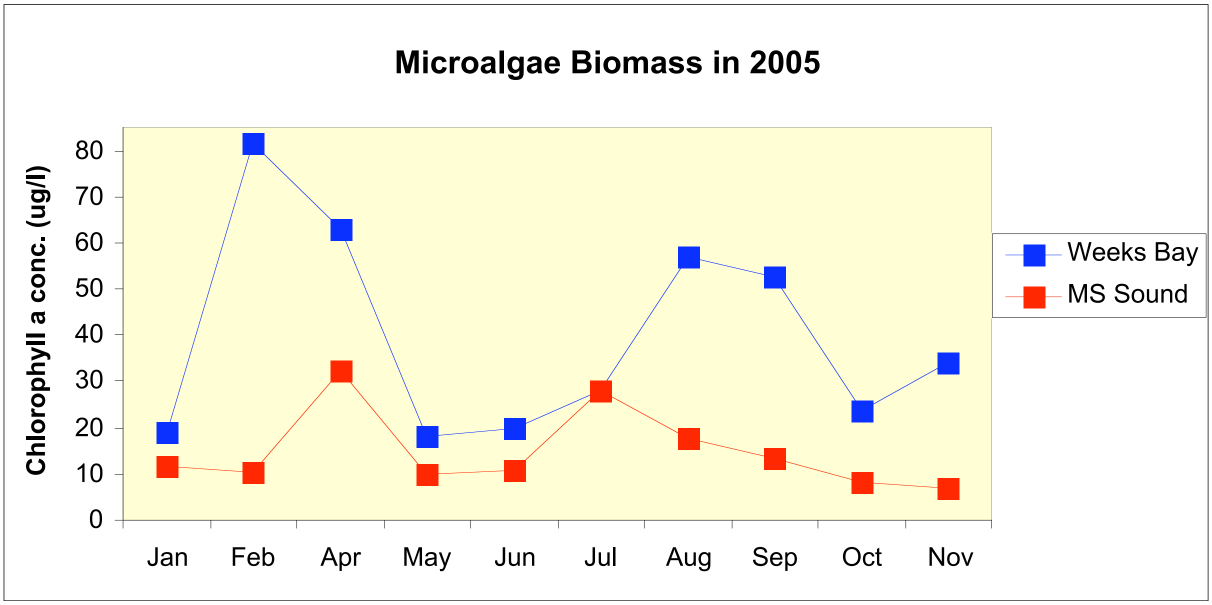

Fig. 4 Annual cycle of microalgal biomass shows geographical differences. Weeks Bay site has generally higher biomass than Mississippi Sound, adjacent to Grand Bay NERR. Sites were sampled monthly over a year (Data: MacIntyre, unpubl.).

Milestone Schedule

Year 1

Month 1	Field work, sample collection, filtering, centrifugation, mercury analysis
Month 2	Nutrient analysis, algae identification, analysis of field equipment data
Month 3	Preparation of instruments for second sampling, analysis of data
Month 4	Sample collection, filtering, centrifugation, mercury analysis
Month 5	Nutrient analysis, algae identification, analysis of field equipment data
Month 6	Preparation of instruments for second sampling, analysis of data
Month 7	Sample collection, filtering, centrifugation, mercury analysis
Month 8	Nutrient analysis, algae identification, analysis of field equipment data
Month 9	Preparation of instruments for second sampling, analysis of data
Month 10	Sample collection, filtering, centrifugation, mercury analysis
Month 11	Nutrient analysis, algae identification, analysis of field equipment data
Month 12	Data analysis, statistical analysis, preliminary report

Year 2

Month 13	Storm tracking, ISCO deployment, sample collection, filtering, centrifugation, mercury analysis
Month 14	Nutrient analysis, algae identification, analysis of field equipment data
Month 15	Preparation of instruments for next sampling, analysis of data
Month 16	Storm tracking, ISCO deployment, sample collection, filtering, centrifugation, mercury analysis
Month 17	Nutrient analysis, algae identification, analysis of field equipment data
Month 18	Preparation of instruments for next sampling, analysis of data
Month 19	Storm tracking, ISCO deployment, sample collection, filtering, centrifugation, mercury analysis
Month 20	Nutrient analysis, algae identification, analysis of field equipment data
Month 21	Preparation of instruments for second sampling, analysis of data, preliminary statistical analysis, preparation of final report
Month 22	Storm tracking, ISCO deployment, sample collection, filtering, centrifugation, mercury analysis
Month 23	Nutrient analysis, algae identification, analysis of field equipment data
Month 24	Data analysis, statistical analysis, final report

Personnel and Project Management

I will be primarily responsible for completing the research described in this proposal, under the direction and guidance of my Ph.D. supervisor, **Dr. Hugh MacIntyre**, Senior Marine Scientist, Dauphin Island Sea Lab, and Assistant Professor, University of South Alabama (joint appointment). I conducted research in the Weeks Bay watershed on mercury bioaccumulation by periphyton assemblages, with funding from a prior 1-year NERR fellowship and am qualified for sample collection and analysis. **Dr. MacIntyre** is an oceanographer with expertise on microalgal dynamics in estuaries.

Throughout completion of the project, I will have guidance from members of my graduate advisory committee, which consists of:

Dr. Eugene Cioffi, Associate Professor, Department of Chemistry, University of South Alabama, with expertise in biogeochemistry of mercury;

Dr. Ruth Carmichael, Dauphin Island Sea Lab, Assistant Professor, Department of Marine Sciences, University of South Alabama, with expertise in biogeochemistry, benthic- pelagic coupling and nutrient dynamics;

Dr. Kelly Major, Assistant Professor, Department of Biology, University of South Alabama, with expertise in the physiology of algae and algae responses to stress and disturbance;

Dr. Robert P. Mason, Professor of Marine Sciences, Department of Marine Sciences, University of Connecticut, with expertise in the fate, transport, and transformation of mercury in the ecosystem.

Additional support will be provided by **Dr. Scott Phipps,** research coordinator at Weeks Bay NERR, and **Dr**. **Mark Woodrey** research coordinator of Grand Bay NERR. Both Dr Phipps and Dr Woodrey have written letters of support for this project.

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