Project title: **The effect of crude oil contamination on microbial communities in salt marshes and potential impact on detritus breakdown**

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Cluster: "Understand" cluster

Objectives:

1. To determine whether the presence of crude oil in salt marshes alters the composition of microbes in the community;
2. To test the hypothesis that the presence of petroleum hydrocarbons in the salt marsh provides an alternative carbon source that negatively impacts the natural breakdown of plant detritus.

Rationale:

Bacteria, Archaea and fungi are critical to the function of all ecosystems. This is mainly because they are abundant and thus have immense cumulative mass and activity. Their role in global carbon cycling has received a lot of research interest in relation to climate change and energy flow. In ocean and coast ecosystems, microbial communities dominate the process of decomposition and transportation of organic matter. In salt marsh ecosystems, microbial decomposition of marsh plants and detritus makes available particulate organic matter (POM) and dissolved organic matter (DOM) that serve as the main carbon source for heterotrophic bacteria in the water and sediment. Heterotrophic bacteria thus pump the carbon flux into the microbial loop and to higher trophic levels and serve as a critical link in carbon-flow in marine ecosystems.

An important problem that has received attention recently is the fact that the majority of POM and DOM are refractory to degradation by general bacteria. Polymeric particles of organic matter such as cellulose, hemicelluloses, lignin and chitin, cannot be taken up directly by general bacteria but need to be first solubilized by bacteria that produce extracellular digestive enzymes. Heterotrophic bacteria then grow on the assimilable DOM produced by POM degrading bacteria.

When a salt marsh is polluted by crude oil from a spill, the composition, structure and functions of the microbial community change. Petroleum hydrocarbon provides a ready source of energy and carbon. Oil-degrading bacteria such as *Pseudomonas* and *Bacillus* can dominate the community and suppress the growth of bacteria that normally degrade plant detritus and chitin. We hypothesize that such an event would result in less assimilable carbon in the DOM pool on which other heterotrophic bacteria normally depend and a disruption on the normal coastal carbon cycle follows. This results in a reduction in the abundance of protozoan and zooplankton that normally feed on heterotrophic bacteria and so forth up the food chain, ultimately affecting the productivity of coastal ecosystems. Furthermore, many recalcitrant chemicals in crude oil may last for a long time in the environment and inhibit metabolic activity of plant detritus- and chitin-degrading bacteria.

We propose to study the effect of spilled crude oil on the microbial community of affected salt marsh in coastal Mississippi and Louisiana. We plan to examine differences in the composition of Bacteria and Archaea between salt marsh sites where visible oil is present and at clean reference sites. We plan to assay for functional differences at the mRNA and activity level of enzymes that breakdown cellulose, hemicelluloses, lignin and chitin. Our long-term goal is to be able to model the effect of crude oil contamination on nutrient cycling in salt marshes along the Northern Gulf. In addition, because we

will be monitoring rates of change in microbial composition as oil is degraded, we will be able to make predictions concerning the rate of salt marsh recovery

Research Plan and Methods

Water, sediment and detritus samples will be collected from at least two salt marsh sites where crude oil has washed ashore and two clean reference sites bi-monthly for the analyses listed below.

Microbial community profiling

The composition and structure of microbial communities will be analyzed by PCR-DGGE using universal primers for ssu-rRNA genes of Bacteria and Archaea. Microbial communities will be compared among sites and over time to determine whether shifts in abundance of the more dominant microbes occur. Sample DNA fingerprints will be compared using the software BioNumerics and microbial diversities will be analyzed using the Shannon index. Principle component analysis and cluster analysis will be used to determine variations and similarities in community profiles among samples. The identity of amplified DNA excised from DGGE gels will be made by sequencing. The objective for doing this work is not only to identify the microbes that are present but also to infer their potential functions in the carbon cycle through *in silica* analyses. For example, the presence and abundance of marine Bacteroidetes, specialists in the degradation of polymeric organic matter pool are of great interest.

Functional gene analyses

Functional genes involved in the decomposition of cellulose, hemicelluloses, lignin and chitin will be analyzed using RT-PCR and qRT-PCR. Total RNA will be extracted from field samples and reverse transcribed prior to amplification. Microbial genes encoding polymer-degrading enzymes such as cellulosae, lignase and chitinase have been widely studied and their related PCR primers are available. For example, genes encoding β 1, 4-glucosidase, xylosidase, laccase and chitinase responsible for the degradation of cellulose, hemicellulose, lignin and chitin, respectively, are well studied and PCR primers and nucleotide probes are available. For this part of the study, we are interested in determining the difference in expression level of these genes at the community level among sites and over time. RNA extracted will be normalized to sample weight or volume (in the case of water samples). RT-PCR analyses will be used to verify whether genes of interest are being expressed and qRT-PCR will be used to quantify expression levels.

Hydrolytic enzyme assays

To determine whether differences in the expression level of genes described above among salt marsh sites are actually biological real at the enzyme function level, we plan to assay activities of enzymes extracted from water and sediment samples. Decomposition of polymeric POM involves many extracellular enzymes released by microbes. For cellulase, we plan to assay for β 1,4-glucosidase and cellobiohydrolase activities using fluorometric methylumbeliferyl (MUB) substrates including 4-MUB-β-D-glucoside and 4-MUB-β-D-cellobioside. Other related enzymes such as xylosidase, laccase and chitinase, can be detected using other fluorometric substrates. In addition, because polysaccharide decaying activity is associated with acquisition of nitrogen and phosphorus, we also plan to assay for alkaline phosphatase and β-N-acetyl-b-glucosaminidaseglucoside using microplate assays.

Budget:

Personnel	
PI, one month summer	7,102
Graduate student, 12 months	18,000
Part-time technician	15,000
Fringe	
PI, one month summer	2,052
Graduate student	900
Part-time technician	5,415
Materials & Supplies	
Research supplies	18,000
Travel	6,800
Total Direct Costs	73,269
Indirect Costs: 46.5%	34,070
Total Project Costs:	107,340

Resume - Shiao Wang

Professional Preparation

William Carey College	Biology	BS	1977
University of Southern Mississippi	Biological Sciences	MS	1981
Louisiana State University	Zoology & Physiology	PhD	1986

Appointments

2008 -	Professor, Dept. of Biological Sciences, USM
1998 - 2008	Associate Professor, Dept. of Biological Sciences, USM
1992 - 1998	Assistant Professor, Dept. of Biological Sciences, USM
1989 - 1992	Visiting Assistant Professor, Dept. of Biological Sciences, USM
1986 – 1989	Post-doctoral Fellow, Biology Division, Oak Ridge National Laboratory

Recent Publications

Carr, M.R., S.Y. Wang, T.I. McLean, C.J. Flood and R.D. Ellender. 2010. Salmonella rarely detected in Mississippi coastal waters and sediment. J. Applied Microbiol. (In press)

Flood, C., J. Ufnar, S. Wang, J. Johnson, M. Carr and R. Ellender. 2010. Lack of correlation between enterococcal counts and the presence of human specific fecal markers in Mississippi creek and coastal waters. Water Res. (In press)

Cao, Z.M., S.Y. Wang, V. Breeland, A.M. Moore and J.M. Lotz. 2010. Taura syndrome virus loads in the hemolymph of *Litopenaeus vannamei* following infection and the relationship to differential mortality. Dis. Aquat. Org. 91:97-103.

Harwood, V.J., M. Brownell, S.Y. Wang, J.E. Lepo, R.D Ellender, A. Ajidahun, K.N Hellein, E. Kennedy, X.Ye and C. Flood. 2009. Validation and field testing of library-independent microbial source tracking methods in the Gulf of Mexico. Water Res. 42:4812-4819.